**FULL DOCUMENT SUBMISSION FOR THE ONLINE SUPPLEMENT**

**Draft Guidance for Industry Duchenne Muscular Dystrophy, Becker Muscular Dystrophy, and Related Dystrophinopathies**

**Developing Potential Treatments for the Entire Spectrum of Disease**

# Main Guidance Sections

1. The Science of Patient Engagement and Patient Experience Assessment
2. Background

**Patient-focused drug development (PFDD) is the systematic approach to ensuring patients’ experiences, perspectives, needs, and priorities are captured and meaningfully incorporated into drug development and evaluation** [89]. The United States (US) Food and Drug Administration (FDA) encourages patient engagement by sponsors throughout the entire product lifecycle. Documenting the experience and perspectives of patients and caregivers can inform an array of decisions related to medical product development and evaluation. Patient engagement supports regulatory science by providing guidance on frameworks, methods, and approaches to measuring patient experience. Patient experience data collection spans approaches such as patient-reported outcomes (PROs) and other patient-relevant outcomes through clinical outcome assessments (COAs), patient and caregiver narratives, and patient-preference information (PPI).

FDA is committed to advancing sponsors’ awareness of the various methods to engage patients and their caregivers. We encourage sponsors to dedicate resources and time to the systematic and robust collection of the patient experience data (PED), including PPI. We encourage these data to be used within both the regulatory context and within the drug development paradigm from start to finish. Along the way, consultation should occur with FDA, patients, caregivers, advocacy organizations, clinical trial sites, and investigators. We encourage sponsors to discuss with the FDA and patient groups as early as possible, the types of patient experience data that may support a regulatory submission and be most suitable from a patient perspective.

1. Existing FDA Guidance

The FDA has been developing a series of guidance documents pertaining to the science of patient engagement. Recent guidance documents have focused on approaches and methods that are applicable to numerous steps of the drug development process. These examples span from agency-attended, patient-focused drug development meetings to implementing fit-for-purpose tools to collect meaningful patient and caregiver input for use in regulatory decision making. The following guidance documents have been mandated by the 21st Century Cures Act and the Prescription Drug User Fee Act (PDUFA) commitments:

1. [Patient-focused drug development (PFFD) guidance and meeting re](https://www.fda.gov/drugs/development-approval-process-drugs/fda-patient-focused-drug-development-guidance-series-enhancing-incorporation-patients-voice-medical)ports

* [Collecting Comprehensive and Representative Input. Guidance for Industry, Food and Drug Administration Staff, and Other Stakeholders](https://www.fda.gov/regulatory-information/search-fda-guidance-documents/patient-focused-drug-development-collecting-comprehensive-and-representative-input) (2018) [23]. This finalized guidance provides an overview of methods to collect robust, meaningful, and sufficiently representative patient input to inform medical product development throughout the drug development process.
  + [Methods to Identify What Is Important to Patients. Guidance for Industry, Food and Drug Administration Staff, and Other Stakeholders](https://www.fda.gov/regulatory-information/search-fda-guidance-documents/patient-focused-drug-development-methods-identify-what-important-patients-guidance-industry-food-and) (2022) [24].
  + This guidance describes methods for the collection and submission of patient-relevant information such as burden of disease and benefits and risk which can be used for medical product development and regulatory decision-making.
  + [Patient Perspectives on Gene Therapy Products (2022)](https://www.fda.gov/media/168183/download#:~:text=The%20FDA%20seeks%20to%20understand,uptake%20of%20future%20marketed%20products.) [90].

A listening meeting covered important issues related to gene therapy risks and benefits, patient and caregiver involvement in study designs and execution, and methods and tools useful to capture PED in gene therapy studies. As an initial accelerated approval for gene therapy in DMD occurred in 2023 with other studies ongoing, the perspectives in this resource are particularly timely and relevant.

1. PFDD draft guidance to FDA

* [**Selecting, Developing, or Modifying Fit-for-Purpose Clinical Outcome Assessments**:](https://www.fda.gov/regulatory-information/search-fda-guidance-documents/patient-focused-drug-development-selecting-developing-or-modifying-fit-purpose-clinical-outcome) This guidance will address methods to measure impacts and prioritize endpoints in a meaningful way. A [public workshop](https://www.fda.gov/drugs/news-events-human-drugs/patient-focused-drug-development-guidance-methods-identify-what-important-patients-and-select) was held in 2018 and 2022 (https://www.fda.gov/drugs/news-events-human-drugs/public-webinar-patient-focused-drug-development-selecting-developing-or-modifying-fit-purpose), and a draft version of this document has been [released](https://www.fda.gov/regulatory-information/search-fda-guidance-documents/patient-focused-drug-development-selecting-developing-or-modifying-fit-purpose-clinical-outcome) [91].
* The Medical Device Innovation Consortium has also published resources as part of their [Science of Patient Input (SPI) initiative](https://mdic.org/project/patient-input-in-clinical-trial-design) on methodologies to systematically identify outcomes that matter most to patients and to establish these outcomes as primary or secondary endpoints for clinical studies [92].
* [**Incorporating Clinical Outcome Assessments into Endpoints for Regulatory Decision Making:**](https://www.fda.gov/regulatory-information/search-fda-guidance-documents/patient-focused-drug-development-incorporating-clinical-outcome-assessments-endpoints-regulatory) This guidance will address methodologies, standards, and technologies that may be used for the collection, capture, storage, and analysis of clinical outcome assessment (COA) data. The guidance will also address methods to better incorporate COAs into endpoints that are considered significantly robust for regulatory decision-making. A public workshop was held to obtain feedback from stakeholders for input on this guidance [93].

1. Other guidance related to patient experience data:

* Patient Preference Information in Medical Device Decision-Making [25].  
  The Center for Devices and Radiological Health launched the Patient Preference Initiative with a guidance document providing recommendations on the voluntary collection of patient preference information that can be submitted as valid scientific evidence of patient and stakeholder perspectives for consideration as part of the benefit-risk assessment during the regulatory review of new devices or products.
* [Benefit-Risk Assessment for New Drug and Biological Products](https://www.fda.gov/media/152544/download) [94].

This guidance lays out important considerations that factor into FDA’s benefit-risk assessments, including how patient experience data can be used to inform the benefit-risk assessment.

1. Duchenne Patient Experience Data Related to Patient Preferences

The Duchenne community has been a leader in providing data to regulators related to patient experience. This has included activities such as providing testimonies about experimental treatments at FDA hearings and conducting rigorous qualitative and quantitative research to collect and analyze patient preference data. The Duchenne community has led efforts to advance patient preference research in rare disease using a variety of stated preference and other survey methods through a community engaged approach [95]. Over the course of 8 years, preference data elicited directly from patients and caregivers have generally demonstrated a tolerance for risk and uncertainty in exchange for a therapy that could stop or slow disease progression [28]. Additional studies have explored parental worries [29], symptom prioritization for treatments [27], meaningful benefit in pulmonary outcomes [30], caregiver vs. patient preferences [30], clinical trial decision making, preferences for emerging gene therapies [31-33], and patient experience with standard-of-care treatment [96]. Qualitative narratives collected from the community have also been analyzed and submitted to FDA [34].

1. Key learnings from studies to date:

**Patients and caregivers are willing to trade off on risks, treatment burden, uncertainty, and treatment benefits, including slowing disease progression.**

* Patients and caregivers generally have similar preferences for treatments, but preferences may vary by age and stage of disease.
* Preferences can differ based on therapeutic intervention.
* Priorities for treatment outside of skeletal muscle include potential therapies targeting cardiac and pulmonary function.

1. Meetings with FDA:

In 2018, “The Duchenne Patient-Focused Compass Meeting” was held in partnership across several US-based Duchenne patient advocacy groups. Modeled on FDA’s externally led PFDD meetings, the Compass Meeting was composed of panels of Duchenne community members (patients and caregivers) to explore “living with Duchenne”. The discussions included clinical trial and therapeutic experiences and access to approved therapies. A detailed report was produced from this meeting:   
[The Duchenne Patient-Focused Compass Meeting Report](https://www.parentprojectmd.org/wp-content/uploads/2018/07/PPMD_Compass_Meeting_Report.pdf) [35].

1. How Patient Experience Data Can Advance Drug Development Programs

FDA believes there is value in patient engagement early and often across the product life cycle.As part of their engagement strategy, sponsors can gather patient experience data that can aid in decision-making at various stages of drug development. Frequent engagement with patients and caregivers over the course of research and product development can keep sponsors apprised of preference changes over time. Continuous engagement is essential because patient experience is not static. Rather, the viewpoint of patients can evolve over time due to a variety of variables, influences, or events. Examples of events that may modify patient preferences and their insights on experiences could include disease progression, approval of other treatments, and changes in standard of care. Throughout the entire product life cycle, we suggest that sponsors consult with the community through direct engagement and research methodologies such as the following:

* Qualitative interviews
* Focus groups or stakeholder meetings
* Patient and caregiver surveys
* Patient preference studies
* Quality of life substudies
* Direct engagement through patient advocacy meetings

These approaches can help inform both sponsor decisions and regulatory recommendations.

**Table 1. Temporal Framework for Patient Engagement and Patient Experience Assessment**

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1. Conclusion/Call to Action

In summary, we strongly recommend companies collect PED across their drug development programs to better understand patient preferences, priorities, and assessments of risk/benefit. Similarly, we encourage sponsors to have early interactions with the agency in order to understand FDA’s intent to consider such data in review. This includes obtaining feedback from the relevant FDA review division on appropriate research designs and clarifying applicable regulatory requirements. Taken together, strategic and thoughtful engagement with both patients and the FDA will offer the best opportunities for successful product development that results in meaningful impact on Duchenne.

Appendix:

### Statement of Patient Experience

Section 3004 of the 21st Century Cures Act directed the FDA to report on the use of PED in regulatory decision-making, focusing on the review of patient experience data and information on PFDD tools. The reporting tool below is now required to be filled out by FDA on approved products as part of the FDA decision memo.

Sponsors should review potential patient experience data that has been published or that could be collected over the course of your development program and submitted within an NDA (refer to Figure 1).

**Figure 1: Statement of Patient Experience**

A close-up of a medical survey

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When reviewing the statement of patient experience, recognize that relevant PED can come directly from trial participants as well as collected from the general Duchenne and Becker population (see Figure 2).

**Figure 2: Statement of Patient Experience Checklist Items**

A diagram of a patient's research

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1. Criteria for Diagnosis in the Clinical and Research Settings
2. Background

Dystrophin-associated muscular dystrophies, caused by pathogenic variants in the *DMD* gene, are allelic disorders having a broad range of phenotypes. The most common and sentinel form is Duchenne muscular dystrophy (DMD). Research over the past 4 decades has led to a better understanding of the clinical features and genetic basis of dystrophinopathies, especially DMD. This knowledge base has generated multiple research efforts to target the *DMD* gene and restore some level of a partially functional dystrophin protein. This, in turn, has led to numerous clinical trials in the past two decades, largely in young ambulant boys with DMD. Establishing the diagnostic criteria for DMD and other dystrophinopathies is important both in the clinical setting and in defining the population for a clinical trial. This updated Diagnostic section of the revised Guidance was developed to provide greater clarity in the current understanding of what is meant by DMD, how the diagnosis is confirmed, and how this can be useful for pharmaceutical companies designing clinical trial protocols.

1. Classic Duchenne Muscular Dystrophy
2. Clinical features

Historically, the diagnosis of Duchenne muscular dystrophy (DMD) was made on the basis of five criteria: (1) typical presenting features and the age of onset in a boy, (2) positive family history of DMD, when present, (3) marked elevation in the serum creatine kinase (CK) level, (4) typical dystrophic features on muscle biopsy, and (5) loss of independent ambulation by age 12 years [7, 97]. The first three of these criteria remain the core clinical features of classic DMD (Figure 3), but the diagnosis of DMD is made using a combination of clinical and laboratory findings. Genetic confirmation has now replaced muscle biopsy in the majority of cases. However, muscle biopsy is still used in the research setting as it is useful for monitoring response to approved or investigational therapies.

Typical presenting features of DMD are delayed milestones or impaired motor function: abnormal walking, including clumsy gait and toe-walking; frequent stumbles and falls; difficulty running, arising from the floor, jumping, and climbing stairs; muscle fatigue—unable to keep up with peers. Calf enlargement (“pseudohypertrophy”) is a classic finding and can range from subtle to marked. About one-third present with delayed speech and communication skills, a harbinger of cognitive impairment [98, 99]. Behavioral issues (eg, poor attention, hyperkinesis, and hyperemotionality) may also be seen. These neurocognitive and neuropsychiatric features are consistent with, but are not necessary, for the diagnosis of DMD. Symptom onset is typically at age 2 to 3 years, with the diagnosis made approximately 2 years later, although the diagnostic odyssey can be further prolonged when symptoms go unreported, are unrecognized or attributed to other causes by healthcare professionals, or when there are financial barriers to completing a specialist referral [100, 101]. Modest gains in motor function may occur until age 7 years, after which all boys with DMD decline. Loss of ambulation (LOA) is a less useful criterion now as current care guidelines recommend early (ages 4 to 6 years) initiation of daily oral glucocorticoid medication, which prolongs ambulation an average of 3 years [101]. The glucocorticoid-related improvements in motor outcomes can complicate clinical trials of *DMD* gene-targeted therapies since they often use motor function tests as the primary outcome measure for young ambulant patients (see also the Natural History section).

Cardiac and pulmonary compromise is typically evident in the second decade, and even with optimal supportive care death now occurs in the third or fourth decade due to cardiac failure. Clinical trials in older patients (second decade and beyond) typically include cardiac and pulmonary assessments, especially in the older nonambulant patients, where it may be a primary outcome measure (refer to the Cardiomyopathy section).

Family history of DMD is a strong predictor that a boy with presenting features of muscular dystrophy will have a DMD trajectory. This remains a useful prognostic factor in diagnosing DMD, but genetic confirmation is still necessary.

Marked elevation in the serum CK level, often to more than 100-fold the upper limit of normal, is characteristic of DMD [102]. Other forms of dystrophinopathy may be more variable, from mild to marked elevations in the CK level, and rarely can be normal. Other forms of muscular dystrophy and myositis also share this nonspecific feature. Thus, an elevated CK level is a necessary but not sufficient test in isolation to establish the diagnosis of DMD. Incidental identification of elevated transaminases (aspartate transaminase [AST], alanine transaminase [ALT]) is commonly encountered and evaluation for muscle disease should be considered before a liver biopsy or other workup.

Prior to the advent of genetic testing for DMD, electromyography and muscle biopsy were mainstays of testing to support the clinical diagnosis. This is no longer the case for the vast majority of patients, and they are no longer a part of the diagnostic criteria in the clinical setting.

1. Genetic confirmation

Genetic confirmation of a pathogenic sequence or copy number variant in the *DMD* gene is a requirement for the clinical diagnosis of dystrophinopathy. A specific type of variant may be a requirement for an approved therapeutic or a clinical trial where the experimental drug is applicable only for those individuals with a certain type of variant, eg, specific deletions for exon-skipping drugs, or a premature stop variant for a read-through drug. Variants in the *DMD* gene are typically consistent with loss-of-function (frameshift or nonsense) but given the very large size of the *DMD* gene, some variants are challenging to identify. Several genetic testing platforms are in use currently to identify pathogenic variants in the *DMD* gene: multiplex ligation-dependent probe amplification (MLPA) and chromosomal microarray (both mainly identify large deletions and duplications), Sanger sequencing (for single nucleotide variants), and next generation sequencing (NGS) of the *DMD* gene or several genes if part of a neuromuscular panel. With increasing use of NGS, whole exome sequencing and whole genome sequencing technologies for diagnosing individual patients and for general population screening, variants of uncertain significance (VUSs) in the *DMD* gene are becoming more common. Investigators should proceed with caution regarding inclusion of individuals with an atypical phenotype and no clear pathogenic variant in trials. VUSs should be re-analyzed using updated in silico databases, eg, ClinVar or the Leiden DMD database. If only a VUS is identified or no variant is identified, muscle biopsy may be necessary to support the diagnosis and for inclusion in trials (see Figure 3: Diagnostic algorithm for DMD and BMD).

1. Genotype-phenotype associations

The identification of a frameshift variant (deletion, insertion) or pathogenic variant in the *DMD* gene (MIM 300377) establishes the diagnosis of a dystrophinopathy, having five allelic phenotypic variants: Duchenne MD (310200) and its milder variant, Becker MD (300376); and more rarely X-linked dilated cardiomyopathy (302045); exercise-induced myalgias with myoglobinuria; and “hyperCKemia” without clinical symptoms or signs. Frameshift variants are typically predictive of a DMD phenotype (approximately 96% positive predictive value) and in-frame variants predictive of milder BMD (approximately 93% positive predictive value). However, there are exceptions to the reading frame rule. Certain out-of-frame deletions in the *DMD* gene are associated with a BMD or DMD phenotype with a slower rate of progression, due to increased dystrophin expression (eg, the 5’-end deletions can lead to BMD instead of DMD, but exon 44 skip-amenable deletions are generally found in DMD with a slower disease progression). Findings from a gene panel may identify variants in other genes which may be potentially clinically significant, and this may need to be considered in clinical trial criteria.

1. The role of muscle biopsy

Muscle biopsy for identification of typical histological dystrophic features is no longer considered necessary to establish the clinical diagnosis of DMD, with some exceptions [36]. DMD is considered a loss-of-function condition with total or near total absence of dystrophin protein. About half of DMD patients show some revertant fibers, and this can lead to a small amount of dystrophin seen on Western blot analysis (typically less than 5% of normal levels) [10]. A 2019 workshop report summarized current and emerging technologies to quantify dystrophin expression [55]. Reports using Western blot analysis have given varied results and quantification of immunohistochemistry staining has been challenging, with limited reproducibility and precision among different laboratories. Newer technologies, such as immunoaffinity liquid chromatography, tandem mass spectrometry and capillary Western analysis [103, 104] may provide greater precision, and have demonstrated substantial overlap in dystrophin expression between DMD (mean 5.4%, range 0.4-24.1% of control level) and BMD (mean 31.7%, range 4-84.5% control level) [104]. These newer technologies will require further validation. Thus, using dystrophin quantification from a muscle biopsy is not definitive for segregating DMD from BMD, but may be useful in a clinical trial setting to identify a change in the level of dystrophin expression in response to an investigational therapy. When a pathogenic variant in the *DMD* gene is not identified in a patient suspected as having DMD, RNA sequencing on muscle tissue may provide the answer, especially with identification of intronic pseudoexonic mutations.

1. Other Allelic Variants of Dystrophinopathy
2. Becker muscular dystrophy (BMD)

The diagnostic criteria for BMD are represented in Figure 3. There is a wider range of phenotypic variability in BMD than in DMD. Many BMD patients exhibit a slightly later onset and slower rate of progression, with LOA after age 16 years with no steroid use [10] and after 19 years with chronic steroid use [101]. Other BMD patients may present in mid to later adulthood with mild proximal weakness, activity-related myalgias, muscle fatigue and mild elevation of CK. Genetic confirmation remains necessary to establish the diagnosis. There remains the risk of significant dilated cardiomyopathy in patients with BMD across this range of severity, sometimes leading to heart transplantation. Identification and characterization of patients with BMD will be important for clinical trials given the broad range of phenotypes.

1. Intermediate form of dystrophinopathy (IMD)

The intermediate form of dystrophinopathy was historically defined as those who lost ambulation between the ages of 13 and 15 years [10]. Now that the vast majority of boys with DMD are treated with steroid medication, and with the diversity of *DMD* mutations, the boundary between DMD and BMD can be considered continuous. This has made IMD largely indistinguishable from DMD and the designation has now been abandoned. However, there remains an indistinct zone between a mild DMD and severe BMD phenotype. Clinical trials will need to address how stringently the criteria are set for a DMD diagnosis.

1. Carrier females

Although true manifesting females with symptoms as severe as DMD males are rare, there is a wide range of clinical variability in females with dystrophinopathy and a higher percentage of all female carriers show cardiac dysfunction on imaging assessments than previously thought. Females with muscle symptoms may have a higher level of X-alleles with a *DMD* mutation due to skewed X chromosome inactivation. Clinical trials involving cardiac therapies may want to consider an arm for females having some element of cardiac dysfunction.

1. X-linked cardiomyopathy

This is uncommon and will not be discussed further.

1. Exercise-induced myalgias with myoglobinuria

This is uncommon and will not be discussed further.

1. HyperCKemia

Patients with asymptomatic elevations in creatine kinase (hyperCKemia) have been identified with in-frame deletions in the *DMD* gene. This topic will not be discussed further.

1. Large scale deletions of Xp21 with contiguous gene syndrome

This involvement of neighboring genes (adrenal hypoplasia, glycerol kinase, and others). Although uncommon, this needs to be considered when a boy has additional clinical concerns beyond those typical of DMD and can be readily assessed with genetic testing. Clinical trials would likely exclude such patients from participation.

1. The Presymptomatic Patient

Population-based newborn screening for DMD is evolving and likely to be implemented more broadly within the coming years as early treatments become available. A nomination to add DMD to the Recommended Uniform Screening Panel (RUSP) was submitted in 2022. The formal evidence review process began in 2023, with the final vote anticipated in 2024. Newborn screening approaches to date identify patients with DMD on the basis of having an elevated CK-skeletal muscle (CK-MM) level on a heel stick blood specimen, leading to genetic testing for conditions associated with such an abnormality, including DMD. The CK screening test is sensitive but nonspecific at birth, so must be followed up with an additional confirmatory test for an elevated CK and/or confirmatory DNA testing soon after birth.

Prenatal diagnosis of DMD or BMD has been available for many years. Prenatal diagnosis is typically done when there is a positive family history of DMD, or when a woman is identified as a DMD carrier via expanded carrier screening. Cell-free fetal DNA from maternal blood may be used for sex determination in the first trimester. Fetal DNA testing from a chorionic villus sampling (CVS) or amniocentesis-derived specimen, which can be performed as early as 10 week’s gestation for CVS, offers the possibility for testing for a *DMD* variant. However, only about half of current DMD cases show a previous family history due to the high spontaneous mutation rate of the gene [105]. The carrier rate of approximately 57%-61% is higher than the reported family history as some of these mothers silently carry the pathogenic variant [106]. Further, population-based screening of either mothers for DMD carrier state, or fetuses for *DMD* gene mutations, remains challenging due to the diversity of variants and difficulty interpreting variants with no family history.

A newborn with DMD is considered asymptomatic, and while young infants with DMD typically show delays in reaching motor milestones and lower scores on the Bayley Scales of Infant and Toddler Development, Third Edition (Bayley-III) assessments of motor function, diagnosis is typically not made until near the age of kindergarten/early school years [107]. As treatments for DMD evolve, it is natural to anticipate clinical trials in these presymptomatic infants. This will optimize the opportunity for a favorable response to these therapeutics, as MRI studies have shown that significant muscle is already lost at young ages [108]. Figure 3 presents a diagnostic algorithm for these patients. A diagnosis of DMD may lead to testing of other male family members, where presymptomatic patients may be identified. In addition, the incidental finding of an elevated CK level may lead to the identification of a presymptomatic patient with DMD or BMD.

**Figure 3: Dystrophinopathy Diagnostic Algorithm**

A diagram of a flowchart

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1. The Current Understanding of the Natural History of Duchenne Muscular Dystrophy
2. Introductory Comments

In the *Draft Guidance for Industry on Common Issues in Drug Development for Rare Diseases*, the FDA counsels sponsors to make an early evaluation of the “depth and quality of existing natural history knowledge to determine if it is sufficient to inform their drug development programs” [38]. Regarding DMD, the understanding of natural history has significantly evolved over the past two decades, primarily due to patient registries, natural history studies, and data acquired from the placebo arms of industry trials that aligns closely with the natural history data [16, 109, 110]. Several clinical trials, albeit unsuccessful (as the pre-specified analysis did not demonstrate the benefit of the experimental treatment) offer insights that can help sponsors select more relevant, clinically meaningful outcome measures and guide the selection of participants, who are poised, in the absence of treatment, to exhibit meaningful disease progression as assessed by the endpoints over the course of the study—a technique known as prognostic enrichment.

Despite the advancements in the understanding of the natural history and identification of the sources of heterogeneity in disease progression, the pressing need for improved therapies for DMD persists. The unmet medical need is emphasized by the ongoing and severe impacts of the disease, despite improvements in the standard of care, and recent FDA accelerated approvals of novel treatments offering modest benefits to some patients. The predominant disease course remains progressive quadriparesis within the first two decades due to dystrophin deficiency and the loss of skeletal muscle fiber, with concomitant development of pulmonary insufficiency from skeletal muscle involvement and cardiomyopathy leading to substantially shortened lifespans even for those receiving optimal care.

It is important to note that, although the majority of this section centers around DMD, subsequent portions of the document will extend natural history information to sponsors exploring research into treatments for BMD and other dystrophinopathies.

1. Overview of Natural History in Duchenne Muscular Dystrophy

Sponsors should note that the progression of DMD, from infancy onwards, can be monitored using diverse evaluation tools and tests measuring developmental delay, functional loss, and other progression parameters. The use of milestones of disease progression and outcome measures in natural history studies and patient registries has enhanced the characterization of the disease’s natural history (refer to Figure 4).

The subsequent schematic (Figure 4) provides a general overview of the stages of disease progression, with violin plots displaying the medians and interquartile ranges of the approximate ages, based primarily on clinical evidence since the widespread use of corticosteroids in DMD clinical management. The schematic illustrates that the loss or delay of clinical milestones is a hallmark of DMD disease progression. Some milestones such as hopping and jumping may never be attainable without corticosteroid treatment in most individuals. The difficulty in performing functions and milestones loss occur in a predictable sequential order, both before and after the loss of ambulation (LOA), with some minor overlap or variation. The timing of the loss of milestones can be associated with the timing of subsequent functional decline later in the disease course.

The schematic also highlights the outcome measures with substantial data supporting their utility in DMD that are commonly used to characterize progression at different disease stages [111]. Note that the schematic does not infer whether specific thresholds or outcomes on those measures are clinically meaningful or have utility as potential clinical endpoints. More insights on specific outcome measures, their correlation to future disease milestones, and inclusion in clinical intervention trials can be found in sections, “Considerations for DMD Outcome Measurement Selection” and “Specific Trial Design and Analysis Issues for Clinical Trials in DMD”.

A chart of different colored shapes

Description automatically generated with medium confidence**Figure 4: Stages of DMD progression, monitored by milestones, and captured by multiple outcomes measures/clinical endpoints**

1. A. The stages of DMD disease progression

Models of disease progression

Several models of disease progression utilized in clinical management can aid in informing clinical development programs. In the DMD Care Considerations (DCC), the progression of DMD is segmented into five primary stages: an early symptomatic phase (when the majority of young children are diagnosed), early and late ambulatory stages, and early and late nonambulatory stages.

The Ambulatory Functional Classification System for DMD (AFCSD) [112] also proposes a 5-stage system; each level is defined with varying degrees of mobility and independence:

* **Level 1,** walking at normal speed with normal postural alignment
* **Level 2,** walking independently without assistive devices or braces but exhibiting abnormal gait patterns, such as tiptoeing or waddling, and impaired postural alignment such as excessive trunk lordosis
* **Level 3,** walking across only short distances using hand-held mobility devices such as a walker or crutches
* **Level 4,** unable to walk, may use a powered wheelchair
* **Level 5,** needs to be transported in a manual wheelchair

Additional models like the UC Davis Duchenne Functional Milestone Model for measuring disease progression [17]and the HERCULES model (Figure 5), offer more detailed insights into clinically meaningful transitions and outcome measures of interest within ambulatory and nonambulatory stages, focusing on outcomes that are important to patients and caregivers, including the ability to stand supported, transfer one’s weight, and bringing hand to mouth. For example, the UC Davis Duchenne Functional Milestone Model categorizes DMD patients into 5 ambulatory stages based on performance of Timed Function Tests (TFTs) predictive of LOA, and four nonambulatory groups derived from upper limb performance on the Brooke scale (Figure 6 below) that correlates to the patient’s self-care and independence.

The HERCULES model also integrates four nonambulatory stages after loss of ability to stand independently, assessing hand-to-mouth-function (HTMF) alongside pulmonary outcome measures indicative of the need for noninvasive ventilation and continuous daytime ventilation, as per the DCC, even though the relationships between some parameters are still awaiting validation [15].

**Figure 5: The HERCULES Model [39]**

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**Figure 6: Performance of the Upper Limb (PUL) (Entry Item)**

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**Box 1: UC Davis Duchenne Functional Milestone Model [17]**

* **Milestone Group 1:** Time to stand from supine <5 seconds (s)
  + *Time to stand from supine* is an important prognostic factor of changes in 6-min walk distance and, more broadly, of disease progression [113]. A threshold value of approximately 5s differentiates patients likely to show stability or improvement from those likely to decline [114].
* **Milestone Group 2:** Time to stand from supine ≥5 and <10s
  + Patients with stand from supine values of 5s or longer are likely to experience decline in function and, possibly, loss of standing ability, but are not at imminent risk for loss of time to climb four stairs or ambulation [115].
* **Milestone Group 3:** Time to stand from supine ≥10s
  + Patients with stand from supine values of 10s or longer are at risk for losing ambulation within the next 2 years [21].
* **Milestone Group 4:** Cannot stand from supine in <30s but can still climb four stairs
  + Patients with loss of stand from supine are at risk of losing ambulation over the next 2 years [21].
* **Milestone Group 5**: Cannot climb four stairs but can still complete the time to run or walk 10 m test
  + These late ambulatory patients who have lost the ability to climb four stairs in ≤30s are at imminent risk of loss of ambulation [21].
* **Milestone Group 6:** Early nonambulatory patients who have a Brooke score of 1 indicating full overhead reach:
  + These patients are at risk of losing full overhead reach as assessed by either the Brooke upper extremity functional rating scale or performance of upper limb measure [116].
* **Milestone Group 7:** Nonambulatory patients with a Brooke score of 2-4, indicating a loss of full overhead reach but retained hand-to-mouth function (HTMF)
* **Milestone Group 8:** Nonambulatory patients who have transitioned to a Brooke upper limb score of 5, indicating loss of unweighted HTMF (but retained hand function)
* **Milestone Group 9:** Nonambulatory patients with a Brooke score of 6, indicating loss of functional use of the hands (unable to pick up objects, drive a power wheelchair hand control, or access technology with the hands)

This overview, while being loosely based on spectrum of disease stages described in the DCC series, integrates insights from the UC Davis and HERCULES models to portray a nuanced representation of the current natural history of DMD. While functional changes are observed at approximate ages, the intent is to illustrate typical disease progression without imposing rigid artificial stages of disease.

DMD is due to generalized skeletal muscle involvement and cardiomyopathy, with pathological processes active concurrently throughout a patient’s life. While the initial focus may be on the loss of ambulatory capacity and gross motor functions in ambulatory boys, signs of neuromuscular deterioration may already be measurable in upper limb, respiratory and cardiac functions.

DMD progression is marked by compensations for weakness and modifications in movement quality and ease; however, the relationship between strength and functional decline is not linear. Changes in strength may cause little change in function, but there are times when subtle changes in strength can trigger significant functional loss quite precipitously, pushing an individual with DMD rapidly beyond a threshold of clinically meaningful disease progression.

Some patients might never accomplish certain early developmental milestones such as hopping, jumping, and running, or achieve them only transiently and at a later age of acquisition. There is heterogeneity in the rate of disease progression among individual patients. However, progression generally follows a fairly typical and consistent fashion in terms of the timing of loss of functional abilities, the compensatory strategies used to perform tasks in the setting of weakness, and the sequential order of the loss of clinically meaningful milestones. An extremely high degree of consistency in progression has been observed across populations and data sources for changes in outcome measures such as TFTs, the 6-minute walk test (6MWT) and the North Star Ambulatory Assessment (NSAA) [117]. Nevertheless, some patients’ progression may diverge from the standard descriptions of the disease progression stages outlined here.

Neonates/infancy (until around 1 year of age):

Clinical discussions of DMD have often omitted neonates and infants, due, in part, to the rarity of DMD diagnosis in infancy, although it is anticipated that the adoption of newborn screening will alter this scenario in the coming years (refer to the Diagnosis section, part IV: The Presymptomatic Patient). Even though this stage is referred to as presymptomatic, manifestations of the disease are present from birth. Predominantly, infants diagnosed through newborn screening or identified via family history exhibit developmental delays when assessed with instruments such as the Griffiths Mental Development Scales (applicable to those aged 6-47 months) [118], and the Bayley Scales of Infant and Toddler Development, Third Edition (Bayley-III), administered from 1-42 months of age [119-121]. Neurocognitive deficits, as gauged by Developmental Quotients (DQ) may be pronounced among boys with specific DMD mutations, although compromised performance on the locomotor subscale is a universal occurrence, independent of the mutation present [122].

Early symptomatic childhood (ages 12-48 months):

The first few years after infancy are characterized by delays in achieving developmental milestones and impaired acquisition of motor skills*.* The attainment of gross motor milestones is typically delayed compared to boys without DMD, and some children may show signs of delayed language development and cognitive impairment. Toddlers and young children, when evaluated with developmental outcome measures such as the Bayley-III and Griffith’s Developmental Scales, have gross motor scores that are lower than those of age-matched controls [119, 120].Subsequent repeated measurements might reveal further declines in gross motor scores compared to typically developing peers. Analogously, cognitive and language scores are also lower compared with typically developing children. As parents and caregivers begin to observe these delays and deficiencies, it usually marks the start of the diagnostic odyssey (refer to the Diagnosis section). However, despite these challenges, young boys with DMD continue to grow and eventually reach most developmental milestones, while their motor skills, albeit below the level of performance of age-matched controls, show improvement over the next few years.

*Key milestones at this stage:*

* Delays or failure to achieve developmental milestones and acquire certain skills (importantly, these could serve as indicators of disease trajectory; refer to Box 1)
* Impaired ability to jump, hop, run, and rise from the floor
* Presence of Gower’s sign when attempting to stand

Young ambulatory (from approximately ages 4-7 years):

During this period, there may be slow gains in some measures while others might exhibit modest functional declines. Any ambulatory function gains are notably slower than observed in typically developing children, often deviating from normative ranges very early in the disease course as illustrated in the 100-meter timed test, 6MWT, and 10-meter walk/run tests. Variations in functional gains or losses are also observable with tools like the North Star Ambulatory Assessment (NSAA). In addition, corticosteroid therapy, commonly initiated during this stage (shortly post-diagnosis), could lead to further gains or stabilization of functional parameters for approximately 6 to 12 months, subsequently reaching a plateau and then a decline, with a decrement from the baseline values at the time of steroid initiation by 30 to 36 months. However, despite improvements in performance due to growth and maturation, physiologic deterioration is ongoing, causing boys with DMD to increasingly fall behind the normative performance levels of their peers who are developing typically.

*Key milestones during this stage:*

* Potential gains in unilateral hopping, jumping, standing on heels, and single-leg standing, particularly post initiation of steroids
* Young ambulatory boys may also exhibit temporary improvements compared to baseline function in certain skills and increased velocity in stand from supine, 4-stair climb, and 10-meter run/walk due to maturation and growth
* However, those with the most severe trajectories may experience a loss in the ability to standing from the floor (eg, stand from supine) and loss of the ability to transition from lying supine to sitting

Late ambulatory (from approximately ages 7-15 years):

This stage is characterized by a swift decline in gross motor and upper limb function, and some pulmonary function parameters, primarily in the early second decade of life. There is marked, progressive muscle fiber loss in the proximal muscles, leading to increasing weakness and the gradual loss of gross motor skills and ambulatory functions, including the ability to stand, climb stairs, and walk (LOA). Skeletal deformities, notably ankle equinus contractures and iliotibial band contractures, may emerge, accompanied by risks of osteopenia and fractures. There is also a deviation in height gain and increased weight gain in comparison with their normally developing peer group. Sponsors should be informed that, during this period, individuals maintaining stable disease (in terms of functional performance) can be distinguished from those likely to experience gradual or rapid progression—as noted by the UC Davis model—based on scores or performances on specific prognostic outcome measures such as TFTs, the 6MWT, and NSAA [17]. For example, a time to stand from supine below 5 seconds can differentiate patients likely to maintain stability or show improvement from those who are likely to experience functional decline; a time to stand from supine of greater than 10 seconds predicts loss of standing ability; and exceeding 10 seconds in a 10-meter run/walk test predicts both LOA within 2 years and the onset of upper limb function loss (refer to Box 1, UC Davis Duchenne Functional Milestones model).

*Key milestones during this stage:*

* Loss of the ability to stand from the floor
* Loss of stair climbing ability
* Loss of ability to stand from a chair
* Loss of ability to walk (LOA) independently (as defined by an inability to perform 10-meter walk/run)

Transition/able to stand and transfer (approximate ages 10-15 years):

As children in the late ambulatory DMD start to experience a rapid decline in ambulatory function, coupled with the initial onset of pulmonary decline and a slight loss of upper limb abilities, there may be a fleeting transitional phase might occur, either between or overlapping the late ambulatory and early nonambulatory stages. Although the age range during which this phase may manifest is wide, the duration of this phase itself tends to be quite brief, typically spanning a matter of months.

As delineated by the HERCULES model, this stage includes individuals with DMD who can no longer walk or run 10 meters but crucially can still stand or transfer bearing most, if not all, of their own weight. Eventually, they may only be able to stand briefly and with assistance. While the HERCULES model (refer to Figure 2) specifies this function as 100% weight bearing (the ability to stand independently), the ability to stand with assistance and partially support one’s weight is meaningful to patients and caregivers, given the burden of unassisted transfers [23].

*Key milestones during this stage:*

* Loss of the ability to take steps (eg, short distances less than 10 meters)
* Loss of the ability to stand in place independently
* Loss of the ability to stand with contact guard support (eg, with support to hips and/or knees)

#### Early nonambulatory (beginning when a boy starts using a wheelchair full-time, usually between ages 10-16 years):

Nonambulatory status is generally defined as the inability to walk 10 meters within 30 seconds. The transition to nonambulatory status is typically delayed by approximately 3 to 3.5 years when daily corticosteroids are used [17]. After LOA, boys need powered mobility and also undergo continued muscular deterioration in both their upper and lower limbs. Skeletal deformities such as limb contractures may arise due to the static positioning of weakened limbs (where joints cannot actively traverse the full range of motion against gravity), with significant fibrotic and fatty infiltration of muscle tissue and sarcomere shortening. While postural maintenance and sitting balance are initially intact, they are progressively lost. For patients without steroid treatment, spinal deformities often become problematic.

Subsequently, there is increasing loss of upper limb function, impacting activities like reaching overhead, dressing independently, and bringing hand to mouth for self-feeding). Both the UC Davis Disease Progression Milestones and HERCULES model add granularity to this stage by distinguishing between patients based upon the degree of losses in upper limb function (on the Brooke Scale or Performance of Upper Limb [PUL] entry items), and in the case of the HERCULES model, incorporating measures of pulmonary function predictive of different ventilation needs.

The UC Davis Disease Progression model (refer to Box 1) differentiates between those with a Brooke scale of 1 (PUL entry of 6), who are at risk of losing full overhead reach, and those with a Brooke score of 2-4 (PUL entry 5 to 2), who have lost full overhead reach but still retain HTMF. According to a patient and caregiver survey, HTMF is the most important function for quality of life among caregivers and parents and the second most important function for nonambulatory patients [124]. Retention of HTMF is also an important criteria for the HERCULES staging system, along with distinctions based on forced vital capacity (FVC) % predicted values related to recommendations for nocturnal noninvasive ventilation (<50% FVC) and continuous day and night diurnal ventilation (< 30% FVC) that are part of the DCC but yet to be validated clinically. Throughout the early nonambulatory stage, declining pulmonary function leads initially to the need for mechanical cough assistance and progressive risk of hypoventilation requiring noninvasive mechanical ventilation during night and, subsequently, both day and night. The ability to shift trunk position independently while seated also holds substantial value for patients’ quality of life. Cardiomyopathy becomes evident by either cardiac echocardiography or cardiac MRI in virtually all patients. Additionally, after transition to a wheelchair, patients tend to gain more weight compared to their normally functioning peer group.

*Key milestones during this stage:*

Upper-limb function (measured by the Brooke scale or PUL entry criteria; see Figure 3):

* Loss of ability to reach overhead
* Loss of ability to touch the scalp
* Loss of moving a weighted hand to mouth (such as an 8-ounce glass of liquid or 200 g weight)
* Loss of an unweighted hand to mouth (associated with loss self-feed ability without adaptations)
* Loss of sitting balance (requiring corrective trunk positioning or trunk shifting to maintain balance over the pelvis while sitting)

Pulmonary function:

* Loss of normal ability to cough/clear airway (necessitating a mechanical cough assistance device)
* Requirement for nocturnal ventilatory assistance (using a bilevel noninvasive ventilation device)

Late nonambulatory (onset ranges from ages 15 years and older, until the third decade of life):

The key milestone that marks the onset of the late ambulatory stage is the loss of unweighted HTMF. Almost all patients who have lost HTMF also have FVC % predicted values below 50% [49]. For patients with cognitive challenges preventing the completion of pulmonary function testing, loss of HTMF serves as a proxy for a 50% threshold of FVC,indicating the need for noninvasive mechanical ventilation. The median age at loss of HTMF in DMD patients not treated with steroids is a median of 15.4 years, compared to 20.5 years in steroid-treated patients.21 In this stage, the weakening of core and upper limb strength make maintaining function and good posture increasingly difficult, necessitating enhanced support from seating systems including power recline and postural support for both the trunk and head. After unweighted HTMF has been lost (Figure 2 and Box 1), upper extremity function is severely constrained, primarily to distal fine motor function and tabletop activities. According to nonambulatory patients in a patient and caregiver survey, repositioning oneself in bed was the most important function for quality of life [124]. The ability to control a wheelchair joystick and access a computer independently are also crucial functions for quality-of-life. Due to losses in chest wall and expiratory muscle function, virtually all patients require mechanical cough assistance, and there is a high risk of hypoventilation during both night and daytime, requiring noninvasive ventilation due to worsening diaphragm muscle function. Risk for dysphagia and aspiration exist, and optimal nutritional management may require the placement of a gastrostomy tube and enteral formula supplementation. Adequate phonation may become an issue, late in the disease course. Older DMD patients have numerous unmet medical needs, with respiratory impairment and cardiomyopathy (heart failure and conduction abnormalities) becoming the chief causes of morbidity and, eventually, mortality. It should be important to note that with optimal care, adults with DMD are increasingly living into the late third to fourth decade, and this has allowed the accumulation of new insights regarding their specific needs.

*Key milestones during this stage:*

Upper-limb function:

* Loss of the ability to place hands on the tabletop
* Loss of independent trunk positioning in bed (loss of the ability to reposition oneself independently)
* Loss of the ability to use a computer/control wheelchair due to compromised distal hand function
* Loss of the ability to operate a joystick/power wheelchair
* Loss of head control

Pulmonary function:

* Need for intermittent mouthpiece ventilatory assistance during the daytime
* Need for full- or near full-time ventilatory assistance

Notes regarding pulmonary and cardiac milestones:

Both progressive limb weakness and decline in pulmonary function are due to skeletal myopathy, and pulmonary decline correlates with skeletal muscle functional measures. However, there is a discordance between cardiac disease progression and skeletal muscle disease progression [124].

In the DCC, the need for mechanical cough assistance and part or full-time ventilatory assistance was based on peak cough flow (PCF) and FVC thresholds, such as FVC < 50% predicted, FVC < 30% predicted, which has been found to lack precision [6]. Pulmonologists are refining the definition of true hypoventilation in individuals with DMD by employing more nuanced measures including data from sleep studies (polysomnography) and in-home measures of hypoventilation (capnography) as discussed in more detail in the Outcome Measures section.

Cardiac deterioration due to progressive cardiomyopathy may not exhibit a consistent correlation with skeletal muscle deterioration [114]. With increased lifespan due to effective ventilation interventions, cardiomyopathy has become the leading cause of death among DMD patients [21, 125]. While the symptoms of cardiomyopathy are difficult to assess in nonambulatory patients due to their limited physical activity, cardiac natural history is increasingly being defined by serial cardiac imaging, especially as MRI has become more widespread. Cardiac MRI is able to visualize fibrofatty replacement of the myocardium through late gadolinium enhancement (LGE) and also provides reproducible measurements of cardiac dimensions and systolic function. Fibrofatty replacement of the myocardium is typically the earliest manifestation of disease and begins on average in the mid-teens. Following the development of LGE, left ventricular (LV) systolic function begins to decline in a process that culminates in the development of heart failure. Arrhythmias may present in later stages of disease as fibrofatty replacement of the myocardium progresses and as systolic dysfunction develops. Although arrhythmia burden generally corresponds with fibrofatty replacement of the myocardium and systolic function, risks of atrial ectopy and arrhythmia, including atrial fibrillation, exist even in patients with preserved systolic function. Moreover, isolated cases of clinically significant arrhythmias have been reported across the spectrum of disease, suggesting the electrophysiologic phenotype may be different than other types of dilated cardiomyopathy (see Cardiomyopathy section of this guidance).

*Cardiac milestones*

* Fibrofatty replacement of the myocardium typically begins at around age 14. This can be visualized by LGE on cardiac MRI.
* Systolic function typically begins to decline following the development of LGE.
* The progressive fibrofatty replacement of the myocardium is associated with worsening systolic function and culminates in the development of heart failure.
* As cardiomyopathy progresses, arrhythmias, including those that are life threatening, develop.

1. Heterogeneity in DMD disease progression: predictability and sources of variability

Boys with DMD of the same age can progress at markedly different rates, which has created challenges for the interpretation of therapeutic trials. However, a clearer understanding of the disease, and the reasons for variability in disease progression is emerging with more extensive data drawn from natural history studies, real world data, and the placebo arms from DMD treatment studies. Increasingly, sponsors are able account for sources of heterogeneity when designing their phase II and phase III studies. The following critical elements are identifiable and can have a large enough effect size that they need to be managed as sources of variability in trial designs.

Disease severity / trajectory class / stage of disease

The stage and severity of an individual’s DMD affects the rate of progression. For instance, baseline levels of function can predict subsequent disease progression in DMD. Typically, higher baseline function, or stabilization of baseline function over the short-term, lead-in period of observation, is associated with slower long-term decline [110, 126].Conversely, a lower baseline function may be linked with a rapid subsequent decline in ambulatory endpoints, especially when patients have passed critical thresholds of strength and function. Among ambulatory boys, the course to loss of ambulation may encompass distinct trajectory classes [68]. Therefore, sponsors should consider that baseline measures of ambulatory capacity or other functional capacities such as upper limb and/or pulmonary function, can and should be used to stratify cohorts in DMD trials in ambulatory boys and to predict changes in an endpoint over time.

The age at the loss of clinically meaningful milestones, serving as a proxy for disease severity, also predicts the age at which future milestones will be lost. For example, the age at LOA can be used to predict the age at which subsequent losses of upper limb functions occur and critical pulmonary milestones are reached [127]. It follows that changes in some clinical outcome measures in response to treatment over the short term can predict subsequent disease progression years later. This has been demonstrated in children on corticosteroid treatment followed for many years [17, 126].

Based on such findings, and through rigorous quantitative analysis of natural history, real-world data, and clinical trial data, prognostic models have been developed that more accurately characterize disease progression and account for the heterogeneity of natural history progression that underlies many of the challenges in DMD drug development [40]. In addition to measures of disease severity and other variables described below—such as genetic mutation and genetic polymorphisms—these models incorporate specific prognostic factors and should ideally include biomarkers such as quantitative dystrophin levels and skeletal muscle fat fraction as measured by MRI. The sections below elaborate more on how these findings and models can assist in selecting optimal outcome measures, determining trial duration, and establishing inclusion and exclusion criteria for enriched cohort selection, all contributing to improved trial design.

Sponsors are advised to mitigate the risk of imbalance in the ages of study participants, which can introduce substantial variability into a trial. Ensuring that the control and treatment arms in clinical trials are matched by both age and functional status is crucial. It is also recommended that consider baseline functional performance—in relation to specific endpoints such as 6MWT, TFTs, or NSAA—since baseline measurements of key outcome measures influence the subsequent rate of progression over time.

Genetic predictors of disease progression

As noted in the Diagnosis section, the maintenance of the *DMD* gene transcriptional reading frame usually predicts the dystrophinopathy phenotype: Frameshift mutations are predictive of a DMD phenotype, and in-frame mutations are predictive of milder BMD [128]. With some exceptions, this correlates to the amount of residual dystrophin in muscle, as less severe phenotypes express more dystrophin in muscle. However, within the DMD cohort, mutations within the *DMD* gene (exon-skippable mutations/deletions, nonsense mutations amenable to stop codon read-through, other deletions, duplications, and point mutations) might alter the course of progression from one patient to another. One study has suggested that there is a trend for children with duplication mutations to perform better than the DMD cohort as a whole [129]. Within those with deletions, there are specific subgroups that appear to be different from each other. For instance, there is a trend towards better baselines, less severe decline in progression as measured by 6MWT, and older age at loss of ambulation in boys with exon 44 skip amenable mutations when compared to those with boys eligible for skipping at exons 45, 51, and 53 [129, 130]. With larger cohorts or extended follow-up, differences between subgroups may become statistically significant. Recent data suggest that these differences in disease severity correlate with dystrophin quantities, even at very low levels (as low as 0.5% of normal by Western blot) [131].

However, while there may be differences between subgroups of patients with specific mutations, the mean 12-month changes in each subgroup falls within a narrow range in comparison to the mean of the whole DMD cohort. Furthermore, some variability will be present within specific subgroups due to the many other sources of heterogeneity listed here.

Certain mutations also appear to cause more dystrophin-related abnormalities in nonskeletal muscles, causing more pulmonary, cardiac, and neurocognitive impairment [132, 133]. Individuals can be categorized based on some of the associated *DMD* mutations:

* **Group 1**: Involves those with mutations only in the region upstream of intron 44, considered Dp427 negative and Dp140/Dp71 positive
* **Group 2:** Comprises those with mutations affecting the region from exon 51 to exon 62 (inclusive and not involving the region of exon 63 or downstream of exon 63) were considered Dp427/Dp140 negative and Dp71 positive
* **Group 3:** Includes those with *mutations* involving exon 63 and/or the region downstream of exon 63 were considered Dp427/Dp140/Dp71 negative.

In Group 1, (lacking only Dp427), approximately 15% of DMD boys had intellectual disability, compared with 25% of boys in Group 2 (lacking Dp427 and Dp140) and 64% of boys in Group 3 (lacking Dp427, Dp140 and Dp71) [134]. Boys with DMD who are lacking Dp140 and Dp71 clearly demonstrate marked reductions in motor function at 5 years of age (reduced mean NSAA score and rise from supine time) and peak motor function (reduced mean peak NSAA score and 10MWR velocity), with a clear cumulative effect of loss of isoforms [135]. Mean peak NSAA score is lower in those with cognitive impairment than those with normal cognition. Differences in NSAA score between isoform groups can be substantial, often exceeding the minimally clinically important difference (MCID) on the NSAA of approximately three points [46, 135, 136]. CNS manifestations such as autism, associated with certain brain isoforms of dystrophin, can also impact the acquisition of motor skills and motor milestones, thereby affecting the measurement of milestones and participation in clinical trials.

Clinical trials of treatments that are not mutation-specific should collect appropriate samples for comprehensive genetic analysis. As noted in the Diagnostics section, certain trial participants might require rescreened with a technique that provides a complete analysis of the *DMD* gene (refer to Diagnostics section).

Genetic modifiers

Genetic screening has identified polymorphisms in several other genes that potentially alter aspects of the response of muscle to dystrophin deficiency and/or medical treatment such as glucocorticoids. These genetic modifiers may influence the onset, progression trajectory, or drug responsiveness of DMD patients, providing insights into crucial biochemical pathways implicated in muscle damage, repair, or response to steroids. These modifiers could increase the understanding of factors responsible for patient-to-patient variability and eventually prove helpful in interpreting clinical trial data. However, conducting genetic modifier studies, as with most genetic-association studies in any human trait, typically requires extensive patient numbers and reliable, sensitive biochemical and/or clinical outcome measures. In addition, challenges in statistical analyses and reproducibility can arise due to differing methods of categorizing cohorts of patients and ethnic differences in polymorphism allele frequencies. To date, identified genetic modifiers include:

* ***Latent TGF-beta-binding protein 4 (LTBP4) polymorphisms****:* A minor allele in a minority of the population appears, in a recessive fashion, to have a protective effect on ambulation, comparable to the effect of steroid treatment, extending ambulation by as much as 2 years [137].
* ***Secreted phosphoprotein 1 (SPP1 or osteopontin) polymorphisms:*** This genetic modifier may alter a patient's responses to corticosteroid management rather than directly affecting the disease [138].
* ***CD40 Exon Variants in the NF-κB and TGFβ Pathways****:* The minor allele at rs1883832, in the 5'-untranslated region of CD40, decreases expression of CD40, a co-stimulatory molecule for T cell polarization. This allele has been associated with earlier LOA [139].
* ***TCTEX1D1 gene polymorphisms:*** Variants might be associated with age at LOA. The minor alleles of two independent variants, which are known to affect the TCTEX1D1 coding sequence and induce skipping of its exon 4, have been associated with earlier LOA [140].
* ***Thrombospondin-1 (THBS1) polymorphisms****:* THBS1 is an activator of TGFβ signaling via direct binding to LTBP4 and an inhibitor of pro-angiogenic nitric oxide signaling. *LTBP4* and *THBS1* encode directly interacting proteins that control TGFβ bioavailability. The longest ambulating DMD patients have been shown to be homozygous for protective alleles at both loci. In one study, protective *THBS1* alleles and a homozygous rs710160 protective genotype resulted in an average delay in LOA of 1.2, 3.5, and 6.8 years for 0, 1, or 2 protective *THBS1* rs2725797 alleles, respectively [141].
* ***ß2 adrenergic (ADRB2) receptor polymorphisms:*** Individuals with DMD expressing the Gly16 polymorphism exhibit systematically lower FVC% p values at any given age compared with patients expressing the Arg16 polymorphism [142].

There may be other genetic modifiers yet to be identified. Sponsors should consult the most recent data to determine whether screening for these genetic modifiers in their clinical trials is prudent for stratification or predefined sensitivity analyses, which can elucidate potential causes of variation in patient outcomes.

Differences in management that can affect the course of DMD

The standard of care administered to DMD patients may have significant implications on the design of trials, depending on the outcomes measured. Sponsors should be aware that current medical management has changed the natural history in DMD, affecting the timing of clinically meaningful milestones in individuals with access to high-quality care. This is primarily attributed to the use of glucocorticoids, management of spine deformity, pulmonary management, and cardiac management.

**Steroid therapy:**Corticosteroids delay the loss of ambulatory milestones, extending ambulation by about 3 to 3.5 years over time, and postponing the deterioration in upper-limb function, allowing patients to continue to raise their hand to their mouths and maintain self-feeding until a later age [17, 127]. Daily steroid treatment with prednisone or deflazacort is more effective than intermittent prednisone alternating 10 days on and 10 days off [70]. However, other intermittent regimens, such as weekend dosing are still commonly practiced. Steroid treatment also impacts pulmonary function, enabling individuals to attain an older age before requiring mechanical cough assistance or noninvasive ventilation, as defined by FVC parameters outlined in the DCC[6, 49].In addition, early treatment benefits often extrapolate to later stages of disease [17]. However, differences in steroid regimen, dosage, and duration may have disparate effects on clinical progression and function [41, 143].The steroid used (prednisone/prednisolone versus deflazacort) may also affect outcomes, with deflazacort associated with less functional decline in patients above 8 years of age who are in the decline phase of the disease [144-146]. Given the individualized nature of DMD management with corticosteroids, differences in efficacy and side effects between the steroid compounds and regimens can also result in differences in dose titration by clinicians and in patient/caregiver adherence.

Enrollment in the trials should either be restricted or stratified according to harmonized corticosteroid therapy. Trials of young children aged 3 years and younger have optionally included steroid management as an inclusionary factor. In those aged 4 years and older, a stable corticosteroid therapy regimen for 6 months has traditionally been an inclusion criterion. However, sponsors should be aware that some ambulatory boys may continue to have functional improvements beyond 6 months on corticosteroid treatment. Note that stable corticosteroid therapy should allow for weight-based dose adjustments when needed.

Beyond corticosteroids, growth hormone and testosterone replacement therapy have the capacity to affect growth and other outcomes.

Alternatives to foundational glucocorticoid standard-of-care therapy have been under evaluation in clinical trials [147, 148]. Some agents being studied as adjunctive therapies to glucocorticoids may soon augment the underlying standard of care.

**Other interventions:**Other therapies such as night splinting, physical therapy, exercise, and other standard-of-care interventions recommended by the DCC can influence functional performance [149]. For instance, the development of contractures can impact mobility and upper limb function, and strategies to prevent and manage contractures have been outlined in the DCC [6]. The effectiveness of these strategies, however, still requires confirmation through well-powered studies [150].

The data are even more limited regarding the effects of exercise, mobility strategies, nutrition, psychological and/or psychiatric care, noninvasive ventilation, or swallowing therapy on DMD progression, although one trial did demonstrate a benefit of recumbent cycle-assisted aerobic exercise in late ambulatory and early nonambulatory DMD [151].

However, there is evidence indicating that several critical standard-of-care treatments can influence survival. For example, spine deformity management, through timely spine surgery for curves exceeding 30-40 degrees, has impacted survival [152]. Pulmonary management has had the most substantial impact on survival [153]., Two recent studies have reported that the implementation of noninvasive ventilation can lengthen lifespans in DMD substantially [125, 154]. As a result, a larger number of young men with DMD are reaching their 20s and 30s, though often with significant disability. The use of glucocorticoids has also been associated with improved overall survival [17].

Cardiac management in DMD, focusing on prevention of progressive ventricular dysfunction through early afterload reduction (eg, angiotensin converting enzyme [ACE] inhibitors, angiotensin II receptor blockers [ARBs], and beta-blockers) may impact long-term outcomes. ACE inhibitors have been shown to have a positive effect on survival in young men with DMD-associated clinical cardiomyopathy by reducing stress on the heart (afterload reduction [155]; refer to the Cardiac section).

Standards of care need to be upheld at every center participating in these studies. Sponsors are advised to meticulously record any concurrent complementary therapy that study participants might be receiving. Some studies are striving to monitor families’ adherence to physiotherapy, home stretching and splinting to account for these variables in potential post-hoc analyses. Ideally, sponsors should control for as many of these factors as possible to reduce potential variability in disease course among trial participants.

Finally, the financial costs of care can limit access to standard of care treatments, and this burden intensifies as boys/men with DMD progress [156]. Insurance and other third-party payers might cover some treatments and types of equipment but not others. Moreover, lower socioeconomic status can lead to disparities in lifelong care patterns. Hence, sponsors should consider support mechanisms for participants from these communities, including ensuring access to standard-of-care therapies, to maintain a consistently high standard of care among all trial participants.

1. Natural history across the spectrum of dystrophinopathy

Most DMD patients treated with steroids lose ambulation by the age of 16 years, but there are individuals with milder dystrophinopathy, including intermediate DMD, BMD, and symptomatic women with dystrophin mutations [157].

BMD usually presents with a later onset of symptoms and progresses more slowly, with wide interpatient variability in severity [158, 159]. Some patients may have a clinical course similar to that of DMD, while other patients may develop mobility impairment and a myopathic gait in their late teens to early 20s [11, 157]. Another subset may remain nearly, or in some cases, entirely asymptomatic, experiencing only mild muscle cramps without overt weakness.

The birth prevalence of BMD is about 1 in 20,000 males [12]. Its overall prevalence in relation to all dystrophinopathy is likely above 25% due to the increased survival. If the cardiac effects of the disease are absent, the median age of death can approach that of a natural lifespan. However, there is considerable heterogeneity, with instances of death from cardiomyopathy sometimes occurring in the teenage years [160].

Finally, some female carriers of dystrophin mutations experience muscle degeneration [161], with symptoms such as muscle weakness, fatigue, pain, excessive tightness, and poor balance [162]. Cardiac involvement is common in carriers with skeletal muscle symptoms [163-165]. While some models have placed women with dystrophinopathies on the milder side of the dystrophinopathy spectrum, emerging data suggest they range from asymptomatic carriers to phenotypes with spectrum of severity that can be similar to that in males with BMD, and in some cases, even DMD [14, 166] (see below for more on clinical trials issues in BMD and other dystrophinopathies).

1. Ongoing natural history study needs

Given the relentless course of DMD and the complexities involved in conducting adequately powered studies for a rare disease, there is a need to establish reliable, and well-matched natural history controls that account for known causes in variability, and that also address key outcome measurement gaps of the disease. For natural history controls to effectively supplement placebo comparisons, the collection of natural history data must adhere a level of rigor meeting FDA standards.

Sponsors are advised to consult the following documents for guidance:

* Rare Diseases: Natural History Studies for Drug Development. Draft Guidance: This recent draft guidance can help inform the design and implementation of natural history studies that can be used to support the development of safe and effective drugs and biological products for rare diseases.
* FDA Guidance for Industry - Computerized Systems Used in Clinical Investigations (2007)
* CFR Part 11, Subpart B – Electronic Records
* ICH Guidelines for Good Clinical Practice – Section 4.9 (Records and Reports), and Section 5.5 (Trial Management, Data Handling, and Record Keeping)

1. Considerations for Outcome Measurement Selection
2. General Comments

Many outcome measures, standardized tools, and devices have been developed for use in clinical settings to categorize the stage and trajectory of individuals with DMD. These tools help monitor clinical progression over time and guide clinical care decisions. Additionally, these outcome measures have applications in clinical trials. For example, emerging there are now data to support the use of specific outcome measures to identify and enrich for specific populations of participants at risk of progression during the course of DMD trials. Implementing these outcome measures during participant screening and for stratification could reduce the risk of an underpowered study that is unable to reach a clear conclusion about a potential therapy’s effectiveness.

Sponsors can use these tools to measure treatment effects, selecting endpoints based on function in a variety of ways. These include performance-based outcome assessments that demonstrate how well a trial participant can perform a specific activity or set of activities (eg, ability to perform an activity or activities [yes or no]; the time needed to perform an activity or activities), or time-to-event for decline or ability loss. With mounting evidence showing that specific changes or thresholds on an outcome measure are predictive of the time to clinically meaningful events, or disease milestones, these outcomes might serve as intermediate clinical endpoints in trials. This might be a more favorable approach than solely relying on timing of functional ability loss as the trial’s primary endpoint. For younger children still acquiring abilities, it may be appropriate to evaluate time to positive outcomes (ie, time to attain an activity or time to reach a certain developmental milestone).

Although existing outcome measures developed for dystrophinopathies or related conditions may be appropriate for defining an endpoint in a clinical trial, FDA remains open to considering proposals for the use of novel outcome measures that can detect clinically meaningful effects in patients. FDA encourages sponsors to propose and, if necessary, develop new tools for measuring primary and secondary endpoints that can validly and reliably assess patients across a broad spectrum of symptoms and disease stages. Sponsors should engage with FDA early during the process of selecting and developing efficacy endpoints.

There are outcome measures that monitor changes in clinically meaningful abilities in a number of functional domains that merit consideration. Sponsors should, when possible, evaluate multiple efficacy endpoints to characterize the breadth of novel therapies’ effects on dystrophin-related pathologies. This includes considering skeletal, respiratory, and cardiac muscle function—even if only one of these measurements is the primary endpoint. As examples, upper limb function along with pulmonary and cardiac statuses can be assessed before LOA—even in younger children—depending on the trial’s inclusion criteria and expected duration. Assessing cardiac- or pulmonary-specific changes might also provide insights into long-term benefits~~.~~

Efficacy endpoints that measure functional change over a broad range of deficits and severities may offer a number of advantages in the development of drugs for dystrophinopathies. Such endpoints may increase the number of patients eligible for enrollment and may decrease possible loss of information from floor and ceiling effects that occur, respectively, when patients become unable to contribute data because they can no longer perform or complete a function fully throughout the study. For similar reasons, FDA encourages sponsors to use endpoints that can assess function across different stages of the disease (eg, combining measures of ambulation and upper body function). Endpoints should have the ability to detect improvement from baseline, as well as decline, to capture the spectrum of possible beneficial drug effects.

The following broad criteria should guide the selection of outcome measures to show treatment effects in clinical trials:

* Outcome measures should be appropriate to and validated for the disease stage, functional capacity, and disease trajectory of study participants.
* They should be appropriate to the treatment target, which could include:
  + The mechanism of action for the potential treatment; and
  + The targeted muscle group/fiber and its medical addressability (ie, whether muscle function has deteriorated beyond a point where it can no longer respond to therapy).
* There should be sufficient natural history data with standard-of-care treatment to allow for the planning and powering of a trial.
* There should be data to support the clinical and contextual meaningfulness of the outcome measure—with patient preference data showing that measure changes over time relate to critical functions and abilities that matter in their daily lives. Sponsors who have gathered such data on a measure or drug development tool are encouraged to seek qualification for the clinical outcome assessment (COA) from the FDA (see Clinical Outcome Assessments (COA): Frequently Asked Questions at https://www.fda.gov/about-fda/clinical-outcome-assessment-coa-frequently-asked-questions#COADefinition).
* The validity and reliability of the outcome measure should have been demonstrated in target populations.
* Measurement of the outcome should be standardized across trials.
* The utility of an outcome measure is increased if it has demonstrated relationships to other outcome measures across the stages of disease (ie, there are data anchoring the measure to timing of loss of future milestones or a continuum of measures).
* Measures should be ideally anchored to clinically meaningful patient-reported outcomes (PROs).
* The measure should have a demonstrated ability to quantify change over the proposed duration of the study.

1. Specific Outcome Measures in DMD

The following section provides a general overview of the types of outcome measures, with some description of when their use may be appropriate.

1. Developmental scales

Developmental scales such as the Bayley-III and Griffiths Mental Development Scales measure the rate of development in children and can identify early developmental delays in children with DMD. The Bayley IIIis a standardized functional measure assessing five domains of infant and young child development (cognitive, language, motor, adaptive, and social-emotional) [119, 120, 122].The tool has been used in infants and young children (up to the age of 42 months) with DMD. There is now a Bayley-4 version which retains the five domains and has been updated with adaptive behavior content from the Vineland Adaptive Behavior Scales–Third Edition (Vineland-3), with social-emotional and adaptive behavior questionnaires that have remote digital web-based administration options via Q-global. Normative data for the Bayley 4 were collected from 2017–2019, and updated reliability and validity studies have been completed [15]. The Griffiths Mental Development Scale is a structured neurodevelopmental assessment that can be used to track progression of disease in infants and young children with DMD in young ambulatory phase (up to 8 years of age) [120, 122].

Most development scales require formal training and certification on the part of the clinical evaluator. The sponsor should consider the availability of language and country-specific validation of each scale in choosing an outcome measure, as well as understand the limitations posed by the end-of-range effects of each scale. Developmental scales may also undergo revision over time and may pose additional challenges in interpretation. FDA recommends that sponsors discuss and reach agreement with the agency on the appropriateness of the use of such scales in clinical trials of young children.

1. Motor measures in DMD

Motor outcomes measures exist across the stages of disease spectrum of DMD. For instance, the Griffiths and Bayley-III scales both have locomotor components that can detect delays in development of motor skills even in young children.

The North Star Ambulatory Assessment (NSAA)

The NSAA, a validated 17-item functional scale specifically developed for use in the ambulatory stages of DMD, can be used to monitor progression in individuals with DMD during the ambulatory period from the age of age of 4 years (or even from 3 years with revision) into the late ambulatory stages in adolescence. FDA has accepted the use of NSAA as a measure of gross motor function in ambulant children in clinical trials [167, 168]. The NSAA has high validity and reliability, as well as increasing validation against other tests (eg, TFTs) across time, defined MCIDs, and predictive capabilities regarding functional motor changes [15, 41, 169-173]. Sponsors can apply different analytical approaches to the NSAA with regards to the knowledge of the MCID, the employment of the shift analysis, cumulative loss of function/cumulative failures, evaluation of actual loss of functions, or time-to-event analysis (time to clinically meaningful disease progression) [46].

In young children and during the early ambulatory stage (ages 4-7 years), the NSAA has been shown to be sufficiently responsive to differentiate disease progression in children with DMD on continuous versus intermittent steroids over time [41]. Sponsors should note that at this stage of disease, there can be improvement in some domains on NSAA, and changes in scores over a duration of 48 weeks may not be of significant magnitude to demonstrate treatment effect. In the late ambulatory stage, on a linearized 100-point scale (based on a Rasch analysis) of the NSAA total score, an approximate 7- to 9-point change has been deemed to be the minimal important difference [41]. However, a recent multicenter trial demonstrated less than a 7-point decline in the linearized NSAA in placebo-treated patients over 48 weeks. For children at earlier stages of disease, it may therefore be advisable to either have trials of longer duration or to stratify trial arms more narrowly by disease trajectory.

Timed function tests

Researchers and care providers also use various timed function tests (TFTs) to assess boys with DMD at similar ages and stages of disease as the NSAA. Note that the UC Davis DMD Disease Progression Model, in Box 1, describes evidence-based thresholds for outcome measures, such as time to stand from supine, which relate to the time to reaching certain milestones of disease, such as LOA. These thresholds could serve as intermediate clinical endpoints in clinical trials.

* Time to stand from supine (or velocity as the reciprocal to time to stand) has been used to monitor disease progression in DMD [174-176],including recently, in a major trial as a primary endpoint [70]. Loss of the ability to stand has been shown to be predictive of time to LOA and time to 10% decline in ambulatory function [48]. Time to stand can be obtained reliably in younger DMD subjects [177] and is a useful endpoint for younger DMD patients. Limitations include the early loss of the endpoint in many boys with DMD, and reduced sensitivity of the endpoint as defined by the ratio of the MCID, which is greater than 3 seconds in DMD, to the mean baseline value [110]. Time to stand from a chair has also been used as a primary endpoint.
* Time to climb 4 stairs represents stair climbing ability—a clinically meaningful function in and of itself that has been used as an endpoint in DMD trials for decades [117, 174-176]. Stair climbing velocity improves until around 7 years of age and then declines. It is predictive of loss of stair climbing ability, LOA, and time to 10% decline in ambulatory capacity [44]. Challenges include standardizing equipment at multiple sites, lack of sensitivity to small changes, and variability that may impact sample size. This has been used as a primary endpoint in one recent trial.
* Time to run/walk 10 meters is another TFT used for decades as a clinical trial endpoint in children with DMD and older [174-176]. It is easily obtained in the clinic and reliable in younger children [177]. The velocity of the 10-meter run/walk increases in DMD up to 7 years of age but not to the same extent as seen in typically developing children. It is reliably assessed and has been validated with other endpoints. It is predictive of future loss of ambulation. A change on the order of 5 seconds or less has been shown to be clinically meaningful [48, 178].

To minimize the effect of outliers in more poorly performing patients, sponsors may wish to consider analyses based on velocity (typically the reciprocal of the timed function) in addition to change in actual time to perform a function. It should be noted that at higher levels of function approaching that of a typically developing child, some changes in time to perform a function may result in large changes in velocity of a timed function test.

The 6-minute Walk Test

The outcome measure most commonly used in the later ambulatory stage of DMD, the 6-minute walk test (6MWT) has been used in clinical trials to evaluate endurance and muscle function in neuromuscular diseases and validated as a clinically meaningful endpoint in ambulant DMD patients with population changes observed over a short period of time (24–52 weeks) [48, 109, 179-181].Much of the experience using 6MWT comes from trials that failed to meet their primary endpoint, although in one trial, the post-hoc analyses suggested a benefit measurable by 6MWT in a subset of participants. One lesson drawn from this was that the trial had enrolled many individuals who were unlikely to progress over the course of the study. Use of narrower 6MWT ranges or use of TFTs (such as more than a 5-second rise from supine) as inclusion criteria could make the 6MWT more useful as a measure [42]. In addition, recent modeling studies suggest ways to improve the 6MWT’s predictive value [43].

100-Meter Run Test

The 100-meter run test (or run/walk test), a fixed distance test of maximal performance (capacity) in younger boys who are asked to run, if they can, at their top speed, has been shown to be sensitive to decline over time [182]. Although the most able boys with DMD may walk almost as well as their age-matched peers, running speed is significantly slower, with normative data that be used to determine percent-predicted 100-meter times to quantify the severity of running impairment in children with a motor deficit. The set course eliminates the ceiling effect seen in other assessments, with excellent test-retest reliability [183]. The 100-meter run test been used as an alternative for the 6MWT in clinical trials studies in younger ambulant children, particularly those aged 4-7 years, who can get distracted over 6 minutes or try to run rather than walk quickly. The children may be more consistently motivated to run as quickly as they can.

Myometry

Myometryprovides a quantitative measure of strength and may be an appropriate endpoint for treatments *that increase or preserve muscle mass and strength*. Several measures can be used, including isometric fixed or handheld devices or fixed isokinetic devices. Manual muscle testing (MMT) was used as the primary outcome measure to demonstrate an effect in the initial prednisone trial, but the outcome measure had a large standard deviation [178]. MMT appears less sensitive and reliable in comparison with quantitative muscle testing (QMT) [177].QMT requires expensive and bulky equipment whereas handheld dynamometry is a more practical and continuous variable. The two muscle groups most reliably assessed in children with myometry are the knee extensors and elbow flexors [48, 177]. In general, children aged 5 years and older may be assessed more reliably with myometry.

The clinical meaningfulness of differences in muscle strength should be supported by the magnitude of the effect observed (both mean effect and distribution of responses) or by the demonstration of a drug effect on an appropriate functional measure. In some instances, a demonstrated effect on muscle strength could be considered an intermediate clinical endpoint and used to support accelerated approval (see the *Guidance for industry: Expedited Programs for Serious Conditions—Drugs and Biologics*) [184].

There has been a concern that lower extremity myometry reaches a floor effect in late ambulatory DMD patients and that upper extremity myometry does not change substantially over 48 weeks in non-ambulatory populations [110]. Percent predicted grip strength shows a more predictable linear change in non-ambulant patients. Some sponsors have added testing of both right and left strengths to decrease some of the variability seen with these tests.

Performance of Upper Limb Test (PUL 1.2 and 2.0)

The use of some outcome measures such as performance of upper limb test (PUL 1.2 and 2.0), which has been validated as a key outcome measure for a disease progression related to the upper limb function and is becoming commonly used as a primary endpoint in trials [45]. While the use of outcome measures to define the ambulatory transitional stage (when an individual is expected to lose ambulation within the next 2 years) can help sponsors enroll participants with a better characterized risk of progression, it is unclear the extent to which an individual’s lower limb function is medically addressable by a treatment (whether there is still a therapeutic window to preserve ambulation). Although, historically, there has been a fine dividing line between clinical trials conducted in the ambulant versus in the nonambulant populations, the use of some outcome measures such as PUL 1.2 and 2.0 can bridge the two populations. In the transitional stage, it should be possible to measure deficits in the performance of upper limb function over time in both late ambulatory as well as early nonambulatory participants.

Importantly, not all motor endpoints are measuring the same phenomenon. There might be fairly high correlation at baseline of, for example, the 6MWT, 100-meter timed test, and TFTs such as the 10-meter run/walk test because all measures are dependent on strength, stride length, and gait cadence. However, the 6MWT is considered a test of endurance and dependent to some extent on self-selected walking pace based on muscle perfusion and metabolism, whereas the 100-meter and the 10-meter timed tests are tests of ambulatory capacity and rely to a somewhat lesser degree on endurance. However, treatments that might improve muscle perfusion or metabolism could affect the 6MWT while not impacting the 10-meter run/walk test or muscle strength to as great a degree in the short term. Consequently, the selection of a motor endpoint for a specific drug program needs to be based on the mechanism of action of the drug as well as the appropriateness to age or stage of disease.

**Box 2: Motor measures by stage of disease**

***In young ambulatory stage***

* The North Star Ambulatory Assessment (NSAA)
* Time function tests (TFTs)
  + Time to stand from supine
  + Time to climb 4 stairs
  + Time to stand from a chair
  + Time to run / walk 10 meters
* 100-meter run test (capacity)
* Myometry measures quantitative strength
  + Manual muscle testing (MMT)
  + Handheld dynamometry (of elbow flexors/extensors; knee extensors/flexors) measuring force in foot-lbs or newtons
  + Quantitative muscle testing (QMT) (isometric and isokinetic) using dynamometer machines that stabilize a joint and measure torque outputs

***In later ambulatory stage / decline stage***

* 6-minute walk test (6MWT)
* NSAA
* TFTs
* 100-meter run test
* Motor function measure (MFM)
* Myometry (MMT, Hand dynamometry, QMT)

***Time to event (eg, time to clinically meaningful disease progression) in ambulatory***

* + 3-point loss on NSAA
  + 2 item loss on NSAA
  + 2 different items on NSAA (not bilateral)
  + 5 second stand from supine
  + 10 second stand from supine
  + Loss of stand from supine ability
  + Loss of 4-stair climb ability
  + Approaching Loss of Ambulation (aLOA) or 10 second 10-meter run/walk
  + Loss of Ambulation (LOA) or inability to ambulate 10 meters

***Community functions measured by digital technology (passive) in ambulatory patients***

* Ambulatory devices (eg, 95th centile stride velocity by ActiMyo/Syde high-performance wearable sensors)
* Walking parameters (longest distance walked continuously over 2 weeks; 95th centile vs. 90th centile vs. 80th centile); temporal gait patterns
* Spontaneous stair climbing velocity at home (identify the stairs; 10% fastest)
* Time spent running
* Falls (in younger patients)

***During transitional stage (in those expected to lose ambulation within next 2 years; may be defined as 10-meter walk > 10 seconds)***

* NSAA
* MFM
* 10-meter walk/run
* PUL 1.2; PUL 2.0 (PUL entry 6 or PUL entry 5)
* Upper limb myometry; quantitative grip
* LOA

***Motor measures in nonambulatory stage***

* Performance of Upper Limb Scale (PUL 1.2 and 2.0)
* Motor function measure (MFM)
* The `Motion & Function Assessment Tool’ (MFAsT)
* Quantitative strength testing using hand-held dynamometry or MyoTools (eg, pinch test, handgrip test, elbow extensors, elbow flexors); use of percent predicted values vs. normative data
* Quantitative measure of reachable workspace, which measures shoulder and elbow movement
* Quantification of elbow, wrist, and digit movement using wearable sensors
* The 9-hole peg test, maneuver
* Egen classification (EK2) scale
* Motor Function Measure (MFM)

***Time to event (eg, time to clinically meaningful disease progression) in nonambulatory patients***

* + Overhead reach (Brooke 1 to 2; PUL entry 6 to 5)
  + Hand to scalp (Brooke 2 to 3; PUL entry 5 to 4)
  + Weighted hand to mouth (Brooke 3 to 4; PUL entry 3 to 2)
  + FVC % predicted (FVC %p) threshold 80% (mild restrictive lung disease by ATS standards)
  + FVC %p 60% (needs mechanical cough assistance)
  + FVC %p 50% (needs evaluation for noninvasive mechanical ventilation)
  + FVC %p 30% (need for daytime mouthpiece ventilation or diurnal noninvasive bilevel ventilation)

Finally, sponsors selecting any of these measures should establish standard operating procedures (SOPs) for their use across each trial site to standardize how outcome measures will be collected. For instance, what training will test reviewers receive for how they work with the trial participant? Will they allow participants to try to perform the activity for up to 30 seconds and then deem them as unable to perform? Will they allow a participant to struggle for 60 seconds, or 45 seconds? How many other effort dependent tests are scheduled during the clinic visit? Such factors may impact both performance and the interpretation of the data.

1. Pulmonary outcome measures

Pulmonary function can be seen as a skeletal muscle outcome measure that includes measures of diaphragmatic impairment for ventilation and abdominal muscle function for airway clearance [50, 185]. In contrast to cardiac function measures, many of the pulmonary outcome measures correlate more closely with functional measures, such as upper limb functional measures [49]. Sponsors developing therapeutics targeting skeletal muscles who are evaluating upper limb outcome measures should monitor pulmonary function measures and additional clinical outcome measures related to functions such as the ability to cough and breathe with or without ventilatory assistance, among others [186].

* **The ability to cough**: Airway clearance ability/cough function requires chest wall and expiratory muscle function and has been documented to be a critical function of importance to patients in patient preference studies in DMD and other neuromuscular diseases. Measures of cough function include***:***
  + Peak cough flow (PCF), easily measured with a flow meter. Among individuals with DMD, a PCF <270 liters per minute (L/min) when well or < 160 L/min if intubated or during an acute illness indicates inadequate airway clearance and the need for a mechanical cough assistdevice. The parameter is effort- dependent and shows high coefficient of variation so this is not used as a primary endpoint in clinical trials.
  + Maximal expiratory pressure (MEP). Static airway pressures such as maximal expiratory pressure is a measure of the strength of the abdominal muscles of respiration important to cough and airway clearance. Similarly, falling below 60 cm H2O MEP indicates inadequate airway clearance and the need for an airway clearance device.

Other than use of these thresholds to identify those with challenges to cough function, it is inadvisable to use changes in peak cough flow and static airway measures such as MEP as outcomes to measure in clinical trials due to poor reliability as assessed by the within-subject coefficients of variation. However, some have used PCF and MEP thresholds as clinically meaningful events.

* **Global inspiratory and expiratory pulmonary measures versus specific inspiratory and expiratory function measures:**
* Forced vital capacity (FVC) and FVC % predicted (FVC%p). Forced vital capacity measurement reflects a global assessment of all respiratory muscles because it requires a full inspiration (reflecting function of inspiratory muscles) and a full expiration (reflecting function of expiratory muscles). FVC % predicted is the most reliable and commonly employed pulmonary spirometry endpoint used in DMD clinical trials [49]. Previously, FVC%p, and peak expiratory flow rate % predicted (PEFR%p) have been shown to be pulmonary function endpoints with consistent declines across the second decade in DMD from ages 10-18 years [49, 187]. Trials should base FVC % predicted values on both height and ulnar length to provide stability of assessment if a transition from ambulatory to nonambulatory status occurs.

Glucocorticoid treatment has been associated with a higher peak absolute FVC, a delay in age at which peak absolute FVC is obtained, and a delay to the time at which patients progress to an absolute FVC below 1L—a level shown to be associated with a 4-fold increased risk of death in one study. Similarly, a prior study reported a median survival of 3.1 years and 5-year survival of only 8% when the FVC fell below 1L [188]. Having an FVC less than 1L remains the best negative predictor of survival in patients with DMD according to a consensus statement from pulmonary medicine specialists [153]. The absolute FVC has the advantage of not being subject to errors in height measurement required for height-based equations but the variable maturational trajectory needs to be considered with absolute FVC. Glucocorticoids have a cumulative effect on growth and preservation of absolute FVC and also the rate at which the critical values of FVC are achieved, such as 30%p and drop below 1L [49].

* Peak expiratory flow/flow rate (PEF, PEFR). PEFR requires both an inspiratory and expiratory maneuver.PEFR has been used as a primary outcome measure for DMD clinical trials, and there are data from the Cooperative International Neuromuscular Research Group Duchenne Natural History Study (CINRG-DNHS), and other studies showing strong correlation between FVC% and PEF% [49, 126, 189]. There are also positive data predicting that measuring peak flow is a potentially useful measure that correlates with quality of life. Clinical intervention with mechanical insufflation/exsufflation device in DMD is driven by a threshold initially identified by clinical experience and has been reinforced in subsequent consensus statements. Chest wall compliance and intrinsic lung function may impact peak flow measures. Use of concomitant medical therapies, including mechanical insufflation-exsufflation (M-I/E) devices and potentially even chest PT, may influence peak flow measures. These are potential confounding influences in measurements of PEFR and other pulmonary endpoints.
  + Maximal Inspiratory pressure (MIP). The static airway pressure MIP obtained through a maximal inspiratory effort maneuver has been used as a measure of diaphragmatic strength [190]. Test-retest reliability of MIP has generally been poor in DMD.
* **Hypoventilation:** The need for ventilatory assistance, as noted earlier, has previously been described in terms of FVC% thresholds that were not precise measures of hypoventilation. More accurate assessments of true hypoventilation can be based on home and laboratory-based sleep studies and include:
  + In-home capnography using transcutaneous CO2: Transcutaneous CO2 monitoring provides time of arterial Co2 above 50 mmHg, and time above 45 mmHg, with a typical increase in CO2 of 10 mm of mercury going from non-REM to REM sleep. (Note that strategies are needed to validate in-home measures of hypoventilation.)
  + Laboratory polysomnography to assess the apnea hypopnea index (AHI), a marker of the number of times per hour that individuals are having difficulty breathing when they go into deep sleep: In individuals with DMD, pulmonologists classify hypopnea as a decrease of nasal airflow of 50% from baseline and a desaturation of 3% in SpO2, in combination with thoracoabdominal asynchrony breathing.

The need for ventilatory assistance can be seen as a pulmonary milestone. Sponsors are also encouraged to collect data to show whether changes or thresholds are associated with the number of pulmonary infections, antibiotic use or hospitalizations.

1. Outcome measures for cardiomyopathy in dystrophinopathies

There is a great need to gather cardiac natural history data and to standardize the measurement of cardiac biomarkers, such as cardiac MRI measures including LGE, left ventricular (LV) ejection fraction (LVEF), progression of systolic dysfunction, and LV strain (please see Cardiac section). Note that cardiac medications alter the trajectory of LGE, strain, and heart failure.

1. Digital technologies and wearable devices

In addition to the standard performance-based measures performed in a clinical setting, during the last several years, novel digital measures that permit objective, continuous measurements of functional ability during daily life have increasingly been used—particularly since the COVID-19 pandemic—has made visits to the clinic problematic [52]. These include both community functions measured passively by digital devices that are worn by the subject and other tools and measures that are home-based but require active participation with a remote interfaced clinical evaluator to perform assessments [51, 191]. These may include: community functions measured passively by digital technology, such as the ActiMyo/Syde and other high-performance wearable sensor devices, which can provide a capacity measure of 95th percentile stride velocity (the measurement of which was recently qualified as a primary endpoint by the European Medicines Agency) [51, 192-194]; active home-based clinical evaluator-interfaced assessments such as home-based spirometry with confirmation of appropriate effort and acquisition of a flow loop consistent with American Thoracic Society guidelines [53, 195, 196]; or home-based video assessments of defined functional tasks providing scoring of quality and ease of movement and compensatory movements used for patients with variable degrees of proximal and distal weakness, such as the Duchenne Video Assessment [54, 197].

Finally, sponsors should be aware that some technology-based assessments, initially evaluated in the laboratory-based environment, are being transitioned to the community. These include:

motion-analysis / gait analysis [198, 199], and measurement of energy cost of locomotion using a portable metabolic cart. Reachable workspace, isolated to upper limb, has been explored in DMD and other muscular dystrophies [200]. In addition to identifying changes in quantitative reachable workspace in the upper limb, the Kinect-based reachable workspace relative surface area (RSA) and PUL assessment demonstrated a treatment effect of a therapeutic that impacted DUX4 expression (double homeobox 4 protein) [201]. On this basis, reachable workspace is now being used as the primary endpoint for an upcoming clinical trial in facioscapulohumeral muscular dystrophy (FSHD). Other paradigms for measuring reachable workspace allow trunk compensations [202].

FDA has recently released draft guidance with recommendations to sponsors, investigators, and other stakeholders on the use of digital health technologies (DHTs) to acquire data remotely from participants in clinical investigations evaluating medical products. See *Digital Health Technologies for Remote Data Acquisition in Clinical Investigations* [203].

1. Generic and DMD-specific PROs

Patient-reported outcomes (PROs) including those measuring activities of daily living, can also be designed to assess the abilities and experiences of patients across a spectrum of disease stages and severities. PROs can be useful to assess the clinical meaningfulness of an objective finding of relatively small magnitude and to contribute to assessments of benefit and risk. The selection, design, and use of PROs have been described in a series of FDA guidance documents, including:

* *Patient-Reported Outcome Measures: Use in Medical Product Development to Support Labeling Claims [93]*
* *Patient Preference Information—Voluntary Submission, Review in Premarket Approval Applications, Humanitarian Device Exemption Applications, De Novo Requests, and Inclusion in Decision Summaries and Device Labeling [204]*
* *Core Patient-Reported Outcomes in Cancer Clinical Trials Guidance for Industry DRAFT GUIDANCE [205]*
* *Principles for Selecting, Developing, Modifying and Adapting Patient-Reported Outcome Instruments for Use in Medical Device Evaluation: Guidance for Industry and Food and Drug Administration Staff, And Other Stakeholders [206]*

Ideally, a PRO should measure abilities and functions that are meaningful or relevant to a population, and there should be evidence from studies that the chosen PRO is a valid measure of the ability or function of interest. The instrument should be able to detect change and differences in scores when used by populations or groups. It should also be reliable—with evidence that it is stable when no change is expected, with internal consistency across items in the PRO that contribute to the score. A PRO should have an adequate range to show when there is a change or a response (without large floor or ceiling effects). Finally, the instrument should not be a burden for a patient to use. A well-designed PRO should limit redundant questions and needs to be performed within a reasonable recall period while the patient can still validly recall the information requested. Note that in cases where an individual with DMD is unable to report for himself or herself in a valid manner (eg, a young child below the age of 8 years), the sponsor should base PROs on what a caregiver or other observer directly sees during a patient’s daily activities. Another consideration is that DMD patients may over-report their function, suggesting another benefit from engaging parent/caregivers as reporters of function.

A number of generic PRO instruments have been repurposed for use in DMD with varying success. Health-related quality-of-life measures such as the PODCI, which uses a degree-of-difficulty construct [17, 49] appear more highly correlated with impairment measures of disease severity than the PedsQL, another generic PRO which has not performed well in clinical trials in DMD [47, 207-211]. Others include the PROMIS (pediatric scales), NeuroQoL [212], Individualized Neuromuscular Quality of Life (INQoL) [213], health state utility values such as the EQ-5D [214, 215], the Pittsburgh Sleep Quality Index [216-219], and the Pediatric Evaluation of Disability Inventory, which has been revised as a computer adaptive test (PEDI-CAT).

However, it is clear that quality of life (QoL) and health-related quality of life (HRQoL) are related but distinct constructs as rated by children with DMD and their parents. Further research is needed to elucidate factors outside HRQoL that contribute to overall QoL.

For clinical trials, instruments specifically designed for dystrophinopathies do have distinct advantages. Disease-specific health indices provide a mechanism for serially monitoring the multifactorial disease burden of patients with rare disease, representing both how a patient feels and functions. They can be designed to include a limited number of items that assess the most critical aspects of the daily life and physical functioning in those with DMD or BMD. They can also be designed to be more responsive, with increased sensitivity to detect small clinically-relevant therapeutic changes, with higher precision and content validity for the target population. They can be simpler to use than broader nonspecific measures of health, excluding nonrelevant issues and questions and creating a lower burden for the patient. Such disease-specific PROs are better suited for clinical trials to measure disease burden and relevant therapeutic gains over time, with an improved ability to show whether therapy has patient-relevance.

Emerging examples of disease-specific PRO instruments in DMD include the following:

* DMD Lifespan Mobility Scale [220]
* The PedsQL 4.0 DMD Module Scale [221]
* DMD Health Index (DMDHI)
* DMD Caregiver-reported Health Index (younger age to 18 years of age)
* Egen Klassifikation 2 (EK2) scale [187, 222-224]
* DMD Upper Limb PROM [225]
* DMD Quality of Life (DMD-QoL) [226, 227]

In a review of instruments measuring caregiver quality of life conducted by consensus-based standards for the selection of health measurement instruments (COSMIN) group [228], the best available instrument in the context of DMD was the PedsQL Family Impact Module [229]. Further work must investigate this and other instruments’ measurement properties in DMD caregivers and the development of new tools.

The lack of PROs that are useful in nonambulatory individuals, particularly those with cardiomyopathy, is a major gap in the field. The development a PRO for the nonambulatory population would represent a significant advance and should be of utmost priority.

1. The clinical meaningfulness of changes in outcome measures, endpoints, and milestones

The most critical factor in selecting a tool or measure for clinical trials is how a given change in that measure translates to how a patient feels and functions—and whether it is a predictor of future prognosis.

There are several approaches sponsors could use to determine the clinical meaningfulness of an outcome-measure change as an endpoint in their clinical trial:

* Anchor-based methodology with a change anchored to patient focus group input (or physical therapists/clinician focus group input) or PRO measures or other functional scale (eg, NSAA linked to Functional Motor Scale [FMS])
* Consensus-based approaches, where a sponsor surveys a group of patients or caregivers or survey experts with a questionnaire about what they consider a meaningful change. Note, survey selection criteria should consider whether participants have relevant and recent experience with the stage of progression being assessed
* Minimal clinically important difference (MCID) estimation (statistical measures / wobble). Three statistical approaches are used which are all distribution-based:
  + Distribution based (0.5\*standard deviation [SD]); 0.5 SDs of the baseline values
  + Distribution based (structural equation modelling [SEM]); obtained from mixed-effects models fit to patients' individual trajectories
  + Distribution based (1/3 SD); minimum value expected to Indicate true change versus scale or group variability
* Prognostic utility, or the use of a given change in a measure as a prognostic indicator of future disease progression, in terms of progress to key future milestones.

1. Biomarkers in Duchenne Muscular Dystrophy
2. General Comments

The FDA the Duchenne and Becker communities are aligned in their objective to develop biomarkers and surrogate endpoints that can swiftly provide meaningful data indicative of drug function, thereby shedding light on potential biological activity that could modify the disease trajectory.

Sponsors should recognize that biomarker development in DMD and BMD is continuously evolving. They should consider incorporating some of the biomarkers described in this section into their clinical development programs as endpoints to support an NDA for their products in development. Additionally, biomarkers could be included in a predefined sensitivity analyses that can advance the field towards a consensus on the biomarker’s utility, potentially reducing costs and speeding the time required to develop subsequent products.

This section describes various biomarkers with possible prognostic or predictive value in determining a patient’s prognosis or likelihood to benefit from a particular treatment. More attention is given to pharmacodynamic (monitoring) biomarkers that reflect post-treatment responses. In comparison with pretreatment values, a treatment-responsive/monitoring biomarker, backed by sufficient data, could potentially serve as a *surrogate endpoint biomarke*r. that could substitute for a clinical endpoint (for accelerated but not a full approval) by providing early and precise predictions of clinical endpoint outcomes (clinical improvement or lack of improvement, harm or lack of harm) and other effects of treatment on this biomarker [184].

Designating a surrogate endpoint biomarker requires agreement with regulatory authorities. A comprehensive evaluation of available data, which includes epidemiologic, therapeutic, pathophysiologic data, or other scientific evidence, must demonstrate that a biomarker can forecast changes in clinical endpoints and may be considered for an accelerated approval decision [230].

Surrogate endpoint markers can serve as the primary endpoint in “adequate and well-controlled studies.” If there is a well-established relationship between the surrogate marker and clinical outcome, such trials can provide evidence for conventional marketing approval. Conversely, if the relationship between the surrogate marker and clinical outcome is not firmly established but is “reasonably likely” to predict a clinical outcome, a positive effect on the surrogate endpoint could lead to an accelerated approval.

In DMD, biomarkers that accurately report on both the health and amount of skeletal muscle might be useful at different stages of the clinical trial process as prognostic, predictive, or pharmacodynamic biomarkers. While use of biomarkers in BMD and other dystrophinopathies in clinical trials is not as well established, this section endeavors to highlight what is known.

The biomarker segment of this guidance is split into two subsections. This initial one addresses biomarkers found in muscle tissue. The biopsy-based biomarker used predominately in DMD trials, namely, dystrophin, is widely accepted by field experts as the appropriate pharmacodynamic biomarker for therapies targeting its expression. A significant treatment-related expression can verify the mechanism of action and facilitate dose selection in subsequent trials. While the main molecular determinant differentiated DMD from BMD phenotypes is the level of dystrophin expression, natural history studies in DMD patients alone, it is clear that those who express even very low levels of dystrophin have a slower disease progression [131, 231]. Biopsy-based biomarkers may nonetheless be unattractive for use in large phase III studies due to the invasive collection method.

The subsequent section explores less invasive methods to monitor changes in the muscle. Some of these methods quantify proteins, protein fragments, or genetic materials in the blood or urine; these are exploratory but merit greater investments due to their convenient measurement. Some imaging techniques are much further along, especially the direct imaging of skeletal muscle using magnetic resonance imaging (MRI), or spectroscopy (MRS) that hold potential as efficacy-response biomarkers and surrogate endpoints.

Sponsors should remain informed that since the issuance of this document, the scientific community may have reached consensus on the utility of any of these exploratory biomarkers. Engaging with the FDA regarding potential biomarkers is encouraged. Meanwhile, demonstrating an effect on an exploratory biomarker could lend supporting evidence for a claim of disease modification in an NDA. When couple with other evidence suggestive of clinical benefit, sponsors could assist in establishing the use of a biomarker as a surrogate endpoint (please refer to *Qualification Process for Drug Development Tools Guidance for Industry and FDA Staff,* [*https://www.fda.gov/regulatory-information/search-fda-guidance-documents/qualification-process-drug-development-tools-guidance-industry-and-fda-staff*](https://www.fda.gov/regulatory-information/search-fda-guidance-documents/qualification-process-drug-development-tools-guidance-industry-and-fda-staff)*).*

1. Quantification of Dystrophin as Biomarkers
2. General comments

Dystrophin organizes and stabilizes the sarcolemma ensuring the efficient distribution of contractile forces and maintaining myofiber structural integrity and function. Furthermore, dystrophin acts as a scaffolding protein vital for the proper localization (and consequently function) of signaling molecules, including neuronal nitric oxide synthase (nNOS) and alpha-1- and beta-1-syntrophin. The use of dystrophin in muscle as a diagnostic (and prognostic) biomarker was discussed in the Diagnosis section.

The accurate quantification of dystrophin in muscle tissue can provide crucial support for the clinical development of dystrophin-restorative treatments. Demonstrating a significant effect on dystrophin levels could, for instance, provide proof of concept that a dystrophin-restorative therapy indeed increases dystrophin production. It is important to highlight that dystrophin quantification played a pivotal role as a surrogate biomarker that facilitated the accelerated approval of multiple antisense oligonucleoside drugs (AONs), including eteplirsen, golodirsen, casimersen, and viltolarsen [3, 19]. Nonetheless, subsequent debates surrounding the quantification methodology and validation warrants careful consideration [232].

1. Considerations related to muscle biopsies

To accurately measure either dystrophin or utrophin in skeletal muscle, meticulous attention is essential regarding specimen collection. This encompasses target muscle selection, method of biopsy, and specimen handling and preparation. Sponsors should be aware of these issues, and specifically address efforts to reduce variability in both procedure and laboratory practices among participating sites to minimize errors. All proposed quantification methods should elucidate the sensitivity and reliability of the suggested assays. Besides these technical issues, sponsors should deliberate on the ethical considerations of biopsies in clinical studies, especially the rationale for conducting repeated biopsies to assess changes in relative dystrophin levels from pretreatment muscle samples.

Ethical concerns of biopsies in children

Sponsors should recognize the ethical dilemmas associated with performing multiple muscle biopsies on patients with a degenerative neuromuscular disorder. In some cases, mandating a muscle biopsy could deter participation in a trial. For example, one recent study found that while parents and patients were amenable to have a biopsy for a dystrophin-restoring therapy in an open label trial, their willingness considerably diminished for a placebo-controlled trial [56]. Conversely, another study indicated that the muscle biopsy requirement was not a major deterrent for participants in studies of GT products, though, it is important to note the high anticipation of benefit from GT products [32]. An inherent risk with biopsies is the necessity for anesthesia, particularly for the pediatric population. This risk is increased in protocols requiring multiple biopsies.

Given the invasiveness of the procedure, sponsors should consider whether biopsies are necessary when planning a clinical trial with an aim to restrict their frequency. If a biopsy is required, caregivers have underscored the importance of receiving their child’s results [56]. Therefore, it is paramount that the biopsy provides a useful specimen and that an appropriate post-treatment interval has been chosen. Sponsors should have an SOP for the acquisition, handling, preparation and transportation of biopsies (see below). Biopsies should not be performed at clinical sites where the specimens may be mishandled. If biopsies are required, sponsors should consider selecting only clinical trial sites that have performed and handled muscle biopsy before or to design trials in which the biopsies are performed exclusively at proficient sites. Finally, sponsors of trials in which muscle biopsies are performed should pledge to provide timely feedback regarding biopsy analysis to both the trial participants and the wider DMD community.

Criteria of an appropriate biopsy for dystrophin quantification

***Site (muscle group) and method of biopsy:*** At baseline, the amount of dystrophin varies by donor, mutation, and muscle group. For this reason, baseline muscle biopsies are essential in documenting changes induced by novel therapeutic agents targeting dystrophin or utrophin. Determination of dystrophin or utrophin content depends on the method utilized and the denominator used (eg, total protein or RNA content, myofibrillar protein content, unit membrane area). Many DMD patients show revertant fibers (endogenous clonal exon skipping), varying by mutation and muscle group.

The biopsy sites should be chosen to maximize the information on dystrophin or utrophin expression pre- and post-treatment. Because DMD is a disorder marked by progressive replacement of contractile muscle by fat and fibrosis, care must be taken to ensure that muscles chosen for biopsy contain sufficient myofibers for meaningful analysis. Sponsors should describe the methodology used to assess site selection, which may include both clinical and radiographic (MRI or ultrasound) assessments.

Two alternative methods for muscle biopsy exist. The traditional open biopsy allows direct visualization of muscle, and, in general, can ensure sufficient tissue for several rounds of complementary forms of analysis (as discussed below). Needle biopsies, which are generally considered less traumatic and leave a smaller scar, may be suitably performed with a Bergstrom needle or a more modern device, such as the self-contained vacuum-assisted biopsy system [233, 234]. Spring-loaded biopsy gun devices generally do not obtain sufficient samples for the analyses below, and do not generally preserve muscle architecture for immunohistochemical assessment. Needle biopsies may be performed under ultrasound guidance.

***The handling of the biopsy:*** *This methodology represents agreed upon good practice at the time of writing this document. However, sponsors are encouraged to utilize the best current methodology at the time of conducting their trial [*57].

* In all cases, biopsies should be performed by physicians familiar with the proper intra-operative handling of muscle specimens to avoid artifacts (excessive stretching, torque).
* Tissues should be flash/snap frozen in isopentane cooled to the temperature of liquid nitrogen as soon as possible after surgery.
* Care should be taken to avoid the use of tissue-embedding media that compromise biochemical analyses involving gel electrophoresis (immunoblots, mass spec).
* Flash-frozen tissues should be stored in prechilled (dry ice), small, airtight, screw-top tubes. Hydration of the container (including ice frozen in bottom of tube) may prevent desiccation artifacts (freeze drying) with extended storage.
* Shipment and transport with temperature monitoring of biopsies from clinical sites to laboratory of analysis: Great care should be made in selecting the courier confirming their expertise in low temperature-controlled shipments and have significant demonstrable history of shipping clinical materials. Sponsors should consider taking two small biopsies that are frozen separately, sending one specimen, and once it has arrived, safely sending the other specimen.
* Samples must not be allowed to thaw at any point, as freeze-thaw cycles decrease intact dystrophin or utrophin content as an artifact.
* Lab qualification issues: Sponsors should only utilize laboratories that are qualified to handle muscle biopsies.

Minimizing variability and sampling errors:

* Sponsors should be aware that a potential limitation of muscle biopsies and quantitation of dystrophin or other myofiber proteins can be the age-related replacement of muscle with variable fibrosis and fat in DMD patients. Dystrophin is only expressed in myofibers, and the gradual age-related loss of myofibers in DMD patient muscle complicates the interpretation of dystrophin rescue. However, the above-mentioned procedures are intended to help minimize such complications.
* Some experts have proposed the use of imaging to guide the biopsy to make sure that the specimen contains an adequate sample of myofibers rather than fibrotic tissue.
* Muscle biopsies from BMD patients, female DMD-carrier patients, and DMD patients often show variability in expression of dystrophin both in neighboring myofibers, between different regions of the same biopsy, and between different biopsies. Histopathology can also be variable within these same biopsies.
* Therefore, quantification of dystrophin expression in DMD biopsies requires rigorous protocols with adequate controls, extremely careful sample handling, and careful examination of a large number of fields (or the entire biopsy cross section) for quantitative analyses. Fit-for-purpose automated analyses are preferable to grading by pathologists; in the latter case grading should be performed by experienced pathologists or readers blinded to the treatment assignment of the patients. Similar issues with variability in utrophin staining should also be expected due to differing regions of myofiber regeneration.
* Sponsors should be familiar with the most current methods to minimize variability and sampling errors when evaluating biochemical efficacy in clinical trials.

1. Dystrophin analyses

Appropriate control tissues

For nearly all of the analyses, consideration of the expression or localization of dystrophin relies upon comparisons to normal control tissues. Because dystrophin levels may vary between different muscle tissues, the reference healthy samples would ideally be from the same muscle as the trial biopsy, although this is often difficult to do in practice. It is imperative that pooled samples be used as controls for immunoblots, or average values from multiple control samples for immunofluorescent methods, because dystrophin levels also differ significantly between individuals [235, 236]. Finally, because of significant gender differences in dystrophin expression between males and females [236], male control tissues should be used.

Broadly disseminated techniques

At present, the two most commonly used methods to quantify dystrophin are immunofluorescence (or immunohistochemical analysis) and Western blot (immunoblot). Immunofluorescence can be used to determine the percentage of muscle fibers that express dystrophin, whether dystrophin is properly located at the fiber membrane, and the levels at which dystrophin is expressed in these fibers. Western blot can show both the total amount and the size of dystrophin in the specimen. The methods are complementary, and protocols that allow standardization of the methodologies across laboratories have also now been published [236-240]. While neither technique provides a complete account of dystrophin restoration, both methods can show increases of dystrophin expression over baseline, although, to date, FDA has only accepted Western blot as a dystrophin-expression surrogate endpoint for regulatory approval. Emerging techniques include mass spectrometry and capillary immunoassay. A comparison of methods is found in the Table below.

**Immunofluorescence (IF) or immunohistochemical (IHC) analysis by type:** Many pathology laboratories routinely analyze dystrophin expression via IF or IHC protocols but only report semiquantitative results. Necessary methods to quantitate dystrophin in a manner able to support clinical trials and drug development have not been broadly disseminated, in part because of a reliance on confocal imaging techniques in earlier studies [237, 240]. Standardization of immunofluorescence methods across laboratories has not yet been widely established, but the availability of methods accessible to all researchers may facilitate such adoption [236].

An advantage of IF or IHC methods is that they examine both relative levels of dystrophin and correct localization at the sarcolemma. Standardized IF analysis may also be more sensitive than standard Western blotting [237]. Immunostaining quantitation of relative dystrophin levels should be done by specific referral laboratories with extensive documented experience with dystrophin quantitation methods and demonstrated reproducibility and precision—both intra-assay (eg, between sections of a biopsy) and inter-assay (eg, between experiments or technicians).

One approach to IF quantification is to assess the percentage of dystrophin-positive fibers (PDPF). For this to be valid, the criteria applied to call a fiber dystrophin positive should be themselves quantitative, explicitly described, and predefined. IF quantification also allows overall assessment of fiber dystrophin intensity, which may not correlate directly with PDPF; for example, in patients with BMD, the percentage of fibers defined as positive may approach 100%, but the overall intensity may be 50% or less [236].

**Western blot**: Western blot (or immunoblot) is a standard method of quantifying the amount and size of a protein. Quantification of dystrophin, however, presents challenges that largely arise from the fact that it is a large molecular weight (427kD), low abundance protein. This leads to frequently encountered technical challenges with consistency and reliability of multiple steps of the protocol, including solubilization, electrophoresis, transfer (blotting), immunodetection, and quantitation.

Methodology: One often-published method is to use cryosections (lacking any embedding media; 20-50 10 micron) collected in prechilled small tubes, with rapid solubilization in low volume high SDS buffer, immediate electrophoresis on gradient Tris-acetate gels, and normalization of dystrophin content to myofiber proteins in the same blots or post-transfer gels.

Despite the demonstration of a high degree of reproducibility among labs using this general approach [237], there has been a growing consensus that normalization of dystrophin content to other proteins (such as alpha-actinin) is inadequate, as variability among samples of these proteins has been observed. For this reason, it is preferred to perform quantification using a normative dilutional curve on each blot consisting of pooled normal samples spiked into muscle specimens with no dystrophin expression [231].

Emerging technologies

**Capillary electrophoretic immunoassay:** Capillary immunoassay methods are potentially faster and more easily quantifiable assessments of dystrophin (or other protein) expression, using much smaller sample sizes. Early results regarding quantification have been promising, albeit tempered by challenges in accurate sizing of the dystrophin protein in relation to device standards [235].

**Mass spectrometry**: Mass spectrometry methods show potential advantages of high reliability, accuracy, and sensitivity. Mass spectrometry methods typically require the addition of stable isotope labeled peptides to the solubilized human muscle sample. One recently reported exploratory method uses stable isotope labeled mouse muscle mixed with human muscle biopsy samples, leading to highly accurate and reliable quantitation of dystrophin over a large dynamic range [241]. This method can be modified to quantify mini or micro dystrophin following gene therapy [242].

Benchmarking to immunoblot and immunostaining has been done in preclinical trials of exon skipping and has shown concordance between all methods [243]. The major distinctions are that the reliability of the mass spectrometry method appears considerably better than immunoblotting or immunostaining, due to the many multiple quantitative measures (peptides) per test, and the high resolution and quantitative precision of the mass spectrometers. The disadvantage of mass spectrometry is that it does not provide information on the location within the muscle fiber. This approach, however, has met FDA guidelines for method qualification.

**Table 2: Dystrophin quantification method overview**

A close-up of a medical chart

Description automatically generated

Current limitations for all methods

All methods in current use allow only relative quantitation and not absolute quantification of dystrophin levels. Since dystrophin levels vary between healthy individuals, using the same control reference samples–whether pooled in immunoblotting or averaged in IF analysis–is necessary to extrapolate relative quantitation. This allows dystrophin quantification to be presented as a percentage of normal control samples.

Sponsors are encouraged to consider methodologies that allow for standardization. Ideally, the percentage of positive fibers as well as the relative dystrophin levels should be assessed. Sponsors considering including such measures in a clinical trial are encouraged to discuss their plans with FDA.

Other considerations

**Blinding:** Simultaneous testing of pre- and post-treatment biopsies is expected to minimize variability in results. It is imperative that analyses be performed and analyzed by staff blinded to the treatment status of the sample.

**Minimizing variability due to muscle biopsied:** Whenever possible, dystrophin expression (and any RNA-based analysis, such as exon skipping efficiency) should be compared within an individual using the same muscle groups in a pre- and post-treatment biopsy.

**Careful consideration of patient genotype and potential impact on phenotype:** The commonly cited “reading-frame rule”—which describes DMD as due to out-of-frame and BMD as due to in-frame mutations—is, when based upon analysis of the genomic mutation alone, only 90% accurate in predicting DMD [244]. A range of mutations considered predictive of a severe DMD genotype may result in BMD, typically due to alteration of splicing of the *DMD* transcriptto allow sufficient dystrophin expression to attenuate phenotype, and pretreatment biopsies from such patients would show significant dystrophin expression. Because even low-level dystrophin expression can modulate disease severity, in order to use dystrophin expression as a surrogate biomarker, sponsors should consider enrollment criteria that are not based on genotype alone but incorporate sufficient clinical parameters to make disease amelioration due to pre-existing endogenous dystrophin expression unlikely.

Clinical meaningfulness of dystrophin expression as a biochemical outcome measure

The amount of dystrophin restoration necessary to achieve clinical benefit is unclear at present and may depend upon the disease stage at treatment initiation and state/health/fragility of the muscle. Nevertheless, accumulating evidence supports the idea that even low levels of dystrophin expression may confer clinical benefit [131, 245].

It has been established that dystrophin levels correlate with the prognosis seen in female DMD carriers [246] expressing normal dystrophin [247], and in many but not all male BMD patients expressing abnormal but at least partially functional dystrophin [158]. While the amount of dystrophin restoration that can be achieved therapeutically is yet to be seen, the broad consensus is that similar levels of dystrophin restoration would be likely to result in clinically meaningful benefit.

However, the correlation is unlikely to be perfect between what may be seen as a result of therapeutic *de novo* dystrophin introduced in DMD patients later in life and what has been reported in female carriers and BMD patients, where some dystrophin is present from birth. The therapeutic benefits of dystrophin restoration may depend upon the age at treatment initiation, the health of the muscle in the patient receiving treatment, and the functionality of the version of dystrophin that is introduced. As an example, versions of dystrophin engineered to fit within adeno-associated vector (AAV) vectors do not have exact correlates in BMD patients, so their functional benefit cannot be predicted with certainty based upon levels of expression in BMD patients. Nevertheless, in a medically addressable population, some degree of dystrophin restoration is reasonably likely to result in some clinical benefit, although the effect size and timing of clinical response are unclear at the time this guidance is being written.

1. Muscle Biopsy Biomarkers: Exon-Skipping Detection to Confirm Mechanism of Action in the Exon-Skipping Field

DMD predominately results from mutations in the *DMD* gene causing a reading frame shift that leads to premature translation termination. Exon skipping approaches alter splicing of the dystrophin pre-mRNA, which restores the reading frame. In most cases, this restoration allows translation of an internally truncated yet functional dystrophin protein. Such exon skipping can be achieved using AONs [248], or AAV-encapsidated U7snRNAs expressing an RNA antisense sequence [249-251].

A commonly used parameter to assess and compare the efficacy of various antisense molecules is the exon skipping percentage. This is defined as the percentage of transcripts in which the targeted exon is skipped compared to the total number of dystrophin transcripts (skipped and non-skipped). There appears to be a correlation between exon-skipping percentages and dystrophin restoration, taking into account that quantification by both methods has only been achieved by highly specialized centers. Thus, the measurement of exon skipping at the RNA level is an important assessment in confirming the capability of AONs’ ability to successfully modify the intended gene target.

Given the low abundance of dystrophin mRNA, the efficacy of AONs in inducing exon skipping has mainly been gauged at the transcriptional level. This is done using the semi-quantitative nested reverse-transcription polymerase chain reaction (RT-PCR) or quantitative PCR (qPCR), each with differing protocols and amplification cycles. Digital droplet PCR (ddPCR) offers an alternative method that can facilitate absolute quantification of the various transcripts. This might be more suitable for clinical trial samples [252]. Additionally, RNA sequencing (RNA Seq) can quantify exon skipping, and has the benefit of allowing unbiased assessments of off-target skipping. This offers further evidence for the safety of exon skipping approaches [253].

Because of the different dynamics of transcripts and proteins, the exon skipping levels may not directly correlate to dystrophin levels. Nevertheless, this is another pharmacodynamic marker that can confirm whether exon skipping has at least occurred after treatment with AONs.

Sponsors considering including such measures in a clinical trial are encouraged to discuss their plans with FDA.

1. Non-Biopsy Based Biomarkers
2. General comments

This subsection deals with two classes of exploratory biomarkers: noninvasive imaging modalities, and substances that can be measured in the blood and urine. Both classes of biomarkers in development could have considerable advantages over muscle biopsies in that they sample large groups of muscles, and thus do not suffer from the sampling errors that can be encountered with muscle biopsies, particularly if adequate care is not taken to follow appropriate procedures. While at the time of writing, we recognize that sponsors may need to rely upon established methodologies in their registrational studies, we would also encourage them to explore the use of less invasive biomarkers in their clinical development programs.

1. Imaging modalities

Imaging can be used to noninvasively monitor multiple aspects of disease in muscular dystrophy. As a result of considerable research in recent years, muscle fat infiltration measured using MRI or MRS has the documented potential to serve as an efficacy-response biomarker and surrogate endpoint.

Fibrofatty replacement of muscle tissue is a hallmark of DMD. MRI and MRS are well-suited to the differentiation of muscle and fat, typically quantified as muscle fat fraction, or the proportion of MR signal coming from fat. Muscle fat fraction measured using MRI is significantly correlated with proportion of fat tissue obtained via muscle biopsy [254]. MRI and MRS are noninvasive and can accommodate both small and large patients, and muscle fat infiltration can be accurately and reproducibly measured across multiple sites and vendors [255]. However, protocols should be planned to allow field of view to be matched to an individual’s body size for optimum quality. In young boys with DMD, motion artifacts are more likely than in older subjects, and studies should plan to account for this by minimizing the length of individual scans, incorporating redundancy into acquisitions, or providing distractions such as videos for subjects to watch during scanning. Additionally, while MR is feasible and meaningful even in the late nonambulatory phase, positioning and data acquisition can be more challenging in nonambulatory individuals [60, 61].

MR measures of fatty infiltration, which include both fat fraction and the transverse relaxation time of muscle tissue [58], are closely related to functional performance, indicating that these measurements are clinically meaningful. A recent systematic literature review synthesized these studies, finding that there were moderate to excellent correlations between MR measures and function across 17 studies meeting inclusion criteria [59]. Additionally, there is a strong predictive relationship between muscle fat infiltration and future disease progression, and multiple investigators have reported that muscle fat fraction is predictive of LOA [58, 60, 62, 63, 256, 257] and loss of HTMF [60].

MR measures of fatty infiltration are sensitive to disease progression and therapeutic intervention in both DMD and BMD [108, 258-265]. Multiple investigations have shown that the effect size or standardized response mean is higher for MR outcomes than standard functional measures, and that significant MR changes take place in the absence of measurable functional changes in DMD and other muscular dystrophies [108, 258, 266, 267]. Thus, these biomarkers may detect treatment effects in smaller samples or younger cohorts than functional outcome measures, making them well-suited to early readout of therapeutic studies.

Emerging imaging methods

Depending on a candidate therapeutic’s mechanism of action, other quantitative MRI or MRS techniques might offer valuable response biomarkers. For example, the anti-inflammatory effects of corticosteroid therapy have been detected by measuring the intramuscular sodium concentration [268, 269] or the transverse relaxation time (T2) of muscle tissue or water [258]. Blood-oxygen-level-dependent (BOLD) imaging could be useful in the evaluation of therapies targeting nNOS [270, 271]; and phosphorus-31 MRS may have utility for measuring therapies that aim to improve mitochondrial function [272].

Both quantitative muscle ultrasound and electrical impedance myography (EIM) may be useful as measures of muscle changes associated with dystrophic pathology. These measurements are also noninvasive and can be measured in a neuromuscular clinic setting. Both EIM and ultrasound are sensitive to disease progression [273-275] and correlated with function in DMD [276-281]. However, further evidence of their prognostic value is needed to evaluate their potential as surrogate endpoints in DMD. Sponsors considering including such measures in a clinical trial are encouraged to discuss their plans with FDA.

MRI/MRS: role in clinical trials

MRI and MRS biomarkers are strongly related to functional ability and predictive of future functional changes. In addition, these biomarkers are highly sensitive to disease progression and therapeutic intervention. These qualities make them well-suited for implementation in clinical trials. Sponsors are encouraged to combine MRI/MRS outcome measures with functional outcomes in treatment trials to provide further evidence demonstrating that MRI/MRS measures can serve as an efficacy-response biomarkers and surrogate endpoints to accelerate clinical development.

1. Serum and urine accessible biomarkers

Biomarkers in the blood or urine hold promise as measures of muscle health. The blood biomarkers that have been explored to date include both protein and RNA, while urine biomarkers are primarily metabolites. However, there are potential challenges to their use [64]. The most commonly used serum biomarker, creatine kinase (CK), is not suitable as a primary endpoint in the ambulant population, as its value may vary depending not only on muscle mass but activity of the subject, with exertion causing significant elevations in comparison to baseline in DMD patients.

Some circulatory biomarkers potentially contain signals coming not only from the affected muscles, but also from other cells involved in response to the muscle damage, including inflammatory cells, motor neurons and fibrosis. The blood and urine biomarkers that originate in skeletal muscle may suffer from the fact that they may reflect the amount of skeletal muscle as well as the health and integrity of the muscles. Changes in biomarkers may be related to maturation, loss of muscle mass, or ubiquitous corticosteroid therapy [65]. However, with increasing data characterizing the performance of circulating biomarkers being gathered rapidly, the potential utility of some of these markers to monitor treatment response may become more apparent. These are discussed in greater detail in the appendix. Sponsors could potentially benefit by including measurement of some of these in their development plans.

Other circulating biomarkers could be useful for monitoring the safety of an experimental treatment. For instance, there are data to support the use of serum glutamate dehydrogenase (GLDH) as a liver injury biomarker in patients with DMD (see Appendix: Additional Exploratory Biomarker appendix) [282].

Sponsors considering including exploratory biomarkers in their trials should focus on the context of use for a circulating biomarker, and specifically address how that biomarker response will depend on the mechanism of action. Sponsors should be aware that it will be difficult to show a biomarker is predictive of clinical benefit without efficacious treatments. Only once a biomarker is independently validated can it serve as an endpoint in a registration trial. However, with a broader collection of these biomarkers, it may be possible to link changes in several biomarkers to milestones such as the capacity to perform an activity. It may then become possible to apply tools which model longitudinal trajectories and survival (such as the Duchenne Regulatory Science Consortium [D-RSC] clinical trial simulation models) to biomarkers in the same way as they are being used for outcome measures in natural history studies.

Recommendations regarding serum and urine biomarkers:

Sponsors should endeavor to collect serum and urine specimens at time points during the trials with the appropriate ethical agreement for use in future biomarker development as research materials. Potentially any samples eventually identified to be samples from the placebo group could be biobanked and be made available to assist other entities developing new biomarkers.

Sponsors should be aware that development of clinically useful biomarkers requires several steps [283]. Sponsors considering development of a biomarkers are referred to recent draft guidance that describes the evidentiary framework to be used to support biomarker qualification under the 21st Century Cures Act (see *Biomarker Qualification: Evidentiary Framework Guidance for Industry and FDA Staff*, at

<https://www.fda.gov/media/119271/download>).

A more detailed review of the experimental biomarkers is provided in the Additional Exploratory Biomarker appendix.

Appendix: Additional Exploratory Biomarkers

A detailed review of updates since the previous guidance:

1. Imaging

Imaging measurements, particularly MRI, have emerged as highly promising biomarkers for clinical trials in DMD in recent years. Because it is noninvasive, imaging can be used at many time points. The most promising imaging biomarkers, which will be the focus of this discussion, capture the progressive replacement of muscle with fatty and fibrotic tissue – these markers include MR-measured fat fraction as well as ultrasound-measured echo intensity and backscatter. Additional imaging biomarkers of muscle tissue inflammation, edema, atrophy, hypertrophy, fiber arrangement, or energetics may be valuable in some clinical trials in DMD and are discussed briefly.

MRI and MRS provide highly detailed and quantitative information, which can be localized to individual skeletal muscles. Healthy muscle can be distinguished from diseased muscle, and the infiltration of fat and fibrosis can be monitored and quantified. The primary limitation of MR approaches is that they are costly, and the evaluation is time consuming, as compared to other imaging approaches. Nonetheless, the power of MRI/MRS has made it increasingly important for DMD studies.

MRI is the modality of choice when high-resolution/high-contrast images of soft tissue are required. MRI is a noninvasive technique that does not use ionizing radiation and provides outstanding volumetric coverage of tissue; instruments are widely available, and the technique can be run quantitatively and standardized across sites [255]. MRS is a class of techniques used to measure the biochemical properties of tissue. The fundamental hardware required for MRS is identical to that used in MRI, which makes MRS a high-value ancillary study to MRI. A fundamental strength of MRS is the increased specificity for measurement of distinct tissue constituents. An example relevant to DMD is the high-fidelity separation of tissue water and fat signals, which typically co-contribute to standard MRI signals collected from skeletal muscle of DMD individuals. MRS techniques have been applied to investigate cellular metabolites typically using the most abundant magnetic isotopes of hydrogen (1H), carbon (13C), and phosphorus (31P). MRS has been used to improve diagnosis, to better define the natural history of a disease process, and in some studies to monitor the response to therapy [264, 265, 284-291]. While most MRI/MRS investigations of DMD skeletal muscle have focused on the lower extremities, investigations of shoulder and upper extremity muscles as well as the respiratory muscles have increased in recent years [60, 61, 260, 292-298]. Finally, the fact that MRI/MRS measures are obtained with the subject at rest greatly reduces the impact of motivational issues that confound many functional measures. Taken together, these attributes make MRI/MRS attractive techniques for longitudinal investigations of rare disease in human pediatric subjects.

MRI/MRS: Emerging biomarkers of human muscular dystrophy pathology

Numerous studies have demonstrated the ability of MRI to detect alterations in skeletal muscle structure in patients with muscular dystrophy [299-311]. Indeed, due to its excellent soft tissue 3D imaging capability and the ability to perform longitudinal measures of muscle mass, Most MRI investigations historically relied on T1- weighted images and the contrast generated by fatty tissue infiltration to visualize the pattern of muscle involvement in muscular dystrophy patients and used a grading system to categorize disease severity [305, 312]. However, considerable efforts over the past decade have provided strong evidence supporting the use of quantitative MR measurements as a sensitive surrogate outcome measure for clinical trials.

**MR measurement of fatty infiltration:** Both MRI and MRS can robustly and accurately quantify intramuscular fat fraction (FF). In addition, fat fraction is closely related to the transverse relaxation (T2) time of muscle-measured multi-echo MRI (sometimes called bulk or global muscle T2) [58] and to qualitative Mercuri grading [313]. Finally, the replacement of muscle with fat can be tracked by measuring the muscle area occupied by nonfatty tissue, sometimes called contractile area [314-317]. A number of studies have found moderate to strong correlations between muscle fat infiltration or its surrogates and measures of functional ability [108, 257, 292, 295, 299, 301, 302, 304, 306-309, 317-329]. A recent systematic literature review synthesized these studies, finding that there were moderate to excellent correlations between MR measures and function across 17 studies meeting inclusion criteria [59]. More recent work has examined the predictive relationship between MR fat fraction and functional ability, and multiple investigators have reported that muscle fat fraction is predictive of LOA [58, 62, 63, 257] and loss of HTMF [60].

Considerable recent work has supported the responsiveness of MR measures of fatty infiltration to disease progression and therapeutic intervention in both DMD and BMD [108, 258-265, 330]. These investigations have frequently highlighted the sensitivity of MR biomarkers. Multiple investigations have shown that the effect size or standardized response mean is higher for MR outcomes than standard functional measures. Thus, these biomarkers may detect treatment effects in smaller samples than functional outcome measures, making them well-suited to early readouts of therapeutic studies. Several studies have shown significant MR changes taking place prior to any measurable functional changes in DMD and other muscular dystrophies [108, 258, 266, 267].

**MR imaging of inflammation:** Other investigations have focused on imaging strategies that are sensitive to muscle damage and inflammation to visualize early dystrophic muscle pathology, which may be particularly important in younger boys with DMD. Short-tau inversion recovery (STIR) sequences can be used to visualize regions of increased signal intensity or muscle inflammation in dystrophic muscles of even very young boys with DMD in the absence of fatty tissue infiltration [302, 331], and has been directly linked with inflammatory markers (serum and tissue) in FSHD [332]. Na+ imaging has also showed that areas of hyper intensity on STIR images from skeletal muscle in DMD subjects are directly related to muscle edema; [269, 333]. Na+ imaging also detects a decrease in inflammation with corticosteroid therapy [268]. Additionally, in quantitative T2 (transverse relaxation time) weighted imaging, both MRI [334-339] and MRS [309, 340] have been used to measure the T2 of water, which is elevated in inflammation, muscle damage, and edema and decreased with corticosteroid treatment in DMD [258].

**MR imaging of fibrosis:** A significant challenge for MR and other noninvasive imaging modalities is the quantification of fibrosis. The observed MR signal intensity associated with fibrosis undergoes a characteristic rapid decay due to the extremely short T2s of water molecules associated with collagen.

Cardiac MRI studies in DMD subjects have reported an age-related decrease in myocardial T2 compared to controls [286, 303] and an increase in myocardial T2 heterogeneity [341]. Similar results have been observed in animal models with diabetic-induced cardiac fibrosis [342, 343]. The decreased T2 has been hypothesized to represent an increased fraction of water molecules “bound” to collagen and other fibrotic tissue. Similar age-related decreases in muscle water T2 were seen in both calf and thigh skeletal muscles in boys with DMD, but not healthy controls. This decrease in T2 is typically masked in skeletal muscle imaging by the large amounts of fatty tissue deposition [340]. Ultrashort echo time (UTE) imaging shows promise as a potential method to quantify fibrosis in skeletal muscle, but technical hurdles remain before this technology is suitable for widespread clinical use [344].

Ultrasound

Ultrasound (US) is a noninvasive imaging technique that can provide rapid anatomical and functional measurement of human tissue, and places low demand on the subject [345]. As such, it is well suited for imaging in young children [274, 331] as well as older individuals. US imaging has been extensively applied to investigate cardiac abnormalities associated with DMD (see Cardiomyopathy section]. Muscle atrophy and intramuscular fibrosis and fatty infiltration can be visualized using US of skeletal muscle [346, 347]. US density analysis of skeletal muscle provides a sensitive method for distinguishing between healthy children and children with neuromuscular disorders [348]. Quantitative muscle ultrasound has been applied to study DMD by quantifying echo intensity and backscatter and muscle thickness. In recent years, additional analyses of ultrasound signal have shown promise for quantifying dystrophic pathology [277, 278, 280, 349]. Muscle ultrasound parameters are significantly correlated with age in DMD, reflecting increased muscle pathology with disease progression [348, 350]. These measures are also sensitive to disease progression longitudinally in DMD [274, 275]. Finally, increased echo intensity and backscatter are associated with muscle function, which has been measured using ambulatory status, functional grading, muscle strength measurements, and standard functional assessments such as the 6-minute walk test (6MWT) [274, 276-280].

Electrical Impedance Myography

Electrical impedance myography (EIM)provides a reliable and noninvasive approach for quantifying tissue composition and compartmentation and as such has relevance for assessment and monitoring of neuromuscular disease pathology with and without therapeutic intervention [274, 351, 352]. Recently, the high sensitivity to disease progression of EIM has been demonstrated [273]. EIM 50 kHz phase measurements have been reported to correlate well with standard functional measures in DMD such as the NorthStar Ambulatory Assessment test (rho = 0.83, p = 0.02) [281].

DEXA

Dual energy X-ray absorptiometry (DEXA) is a technique that can be used to estimate body composition, including bone mineral density and body lean soft tissue, and indirectly provides an estimate of fat content. Studies of DEXA in DMD subjects have found decreased regional lean mass, increased regional fat mass, and decreased strength — but DEXA cannot distinguish between muscle and fibrosis [353]. Nevertheless, there may be a role for DEXA to help normalize muscle mass for the accurate measurement of serum biomarkers.

Muscle imaging: Future directions

While MR measurement of muscle fatty infiltration, in addition to measurement of inflammation, have undergone substantial development in recent years, other methodologies show promise in DMD. Specifically, MR diffusion tensor imaging (DTI) has been used to measure changes to muscle structure with muscular dystrophy but has not emerged as a leading candidate biomarker for DMD, with some investigations concluding that it does not differentiate DMD and control muscle [269, 290, 338, 354-357]. Muscle blood flow, known to be mediated by nNOS localization which is impaired in DMD and BMD, has been investigated using BOLD MR imaging. Finally, muscle metabolism can be measured using 31P-MRS, both at rest and during exercise [292, 335, 338].

Muscle imaging biomarkers, particularly MR measures of fat fraction, have shown substantial promise in DMD. These biomarkers capture disease involvement differently than biomarkers measured in biopsy or serum samples. Future studies may benefit from the inclusion of composite biomarkers—for example, a combination of serum markers and MR fat fraction, or ultrasound and EIM [358]. Additionally, while MR fat fraction is increasingly included in clinical trials in DMD, future studies may consider measures of inflammation or metabolism depending on each drug’s mechanism of action.

Exploratory biopsy-based biomarkers

Utrophin Expression Analysis

Utrophin is an autosomal paralogue of dystrophin that plays a similar role in prenatal muscle. Postnatally, utrophin is largely but not exclusively restricted to the myotendinous and neuromuscular junctions, and its overexpression has been proposed as a therapeutic approach to DMD.

Utrophin is upregulated in DMD muscle, with evidence that greater utrophin expression correlates with disease severity [243]. Utrophin overexpression has been proposed as a potential therapeutic approach for DMD and BMD, but, at the moment, the role of utrophin and its therapeutic value is questionable.

The methodologies employed to quantify the expression of utrophin or associated proteins such as the sarcoglycans and nNOS closely mirror those described above for dystrophin, although there are some technical challenges described in the literature [359]. In designing clinical trials of utrophin upregulation therapies, sponsors should address considerations raised for dystrophin quantification, above.

Serum and urine biomarkers

Sampling blood and urine in DMD may indicate the health and integrity of skeletal muscles. Biomarkers in the blood or urine potentially contain signals coming not only from the affected muscles, but also from other cells involved in response to muscle damage, including inflammatory cells and motor neurons, and fibrosis. The blood biomarkers that have been explored to date include both protein and RNA, while urine biomarkers are primarily metabolites [360-363], RNAs (miRNA and mRNAs) [364-367], and proteins [368-372].

Proteins, protein fragments, and metabolites

Blood-circulating protein biomarkers are the most studied biomarker for DMD to date. A large number of these have been identified in the discovery phase by different labs using different cohorts and high-throughput methods, including antibody beads array [370], mass spectrometry-based proteomics methods [373], and aptamer panels [371, 372]. What is missing for most is a clear context of use—defining their potential clinical utility for diagnosis, defining disease progression or prognosis, or monitoring response to corticosteroids treatment and dystrophin replacement therapies. However, data collected in serum samples from DMD patients enrolled in natural history studies or clinical trials suggest that a number of these circulating protein biomarkers reflect alterations in muscle such as sarcolemma instability, muscle injury, inflammation, muscle regeneration, and fibrosis.

Fibrosis biomarkers assays are another platform under evaluation in DMD that enable the identification of specific protein fragments, or ‘neo-epitopes’, produced when proteins are subject to post-translational modifications (PTMs) (eg, cleavage, glycosylation, or citrullination), that are related to defined (patho)physiological processes during morphological deterioration [374-376]. The resulting specificity between the parent protein and the relevant PTM gives rise to modified peptides that are associated with specific (patho)physiological processes in cancer, fibrosis, or neuromuscular degeneration.

miRs (miRNAs and mRNAs) are short (~22 nucleotide) RNA molecules that function in the post-transcriptional regulation of gene expression by inducing mRNA degradation or translational inhibition. A set of miRs, called dystromirs in some studies, have been identified in the serum of DMD patients, as well as that of DMD animal models, at copy numbers that are significantly different from healthy subjects or control animals. miRs may have advantages over proteins or metabolites as serum biomarkers [367, 377, 378]. Quantitative RT-PCR serves as a rapid, sensitive, and accurate method of detection of these small RNA molecules. Since they may be actively exported from muscle cells, serum levels of miRNAs could be less sensitive to the effects of physical activity than creatine kinase (CK).

Determining the context of use

A number of cross-sectional studies have looked at a range of serum and urine biomarkers and their potential to discriminate between healthy and DMD patients. These studies listed matrix metallopeptidase 9 (MMP-9) [379], fibronectin [380],muscle protein fragments in serum and urine*,* succinate in *mdx,* prostaglandin D2 [381], and 3-methyl-L-histidine [382]. Some proteins may be markers of the disease repair process and tissue remodeling. Although the associations are evident, a clear context of use for these biomarkers is still not clear.

Some of the muscle injury biomarkers in discovery appear to behave similar to CK, which depends on muscle mass and may be affected by exercise, muscle damage, and age. Biomarkers of muscle injury and progression naturally increase with age, so levels will be age dependent (which has implications for comparison in different age groups). While these may flag the deterioration of muscle seen in DMD, they may not correlate with performance and may not have prognostic value. However, some may provide additional information (including response to treatment and disease progression).

For instance, recent studies have enabled the identification of potential prognostic biomarkers. One analyzed a large panel of pre-selected biomarkers in a large retrospective multicenter cohort. Modelling of the data enabled the identification of proteins associated with wheelchair dependency after correcting for age and treatment with steroids. Interestingly, a time-to-event analysis suggested that some of these proteins and miRNA (such as malate dehydrogenase 2 [MDH2], KRT10 [a keratin 10 miRNA], DES, myosin light chain 3 [MYL3], collagen type I alpha 1 chain [COL1A1], electron transfer flavoprotein A [ETFA], C4b-binding protein alpha chain [C4BPA]) may be predictors of a clinically meaningful milestone such as LOA [369]. Another recent study showed how on serum creatinine levels are associated with performance (as measured by NSAA, 6MWT, Vignos, and the 10-meter walk test) mostly in a cross-sectional comparison but also in small longitudinal sub-groups [362, 383]. Another recent study identified nine blood protein biomarkers related to muscle mass that correlated with disease milestones, functional tests, and respiratory capacity [384].

While at the time of writing this guidance all of these biomarkers remain exploratory, sponsors are encouraged to screen for these potential biomarkers in longitudinal studies, especially studies aimed to define the context of use. Recent studies have shown how biomarkers can be used to identify exposure to corticosteroids [65, 385] and competing analogs [385]. Future studies in DMD and BMD patients are needed to understand the clinical utility of such markers and evaluate their predictive value of clinical outcomes. However, it will be difficult to show that a biomarker is predictive of clinical benefit without more efficacious treatments.

Safety biomarkers

Finally, there is also a need to identify biomarkers to aid in the detection of drug-induced injury. Preclinical evidence for a panel of biomarkers including MYL3, serum troponin I (sTn1), fatty acid binding protein 3 (FABP3), and creatine kinase measured by a mass assay (CKm) show that the panel outperformed or added value to the conventional skeletal-muscle-injury biomarkers CK and aspartate transaminase (AST). However, a demonstrated clinical context of use remains to be defined for the MIP biomarker panel analytes [386].

More recently, it has become clear that biomarkers to monitor drug toxicity (eg, liver injury) may be needed in DMD treatment and trials of experimental therapies. Standard liver function tests such as the transaminases ALT and AST may be significantly elevated in patients with underlying muscle impairments in the absence of hepatocellular injury, as they are also released from muscle, and are uniformly elevated in DMD patients. Recent evidence from a phase II clinical trial demonstrated that serum glutamate dehydrogenase (GLDH) is a liver-specific alternative diagnostic biomarker of the onset of hepatocellular injury [282]. Consequently, it is recommended that sponsors consider including monitoring GLDH as a biomarker of drug-induced liver injury in clinical trials for new therapies to treat dystrophinopathies such as DMD.

1. Specific Trial Design and Analysis Issues for Clinical Trials in DMD

As noted in 2019’s *Draft Guidance: Rare Diseases: Common Issues in Drug Development Guidance for Industry*, the overall goals of a drug development program is to demonstrate the effectiveness of a drug in treating or preventing a disease or condition, assess the magnitude and frequency of that effect, and the risks of the drug, thereby enabling a benefit-risk assessment and appropriate labeling [38]. One of the statutory requirements for drug marketing approval is “substantial evidence” that the drug will have its claimed effect [184]. This requirement is the same for all drugs regardless of whether they are for common or rare diseases. However, as stressed in the previous FDA Guidance on dystrophinopathies, it is appropriate to exercise flexibility in applying the statutory standards to drugs for serious diseases with unmet medical needs, while preserving appropriate guarantees for safety and effectiveness [387].

1. Key Learnings from Past DMD Trials

Sponsors involved in rare disease drug development often encounter unexpected challenges in the design, execution, and evaluation of clinical trials. Gathering “substantial evidence” can be a complex task due to:

* The logistical and statistical challenges of enrolling and conducting trials with small, dispersed, and heterogenous patient populations
* Limited natural history data characterizing the disease
* Limited experience working with novel outcome measures and limited standardization of those measures across different cohorts
* Uncertainty in choosing endpoints that reliably show a clinical effect within the specified period

The journey of drug development in DMD exemplifies these challenges and offers valuable insights for the design and evaluation of future trials in dystrophinopathies such as DMD and BMD [66, 67].

There can be tension between the community’s desire to enroll a wide spectrum of individuals representing various disease stages and phenotypes, versus the need for inclusion and exclusion criteria that prioritize enrolling individuals most likely to experience progression to a specified endpoint over the course of the trial without effective treatment. In hindsight, a number of DMD trials could have fine-tuned their inclusion/exclusion criteria for enrichment of patients on the optimal short-term trajectory, while also capturing a larger subset that could have potentially shown a treatment effect [68, 69].

In some cases, trials might have had durations that were too short to demonstrate efficacy with the selected clinical endpoint, even though there was biomarker evidence or intermediate clinical outcomes that suggested a treatment effect in post-hoc analyses. While findings from a post-hoc analysis might not directly facilitate regulatory approvals, they can be used to refine the outcome measure selection and optimal target populations for subsequent research. For instance, current data demonstrates that a change in near-term outcomes, such as changes on TFTs such as “time to stand from supine” could, in a predefined analysis, serve as an intermediate clinical outcome predictive of down-stream outcomes such as LOA (see Box 1) [69].

One of the most significant lessons from prior DMD trials is that age is not the optimal criterion for enrichment of patient trajectories. Instead, the focus should be on baseline disease severity. characteristics. Recognizing that, on average, loss of milestones occurs near certain ages, sponsors conducted trials that enrolled similarly aged boys that failed to demonstrate clinical efficacy because the participants were at different stages of disease. Thus, some trials randomized more patients likely to remain stable to placebo and those with a more rapid disease trajectory to the treatment group, or there were imbalances in patients’ rates of progression based upon phenotype.

Registrational studies may have benefited from prognostic enrichment by enrolling participants poised to progress to a trial endpoint during the study, provided their condition was still medically addressable (could be aided) by the treatment in question. Utilizing outcome measures to stage and stratify participants can also help discern the effect of treatment among subgroups with distinct disease trajectories [69].

The recent DMD gene therapy approval of delandistrogene moxeparvovec-rokl (during the preparation of these guidelines) serves as a case in point. The Agency extended accelerated approval for a specific age group based on limited efficacy data suggesting that increased micro-dystrophin expression correlated with functional benefit (an improvement in NSAA total scores). However, for older trial participants, discerning functional outcomes was complicated by an imbalance in baseline disease trajectories between the treatment and placebo cohorts. Clear demonstration of a therapy's impact on the disease trajectory, especially for those anticipated to decline, remains paramount. Yet, stability in function might also indicate a positive therapeutic response—one that is clinically meaningful in this community.

Given the urgency and importance of bringing efficacious drugs to the market, sponsors are encouraged to collaborate closely with the FDA. These engagements can help tailor their drug development strategies to best gain experience and document safety across different DMD groups and demographics, as well as other dystrophinopathies, considering both pre- and post-marketing commitments.

1. Key Features of DMD Trial Design and Analysis

Sponsors of clinical trials of investigational products for DMD are reminded that the diagnosis of DMD should be based on the clinical phenotype with dystrophin mutation (as described in the Diagnosis section) rather than upon the presence of an out-of-frame dystrophin mutation as there are individuals who do not follow the open reading frame rule. Issues related to performing trials in BMD and other dystrophinopathies are described later in this section.

While there is widespread support in the DMD community to move away from placebo-controlled designs, FDA recommends randomized placebo-controlled trials as the most efficient way to demonstrate efficacy of drugs for rare and common disorders. Nevertheless, there may be some circumstances in which use of external or historical natural history controls could contribute evidence to support approval (see below).

As pointed out in the previous guidance, trials in DMD should be conducted under the oversight of a data monitoring committee (DMC). The DMC should look for emerging safety signals at frequent intervals and, if necessary, advise the sponsor regarding appropriate measures to ensure that patients are not placed at unreasonable risk of harm [388]. To the extent possible, sponsors should gather safety data on the use of their drug or experimental treatment across the spectrum of disease, ages, phenotypes, and functional abilities.

A well-designed drug development program can gather safety data in as wide a population as possible, and efficacy data in registration trials that feature prognostic enrichment. One approach would be to conduct more than one trial, evaluating the product in participants at different stages of DMD. Alternatively, the sponsor can design a registrational trial that has broad inclusion criteria, stratifying participants based on severity measures or disease severity prognostic modeling methodology, with a prespecified subgroup analyses of the primary endpoint in the prognostically enriched strata. This would allow the collection of safety data for a broad product label and allow the sponsor to more quickly obtain sufficient data and results to support the filing of an application for broad regulatory approval.

Sponsors are encouraged to include siblings if they meet entry criteria and to be assigned to same treatment arm. They can be excluded from primary analysis if there is concern for genotype specific safety or efficacy effects.

Whether in a randomized controlled trial, or a study using an external control, control groups should be well matched to the treatment group across important baseline and prognostic variables to account for the sources of heterogeneity in disease progression. However, the risk is particularly great that differences in patient characteristics (including age, disease stage, and genotype) or concomitant treatments between a trial population and the external control population could lead to differences in outcomes that are unrelated to the investigational treatment. (See the subsection: *Heterogeneity in DMD disease progression: predictability and sources of variability.)*

1. Standards of care for concomitant therapies to consider in clinical trial design:

* The requirement of steroids as foundational treatment: Stable doses of glucocorticoids are often required as standard of care. If a steroid naïve arm is employed in a trial, this is usually only allowed for 6 months due to ethical considerations and the widespread acceptance of glucocorticoids as standard of care [17]. Differences in concomitant corticosteroid therapy are carefully considered including:
  + Duration of stable use: Trial participants should be on at least 6 months stable corticosteroid therapy as there can be improvement in some outcome measures in individuals for up to 6 to 12 months after initiating corticosteroid therapy. Note that steroid initiation can be age/disease progression dependent.

Stable steroid dose: While stable glucocorticoid doses are usually required, allowances (but not requirements) are usually provided for weight-based dose adjustments when weight increases. Note that transient improvement in functional testing shortly after dose adjustment could create timing issues when measuring study endpoints.

Dosing regimens (daily versus intermittent): Emerging accelerometry data suggest that individuals on intermittent corticosteroid regimens perform differently on days when they are taking the steroids and days when they are off the steroids. This should be standardized in the assessment schedule of patients, to the extent possible. High dose weekend regimens used by some individuals may also pose complications for trial design.

Data from a recent randomized clinical trial demonstrates that daily regimens of deflazacort or prednisone were more effective than a 10-day-on-and-10-day-off regimen in terms of greater rise from the floor velocity [70].

Sponsors should systematically collect data on participants’ use of other concurrent medications including growth hormone administration, as well as on their contracture management or prevention, the frequency and content of physical therapy treatments, and pulmonary interventions and cardiac management (see Cardiomyopathy section).

Data collection should be standardized across trial sites as missing data on these sources of heterogeneity at baseline can complicate the interpretation of findings.

1. Duration of trials/duration of time needed to see clinical benefit

The duration of a registrational trial is dependent upon a number of factors, including the number of participants, the age of the patients, the primary endpoint selected, the degree of prognostic enrichment for the population at significant risk of progression based on that endpoint, and the expected effect size of treatment. Given the great unmet need for improved therapy in DMD, even treatments that only modestly reduce disease progression over time could be meaningful to patients and caregivers and could merit evaluation in a registrational study. If the duration of the trial is too short, it may not be possible to provide substantial evidence of efficacy for such a treatment using specific outcome measures, but it might be possible to demonstrate treatment effect in a longer study. This can be related to a treatment’s mechanism of action. For instance, recent clinical trials using first generation AON dystrophin restoration strategies which produce low levels of dystrophin and peak levels of dystrophin after the first year of use have not shown the use of the endpoints such as the 6MWD, timed function tests, or NSAA to be sufficiently sensitive to changes in disease progression over 48 weeks. Longer duration trials, 18 months or longer may potentially show more meaningful change with these measures. However, these outcome measures may be used in trials of shorter duration when using different treatment modalities that have more immediate effects [70].

Sponsors are encouraged to discuss the selection of endpoints and duration of trials with FDA once a decision has been made to move forward with a registrational trial for their product.

1. The Use of Modeling, Natural History Data, and Real-World Data in External Control Arms and to Enrich Placebos

Recent guidance from FDA describes increased flexibility regarding the types of data and evidence that can meet the substantial evidence requirement for new drug approvals in rare diseases such as DMD [37]. This could include unequal allocation to treatment versus placebo in a randomized controlled trial and dose comparison trials. In some circumstances, trials with a single arm and an external control may be acceptable to support an NDA (refer to *Demonstrating Substantial Evidence of Effectiveness for Human Drug and Biological Products: Draft Guidance for Industry.* <https://www.fda.gov/regulatory-information/search-fda-guidance-documents/demonstrating-substantial-evidence-effectiveness-human-drug-and-biological-products>).

The *2018 DMD Guidance and 2019 Rare Disease: Natural History Studies in Drug Development Guidanc*e also describe circumstances when using external controls matched for disease severity factors (such as genotype and baseline severity measures) can be deemed to constitute adequate and well-controlled studies that may contribute to evidence of efficacy to support approval [2, 38]. Because of the many potential sources of bias, such studies have generally been seen as persuasive only when drug effects are large on objective (categorical) endpoints that are less susceptible to bias.

However, a baseline control study design can be used when the pathophysiology is well understood, for instance, tumors are known to have a high probability of progression in a defined time. Similarly, in DMD, there may be critical thresholds in a structural marker (a certain percentage of fibrofatty replacement on MRI) or functional performance scores that have been demonstrated to have high probability of progression to a milestone, such as LOA within a defined period.

To reduce bias in a historically controlled study, it is critical that the patient characteristics in the population in the external control arm are matched very closely to those in the treatment arms in terms of disease stage, disease trajectory and mutations. It is advisable for study sponsors to preview their external control matching criteria with FDA to assure alignment/agreement on general principles. Any concomitant treatments and therapies that affect the primary endpoint should be based on contemporary standards of care and should not be substantially different between the external control population and the trial population.

In addition to data from the placebo arms of different studies or from prospective natural history studies collecting standardized outcome measures, an external control could use real world evidence (RWE) or

clinical evidence derived from analysis of contemporary real-world data (RWD), although use of such potential data in original NDA or BLA submissions has yet to be fully embraced by regulators, and any such pursuits should be reviewed with FDA before doing such. RWD are data relating to patient health status and/or the delivery of health care that are routinely collected from a variety of sources, including data derived from electronic medical records (EMR), product or patient registries, and data that is patient-generated or from mobile devices. Including data from EMR will require standardized outcome measures collection at more centers treating DMD or data curation using only data from centers of excellence providing standardized care with standardized data collection—such as the Parent Project Muscular Dystrophy Duchenne Certified Care Centers.

As noted in FDA’s “*Framework for FDA’s Real-World Evidence Program*,*”* RWD, when used together with “statistical methods, such as propensity scoring, could improve the quality of the external control data that are used when randomization may not be feasible or ethical, provided there is adequate detail to capture relevant covariates.” Sponsors should be aware that since the external control arms will lack the placebo effect that occurs in the placebo-controlled arm of a randomized controlled trial (RCT) when the individual believes they may be on a treatment. Modeling adjustment could be used to account for the placebo effect.

There are a number of other ways RWD/RWE can support regulatory decision making:

* When an external control arm is not possible or advisable (if a treatment effect is likely to be modest), it may still be possible to use RWD to enrich placebo groups that will decrease exposure to placebo.
* RWD can be used in hypothesis generation and to assist in trial design by assessing the frequency of an endpoint within different potential study populations.
* RWD can be used to assess real world treatment effects, evaluating longer-duration treatment effects (beyond 12-18 months) that are not possible to collect in clinical trials, or the comparative effectiveness of marketed drugs.
* In rare diseases, FDA has endorsed the use of RWD to fulfill phase-IV post-marketing requirements. RWD evidence could provide confirmatory support of an approved New Drug Application (NDA) in rare diseases (not just new indications). However, a recent cross-sectional study has reported that the data that can be extracted from EMR may not be able to fully replace all aspects of post-approval confirmatory trial requirements [389].
* Researchers can use RWE to extrapolate the benefits of a marketed treatment to non-studied populations for payers who, despite wide labelling, may resist paying for treatments for groups that were not studied as part of the treatment’s NDA package.
* Creating derivative models for post-marketing evaluation (using EMR data); care elements; claims data.

Sponsors are referred to the following page on the FDA internet site containing links to a number of guidance documents and other supportive documents on the collection, use and submission of RWE/RWD: <https://www.fda.gov/science-research/science-and-research-special-topics/real-world-evidence>. In addition, FDA is preparing guidance on designing studies that use RWD, specifically externally controlled trials and randomized controlled trials conducted in clinical practice settings.

1. Prediction models used to measure treatment effect

Sponsors are encouraged to use enrichment strategies with inclusion criteria to select a population at a stage of disease most likely to be modified by a drug over the duration of the trial. Sponsors are referred to FDA’s 2019 *Guidance for Industry: Enrichment Strategies for Clinical Trials to Support Determination of Effectiveness of Human Drugs and Biological Products*. <https://www.fda.gov/regulatory-information/search-fda-guidance-documents/enrichment-strategies-clinical-trials-support-approval-human-drugs-and-biological-products>. In addition, the FDA has descriptive documents online about model informed drug development (see <https://www.fda.gov/drugs/development-resources/model-informed-drug-development-pilot-program>).

Specific prognostic factors that may be useful to help with analysis of treatment effect in DMD include:

* Dystrophin level
* Fat fraction (skeletal muscle MRI)
* Other biomarkers
* Functional status at baseline (based on clinical endpoints)
* Genetic factors (gene mutation and genetic polymorphisms)
* Or a combination of prognostic factors (greater than the sum of the parts)

1. Innovations in Trial Designs

Innovative trial designs may help meet expectations of the DMD community to account for fewer participants who are available to be in the trial, and to place fewer patients on placebo or shorter duration exposure to placebo. These include delayed placebo (or run-in trials, in which natural history data are used in the run-in to the trial off treatment) and roll-over trials, among other approaches.

In a roll-over (or cross-over) trial, participants who reach a non-categorical endpoint could be rapidly rolled over to the treatment arm. Non-categorical endpoints could include a surrogate marker or an intermediate clinical endpoint, such as a time-to-event endpoint that is predictive of a progression to a categorical and clinically meaningful endpoint or disease milestone such as loss of ambulation, or loss of HTMF. Participants in the trial who demonstrate clinically meaningful disease progression on an endpoint or multiple endpoints could then be crossed over onto treatment before an irretrievable loss of a critical function. This might serve to decrease the duration of placebo exposure.

Another approach to minimize the time off drug in between trials in DMD studies would be phase I/II to phase III seamless trial designs that plan to proceed directly from dose escalation studies into clinical efficacy studies once a dose has been selected.

Combination trials, including use of more than one experimental therapy are also possibilities, though such trials are complex, and it is advisable to seek regulator guidance before pursuing. See *Codevelopment of Two or More New Investigational Drugs for Use in Combination* (<http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM236669.pdf>).

The DMD community also has been working with FDA on a Master Protocol for a potential DMD Platform Trial, an *Adaptive Master Protocol to Evaluate Investigational Treatments for Duchenne Muscular Dystrophy (DMD)* in order to randomize to treatment protocols, reduce proportions randomized to placebo and share placebo patients in order to accelerate the development of new therapies (https://ctti-clinicaltrials.org/wp-content/uploads/2021/07/CTTI\_Master\_Protocols\_New\_Resources\_Webinar\_Presentation.pptx).

Adaptive trial designs can allow sponsors to evaluate and drop doses or study arms shown to be less effective, and, in some cases, make other adjustments to their clinical trial design. For more on current regulatory thinking on adaptive trial design, sponsors are referred to FDA’s 2019 *Guidance for Industry on Adaptive Designs for Clinical Trials of Drugs and Biologics.*

1. Improving Diversity, Equity, and Inclusion: Racial Distribution of Trial Participation and Diversity of Participation in Natural History Studies

The DMD community and FDA have promoted enrollment practices that would lead to clinical trials that better reflect the populations likely to use potential therapies, but some populations remain underrepresented [390]. Such disparities could result in treatments that perform differently in the real world than in the clinical trial setting.

In 2020, FDA released new guidance for industry that could help sponsors increase the diversity of populations enrolled in clinical trials: *Enhancing the Diversity of Clinical Trial Populations — Eligibility Criteria, Enrollment Practices, and Trial Designs*. Sponsors are encouraged to provide support to families from lower socioeconomic strata so that they can access clinical trials. In addition, sponsors should evaluate trial designs and visit schedules from a standpoint of economic impact and logistical ability for lower-income families to participate.

1. Extrapolation of Results to Non-Studied Populations

Extrapolating data of efficacy from one stage of disease to another could depend upon the mechanism of action. However, drugs designed to improve the quality and health of the muscle would be expected to benefit the patients with DMD at any stage of the disease. In DMD approvals to date, FDA has demonstrated that it is not necessary to test a drug at every stage of DMD to justify a broader indication. Nevertheless, having some secondary endpoint data showing an effect in other disease stages could help secure reimbursement from third-party payers.

1. Specific Clinical Trial Considerations in BMD and Other Forms of Dystrophinopathy (Becker Muscular Dystrophy, Intermediate DMD, and Female Dystrophinopathies)

There is a significant body of natural history data to support the design of clinical trials in BMD, as well as in those with what used to be known as intermediate DMD and other dystrophinopathies [11, 12, 72-74, 391]. BMD is defined by dystrophin mutations associated with continued ambulation beyond 16 years of age without steroids or continued ambulation at 19 years of age and older with steroids.

Non-genetic targeted therapies may ameliorate the pathogenesis of disease in diverse populations with milder forms of dystrophinopathy and broader inclusion criteria should be considered by sponsors targeting these milder forms of dystrophinopathy. For example:

* Adults (aged ≥ 18 years) with a documented in-frame dystrophin mutation and phenotype consistent with BMD, and history of being ambulatory beyond 16 years of age without steroids; history of being ambulatory beyond 18 years of age with steroids, OR
* Adults (≥ 18 years) with an out-of-frame dystrophin mutations and/or genetic polymorphisms known to be associated with older age at loss of ambulation (eg, exon 3-7 deletion, exon 44 skip amenable mutation, etc.) and history of being ambulatory beyond 16 years of age without steroids or history of being ambulatory beyond 18 years of age with steroids, and a milder phenotype consistent with BMD as determined by the site principal investigator, OR
* Adolescents (< 18 years) with genetic confirmation of an in-frame dystrophin mutation not previously associated with a DMD phenotype, and with a milder phenotype consistent with BMD as determined by the site primary investigator [11].

Specific endpoints such as North Star Assessment for Dysferlinopathy (NSAD) may be used in addition to the NSAA in individuals with BMD. Community functions measured by digital technology (passive) in ambulatory patients with mild dystrophinopathy (eg, 95th centile stride velocity) avoid ceiling effects and hold promise and should be considered as exploratory endpoints.

The very slow progression in BMD and other dystrophinopathies will affect trial design. Biomarkers may be essential in the early stages of drug development for these patients. If conducting a 12-month or 18-month trial, sponsors may need to identify biomarkers predictive of improved function, to be able to show a difference from placebo-treated population. Including such evidence of a change in biomarkers, such as structural changes on imaging, may also contribute support to the NDA.

1. Cardiomyopathy
2. Introduction

The purpose of this guidance is to assist sponsors in the clinical development of therapeutics (biologics and pharmaceutics) for the treatment of dystrophinopathy related cardiomyopathy and also to provide guidance regarding cardiac monitoring and evaluation for therapeutics targeted at noncardiac skeletal muscle. While the majority of the document will focus on Duchenne muscular dystrophy (DMD), the topic of Becker muscular dystrophy (BMD) and carrier-related cardiomyopathy will also be addressed specifically where appropriate.

Specific guidance regarding cardiac disease is indicated based on evolving natural history studies demonstrating the impact of cardiomyopathy on clinical outcomes in DMD as therapeutic advancements in the treatment of the skeletal muscle components of the disease and multidisciplinary care have extended life expectancy. The guidance will address the selection of cardiac biomarkers that have the potential to become surrogate endpoints of disease progression in clinical trials.

This guidance will also serve as a platform for further discussions among the various stakeholders, including patients, caregivers, the Food and Drug Administration (FDA), research sponsors, academia, industry, and the public.

1. Background
2. Cardiomyopathy natural history

The last three decades have witnessed significant improvements in the care of patients with Duchenne muscular dystrophy (DMD). These improvements have translated into greater long-term survival as patients are now consistently living into their late 20s or early 30s [392]. These improvements were largely attributable to advances in respiratory care, widespread use of steroid therapy, application of guideline-directed therapy for cardiomyopathy, and the development of a multidisciplinary care treatment paradigm [17, 393, 394]. As long-term survival has improved, the cardiac manifestations of disease have become increasingly apparent, despite application of conventional therapy, and cardiac disease is now a leading cause of death in DMD [8, 9, 395-397].

Cardiac disease progresses in a similar, albeit delayed, manner to skeletal muscle disease. Subclinical cardiac injury results in fibrosis then fibrofatty replacement of the myocardium, which ultimately results in progressive systolic dysfunction and heart failure [75]. Subclinical cardiac injury is evident through the development of troponin leak and via several cardiac imaging biomarkers [398]. Evolving cardiomyopathy can be detected through the use of cardiac MRI (CMR) and strain imaging. Late gadolinium enhancement (LGE) can be found in the left ventricular myocardium in segments that correspond to areas of fibrofatty replacement of the myocardium on autopsy and the degree of LGE correlates with strain abnormalities [399-401]. The development of LGE is notable given this appears to signify a transition from a period of subclinical injury to a period of progressive fibrofatty replacement of the myocardium and corresponding decreases in cardiac systolic function [76, 77]. These data suggest LGE is a quantifiable imaging biomarker that identifies fundamental and irreversible changes in myocardial tissue that correspond to an important clinical period where cardiac function begins to decline.

As in the case of skeletal muscle disease, the time to development of cardiomyopathy and the progression of disease is variable. As noted above, the earliest manifestation of cardiac disease appears to be the development of LGE and troponin leak. Troponin leak has been detected in children younger than 10 years of age, although the characterization of the frequency, severity, and prognostic significance of this biomarker are only just being established [398, 402]. The natural history of LGE and its relation to long-term outcomes is better understood [76, 77]. LGE typically develops around the age of 14 years, although boys as young as 6 years of age have been found to have LGE, and ~15-20% of patients will have LGE prior to 10 years of age. Following the development of LGE, systolic function decreases by ~1-2%/year, although this progression seems to be mitigated by the use of steroids [76, 403]. The slow rate of progression is especially notable when considering using LVEF as an imaging biomarker in unselected populations with DMD. It can take over 10 years for ejection fraction to fall from 55% to below 45%. Ultimately, the progression to severe dysfunction becomes manifest as heart failure; however, the slow rate of progression creates a challenge for clinical trials that attempt to slow progression to heart failure. In addition, the unique phenotype present in DMD cardiomyopathy dictates that typical symptom assessment tools and biomarkers do not translate directly [404].

Existing heart failure assessment tools and heart failure scores incorporate symptom assessments, vital signs, and comorbidities that may not be applicable to DMD. Symptom assessments and disease manifestations are particularly difficult to assess given patients are nonambulatory, breathlessness is commonly present due to respiratory insufficiency, and edema is multifactorial as patients are nonambulatory and may primarily use a wheelchair for years prior to onset of heart failure. For example, the widely used New York Heart Association (NYHA) classification is dependent on symptom response to physical activity. Similarly, use of normative values for serum biomarkers (eg, natriuretic peptides) from non-dystrophinopathy patients to understand the presence or progression of heart failure is fraught, and dystrophinopathy specific values should be used [404, 405]. Thus, by the time heart failure is overtly symptomatic, patients may be end-stage and the effectiveness of disease-modifying therapies may be lessened.

These insights into the natural history of DMD cardiomyopathy as well as the benefits of early, disease-modifying therapy have resulted in a shift in the cardiac management of DMD. Medical therapy is no longer delayed until the onset of heart failure symptoms in the setting of a reduced ejection fraction, but rather is now focused on early initiation of disease-modifying therapy including steroids, angiotensin converting enzyme (ACE) inhibitors, and mineralocorticoid antagonists [395, 406, 407]. The use of prophylactic therapy and the heterogeneous nature of the introduction of these therapies should be considered when assessing previously published cohort data [78].

The progression from LGE to heart failure appears similar in BMD and among symptomatic female carriers, albeit on a delayed trajectory as LGE develops in the late teens to 20s and systolic dysfunction progresses slower [164, 408, 409]. While the nomenclature does have some prognostic significance, the individual variability inherent to each suggests that phenotypic manifestation of cardiac disease are more relevant than classification as DMD or BMD as the therapeutic approach appears similarly relevant [410, 411]. Thus, a given patient’s cardiac phenotype (eg, presence of LGE or systolic dysfunction) appears more relevant than age or dystrophinopathy sub-categorization.

Cardiac rhythm

Atrial and ventricular ectopy are common and the frequency and severity generally parallel the progression of systolic dysfunction [412-414]. However, it remains unclear which arrhythmias are responsible for sudden death. The existing data are further complicated by the difficulties in adjudicating the causes of sudden out-of-hospital death in patients with significant respiratory disease [9]. Studies assessing the risk are ongoing and this will likely be answered only through the use of implantable loop recorders, medium term wearable ambulatory rhythm monitors, or implantable cardioverter defibrillators (ICDs).

Guidance to sponsors regarding natural history

The last decade has seen studies document the earliest manifestations of disease and disease progression with increasing granularity. These have provided a wealth of data to document long-term outcomes, while underscoring a few consistent themes:

* Cardiomyopathy is detectable prior to the onset of systolic dysfunction, AND early institution of guideline directed therapy can be effective in slowing the progression of disease.
* Existing heart failure symptom and severity scores consistently underestimate the progression of DMD cardiomyopathy.
* Established imaging biomarkers (left ventricular strain and LGE) may identify patients with early cardiomyopathy more likely to have cardiac disease progression (prognostic enrichment) in the context of a clinical trial.

1. DMD Cardiac Assessment, Trial Designs, Potential Outcome Measures
2. General comments

The number of cardiac-specific clinical trials in DMD has been limited. As the impact of cardiac disease on long-term outcomes has become evident, there has been growing interest in assessing the impact of existing skeletal muscle therapies on cardiac function as well as cardiac focused trials. Non-DMD cardiac trials have relied on clinical endpoints including heart failure hospitalization and mortality. This approach is effective in cardiac pathologies where cardiac event rates are high and the patient population is large. This methodology proves challenging to apply in rare diseases, especially those with multisystem involvement like DMD where mortality may be multifactorial and disease progression is slow [403]. Furthermore, the pathophysiology of disease may dictate that early therapy is required in order to maintain organ function, especially when the disease process fundamentally alters tissue characteristics as in DMD where fibrofatty infiltration of the myocardium ultimately occurs [75, 415-417]. That is not to say mortality and hospitalization endpoints should be ignored, but rather that a dual-pronged approach may be necessary given the expected low event rate of mortality and morbidity over the course of a 1- or 2-year study: one focused on slowing fibrofatty replacement of myocardium in order to maintain cardiac function; and the second to maximize event-free survival [418]. For example, should a trial generate data demonstrating that a treatment delays fibrofatty replacement, it may be necessary to use a natural history comparator to confirm a reduction in clinical events, given the challenges of maintaining a placebo. Given the extended timeline of cardiac disease progression, it may be advantageous to use composite endpoints that examine the totality of disease burden, by marrying functional clinical data, including measures of pulmonary function or skeletal muscle function (such as upper limb function measures) with cardiac biomarkers. For example, this would allow therapies to demonstrate clinically meaningful benefit in measures of upper limb function as a functional endpoint in addition to slowing LGE progression or development of cardiac dysfunction.

To date, clinical and research studies have focused on the use of cardiac imaging biomarkers including strain, LGE progression, and left ventricular ejection fraction [395, 407, 419-421]. These studies have varied in their inclusion criteria, although the majority have focused on early stages of disease where LGE is present and systolic function is preserved or mild/moderate systolic dysfunction is observed, but patients are not yet symptomatic. This acknowledges the concern that in late-stage disease (where fibrofatty replacement of myocardium, including transmural LGE, is significant) response to therapy will be limited.

1. Cardiac endpoints

Cardiac systolic function, dimensions, and exploratory imaging biomarkers

Assessing systolic function traditionally relied heavily on echocardiography; however, this approach may be problematic in DMD. First, echocardiography does not detect early manifestations of disease, especially fibrofatty replacement of the myocardium. Furthermore, reliable assessment of cardiac dimensions and systolic function is dependent on adequate ultrasound windows [422]. These windows are often not present in DMD, especially at later ages where chest wall deformities and obesity may prohibit accurate, reproducible assessment of cardiac function by ultrasound. Thus, cardiac MRI is the preferred method for quantitative assessment of cardiac systolic function and cardiac measurements. Cardiac MRI does, however, have its challenges as it may be difficult or impossible for patients with advanced skeletal or respiratory disease to lie recumbent for prolonged periods. Thus, echocardiography remains an important adjunct for assessing function. However, selection of cardiac outcome measures for clinical trials is complicated by the limited natural history data regarding end-stage heart failure, especially cardiac event frequency and event rates.

To date, the limited natural history data has made the consideration of cardiac biomarkers for accelerated approval challenging. However, as reproducible, longitudinal, quantitative assessment of DMD-related cardiomyopathy has become more widespread, this approach should be reconsidered. The relationship between the development of LGE and subsequent progression of systolic dysfunction has been well described and there is a clear relationship between severity of systolic dysfunction and mortality in the current era [8, 396]. Should consideration be given to using imaging biomarkers like LGE progression to identify potential therapeutic effect, long-term follow-up to demonstrate the impact on ejection fraction, incident heart failure or heart failure will be needed as mentioned above.

With these considerations in mind, the following are proposed for cardiac monitoring:

1. Cardiac MRI: This provides the most reproducible assessment of cardiac dimensions and biventricular systolic function as it is less affected by body habitus and scoliosis. Measures of wall strain identifies subclinical cardiomyopathy progression preceding cardiac dysfunction in boys with DMD. LGE can also detect areas of cardiac fibrosis and fibrofatty replacement before changes in systolic function. This method is currently preferred for assessing cardiac function due to the reproducibility of quantitative assessment and the ability to assess LGE [422].
   1. Limitations: Its use is limited in young patients (<8 years of age), claustrophobic patients, and those with developmental delays unable to lay flat. Older patients with significant pulmonary insufficiency, kyphoscoliosis and/or contractures may also be unable to tolerate the positioning required for CMR.
2. Echocardiography: This provides a quantitative measurement of left ventricular systolic function, left ventricular dimensions, and valve function. Quantitative assessment of right ventricular function and dimensions is more limited. This method should be considered an adjunct imaging test as it allows assessment of large changes in systolic function from a safety perspective, is widely available, and may allow qualitative/semi-quantitative assessment of cardiac function for studies that focus on clinical outcomes in patients unable to tolerate CMR. Echocardiography will also be fundamental to providing trial access in populations who are unable to tolerate CMR (eg, older patients with scoliosis), as long as trial design reflects the limitations inherent to echocardiography in patients with DMD due to image quality and reproducibility.
   1. Limitations: Reproducibility of quantitative cardiac assessments becomes more challenging with age due to changes in body habitus and chest wall.

***Electrophysiologic Assessment***

Current data suggest the predominant cause of cardiac mortality is heart failure; however, sudden cardiac death is also an appreciable cause of mortality [9, 392, 395, 406, 414, 423, 424]. Sinus tachycardia, abnormal heart rate variability (HRV), and atrial ectopy are early manifestations of the disease [424, 425]. Ventricular ectopy, including ventricular tachycardia, atrial fibrillation, and ventricular fibrillation are later manifestations of disease and generally occur as systolic function decreases [412, 413].

1. Electrocardiogram (ECG): Electrocardiography may detect the rhythm disturbances noted above, however, it should be used primarily to detect, evidence of myocardial injury, changes in cardiac conduction and repolarization (corrected QT interval), especially in response to therapy. Serial ECGs should be standard of care for any DMD related trial.
   1. Limitations: Does not obviate the need for ambulatory monitoring to detect rare or episodic arrhythmias and thus consideration should be given for ambulatory monitoring.
2. Ambulatory rhythm monitoring: Holter monitors and extended cardiac rhythm monitors (eg, Ziopatch) may be used to detect potentially life-threatening arrhythmias. The indications for ambulatory monitoring will vary by the trial. Some form of ambulatory rhythm monitoring should be strongly considered in cardiac specific trials and in trials including patients with at least moderate systolic dysfunction. Implantable loop recorders may also be considered.

Serum Biomarkers

To date, identification of early evidence of cardiomyopathy has been limited to imaging biomarkers including strain and LGE. More recently, high sensitivity troponin has emerged as a potential biomarker, however, longitudinal assessment of levels and the relation to disease progression and clinical outcomes are needed given that recent reports have been mixed [398, 402]. The presence of elevated levels in asymptomatic patients with preserved systolic function and LGE should also be noted when considering this as a safety marker. Given the current state of the field, this is best considered an exploratory marker pending further study [426]. Preliminary data on additional biomarkers is also available, although data, especially longitudinal data, is limited and thus the utility of these biomarkers in DMD remains unclear [78].

1. Cardiac medications

The use of guideline based cardiac therapy should be required at baseline. In particular, prophylactic use of renin-angiotensin-system (RAS) inhibition through angiotensin-converting enzyme (ACE) inhibitors, or angiotensin receptor blockers (ARB), at baseline should be addressed based on the age or cardiac phenotype of the patient (eg, systolic dysfunction is present). The use of other classes of medication, including beta-blockers, should be accounted for at baseline as these are typically initiated following the onset of systolic dysfunction (LVEF <55%) per the Care Considerations and American Heart Association Guidelines [15, 427]. The use of additional medication classes including mineralocorticoid receptor antagonists (MRA) should be noted given preliminary evidence that they may affect disease progression [407, 419]. Valsartan-sacubitril is now viewed as first line RAS inhibition for adults with heart failure and is now being used more in other populations like DMD. Finally, sodium-glucose cotransporter-2 inhibitors (SGLT2i) have become standard of care in patients with non-DMD dilated cardiomyopathy (DCM) heart failure with reduced ejection fraction (HFrEF) patients, although their use has been limited in DMD patients to date. These medications should not preclude inclusion in a trial, but the variability of uptake of newer heart failure therapies in DMD should be considered as part of trial design, especially when natural history studies are being considered.

1. Renal function

Measuring accurate renal function is challenging in the setting of neuromuscular disease and muscle wasting, including in DMD, where serum creatinine is typically less than measurable in commonly used assays. Since many cardiac medications and contrast agents used for imaging can adversely affect kidney function, renal function should be monitored in any trial. Cystatin C is a non-glycosylated protein that is unaffected by muscle mass, and it can be used to monitor renal function in DMD [428, 429].

1. Trial design

Many of the challenges inherent to noncardiac trials apply (see additional sections in noncardiac section), though specific considerations for cardiac trials should be noted.

Age-based natural history data is dependent on the timing of cardiac therapies and steroid therapy, each of which has been shown to affect the development of heart failure [395, 406, 430]. While steroids have become standard of care, the application of cardiac therapies remains highly variable [78, 423]. Thus, an approach that specifically accounts for the stage of cardiac progression (eg, LGE vs LGE with mild systolic function vs LGE with moderate systolic dysfunction, etc.) and cardiac therapy, rather than an approach based on diagnosis (DMD vs BMD vs intermediate) or age, is preferred. This approach is especially important when considering natural-history-based controls to ensure current standards of care are being applied to each population and that the cardiomyopathy stage is equivalent.

Trial sponsors should be familiar with evolving practice, including the application of both prophylactic therapy and therapy following the onset of systolic dysfunction, and account for these differences in any trial design. The importance of this topic has been underscored in recent multi-stakeholder meetings [79]. The topic is perhaps more relevant in the setting of DMD given the rarity of disease and heterogeneity of clinical practice, especially early in disease [80]. The challenge may only grow in coming years as newer heart failure therapies are adopted variably across clinical practices (eg, sodium-glucose cotransporter-2 [SGLT2] inhibitors). While consistency of background therapy would provide benefits from a clinical trial perspective, mandating specific therapies and doses poses challenges from an economic, logistic, and recruitment perspective. Each of these factors also contribute to discussions of equity in rare disease, especially access to trials. A pragmatic approach specifying a minimum background therapy by class and in accordance with the stage of cardiomyopathy may provide the balance needed. Consensus recommendations given the range of reasonable solutions are needed while incorporating the voices of the relevant stakeholder including but not limited to patients, families, clinical providers, industry, and government representatives.

Standardization of cardiac measurement

Given the overall paucity of granular, longitudinal cardiac data in DMD, standardization of cardiac assessment in clinical trials is fundamental to moving the field forward. A harmonized approach to imaging evaluation and rhythm monitoring will not only provide valuable data on potential efficacy in a given trial, but also would supplement existing natural history data. This approach requires a commitment to expedient peer-reviewed publication and presentation of data and may increase the feasibility and applicability of studies which may attempt to use natural history controls in the future.

Currently, the area with most heterogeneity of practice is cardiac imaging, specifically CMR. Ensuring harmonization of practice in the frequency of cardiac imaging and sequences used would be useful. Creation of an imaging charter would be beneficial in this regard. This would provide clarity on numerous logistical considerations, but in particular should include guidance on the need for centralized imaging interpretation, frequency of cardiac imaging, and the development of manufacturer-independent CMR protocols.

Cardiac monitoring and evaluation in noncardiac trials

Assessing the cardiac phenotype is indicated for noncardiac trials in DMD given the potential for adverse cardiac events and also to provide a baseline for long-term monitoring. Age, skeletal muscle function, and respiratory status all impact the ability to provide reasonable imaging. For children <8-10 years of age, CMR without sedation may be a challenge and echo would be standard of care. Furthermore, the quality of the images is typically not impacted by factors that lead to poor echocardiographic windows with age. The frequency of cardiac imaging may vary based on trial design. But at the very least, each study should include standard of care imaging based on the cardiomyopathy stage of the patients enrolled.

Patient access to clinical trials

Ensuring access to trials to an ethnically, economically, and geographically diverse population is fundamental to promoting public health generally and in dystrophinopathy specifically. To achieve this, the field must take an inclusive, patient/family centered approach to trial practices and designs. Cost of care should not be a barrier to participation in a trial; therefore, sponsors should consider providing access to standard-of-care therapies and monitoring to maintain a consistently high standard of care among all trial participants. A patient-centered approach also minimizes placebo exposure where possible, minimizes trial exclusion based on multi-system disease and prior therapies, and readdresses eligibility criteria where possible between early phase II trials and phase III trials, while ensuring patient safety. This approach will broaden the access to novel therapies and will help to understand the efficacy of therapies in late stages of disease, which may have been excluded from initial trials due to concerns that muscle injury has become irreversible. Multicenter collaboratives are also currently forming to monitor long-term cardiac disease progression raising the possibility of incorporating real-world data into trial design and long-term safety and efficacy monitoring. These are expected to be integral to long-term safety and efficacy evaluation, but also may be an avenue to enable greater access to novel therapies for a diverse population.

Enrolling patients with cardiac disease, or those at risk for cardiac progression in the near term, is fundamental to understanding the efficacy of new cardiac therapies. This generally translates to patients who are teenagers or young adults, the majority of whom are nonambulatory and have some degree of respiratory insufficiency. These factors pose significant logistical challenges and burdens to participants and caregivers for enrollment as frequent visits to geographically distant centers are challenging. Thus, flexible trial design which minimizes the frequency of study visits in order to ensure safety of therapy, but which allows appropriate monitoring should be pursued. This may include the use of digital health technology and remote monitoring.

1. Conclusions

Advancements in neuromuscular and pulmonary care have improved long-term survival in DMD. Novel neuromuscular therapies, including gene therapy, carry the prospect of further clinical gains in the coming years. To solidify and expand on these gains, a pathway is needed for developing and testing new cardiac therapies in DMD. As our understanding of DMD-related cardiomyopathy has grown, it has become evident that existing methods to define heart failure and heart failure severity have significant limitations in DMD due to multisystem disease. DMD-related cardiomyopathy is characterized by slowly progressing, yet irreversible fibrofatty replacement of the myocardium. The prolonged course of the cardiomyopathy, in conjunction with the rarity of DMD, and the competing causes of mortality are significant barriers to utilizing typical heart trial methodologies and outcomes for DMD-related cardiac trials. A consensus roadmap is needed which bridges the gap between the relevant stakeholders. The roadmap should define the similarities and differences between DMD related cardiomyopathy and non-DMD dilated cardiomyopathy, provide a common framework for defining cardiac disease progression and development of heart failure in DMD, and the characteristics needed to consider cardiac biomarkers for accelerated approval in DMD, including how to assess long-term safety and efficacy over a long period.

1. Gene therapy for DMD and other dystrophinopathies: approaches, patient-centered considerations, and development pathway
2. Introduction

This section of guidance provides recommendations to sponsors developing gene therapy (GT)products intended to treat DMD in pediatric and/or adult patients. Note that while other sections in this guidance also provide recommendations for sponsors considering developing products for BMD, which results from an in-frame deletion, BMD has not been a target population for most of the GT approaches in clinic to date. However, there is a subset of individuals with in-frame deletions who either make so little dystrophin or such nonfunctional dystrophin that they progress with a more DMD-like phenotype (ie, loss of ambulation in early teens). These individuals should qualify as candidates for GT. There are also individuals on the mild end of the DMD spectrum with slow disease progression (see Natural History section) who may be candidates for treatment with dystrophin-restoring GT products if they have little or no expression of dystrophin.

1. Background

The information in this section of the guidance is intended to assist sponsors in designing clinical development programs for such GT products, in light of the issues raised in the other sections (natural history, clinical trials, etc.) of this document. While these and other general research and development principles apply to the development of GT programs, there are challenges unique to GT such as:

* Technical challenges related to target cells (delivering gene therapy to skeletal and cardiac muscle tissue):
  + As skeletal muscle fibers grow and undergo repair, cells constantly turn over. As cardiac cells are nondividing, treatment may be for life. Evaluating the effect and duration of any GT on the heart is critically important.
  + Dystrophin is also expressed in the CNS and in smooth muscle. The impact of treating or not treating those tissues is not currently known.
* Technical challenges related to the vector:
  + The most commonly used vectors in GT products currently in clinical trials for DMD, adeno-associated virus (AAV) vectors, have a limited carrying capacity of ~4.7 kb, whereas the Dp427 muscle isoform of dystrophin is encoded by an ~11.4 kb cDNA. Consequently, several current GT constructs use an abbreviated gene to produce a micro- or mini-dystrophin[[1]](#footnote-1) rather than the full protein [84, 85]. Future GT products with a greater packaging capacity or which affect their action via gene editing or altered RNA splicing may not have the same constraints and may overcome some of these limitations. However, these other approaches may present their own sets of challenges.
  + Delivery of transgenes to skeletal muscle satellite cells with AAV vectors presents a challenge if AAV vector genomes are lost in dividing satellite cells. In other words, there are theoretical concerns that if a gene product that is being delivered by the AAV does not modify the DNA of the satellite cells, then its effect may be lost—although this has not yet been observed in animal models or in clinic with micro-dystrophin. As the durability of expression of the transgene products has not yet been established, it is possible that therapeutic effects could wane over time without retreatment. Achieving a very high-level expression may prolong durability of expression and effect to an extent. An approach that might safely permit repeat dosing of products using these vectors has not yet been established (see below). Additionally, although high-level expression and redosing to maintain it may be advantageous for skeletal muscle, it is not yet established whether extremely high-level expression will be well tolerated in cardiomyocytes.
  + In the case of gene editing constructs (including CRISPR Cas9 and other platforms) recently published studies suggest that it may be possible to infect and alter genes in muscle satellite/precursor cells in murine and humanized murine models with various AAV serotypes [431].
  + In the case of RNA splice-altering constructs (including U7snRNA vectors), potential therapies may be directed toward expression of a nearly full-length dystrophin based upon targeting rare or even private DMD mutations (ie, bespoke vectors). Such an approach, directed toward skipping of a duplicated exon 2, has been shown to be safe and specific in preclinical studies, and has shown promise in a preliminary clinical trial, leading to expression of full-length dystrophin. In the development of such vectors, and particularly for bespoke therapies (made for single individuals), standard DMD animal models cannot be used to demonstrate efficacy. The absence of appropriate animal models for establishing dosing levels may need to be addressed by rational inferences from other similar vectors [431].
* Safety considerations and other limitations due to immune responses directed against the vector capsid, viral DNA and the expressed transgene product could limit administration of GT products to some individuals [432]. This includes re-administration to previously treated individuals or by a product using the same vector—although different immune suppression and antibody clearance strategies to mitigate these risks are under evaluation.

1. Considerations for Chemistry, Manufacturing, and Controls

The general chemistry, manufacturing, and controls (CMC) considerations for product manufacturing, testing, and release of GT products for DMD are the same as those described for other GT products. However, as with other rare diseases, the smaller study populations of individuals with DMD may result in the need for fewer manufacturing runs, which can make it difficult to establish the critical process parameters necessary for ensuring critical quality attributes with these emerging technologies. In such cases, the approach to CMC pre- and post-approval will be iterative and incorporate evolving product and process understanding and site evolution. Sponsors developing GT products for DMD are directed to the recent guidance documents on Human Gene Therapy for Rare Diseases [87] and Human Gene Therapy for Neurodegenerative Diseases [433] for a review of these considerations. Sponsors are also strongly encouraged to contact the Office of Tissues and Advanced Therapies (OTAT) in the Center for Biologics Evaluation and Research (CBER) prior to investigational new drug application (IND) submission and during product development to discuss their product-specific considerations.

1. Considerations for Preclinical Studies

GT products can pose risks to subjects due to features such as the potential for prolonged biological activity after a single administration which will produce an immune response. Toxicities observed in systemic gene therapies may be dependent on several factors including AAV serotype, vector composition, target tissue, vector or encapsidated DNA-related impurities, dose and route of administration, disease indication, clinical protocols, and patient screening regimens. It is therefore important that sponsors conduct a thorough preclinical program to characterize the product’s benefit-risk profile. As discussed in the Science of Patient Engagement section of this guidance, patient engagement is a key component of characterizing benefit/risk and should be included in the process. Many of the research and patient safety issues that would traditionally be evaluated in early phase GT clinical trials are best addressed in a preclinical setting. For instance, if feasible, preclinical studies should identify a biologically active dose range; the dose-escalation schedule and dosing regimen that will be taken into clinical trials; and potential toxicities and physiologic parameters that will help guide clinical monitoring for a particular investigational product. For dystrophinopathies, it should be possible to identify a starting dose with therapeutic effect and where that therapeutic effect lies in terms of vector-genomes per kilo during bench-side experiments by doing dose escalation in animal models (in rodents and other animal models). Additional details for general considerations in preclinical studies are available in separate guidance documents (*Preclinical Assessment of Investigational Cellular and Gene Therapy Products; Guidance for Industry* [88], and *Human Gene Therapy for Rare Diseases [87]*).

Based on the experience with GT products to date, recommendations highlighted in *Human Gene Therapy for Rare Diseases* may be particularly critical in the development of a preclinical program for new investigational GT products for DMD. Sponsors are encouraged to consider performing the following:

* Preclinical in vitro and in vivo proof-of-concept (POC) studies to establish feasibility and to support the scientific rationale for administration of the investigational GT product in a clinical trial:
  + Note that in DMD, there may be more than one target tissue. While skeletal muscle has been the primary target for most DMD GT products to date, we encourage sponsors to assess the activity of the transgene products in other tissues affected by dystrophinopathy, such as the heart. To date, there has been a lack of uniformly appropriate evaluation of the cardiac impact in animal models.
* Biodistribution studies to assess the distribution, persistence, shedding, and clearance of vectors and expressed transgene product: In DMD, sponsors may also benefit from evaluating alternative vectors, or improved AAV serotypes to identify those with better muscle tropism. Studies could also evaluate whether alternative routes or strategies of viral delivery achieve more efficient transduction to the muscle tissue without overburdening nontarget tissues (especially the liver).
* Toxicology studies as described in the *Guidance for Industry–Preclinical Assessment of Investigational Cellular and Gene Therapy Products* [88].
  + Note, however, that there are limitations to preclinical toxicology studies in gene therapy. Due to the differences in immune systems across species, preclinical toxicology has not predicted some of the adverse events seen in clinical trials of DMD.

1. Considerations for Clinical Trials

FDA recognizes the substantial unmet medical needs for individuals with DMD. The Agency is also aware that in recent years, the natural history of DMD has become well characterized, and sources of heterogeneity, in terms of rate of progression, better understood. Thresholds for outcome measures (such as timed function tests (TFTs) have been identified that can help categorize the trajectory of disease for individual patients. Sponsors of GT products for DMD should be aware these natural history data can potentially provide critical information to guide every stage of drug development from drug discovery to determining effectiveness and safety of the drug in treating a disease (*Rare Diseases: Common Issues in Drug Development; Draft Guidance for Industry, February 2019*. <https://www.fda.gov/media/120091/download>.)

The following elements are recommended for consideration during clinical development of investigational GT products intended for treatment of DMD.

1. Considerations for early phase trials and dose selection

As mentioned earlier, the design of early phase clinical trials of GT products differs from the design of clinical trials for other types of pharmaceutical products because of the distinctive features of GT products. FDA recommends rigorous follow-up of the issues explored in preclinical studies as sponsors move GT products for DMD into early phase trials. Further guidance is described in *Considerations for the Design of Early-Phase Clinical Trials of Cellular and Gene Therapy Products [434].*

* Since study participants may have only one chance to receive the product due to the development of an immune response that may preclude retreatment with the investigational agent, study treatment should start with a potentially therapeutic dose for participants with DMD, in contrast to traditional small molecule or biologic dose escalation approaches. Sponsors should be aware that caregivers and patients may be reluctant to enroll in an early phase clinical trial or provide consent and assent to participate in an initial dosing arm of a new GT product if they fear that they may be given a suboptimal dose and thus have more potential for risk than benefit [32]. As noted in other guidance, sponsors should use all available sources of preclinical and available clinical information to select, to the extent possible, an initial human dose for their GT product that is not only reasonably safe but that can achieve a physiological change anticipated to provide benefit.
* Dose exploration may still be needed to identify an optimal therapeutic dose and to identify potentially safe and therapeutic dose(s) when the study drug is administered for individuals of different ages, at different stages of disease, and with different physical characteristics (in, for instance, nonambulatory individuals who have lost most muscle mass, where dose proportionality to muscle mass may be a factor in safety). As higher doses of GT products with AAV vectors are used to achieve efficient transduction to all the skeletal muscles, there is a greater risk of immune response to the vector. Sponsors are encouraged to monitor immunogenicity and to interrogate programs for evidence of complement activation as a safety measure.

1. Study design

As stated in other GT guidance, if the effect size is large, depending on the study design, conduct, and other results, a first-in-human trial of a GT product for DMD may provide sufficient evidence of effectiveness to support a marketing application. Sponsors developing such products therefore should consider designing their first-in-human study to be an adequate and well-controlled investigation. However, at the time of first-in-human study, the manufacturing and analytical method may still be in initial phases, and development of potency assays may require more time. Therefore, in a case where it is possible for a sponsor to file after a first-in-human study, the Agency may consider waivers from the quality requirements for GT for Biologics License Applications (BLA). (Note: this is a forward-looking statement, and sponsors are encouraged to discuss the issue with the Agency.)

As described earlier, the understanding of the natural history of DMD, the causes of heterogeneity in rates of progression, and use of prognostic modeling have progressed to the point where innovative trial designs may be feasible to expedite clinical development, if the effect of treatment with a GT product is expected to be large, self-evident, and closely associated temporally with the intervention. In situations where that is not likely to be the case, randomized, placebo-controlled clinical trials may be the most efficient means of obtaining persuasive evidence of effectiveness.

Challenges performing placebo-controlled studies

FDA recognizes that there can be challenges performing placebo-controlled studies with GT products, particularly in the context of DMD:

* It can be difficult to maintain double-blinding in a placebo-controlled trial with some GT products. For instance, while sponsors may consider recommending antiemetics to participants in both arms of a trial, vomiting has been quite common in treatment arms of GT products that use AAV vectors. In addition, there is a chance that participants may be unblinded through routine monitoring by their own clinicians, if treatment is associated with reductions in CK values and changes in liver enzymes. There are clear ethical concerns regarding keeping participants and their caregivers blinded to elevations in AST and ALT requiring treatment.
* There may be further ethical concerns when the duration of exposure to placebo in a GT product is prolonged (beyond 12 to 18 months). If a study has enrolled a population at elevated risk of disease progression, there may be ethical issues with randomizing children. For instance, this is especially the case for individuals with late ambulatory DMD who are likely to lose functions including LOA or nonambulatory participants who may lose other functions critical for quality of life that cannot be recovered.
* In the case of GT products with an AAV, although rare, there is also an increasing risk over time of participants on placebo seroconverting to AAV while they are on placebo, and potentially not benefiting during a cross-over phase of the study. Note that there is also a risk of viral vector shedding, which means that if there is more than one individual with DMD residing in the same household, there is a risk of seroconversion among siblings. If both siblings meet the study inclusion criteria, sponsors should either treat both siblings together (randomizing each individual to the same arm). If only one sibling is able to participate, it is recommended that the family keep the siblings apart for a period of at least 90 days to prevent seroconversion. Such separation is very burdensome for families, so sponsors should consider alternative mechanisms for alleviating this concern.
* In addition to the risk of progression, participants on placebo in a trial for DMD may be subjected to other risks, including, potentially, a higher dose of corticosteroids than they would typically use, other immunosuppressants, and/or invasive procedures such as biopsy to monitor transgene products.
* Some GT products may be directed toward individuals with a specific genotype (such as a duplication of a single exon). In such cases, the rarity of the patient population among the DMD population may preclude a placebo-controlled design.

Use of natural history data and prognostic models

As described in the general Clinical Trials section of this guidance, FDA believes that in DMD, it may be possible to use natural history data and prognostic models to, at the very least, decrease exposure to placebo. This could include time-to-event (defined as time to clinically meaningful disease progression as discussed in the Clinical Outcome Measures section) designs, where placebo recipients are unblinded and placed onto treatment after reaching a predetermined intermediate clinical endpoint or after a change in outcome measure (such as a TFT) that prognostic models indicate will lead to loss of a clinically meaningful function in the absence of an intervention. If a time-to-event criteria is utilized for roll over to alternate treatment, blinding should be maintained and potential placebo exposure should be of a sufficient duration to allow multiple endpoints to be evaluated. Sponsors are also encouraged to discuss with FDA whether other innovative trial designs (eg, adaptive designs, enrichment designs, dose-controlled studies, or historical controls) may be justified and facilitate product development, as well as reduce or avert placebo cohorts.

Concomitant medication(s):

As noted in earlier sections of the guidance, pharmacological corticosteroid therapy is the standard of care for DMD. The dose of any concomitant medication in a clinical trial of a GT product should be stable over a specific period and specified in the clinical protocol. Historically, for corticosteroids in DMD, that period has been at least 3 months. However, there is emerging evidence that shows that individuals with DMD aged 4-7 years who have never been on steroids before, may show improvement on some outcome measures, such as the NSAA, for periods of beyond 6 months, and TFTs for periods of up to 6-12 months [17, 70]. We therefore recommend that sponsors require a 6-month duration of stable steroid treatment before study treatment in participants aged 4-7 years. Note that GT does not obviate the need for standard of care corticosteroid therapy. However, the optimal dosing regimen of corticosteroids post gene therapy has not been established.

The immunosuppressive dose of corticosteroid administered at the start of a GT trial is substantially higher than the recommended dose in DMD management. Higher corticosteroid doses above standard of care doses may have a temporary positive impact on clinical endpoints over and beyond what is seen with typical standard-of-care daily doses or with chronic intermittent dosing. Sponsors should also consider including in their trial protocol, a standardized approach to how steroid regimens are tapered after the initial higher loading dose. This can be important when the study’s endpoints are evaluated if clinicians have tapered their patients at variable rates. For instance, sponsors could consider a tapering regimen of two months minimum, during which time steroids would then be tapered in both the placebo group and the treatment group. Note that the rate of viral clearance, which could vary by vector, or the presence of other indicators such as liver marker abnormalities, might be determinants of tapering as well.

Pilot studies of alternative immunosuppressive regimens

While it is possible that transgene expression and consequent treatment effect may decrease over time in skeletal muscle, repeat administration of viral vector-based GT products is at this time unlikely to be safe, feasible, or effective since subjects given a GT product will experience a strong immune response, with extremely high titers of neutralizing antibodies. However, sponsors may consider performing pilot studies to evaluate whether other strategies—such as plasmapheresis, the coadministration of IgG peptidase (IdeZ), or different immunosuppressive regimens that suppress antibody production, T cell responses, and the complement response cascade—could counter the immune response and allow repeat administration of the GT product. Assessment of immunogenicity and its clinical manifestations (loss of treatment effect and toxicity) will be even more critical in the setting of repeat administration. Studies could also assess whether pretreatment with other immunomodulatory regimens could eliminate antibodies in a patient on the cusp of a positive threshold [435].

In all cases, however, we encourage sponsors to discuss clinical development plans with FDA early on.

1. Study population

Selection criteria for the study population in DMD should consider existing preclinical or clinical data to determine the potential risks and benefits for the study participants. In addition, sponsors should consider whether the proposed study population is likely to provide informative safety and/or efficacy data.

As for any other gene-targeted treatment, the sponsor should perform genetic test(s) to confirm that all potential clinical trial participants are harboring *DMD* gene mutations. Evaluations should also be performed to determine the clinical phenotype and DMD stage of all potential participants, to assess their risk of progression to the selected endpoint(s) during the course of the study. Note that in DMD, there could be some mutations amenable to treatment by the GT product that are associated with a slower course of disease progression in some but not all individuals. In such individuals, sponsors are encouraged to include those at risk of progression based upon their clinical phenotype at the time of enrollment.

Sponsors should be aware that while the potential benefit of a GT product may be greater at younger ages (for instance, in ambulant boys aged 4-7 years), at these ages, children are maturing in their ability and the refinement of their small and large motor function. In such a population, over the duration of a short trial (eg, 6-12 months), it may be difficult to distinguish whether clinical improvements or improvements in outcome measures such as NSAA are due to maturation, the introduction of high dose steroids, or the effect of treatment.

Guidance pertaining to inclusion of late ambulatory participants between the ages of 8-13 years, particularly those on a trajectory towards more rapid disease progression, and older individuals in the nonambulatory population is included in the Clinical Trials section of this guidance.

While sponsors may choose to focus on the enrollment of participants at a particular stage of DMD for the trial’s primary efficacy analysis, including different age groups and disease stages provides an opportunity to gain a better understanding of a potential therapy’s safety and gather preliminary data on how it might work across the entire spectrum of the disease. Sponsors choosing to do this are encouraged to stratify such participants or, if necessary, based upon the endpoint, develop a pre-specified statistical analysis plan that does not include them in the final analysis of the primary endpoint. Demonstrating safety in a broader population would also lend support to a wide labeling for the product.

Pre-existing antibodies to any component of the GT product may pose a potential risk to patient safety and limit its therapeutic potential. Antibodies to the gene therapeutic agent also limit the potential for readministration of the product. For these reasons, sponsors may choose to exclude patients with pre-existing antibodies to the GT product. Note that the risk of pre-existing antibodies to AAV-vectors increases with age, which could present a challenge for older age groups.

However, the limitations of the neutralizing antibody assays that are currently used to assess this issue should be noted. There is a need to strengthen the armory of assays to increase the understanding of the immune responses, and to help evolve immunosuppressant therapy. This includes standardization of screening assays for neutralizing or binding antibodies to specific serotypes/capsids, defining the specificity and sensitivity of the assays (and the biological interpretation of seropositivity thresholds), as some positive results could be due to suboptimal assay specificity. To the extent possible, sponsors should explore the parameters of any experimental assays in the preclinical setting due to safety concerns. Sponsors are encouraged to refer to FDA guidance on developing companion diagnostics [436].

Although previous treatment with a GT product may be a reasonable exclusion criterion for another GT product using the same vector, FDA recommends that sponsors do not exclude those previously treated with other types of treatments, such as a cell-based therapy, after a reasonable washout period, unless there is a clear scientific rationale for doing so. Conversely, individuals with progression of DMD to the study’s primary endpoint despite treatment with a GT product, should be eligible for participation in other trials after a reasonable washout period, although not in a trial of an AAV GT product where there may be a clinically significant immune response due to seroconversion.

1. Safety considerations

The safety considerations for clinical trials of GT products for DMD are essentially the same as described in the guidance on Gene Therapy for Rare Diseases.

The safety and incidence of severe adverse events with GT products may be dependent on several factors, including AAV serotype, vector composition, target tissue, vector or encapsidated DNA-related impurities, dose and route of administration, clinical protocols, and patient screening regimens. While there may be class-wide concerns (and the experience of other GT product programs should inform the safety monitoring of products in the class) each GT product’s safety profile should be individually evaluated, taking into consideration these variables, including for the purpose of establishing clinical monitoring requirements. Risk mitigation strategies may borrow from the experience of other programs.

Micro-dystrophins may present as neoantigens in some patients, depending on their mutation and the sequences contained in the micro-dystrophin. There is evolving evidence that in subjects with large deletions (or certain deletions in the N-terminal region of the gene), the transgene product itself may be seen as a foreign protein and elicit a potentially dangerous immune response such as immune-mediated myositis. This is an area of concern that requires careful consideration and close monitoring. It may be necessary to exclude participants with large deletions from some studies, and rather enroll them into a study with a different immunosuppressive regimen. Alternatively, sponsors could consider a tiered approach to the study of patients with at-risk mutations with lower risk mutations treated initially in a stepwise fashion. Note that if vectors other than AAV are used to insert a full-length *DMD* gene, immune responses to the transgene will also need to be monitored closely, particularly in individuals with very large deletions, although for certain mutation classes amenable to RNA splice altering therapies, such as single-exon deletions or deep intronic pseudoexon mutations, the restoration of full-length dystrophin expression is unlikely to be perceived as a neoantigen due to measurable levels of endogenous normal splicing.

If there are specific gene mutations that might pose additional immunologic risk, multiple trials with different immunomodulating regimens should be considered to address participants with those mutations more safely.

Sponsors should consider including immunologists in the trial’s Data Safety and Monitoring Boards who could consider questions of immunogenicity and safety, in particular. In addition, if there is what appears to be an immune response after administering a vector, there should be a suggested protocol in place for what lab tests should be drawn, and what specialists should be called in to review the case, rather than leaving this up to the discretion of the principal investigator.

Cardiac specific concerns:

Given the cardiac impact of gene therapies that has not been adequately modeled in preclinical studies, it's crucial that all studies of gene therapy (GT) products thoroughly evaluate the cardiac implications in patients. This entails identifying heart risks at baseline and consistently monitoring for changes in heart function and health over time. Although GT products treating the skeletal muscle might have different or even no effects on the heart, it's worth noting that treatments improving skeletal muscle function could indirectly place a greater load on the heart (refer to the Cardiomyopathy section).

Recent reports have highlighted episodes of myocarditis, some leading to outcomes such as fatal cardiogenic shock. In light of these events, sponsors are urgently advised to adopt protocols and systems that minimize the risk of these occurrences and ensure optimal care when they transpire.

**Some key recommendations for industry**

1. **Have protocols in place:**
   * Conduct thorough cardiac evaluations prior to administering therapy.
   * Adopt stricter cardiac monitoring mechanisms for early detection of cardiac injuries.
   * Implement enhanced symptom management strategies.
   * Establish proactive measures to prevent severe outcomes.
2. **Data Sharing**:
   * Rapid data sharing is crucial, especially when acute events occur. Sharing data across centers and with families accelerates the learning process.
   * Utilizing an integrated approach can aid in developing clinical care models that are adjustable in real time.
3. **Cardiac Injury Diagnosis and Monitoring**:
   * Employ baseline measures like echocardiography, MRI, and troponin [426] to assess cardiac risks.
   * Use rhythm monitoring to detect abnormal rhythms that might stem from cardiac injuries.
   * Recognize and monitor for potential cardiac risks from gene therapy:
     + **Very Early** (first day - weeks): Immune reaction to the virus.
     + **Mid-term** (weeks – months after therapy): Immune reaction to neo-dystrophin.
     + **Late** (years after therapy): Early cardiac progression due to initial injury as well as potential cardiac damage due to increased strength and activity
4. **Emergency Planning and Treatments**:
   * Consider antibody-based therapies, regularly employed in heart transplant scenarios, to prevent antibody-mediated rejection.
   * Anticipate the possible requirement of Ventricular Assist Device (VAD) therapy. Pre-existing consent for VAD installation is necessary, because it may not be possible for decisions to be hastily made during rapidly-evolving scenarios.
   * Establish logistics for patients located in areas without access to these interventions.

In addition, the Advanced Cardiac Therapies Improving Outcomes Network (ACTION network) is actively developing protocols to have in place prior to GT administration, whether in clinical trials or clinic, in dystrophinopathies, emphasizing diagnosis, therapeutic responses, and patient consent in advance of critical interventions.

In conclusion, sponsors must keep abreast with evolving insights and recommendations to ensure patient safety and therapeutic efficacy.

Sponsors should also note that it will be critically important to involve caregivers in safety monitoring, particularly those with very young or nonverbal children who might participate (see below).

Since genetic manipulation could cause serious long-term adverse effects, including a risk of mutagenesis, oncogenicity, and germline transmission that may not be apparent during development or at the time of an initial licensure, FDA has provided sponsors with recommendations regarding the design of long-term, follow-up studies. While the appropriate duration of long-term follow-up may depend on preclinical findings and the specific disease process, among other factors, sponsors should expect to continue to monitor patients for adverse events possibly related to GT for up to 15 years. This would include a minimum of five years of annual examinations, followed by 10 years of annual queries of study subjects, either in person or by questionnaire (see *Guidance on Long-term Follow-up of GT Products*) [437]. Please note that GT product recipients should not be restricted from enrolling in other clinical trials or accessing other experimental or approved therapies during this period.

1. Efficacy endpoints

Guidance pertaining to the choice of outcome measures and selection of endpoints, which is dependent upon stage of disease, is essentially the same for GT products as for other medical products; and has been described earlier in this guidance (see Natural History, Outcome Measures, and Clinical Trials sections).

With regards to using a transgene product as a biomarker or a surrogate marker, in animal models, there is clear evidence that increased expression of internally truncated dystrophin proteins may be associated with functional gain. The preclinical models are expected to have predictive value on the biological function in humans. This is also supported by natural history data in BMD. How much, if any, function other transgene products may provide has yet to be demonstrated in humans. In addition, the proteins from different GT products could have different bioactivity from one another and from the internally truncated dystrophin produced by exon-skipping AON therapies, which may warrant additional considerations both pre-approval and post-marketing.

Sponsors are encouraged to gather supportive evidence from other biomarkers, for instance, imaging, or circulating biomarkers that suggest a change from typical disease progression in treated individuals.

Sponsors are advised to gather evidence on the durability of transgene products in muscle, although issues of durability may differ greatly, depending on the product or therapy. Sponsors should be prepared to characterize the degree of muscle turnover, and whether there is evidence of continued expression in regenerated muscle. There may be trial participants with DMD who undergo a needle biopsy years after treatment for reasons related to their own clinical management, and sponsors could make arrangements to analyze levels of transgene product in such specimens. Sponsors that are considering incorporating such assessments in their development plan should collect an adequate number of samples to make a scientifically meaningful interpretation of the findings. There is also a possibility that the durability of the transgene product may differ by the product being expressed (eg, dystrophin versus micro-dystrophin), target muscle, and by the age/state of progression at which the individual was treated.

1. Patient engagement/patient-focused GT product development

As noted earlier in the guidance, patient-experience data should be woven into the initial selection of efficacy endpoints rather than added as an afterthought. A number of patient-reported outcome measures have been previously discussed for possible inclusion as endpoints. Patient experience data may also provide evidence of the clinical meaningfulness of an outcome. Studies in DMD have demonstrated how patients and caregivers weigh a particular outcome and/or benefit against any possible risks associated with the GT product (see the Science of Patient Engagement and Patient Experience Assessment section earlier in this guidance).

Sponsors drafting informed consent documents should consult with the DMD community. Note that this guidance has included a model informed consent policy in the guidance appendices (see page 110). This includes special considerations for gene therapy.

Patients and caregivers should be informed, in plain language, about:

* The vector, including the possibility that there may only be one opportunity to be treated by an AAV-vector delivered gene therapy, at present
* Potential adverse events including death (noting that as information becomes available that there would be updates to the IRB and the informed consent) in proportion to the level of risk
* Biopsy requirements, and the after-effects of biopsy
* What is known or not known about durability of the effect
* The potential that the trial will involve long-term follow-up, which FDA recommends could be anywhere up to 15 years
* The impact undergoing this clinical trial will have on the participants’ options for future clinical trials and disqualification for certain other types of treatments in development, potentially including other GT products.
* The process in place for communicating results (including biopsy results, etc.) to individual participants upon request once aggregate data has been made public, as time sensitive and disease impacting decisions may need to be made on the participants path of care.

In addition, the DMD community is well-developed among rare disease communities in terms of resources for education and community mobilization. Sponsors should consider working with the community advocacy organizations to develop materials that show caregivers and study participants how to proactively monitor and have an action plan should an adverse event occur. Caregivers and study participants should be fully informed before going into trials of the problems that may occur or arise, and whether they have any ability to watch for events.

1. Expedited programs

As described in the guidance document *Expedited Programs for Serious Conditions––Drugs and Biologics* [438], there are four FDA longstanding programs intended to facilitate and expedite development and review of new drugs to address unmet medical need in the treatment of a serious or life-threatening condition: fast track designation, breakthrough therapy designation, priority review designation, and accelerated approval. In addition, as part of the 21st Century Cures Act, a fifth expedited program, the regenerative medicine advanced therapy (RMAT) designation, was added for cell therapies, therapeutic tissue engineering product, human cell and tissue product, or any combination product using such therapies or products intended to treat, modify, reverse, or cure a serious or life-threatening disease or condition.

In principle, the same criteria that qualify a drug therapy for these expedited programs should apply to GT products. With regards to accelerated approval, GT products, including gene editing technologies, that demonstrate clear benefit in terms of intermediate clinical endpoints (see the outcome measure section earlier in the guidance) or that lead to change in a biomarker that is reasonably likely to predict clinical benefit may qualify for accelerated approval. This could include if there is substantial imaging evidence (stability on MR) that muscle quality is preserved and stable on treatment, if there is evidence that the treatment has altered a critical disease pathway at the cellular level, or if the mechanism of action directly affects the etiology of the disease.

While a change in functional dystrophin expression could qualify as a surrogate marker for approval of a GT product, at the time of writing, it is not yet clear that the transgene expression of mini- or micro-dystrophin, on its own, would qualify as reasonably likely to predict clinical benefit. In addition, the effects of mini- and micro-dystrophins could differ from each other. Nevertheless, deficiency of functional dystrophin is the proximate cause of the symptomatic and functional consequences of dystrophinopathies, which justifies particular interest in dystrophin as a surrogate endpoint for accelerated approval, and potentially in mini- and micro-dystrophin (once there are supportive data on function) as candidate surrogate endpoints. Complementary biomarker data or intermediate clinical evidence of benefit in treated patients could lend support for accelerated approval.

As this guidance went to press, one gene therapy product, delandistrogene moxeparvovec-rokl, was granted an accelerated approval with a label limiting its use to younger DMD patients (ages 4-5 years). This was based on limited data from a placebo-controlled trial suggesting that increased micro-dystrophin expression at 12 weeks correlated with functional improvements (NSAA total scores after one year of follow-up) in this particular age group. Continued approval for this indication may be contingent upon verification and description of clinical benefit in clinical trials—particularly in older individuals with DMD. It is also worth noting that insurers and other third-party payers may be more likely to provide reimbursement for a GT product with supportive biomarker and outcome measure data.

At the time of writing, unlike most drugs, re-dosing is not yet possible for GT products. This means that a GT product may be a one-time treatment (gene therapy cannot be withdrawn and subsequently readministered), which could change the risk versus benefit calculus. Individuals with DMD may also only have one opportunity to benefit from a GT product using a specific vector, and treatment decisions will likely be based upon their individual disease trajectory profile. However, each GT product’s regulatory review should be based upon its own merit.

Finally, sponsors and regulators should consider patient/caregivers preferences regarding how to balance the uncertainty of benefit with this uncertainty of risk on a product-by-product basis, throughout the development process and during regulatory review.

Appendix: Informed Consent in Gene Therapy Trials

Considerations for Informed Consent in

Gene Therapy Clinical Trials

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Informed consent is an integral and important component of clinical research. It is important that prospective research subject understand the research nature of a trial they are considering, as well as their rights, responsibilities, and the implications of participating in a study. Because of the complexity of gene therapy, which may not be appreciated or understood by the general public, it is important to ensure prospective research participants fully understand the procedure, and the potential risks and benefits of this approach.

FDA regulations [(21 CFR part 50)](https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?CFRPart=50&showFR=1)

(https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?CFRPart=50&showFR=1) at FDA 21 CFR 50.25(a) and The Federal Policy for the Protection of Human Subjects, usually referred to as [the Common Rule](https://www.hhs.gov/ohrp/regulations-and-policy/regulations/45-cfr-46/revised-common-rule-regulatory-text/index.html) at 45 CFR 46.116 (which applied to federally-funded research) describe requirements for informed consent. All of the elements described, both as “Required” and “Additional Elements” that apply to the study must be addressed in the informed consent.

A common complaint from prospective and enrolled research subjects about informed consent is the amount of medical jargon or technical terms, and “legalese” that makes the consent document and process confusing and difficult to understand.

While the Common Rule applies only to federally supported research, researchers may choose to include a “key information section” as described below in any consent for research that is not federally funded or supported. Thus, for the sake of clarity and understanding, informed consent for all biomedical research, whether federally funded or not, should adhere to the Common Rule format requirement in [45 CFR 46.116(a)(5)(i)](https://www.hhs.gov/ohrp/regulations-and-policy/regulations/45-cfr-46/revised-common-rule-regulatory-text/index.html#46.116) (https://www.hhs.gov/ohrp/regulations-and-policy/regulations/45-cfr-46/revised-common-rule-regulatory-text/index.html#46.116), which includes:

* Reasonable Person Standard Requirements
  + - The subject or their legally authorized representative must be provided with the information that a reasonable person would want to have in order to make an informed decision about whether to participate, and opportunity to discuss that information
* Key Information – New Section of Consent
  + Informed consent must begin with a concise and focused presentation of the key information that is most likely to assist a prospective subject or legally authorized representative in understanding the reasons why one might or might now want to participate in the research. This part must be organized in a way that facilitates comprehension.

Assent: Because many of the research subjects involved in gene therapy are children assent from the child should be sought. Legally, children are unable to give informed consent until they reach the age of majority, which may vary by state or country. “Assent” refers to a child’s affirmative agreement to participate in research. Mere failure to object should not, absent affirmative agreement, be construed as assent.

[[45 CFR 46.402(b)](https://www.hhs.gov/ohrp/regulations-and-policy/regulations/45-cfr-46/common-rule-subpart-d/index.html#46.402)] (https://www.hhs.gov/ohrp/regulations-and-policy/regulations/45-cfr-46/common-rule-subpart-d/index.html#46.402).

Assent is the process in which children or adolescents are given easy-to-understand information about a clinical trial to help them decide if they want to take part in the trial. The patient is given a chance to ask questions about what will happen during the trial, why it’s being done, and what they will be asked to do. Formal consent, however, to enter the trial comes from the parent or guardian.

When judging whether children are capable of assent, the investigator and the IRB needs to take into account the ages, maturity, and psychological state of the children involved to judge the capacity of the children to be involved in a proposed research activity to assent.

Oral consent, using a script, may be appropriate for younger children (eg, 7 – 11 years of age), while written assent can be appropriate for older children. Assent should make the research experience understandable to the child, and include information such as how long the study will take, what kinds of procedures will be involved, whether there will be hospital stays, whether pain or discomfort is likely, etc.

Assent is meant to ensure that children are not forced to be research subjects, even when their parents’ consent to it. When an investigational intervention or procedure involved in the research may provide direct benefit that is likely to outweigh potential risks and is available only in the context of the research, the assent of the children is not a necessary condition for proceeding with the research.

Community focus group/ community engagement:

Engaging with patients and families to assess and improve their understanding of the information in, and format of informed consent can help vastly improve the clarity, readability, and understanding of the consent document. Ideally, community engagement can be used to provide input on what prospective research subjects/families want and need to know, and how best to convey the information in a clear and understandable way.

Special considerations related to gene therapy

Special considerations for gene therapy should be discussed in the informed consent. These include, but are not limited to:

Possible immune response to vector, uncertainty of effect, uncertainty of durability of effects achieved, whether biopsies will be necessary, the possible aftereffects of the procedure, potentially long-term follow-up period that could last years, possible impact on participation in future clinical trials, or disqualification for other treatment options.

Post consent online

Clinical studies that are conducted or supported by a [Common Rule federal agency are required to post one consent form](https://clinicaltrials.gov/ct2/about-site/history#RevisedCommonRule) (<https://clinicaltrials.gov/ct2/about-site/history#RevisedCommonRule>) used in the enrolling of participants to a publicly available federal website. Ideally, whether federally funded or not, sponsors should post a copy of the informed consent to ClincalTrials.gov to help create a repository of consent documents to provide models for improving the overall quality and understandability of gene therapy informed consents.

Information about how to post study -related documents is available on the [ClinicalTrials.gov site](https://prsinfo.clinicaltrials.gov/tutorial/content/index.html#/lessons/EbNabHFSR5PX40GBkpi3Kmk4CmirjZ7k) (https://prsinfo.clinicaltrials.gov/tutorial/content/index.html#/lessons/EbNabHFSR5PX40GBkpi3Kmk4CmirjZ7k).

***Appendix 1:   
  
Working groups membership:***

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|  | **Name** | **Title** |
| **WG #1: Benefit-Risk** | | **Co- chairs** | **Rose Juhasz, PhD** | Director of Oncology Research, Trinity Health, Michigan |
| **Ryan Fischer** | Chief Advocacy Officer, Parent Project Muscular Dystrophy |
| **Members** | John Bridges, PhD | Professor, Department of Biomedical Informatics and Surgery, Ohio State University College of Medicine |
| Ellen Janssen, PhD | Associate Director, Benefit-Risk Assessment, Janssen Pharmaceutical Companies of Johnson & Johnson |
| Colin Rench | Patient |
| Holly Peay, PhD | Senior Research Analyst, RTI International |
| Jodi Wolff, PhD | Vice President, Patient Engagement and Advocacy at Stride Bio |
| Katherine Beaverson | Senior Director, Patient Advocacy Lead |
| Peter Pitts | President and Co-Founder, Center for Medicine in the Public Interest |
| **WG #2: Biomarkers** | | **Co- chairs** | **Kevin Flanigan, MD** | Director, Center for Gene Therapy at Nationwide Children’s Hospital |
| **Eric Camino, PhD** | Vice President, Research and Clinical Innovation |
| **Members** | Annemieke Aartsma-Rus, PhD | Professor of Translational Genetics, Leiden University Medical Center |
| Bill Rooney, PhD | Director, Advanced Imaging Research Center and Associate Professor of Behavioral Neuroscience, School of Medicine, Oregon Health and Science University |
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| Marty Karlin | Rally for Ryan |
| Sheryl Marazzo | 4 Jake's Sake |
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**Table 1. Temporal Framework for Patient Engagement and Patient Experience Assessment**

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**Figure 1: Statement of Patient Experience**

A close-up of a medical survey

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**Figure 2: Statement of Patient Experience Checklist Items**

A diagram of a patient's research

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**Figure 3: Dystrophinopathy Diagnostic Algorithm**

A diagram of a flowchart

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A chart of different colored shapes

Description automatically generated with medium confidence**Figure 4. Stages of DMD progression, monitored by milestones, and captured by multiple outcomes measures/clinical endpoints**

**Figure 5: The HERCULES Model [39)**

Diagram

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**Figure 6: Performance of the Upper Limb (PUL) (Entry Item)**

**A chart with black stick figures

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**Table 2: Dystrophin quantification method overview**

A close-up of a medical chart

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1. The protein created by exon skipping (exon-skipped-dystrophin or ES-dystrophin), whether achieved using oligos, delivery of U7-directed exon skipping, or exon deletion using gene editing, are internally deleted dystrophins of varying sizes and stabilities. One exception is the case of exon skipping prior to exon 5 that is directed at causing translation of dystrophin using the IRES present in exon 5. In this case, the resulting dystrophin (IRES-dystrophin) has an N-terminal deletion. The dystrophin constructs designed to fit in AAV are known as micro-dystrophins. They are both truncated (C-terminal) and internally deleted in order to reduce the size sufficiently to have the cDNA package in AAV. The term micro-dystrophin implies nothing about functionality, which can differ dependent on the components included within the micro-dystrophin. The terminology was developed to distinguish these constructs from mini-dystrophins, which were larger internally deleted (and in some cases truncated) constructs that were designed to fit within adenoviruses or other viruses with larger packaging capacity than AAV. [↑](#footnote-ref-1)