Draft Guidance for Industry

Duchenne Muscular Dystrophy, Becker Muscular Dystrophy, and Related Dystrophinopathies

Developing Potential Treatments for the Entire Spectrum of Disease

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Developing Potential Treatments for the Entire Spectrum of Disease

This draft guidance represents an update of the first draft FDA guidance initially composed by a disease community, with input from industry, sponsors, academia and the Duchenne muscular dystrophy patient community. When finalized, it should represent the Food and Drug Administration's (FDA's) current thinking on this topic. It does not create or confer any rights for or on any person and does not operate to bind FDA or the public. You can use an alternative approach if the approach satisfies the requirements of the applicable statutes and regulations. If you want to discuss an alternative approach, contact the FDA staff responsible for implementing this guidance. If you cannot identify the appropriate FDA staff, call the appropriate number listed on the title page of this guidance.

INTRODUCTION

The purpose of this guidance is to assist sponsors in the clinical development of medical products (ie, human drugs and therapeutic biological products) for the treatment of Duchenne muscular dystrophy (DMD) over the entire spectrum of the disease.

This updated guidance is the result of the first collaboration between the FDA and a disease-specific community in their respective disease area. The FDA invited the Duchenne community (including patients, parents and caregivers, clinicians, academic experts, and industry) to develop the earlier version of the guidance as provided under FDA's interpretation of Good Guidance Practice provisions. Upon receipt of the first iteration of the guidance on June 25, 2014, the FDA opened a docket and held further meetings with the DMD community and other experts, leading to revisions based upon regulatory and statutory requirements and additional published data, released in June 2015 (see https://www.parentprojectmd.org/wp-content/uploads/2021/07/2014_Community_Guidance.pdf).

These activities provided the impetus and laid the groundwork for the FDA to develop its own streamlined guidance for industry on DMD and related dystrophinopathies—the first for a specific rare

disease—focused specifically on the clinical trial process. This guidance was finalized in February 2018 (see https://www.fda.gov/media/92233/download).

Since that time, there have been numerous advances in DMD, including an increase in gene therapy studies and a growing recognition of the need to focus on the deterioration of cardiac function as a separate process from loss of skeletal muscle function. There are updated care considerations guidelines that include the care of adult patients, progress in the FDA's approach to patient engagement and preference studies, genotype/ phenotype correlations and disease progression models, as well as new more sensitive outcome measures. In addition, there was also a need to expand the guidance to include considerations specific to development of treatments for Becker muscular dystrophy (BMD) and other dystrophinopathies. Given all these developments, it was critical that all of the new knowledge be captured in an update to the patient-led guidance document.

Like the previous community guidance, this document addresses and expands upon the FDA's current thinking regarding the consideration that should be given to the patient engagement of the DMD and BMD community. It also reflects the FDA's appreciation that recent evidence from patient registries, natural history studies, and clinical trial cohorts have updated both the understanding of DMD and BMD natural history and the causes for variability in outcomes. It addresses the selection of endpoints for clinical trials in populations with DMD as well as the manner in which disease modification might be demonstrated. Given the use of dystrophin as a surrogate endpoint marker for the approval of several drugs, this document provides up-to-date guidance on the state-of-the-art measurement of the biomarker. This updated guidance also provides similar guidance on the use of magnetic resonance (MR) imaging measures of both skeletal and cardiac muscle and function and encourages sponsors and regulators to consider their use as surrogate endpoints. Finally, the guidance expands beyond the development of pharmacological treatments (drugs) to consider the development of gene therapy products.

I. Background

Dystrophinopathies result from genetic mutations in the *DMD* gene that decrease the amount of dystrophin protein and/or cause dysfunction of the protein. In association with other proteins, dystrophin protects muscle fibers against the mechanical forces of contraction—in the absence of dystrophin, muscle is prone to damage, and progressive muscle degeneration. Downstream pathologies including inflammation and fibrosis interfere with muscle regeneration and cause loss of ambulation, loss of upper limb function and other movement, orthopedic complications, and, ultimately, respiratory and cardiac failure.

The most common and generally most severe dystrophinopathy is DMD, with a birth prevalence of about 1 in 3,500 to 6,000 males. DMD causes delay and/or failure to reach developmental milestones, functional losses in the first decade of life, and a loss of independent ambulation before the age of 13 years in the absence of disease-modifying treatment. In nonambulatory boys and young men, there is gradual loss of upper limb and neck functions, so that grooming, toileting, bathing, dressing, and eating become impaired or impossible to perform by oneself—affecting the quality of life of patients, their caregivers, and families. This is accompanied by weakness affecting respiratory muscles and the heart that contributes to decreased respiratory function and cardiomyopathy—and greatly decreased life expectancy. Heart disease is now the most common cause of death in boys and young men with DMD.

BMD has later onset of symptoms and slower progression. BMD is characterized by wide interpatient variability in severity, with some patients having a clinical course similar to that observed for DMD, while other patients remain nearly, or in some cases completely, asymptomatic. The birth prevalence of BMD is about 1 in 20,000 males. A small percentage of female carriers may also exhibit a range of muscle symptoms from the full Duchenne phenotype to milder skeletal muscle weakness (see more on related dystrophinopathies in the Diagnosis section).

Over the past decade, patient organizations, academia, and industry have worked together to develop several patient registries, disseminate improved standards of care, and explore clinical outcome measures and biomarkers. This experience and data collection has resulted in a greatly improved understanding of the pathogenesis and the natural history of DMD and BMD, including factors that may lead to variability in the course of the disease.

Natural history studies as well as clinical trials have shown that the use of glucocorticoids and the management of spine deformity, and pulmonary and cardiac dysfunctions have altered the timing of some of the clinical milestones of the disease. But with improved medical management have come new complications, and quality of life often suffers. For instance, adverse events known to be associated with glucocorticoid usage include excessive weight gain, growth inhibition, bone fragility with a high risk of fractures, risk of diabetes, behavioral abnormalities, Cushingoid features, change in pubertal progression, and cataracts. Of particular concern is the issue of weight gain since weight gain can compound the physical limitations of a dystrophic myopathy.

At the time of this update, it should be acknowledged, with gratitude, that there have been some advances in treatment since the previous guidance with an FDA-approved corticosteroid drug, and also several FDA-approved DMD-specific exon-skipping drugs that provide some benefit for individuals with specific DMD mutations. These latter agents were approved based on surrogate marker-evidence, and there is increasing evidence of clinical benefit based on longer term observation on treatment, but we urge the sponsors to complete post-marketing placebo-controlled trials in an expeditious manner, thus, better characterizing the extent of this clinical benefit.

However, these advances in no way reverse the underlying condition. Duchenne is characterized by a progressive, irreversible loss of one function after the other, from the loss of standing from the floor, to the loss of ambulation, to the loss of the ability to self-feed, and the inability to breath without assisted ventilation Once a functional capacity is lost in an individual with DMD, it is gone forever. Death can happen without warning, at any moment, even in younger boys. Complications such as cardiomyopathy commonly cause early death in patients with BMD.

There is an urgent unmet need to develop new treatments, especially those that address the underlying cause of dystrophinopathy. With a number of potential therapeutic agents in or entering clinical development, sponsors need formal guidance on how best to demonstrate a treatment's effectiveness and safety in this rare disease and what sort of effect would be clinically meaningful to patients and their caregivers.

II. Guidance Updates

This iteration of the draft guidance contains updates to the sections of the first draft guidance. As with the initial draft guidance, the community chose to place the topic of patient engagement at the start of the document, because it was felt that sponsors should be guided by patient engagement and patient and caregiver preferences from the very start of a product's clinical development. The diagnosis section follows, to help guide sponsors in the selection of patients, and also to prepare for the introduction of newborn screening. This is followed with sections on natural history, outcome measure selection, and a section on biomarkers that has been moved before the updated clinical trials section as biomarkers are increasingly used or used as surrogate endpoints.

The following are key considerations in these updated sections.

A. The Science of Patient Engagement and Patient Experience Assessment

(Formerly the Benefit/Risk Assessment section)

Key considerations in this section:

- *Patient-focused drug development (PFDD) has evolved considerably since the 2014 community-led Duchenne Guidance was released, with FDA providing clearer direction via guidance documents on the collection of data related to patient experiences.*
- *Patient experience data comes in many forms and are intended to provide information about patients' experiences with DMD and BMD. More data related to BMD patient experiences are needed to inform drug development.*
- *Patient and caregiver preferences for treatments have been measured and are well documented in the Duchenne community and can inform all stages of drug development.*
- *Preference data has shown that patients and caregivers have similar preferences and that they are willing to accept risk and uncertainty in exchange for therapies aimed at slowing disease progression.*
- *Sponsors should engage patient groups and FDA on the collection of new patient experience data related to their development programs.*

B. Criteria for Diagnosis in the Clinical and Research Settings

This section provides sponsors with an overview on the diagnosis of dystrophinopathies and differs from the section in the earlier guidance in some key areas.

- *The updated section approaches dystrophinopathies as a spectrum of disorders rather than focusing solely on DMD, adding a list of clinical features for typical DMD and those with later-onset of clinical progression.*
- *While it is emphasized that genetic testing remains the gold standard for diagnosis, it needs to be considered within the clinical context. Muscle biopsy is usually not required in a clinical setting but often still necessary in the research setting.*
- *The discussion on the multiple testing options for genetic confirmation of a dystrophinopathy has been expanded and an algorithm that charts a diagnostic pathway has been added. It also reviews variants of uncertain significance in the DMD gene which have become more common with the advent of next generation sequencing and population screening (expanded carrier screening).*
- *Forward-looking statements have been added regarding newborn screening for Duchenne given that a Recommended Uniform Screening Panel (RUSP) nomination has been submitted in 2022 and implementation would mean diagnosing and managing infants in a presymptomatic stage.*

Another change in the drafting of this guidance, was to combine the working groups drafting the Natural History, Outcome Measures and Clinical Trials sections into one large group, as in the previous guidance, the work of each working group often informed the other. In addition, there has been increasing use and acceptance of DMD natural history data both in the development of disease progression models and as real-world evidence of disease progression and natural history in the absence of novel therapies that can augment or replace data from placebo arms.

C. The Current Understanding of the Natural History of Duchenne Muscular Dystrophy

Key considerations in this section:

- *This section provides an updated overview of DMD natural history concepts, with a new schematic (still in development) that will show the typical progression of DMD. This schematic uses violin plots with the median and range of timing when milestones, (eg, loss of ambulation, loss of standing ability from the floor, etc.,) occur, based on data from large natural history cohorts, as well as some of the key outcome measures used to monitor disease progression across the different stages of disease.*
- *New models of progression such as the HERCULES Model and the UC Davis Model link events and outcome measures to add granularity to characterization of disease stage and trajectory. One important aspect of these models is the identification of a brief transitional stage beginning during late ambulation where individuals are able to either independently stand or stand with assistance and transfer their own weight.*
- *There is now an increased body of natural history data that can better characterize an individual's disease course and the sources of heterogeneity that sponsors can account for in clinical trials.*

D. Outcome Measurement Selection

Key considerations in this section:

- *This section describes outcome measure selection for staging disease, stratifying cohorts and for monitoring disease progression.*
- *Certain outcome measures can be used to identify specific populations of participants at risk of measurable progression and loss of specific functions during the course of a DMD trial. Use of these outcome measures during participant screening and for stratification could reduce the risk of underpowering a study and not reaching a conclusive answer regarding the effectiveness of a potential therapy. It is possible for a study to have broad inclusion criteria, but, with stratification, enrich a group that the study's primary prespecified analysis is based on.*
- *There are more data now showing specific changes in outcome measures that may be clinically meaningful to patients and families at different stages of disease. Performance measured by some tools are predictive of progression to disease milestones and thus may be useful as intermediate clinical endpoints.*
- *In addition to developmental and motor measures, the section reviews the use of pulmonary outcome measures, upper limb function measures, and activities monitored by digital technologies and wearable devices that can track the course of progression during the transitional through loss of ambulation and through the nonambulatory stages of DMD.*

E. Biomarkers in Duchenne muscular dystrophy

Key considerations within this section:

- *Dystrophin quantification has been used in the approval of several genetic therapies in DMD. There are a variety of quantification methods for assessing dystrophin; multiple methodologies could be required to properly reflect the expression and biodistribution of protein.*
- *Sponsors should strive to minimize trauma for patients when including muscle biopsy and develop clear protocols for handling and preparing samples to reduce loss of valuable tissue.*
- *There is ample evidence that MR measures are related to patient function, predictive of future changes in function, and suitable for use in both ambulatory and nonambulatory patients. Sponsors should consider including MR measures in trials to build evidence as a potential surrogate endpoint.*
- *Circulating biomarkers may aid in characterization of response to therapy, but further work is needed to link circulating biomarkers to specific mechanisms of action.*

F. Specific Trial Design and Analysis Issues for Clinical Trials In DMD

Key considerations within this section:

- *This section contains key learnings from past trials, including the chief finding that baseline disease severity characteristics are better than age as criteria for enrichment of patient trajectories.*
- *The section describes key considerations in DMD trial design and analysis, including recommendations on concurrent therapy and duration in order to measure clinical benefit at different disease stages.*
- *The section considers advances in the collection and analysis of natural history data and real-world data and their use in informing the design of clinical trials. Innovative trial designs can also include delayed placebo (or run-in trials, in which natural history data are used in the run-in to the trial) and roll-over trials in order to make trials more efficient and reduce participants' exposure to placebo. For instance, the DMD community has been working on a master protocol for a platform trial that can share placebo patients and reduce the proportion of individuals randomized to placebo.*
- *Finally, a brief discussion of clinical trial considerations in BMD and other dystrophinopathies is included.*

This version of the guidance also contains two new sections, one on cardiomyopathy and the other on gene therapy. Cardiomyopathy is now the leading cause of death among young men with DMD, and some sponsors are looking specifically at heart function in patients with dystrophinopathies, and the use of imaging methodologies to monitor pathogenic changes to the heart. The guidance calls on sponsors to gather evidence linking these pathogenic changes to clinical progression in order to support regulatory acceptance of these imaging biomarkers as surrogate markers.

Finally, at the time of the original guidance drafting, most of gene therapy research was preclinical. Now, with a few years' worth of data in clinic, the community saw a need to engage with the Center for Biologics Evaluation and Research to develop a section that consolidates the existing FDA guidance on gene therapy and provides specific recommendations on patient considerations unique to DMD and related dystrophinopathies.

G. Cardiomyopathy

Key considerations within this section:

- *DMD-related cardiomyopathy is characterized by fibrofatty replacement of the myocardium, with an extended timeline of cardiac disease progression culminating in full thickness fatty replacement of the myocardium. This suggests maximum therapeutic benefit will be garnered only by developing trials focused on BOTH early and later stage disease. A singular focus on trials powered to examine late-stage*

disease in order to incorporate mortality outcomes may miss an important therapeutic window prior to irreversible, fatty replacement of the myocardium.

- *Harmonization of diagnostic evaluation and therapeutics between trial centers is integral to trial design but must be balanced with the need for inclusivity and access. Consensus recommendations regarding potential cardiac biomarkers and their consideration in trial design will not only facilitate effective trial design but would also provide a means to develop a more robust real-world data infrastructure. This infrastructure is currently needed to assess ongoing clinical trials and for future trials, both cardiac and noncardiac.*
- *The understanding of cardiac disease progression has evolved as longitudinal, granular cardiac data has emerged over the last decade. These data and the creation of multicenter networks have made cardiac clinical trials in DMD more feasible. Creation of a roadmap to assess effectiveness of cardiac therapies in DMD will further facilitate the timely development of therapies.*

H. Gene Therapy for DMD and Other Dystrophinopathies: Approaches, Patient-Centered Considerations, and Development Pathway

Key considerations within this section:

- *This section draws upon existing FDA guidance on gene therapy (GT) and considers how sponsors can apply it to the development of GT products for DMD.*
- *Technical challenges for the development of GT products that are unique to dystrophinopathies include the target tissues—both skeletal muscle and cardiac muscle—as well as the size and complexity of DMD gene that a GT would be designed to restore or correct.*
- *Sponsors should consider the implications of the immune responses and safety issues that currently limit the administration, and preclude re-administration, of some of the GT products furthest along in development.*
- *Priorities for preclinical studies include dose selection so that clinical trials start with a dose expected to have a therapeutic effect, as well as early evaluation of the effects of GT on the heart.*
- *While well-controlled placebo-controlled studies are recommended for GT products that are not expected to have large, self-evident effects, sponsors are encouraged to discuss novel trial designs with FDA that limit the time or necessity that a trial participant is on placebo.*
- *The section includes guidance on corticosteroid treatment prior to and during clinical trials, participant selection criteria and safety considerations including long-term monitoring of GT trial participants.*
- *Efficacy endpoints considerations are the same as in trials of non-GT product for DMD. Intermediate clinical endpoints and surrogate endpoints reasonably likely to lead to or predict clinical benefit could be the basis for a GT to be granted accelerated approval. Given the inability to repeat dosing at the present time, there should be some evidence suggestive of clinical benefit, whether through demonstration of high levels of expression of a functional transgene or demonstration of restored expression of the endogenous gene after gene editing, for the proteins produced by gene therapy to be considered a surrogate endpoint meeting the "reasonably likely" standard for accelerated approval. Evidence from other candidate surrogate endpoints (such as imaging) could support an application.*

III. Imperatives and Implications

Finally, the document concludes with a section on the community's imperatives for regulators and sponsors in the development of treatments across the spectrum of dystrophinopathies, based upon

consultation with our community guidance board consisting of patients, caregivers, and other representatives of the DMD and BMD community.

The FDA has acknowledged the concerns expressed by the DMD and BMD community that flexibility be exercised in the review of products for these diseases—recognizing that many patients and caregivers are willing to take greater risks for a treatment that may slow clinical deterioration or delay the loss of functional milestones, each of which is clinically meaningful.

The FDA shares the Duchenne and Becker community's goal to work with industry to get new therapeutic agents onto the market as rapidly and responsibly as possible. This updated guidance for industry is but a step towards achieving that goal.

This guidance is intended to serve as a focus for continued discussions among the FDA, the medical industry, sponsors, academic community, the patient and caregiver community, and the public.

FDA's guidance documents, including this community guidance, do not establish legally enforceable responsibilities. Instead, guidance documents describe the Agency's current thinking on a topic and should be viewed only as recommendations, unless specific regulatory or statutory requirements are cited. The use of the word *should* in Agency guidance documents means that something is suggested or recommended, but not required.

THE SCIENCE OF PATIENT ENGAGEMENT AND PATIENT EXPERIENCE ASSESSMENT

I. 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.[1] The United States 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).

Key considerations in this section:

- *Patient-focused drug development (PFDD) has evolved considerably since the 2014 community-led Duchenne Guidance was released, with FDA providing clearer direction via guidance documents on the collection of data related to patient experiences.*
- *Patient experience data comes in many forms and are intended to provide information about patients' experiences with Duchenne muscular dystrophy (DMD) and Becker muscular dystrophy (BMD). More data related to BMD patient experiences are needed to inform drug development.*
- *Patient and caregiver preferences for treatments have been measured and are well documented in the Duchenne community and can inform all stages of drug development.*
- *Preference data has shown that patients and caregivers have similar preferences and that they are willing to accept risk and uncertainty in exchange for therapies aimed at slowing disease progression.*
- *Sponsors should engage patient groups and FDA on the collection of new patient experience data related to their development programs.*

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, and advocacy organizations. We encourage sponsors to discuss with the FDA, as early as possible, the types of patient experience data that may support a regulatory submission. FDA should continue partnering with the patient community and share information and data with sponsors in the space on their own investments in patient perspective research.

II. 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:

A. Patient-focused drug development (PFFD) guidance

- Collecting Comprehensive and Representative Input. Guidance for Industry, Food and Drug Administration Staff, and Other Stakeholders.[2]
  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 [3]
  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.

B. PFDD draft guidance pending release

- **Selecting, Developing, or Modifying Fit-for-Purpose Clinical Outcome Assessments**: This guidance will address methods to measure impacts and prioritize endpoints in a meaningful way. A public workshop 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.[4]
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  - The Medical Device Innovation Consortium has also published resources as part of their Science of Patient Input (SPI) initiative on methodologies to systematically identify outcomes that matter most to patients and to establish these outcomes as primary or secondary endpoints for clinical studies.[5]
- **Incorporating Clinical Outcome Assessments into Endpoints for Regulatory Decision Making:** 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.[6]

C. Other guidance related to patient experience data:

- Patient Preference Information in Medical Device Decision-Making.[7]
  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 (Draft)[8]
  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.

III. 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.[9] 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.[10] Additional studies have explored parental worries,[11] symptom prioritization for treatments,[12] meaningful benefit in pulmonary outcomes,[13] caregiver vs. patient preferences, [13] clinical trial decision making, preferences for emerging gene therapies,[14-16] and patient experience with standard-of-care treatment.[8] Qualitative narratives collected from the community have also been analyzed and submitted to FDA.[17]

A. Key learnings from studies to date:

1. Patients and caregivers are willing to trade off on risks, treatment burden, uncertainty, and treatment benefits, including slowing disease progression.
2. Patients and caregivers generally have similar preferences for treatments, but preferences may vary by age and stage of disease.
3. Preferences can differ based on therapeutic intervention.
4. Priorities for treatment outside of skeletal muscle include potential therapies targeting cardiac and pulmonary function.

B. 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.[18]

IV. 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
- 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

Research and Discovery	
Early in product discovery, when defining a targeted product profile, patient experience data can ● identify unmet medical needs and burden of disease in Duchenne; ● define the shortcomings of existing standards of care which new products may address; and ● identify symptoms that are most important to treat.	Considerations: ● Engage with patient groups early and often throughout the development process. ● Review existing patient experience data on Duchenne and Becker. ● Generate new data that are needed to inform the development program.
Preclinical/Pre-IND	
At this stage, as sponsors prepare for FDA interactions, PED can inform: ● selection of clinical outcome assessments from existing measures that are meaningful to patients; ● trial design with the potential to reduce trial burden on patients; ● trial participation interest; and ● preferences around drug's formulation, dosing frequency, and routes of administration.	Considerations: ● PED should be collected as early as possible to inform the design of the trial. ● Review relevant clinical outcome assessments from natural history studies and past trials. ● Through patient engagement methods, collect PED that demonstrates meaningfulness of chosen outcome assessments to patients and caregivers.
Clinical Development (Phase 1,2,3)	
PED collected during clinical development can inform: ● understanding early phase trial experiences to inform future development; ● eliciting preferences for treatments (from trial participants and general population), including the types of risks and burdens that may be acceptable in exchange for benefit proposed by treatment profile; ● Quantifying, defining, and explaining the meaningfulness of changes in outcome measures including how these changes impact activities of daily living.	Considerations: ● Sponsors should avoid including too many exploratory measures in order to reduce trial burden. ● Consider qualitative tools such as exit interviews deployed at screening and at the end of the trial to understand the patient experience of those on therapy vs. those on placebo. ● Ensure PRO tools are relevant to the patient population.
Regulatory Review	
Types of PED that may be relevant to NDA submission: ● Patient preference data ● Patient-reported outcome data ● Natural history data ● External natural history control data that matches trial arms ● Qualitative or quantitative PED may also lend insight into the clinical meaningfulness of the change in outcome assessment.	Considerations: ● Preference studies should focus on acceptable levels of risk and uncertainty for the proposed therapy benefit. ● Consider implications for label and potential to include PED.

V. 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

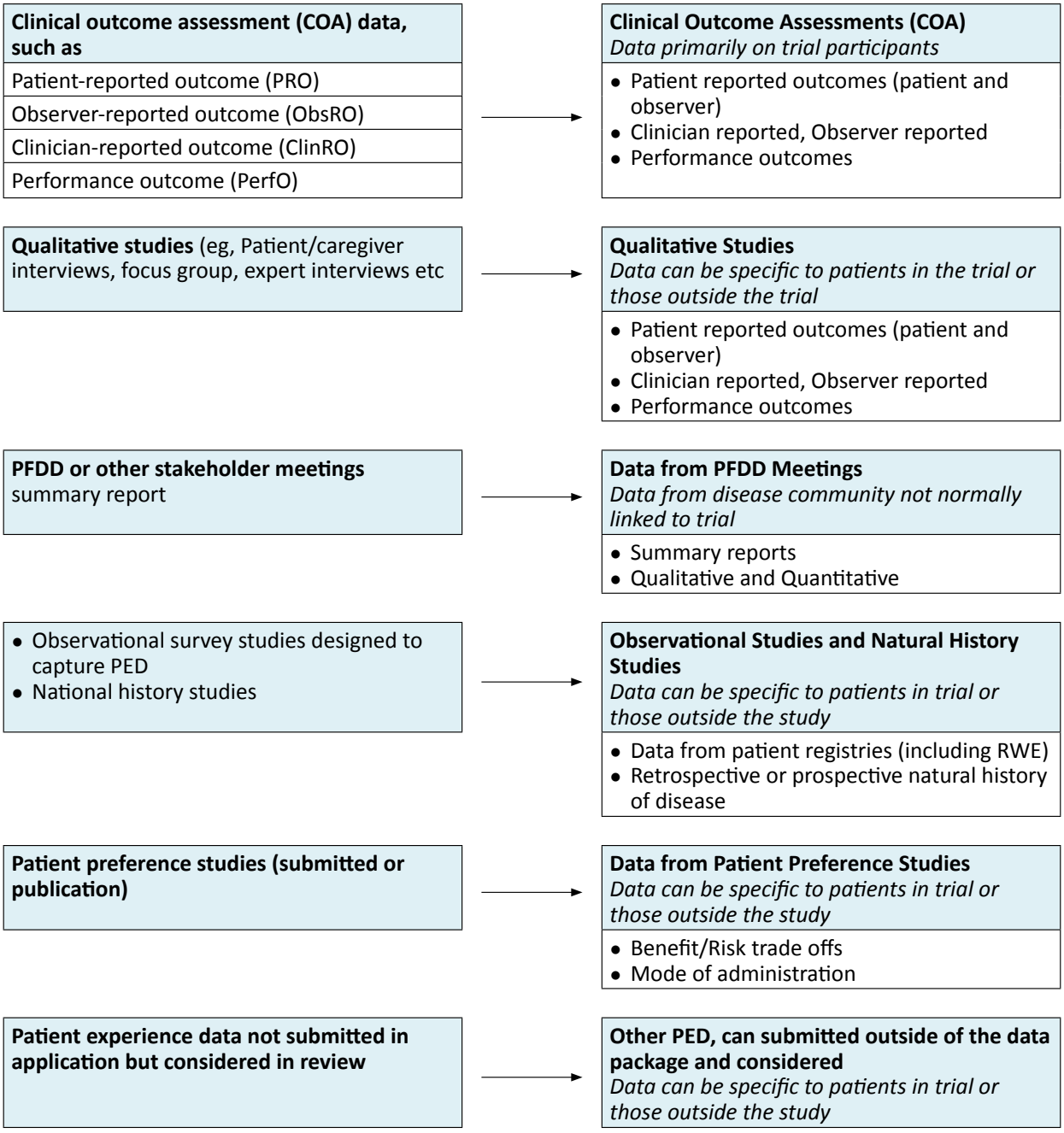
Section 3004 of the 21st Century Cures Act directed the FDA to report on the use of PED in regulatory decision-making, focusing on the review of patient experience data and information on PFDD tools. The reporting tool below is now required to be filled out by FDA on approved products as part of the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

Patient Experience Data Relevant to this Application (check all that apply)				**Section of review where discussed, if applicable**
☐	The patient experience data that were submitted as part of the application include?			
	☐	Clinical outcome assessment (COA) data, such as		
		☐	Patient-reported outcome (PRO)	
		☐	Observer-reported outcome (ObsRO)	
		☐	Clinician-reported outcome (ClinRO)	
		☐	Performance outcome (PerfO)	
	☐	Qualitative (e.g., individual patient/caregiver interviews, focus group interviews, expert interviews, Delphi Panel, etc.)		
	☐	Patient-focused drug development or other stakeholder meeting summary reports		
	☐	Observational survey studies designed to capture patient experience data		
	☐	Natural history studies		
	☐	Patient preference studies (e.g., submitted studies or scientific publications)		
	☐	Other: (Please specify):		
☐	Patient experience data that were not submitted in the application, but were considered in this review?			
	☐	Input informed from participation in meetings with patient stakeholders		
	☐	Patient-focused drug development or other stakeholder meeting summary reports		
	☐	Observational survey studies designed to capture patient experience data		
	☐	Other: (Please specify):		
		If none checked, check here to confirm that patient experience data that were not submitted in the application were not applicable, available, or considered in this review		
☐	Patient experience data were not submitted as part of this application			

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.

Statement of Patient Experience Checklist Items



CRITERIA FOR DIAGNOSIS IN THE CLINICAL AND RESEARCH SETTINGS

I. 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.

Key considerations within this section:

This section provides sponsors with an overview on the diagnosis of dystrophinopathies and differs from the section in the earlier guidance in some key areas.

- *The updated section approaches dystrophinopathies as a spectrum of disorders rather than focusing solely on DMD, adding a list of clinical features for typical DMD and later-onset patients.*
- *While it is emphasized that genetic testing remains the gold standard for diagnosis, it needs to be considered within the clinical context. Muscle biopsy is usually not required in a clinical setting but often still necessary in the research setting.*
- *The discussion on the multiple testing options for genetic confirmation of a dystrophinopathy has been expanded and an algorithm that charts a diagnostic pathway has been added. It also reviews variants of uncertain significance in the DMD gene which have become more common with the advent of next generation sequencing and population screening (expanded carrier screening).*
- *Forward-looking statements have been added regarding newborn screening for Duchenne given that a Recommended Uniform Screening Panel (RUSP) nomination has been submitted in 2022 and implementation would mean diagnosing and managing infants in a presymptomatic stage.*

II. Classic Duchenne Muscular Dystrophy

A. 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.[19,20] The first three of these criteria remain the core clinical features of classic DMD (Figure 1), 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.[21,22] 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.[23,24] 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.[24] 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 (see also the Cardiac 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.[25] 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.

B. 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 1: Diagnostic algorithm for DMD and BMD).

C. 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.

D. 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. 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).[26] A 2019 workshop report summarizes current and emerging technologies to quantify dystrophin expression.[27] 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[28,29] 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).[29] 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.

III. Other Allelic Variants of Dystrophinopathy

A. Becker muscular dystrophy (BMD)

The diagnostic criteria for BMD are represented in Figure 1. 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[26] and after 19 years with chronic steroid use.[24] 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.

B. 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.[26] 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.

C. 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.

D. X-linked cardiomyopathy

This is uncommon and will not be discussed further.

E. Exercise-induced myalgias with myoglobinuria

This is uncommon and will not be discussed further.

F. 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.

G. 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.

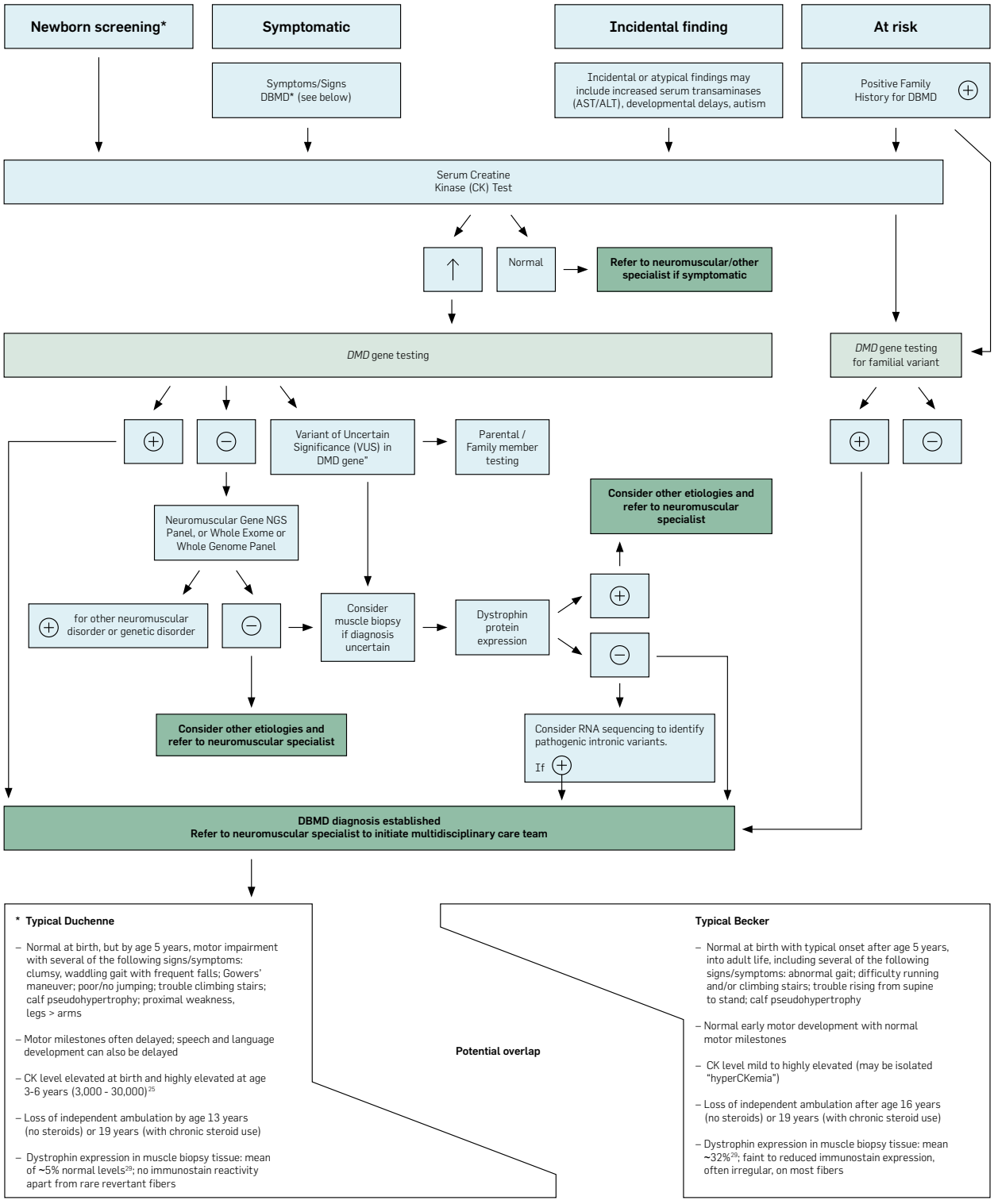
IV. The Presymptomatic Patient

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 formal nomination to add DMD to the Recommended Uniform Screening Panel (RUSP) was submitted in 2022. 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.[30] The carrier rate of approximately 57%-61% is higher than the reported family history as some of these mothers silently carry the pathogenic variant.[31] 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.[32] 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.[33] Figure 1 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 1: Dystrophinopathy Diagnostic Algorithm



* **Newborn Screening (NBS)** –CK-MM is the first tier test and if elevated may include a repeat CK and/or confirmatory *DMD* gene testing or neuromuscular gene panel. NBS is evolving in the United States and will take several years before it is implemented in every state.

THE CURRENT UNDERSTANDING OF THE NATURAL HISTORY OF DUCHENNE MUSCULAR DYSTROPHY

I. Introductory Comments

In the *Draft Guidance for Industry on Common Issues in Drug Development for Rare Diseases*, FDA advises 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."[34] In the case of DMD, over the past two decades, the understanding of natural history has improved dramatically as a consequence of patient registries, natural history studies, and data drawn from the placebo arms of industry trials that corresponds closely to the natural history data.[35-37] There have also been a number unsuccessful clinical trials (where the pre-specified analysis did not demonstrate the benefit of the experimental treatment) that can provide lessons to help sponsors select better, clinically meaningful outcome measures and guide the selection of participants, who, in the absence of treatment, would be most likely to show meaningful disease progression using the endpoints assessed over the course of the study—a process of prognostic enrichment.

Key considerations in this section:

- *This section provides an updated overview of Duchenne muscular dystrophy (DMD) natural history concepts, with a new schematic (still in development) that will show the typical progression of DMD. This schematic uses violin plots with the median and range of timing when milestones, (eg, loss of ambulation, loss of standing ability from the floor, etc.,) occur, based on data from large natural history cohorts, as well as some of the key outcome measures used to monitor disease progression across the different stages of disease.*
- *New models of progression such as the HERCULES Model and the UC Davis Model link events and outcome measures to add granularity to characterization of disease stage and trajectory. One important aspect of these models is the identification of a brief transitional stage beginning during late ambulation where individuals are able to either independently stand or stand with assistance and transfer their own weight.*
- *There is now an increased body of natural history data that can better characterize an individual's disease course and the sources of heterogeneity that sponsors can account for in clinical trials.*

While understanding of the natural history and the sources of heterogeneity in the progression of disease has evolved, the unmet medical need and urgency for improved therapies for DMD is still profound, despite improvements in the standard of care, and recent FDA accelerated approvals of novel treatments that may have a modest effect on the disease for certain patients. Progressive quadriparesis during the first two decades due to dystrophin deficiency and skeletal muscle fiber loss remains the common disease course, with concomitant development of pulmonary insufficiency from skeletal muscle involvement and cardiomyopathy leading to substantially shortened lifespans even among patients receiving optimal care.

Note that while most of this section is focused on DMD, later in the narrative we include natural history information for sponsors interested in conducting research into treatments for BMD and other dystrophinopathies.

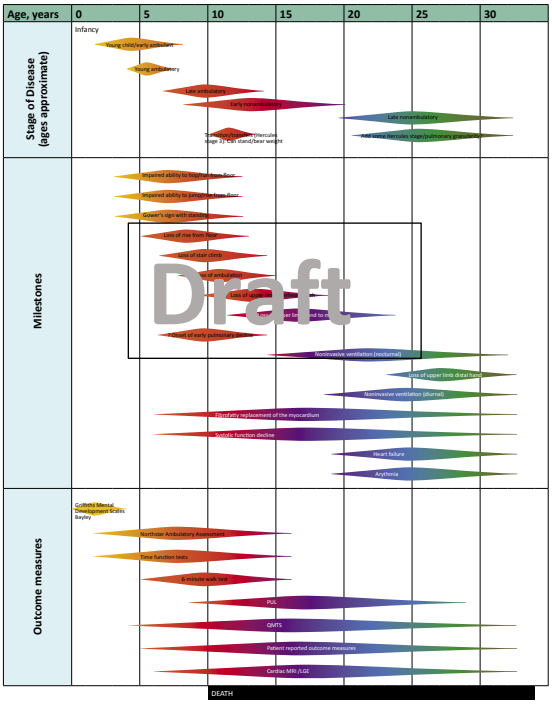
II. Overview of Natural History in Duchenne Muscular Dystrophy

Sponsors should be aware that from infancy on, the course of DMD can be monitored with a variety of testing and evaluation tools that measure developmental delay, functional loss, and other parameters of progression of DMD. The use of milestones of disease progression and outcome measures in natural history studies and patient registries have helped to better characterize the natural history of the disease (see Figure 1).

The following schematic present a general overview of the approximate ages, with violin plots illustrating the medians and interquartile ranges of the stages of disease progression, loosely based on clinical evidence since the widespread use of corticosteroids in the clinical management of DMD. As the schematic illustrates, the loss or delay of clinical milestones is a hallmark of disease progression in DMD. Some milestones such as hopping and jumping may never be achieved in most persons without corticosteroid treatment. Difficulty performing functions and the loss of milestones occur in a predictable sequential order both before and after the loss of ambulation (although there may be some overlap or slight variation in some of the milestones). The timing of the loss of milestones can also be linked with the timing of subsequent functional deterioration later in the disease course.

The schematic also includes the outcome measures where there are more data on their utility in DMD and that are most widely used to characterize disease progression at different stages of disease.[38] Note that the schematic does not address whether specific thresholds or outcomes on those measures are clinically meaningful or useful as potential clinical endpoints. Further details on specific outcome measures, how they relate to (may be predictive of) milestones of disease and their inclusion in clinical intervention trials is discussed in Considerations for DMD Outcome Measurement Selection (page 42) and Specific Trial Design and Analysis Issues for Clinical Trials in DMD (page 72).

Figure 1: Stages of DMD disease progression, tracked by milestones and monitored by outcome measures and clinical endpoints [Still under development, to be shared in an addendum to docket.]



A. The stages of DMD disease progression

Models of disease progression

A number of models of disease progression have been used in clinical management which may help inform clinical development programs. In the DMD Care Considerations (DCC), the course of DMD was divided into five general stages: an early symptomatic phase when most young children are diagnosed, an early and a late ambulatory stage, and early and a late nonambulatory stage. Another 5-stage system, the Ambulatory Functional Classification System for DMD (AFCSD)[39] comprises 5 levels, defined as follows: level 1, walking at normal speed and with normal postural alignment; level 2, walking independently without an assistive device or brace, with evidence of abnormal gait patterns, such as tip-toeing or waddling, and with impaired postural alignment, such as excessive trunk lordosis; level 3, walking across only short distances, using a hand-held mobility device, such as a walker or crutch; level 4, inability to walk and use of a powered wheelchair; and level 5, need for transportation in a manual wheelchair. Other models, such the UC Davis Duchenne Functional Milestones for measuring disease progression[40] and the HERCULES model (Figure 2), add granularity with regards to clinically meaningful transitions and outcome measures of interest within ambulatory and nonambulatory stages, noting outcomes that are important to patients and caregiver, such as those related to the ability to stand supported and transfer weight and bringing hand to mouth. For instance, the UC Davis DMD Disease Progression Model groups DMD patient into 5 ambulatory stages by performance of timed function tests (TFTs) that are predictive of the loss of ambulation, and four nonambulatory groups based upon upper limb performance on the Brooke scale (Figure 3 below) that can be linked to the patient's self-care and independence. The HERCULES model also includes four nonambulatory stages after loss of ability to stand independently. The HERCULES model assesses hand-to-mouth-function (HTMF) together with pulmonary outcome measures, which are markers of need for noninvasive ventilation and continuous daytime ventilation, as per the DCC, although the relationships between some of the parameters have yet to be validated.[41]

Figure 2: The HERCULES Model[42]

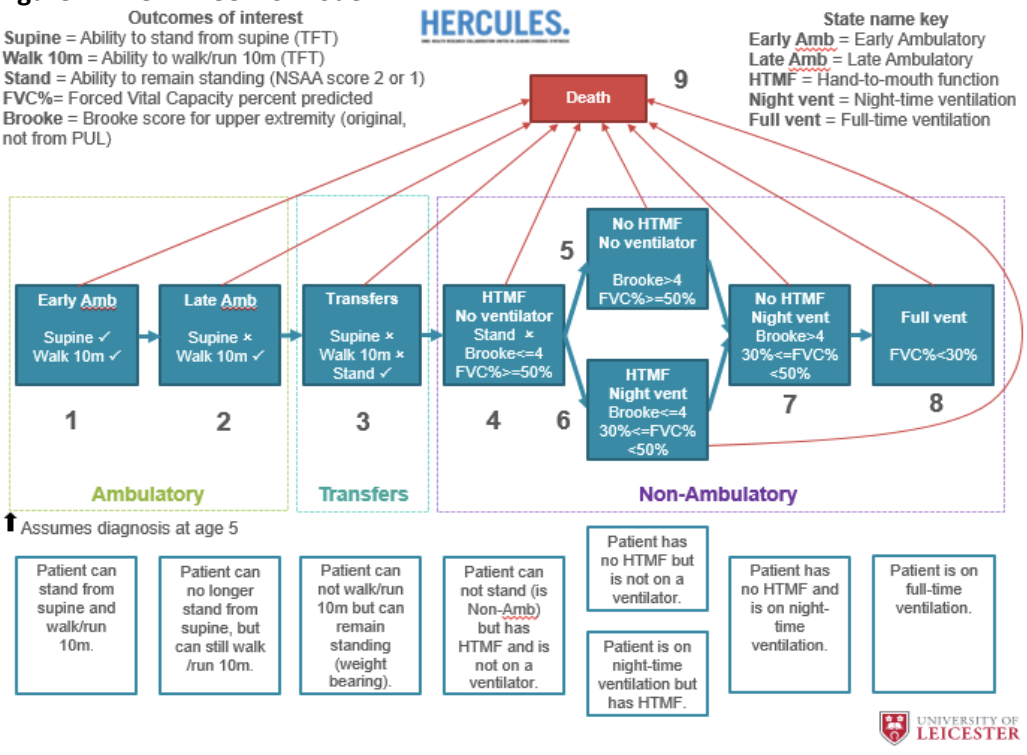


Figure 3 Performance of the Upper Limb (PUL) (Entry Item)

		Target Population				
0	1	2	3	4	5	6
						
No useful function of hands.	Can use hands to hold pen or pick up a coin or drive a powered Chair	Can raise 1 or 2 hands to mouth but cannot raise a cup with a 200g weight in it to mouth	Can raise standardized plastic cup with 200g weight in it to mough using both hands if necessary	Can raise both arms to shoulder height simultaneously w/or w/o compensation	Can raise both arms simultaneously above head only by flexing the elbow	Full overhead reach without compensation
6	5	4	3		2	1
Brooke Score						

Box 1: UC Davis Duchenne Functional Milestone Model for measuring disease progression[40]

- 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 generally of disease progression.[43] A threshold value of approximately 5 s differentiates between patients who are likely to show stability or improvement versus those who are likely to decline.[44]
- Milestone group 2: time to stand from supine ≥5 and <10 s
  - Patients with stand from supine values of 5 s 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.[45]
- Milestone group 3: time to stand from supine ≥10 s
  - Patients with stand from supine values of 10 s or longer are at risk for losing ambulation during the next 2 years.[46]
- Milestone group 4: cannot stand from supine in <30 s but can still climb four stairs
  - Patients with loss of stand from supine are at risk of losing ambulation over the next 2 years.[46]
- 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.[46]
- 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.[47]
- 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)

While loosely based on stages across the spectrum of disease described in the DCC series, the following overview of the current natural history of DMD factors in some of the insights from the UC Davis and HERCULES models. While specific functional changes are observed at different approximate ages, the intent is not to create artificial stages of disease but to portray typical disease progression.

DMD is due to generalized skeletal muscle involvement and cardiomyopathy. The pathological processes involved in DMD are underway concurrently over the course of a patient's lifetime. Though loss of ambulatory capacity and gross motor functions may be a primary focus initially in ambulatory boys, neuromuscular deterioration may already be measurable in the upper limb, and in respiratory and cardiac function.

DMD progression is characterized by compensations for weakness and changes in quality and ease of movement; however, strength and functional decline (strength and function) are not linearly related. Changes in strength may occur with little change in function, but there are also situations where a very small change in strength may move an individual with DMD rapidly beyond a threshold of clinically meaningful disease progression, and they may lose significant function quite precipitously.

Some patients may never reach some of the early developmental milestones such as hopping, jumping and running, and a large proportion may only reach them transiently at a later age of acquisition. There is heterogeneity in disease progression in terms of rate of disease progression in individual patients. In general, though, the disease progresses in 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 of 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).[48] Nevertheless, some patients' progression may not fit neatly into the following stages of disease progression descriptions.

Neonates/infancy (until around 1 year of age):
Clinical descriptions of DMD often omit neonates and infants, partly because the diagnosis of DMD in infancy has been uncommon, although the adoption of newborn screening may change this in the next few years (see page 25). Although this stage is referred to as presymptomatic, the disease is manifested at birth. Most infants diagnosed due to newborn screening or family history will show delayed development if evaluated with tools such as the Griffiths Mental Development Scales, an outcome measure than can be used in the very young (ages 6-47 months)[49] and the Bayley-III, which is administered from 1-42 months of age.[50,51] There may be neurocognitive deficits as measured by Developmental Quotients (DQ) that may be greater among boys with certain DMD mutations, though performance on the locomotor subscale is poor regardless of mutation.[52]

Early symptomatic childhood (ages 12-48 months):
The first few years after infancy are characterized by delays in reaching developmental milestones and impaired acquisition of motor skills. The development of gross motor milestones is typically slower than in boys without DMD, and some children may show signs of delayed language and of cognitive impairment. Toddlers and young children evaluated with developmental outcome measures such as the Bayley-III and Griffith's Developmental Scales have gross motor scores that are lower than age-matched controls.[50,51] Repeated measurements over time may reveal further declines in gross motor scores relative to typically developing children. Cognitive and language scores are also lower compared with typically developing children. As these delays and deficiencies become noticeable to parents and

caregivers, this is the stage at which the diagnostic odyssey typically begins (see diagnosis chapter). Nevertheless, young boys with DMD are growing and will belatedly reach most developmental milestones, and although performance is poorer than age-matched controls, their motor skills improve over the next few years.

Key milestones at this stage:

- Delays or failure to meet developmental milestone and achieve certain skills (note, these could be indicators of disease trajectory)
- Impaired ability to jump, hop, run, and rise from the floor
- Gower's sign with standing

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

During this period, there may be slow gains in some measures but modest functional decline in others. Any gains in ambulatory function are slower than observed in typically developing children and often outside normative ranges very early in the disease course (on 100-meter timed test, 6MWT, and 10-meter walk/run tests). Either gains or losses in functions may be noted on endpoints such as the North Star Ambulatory Assessment (NSAA). In addition, corticosteroid therapy, which is typically initiated during this period (not long after diagnosis), may lead to further gains or stabilization of functional measures for approximately 6 to 12 months, followed by a plateau and then a decline with decrement from baseline values at the time of steroid initiation by 30 to 36 months. However, even while performance improves with growth and maturation, physiologic deterioration is ongoing, and boys are increasingly falling behind normative performance levels of their normally functioning peer group.

Key milestones during this stage:

- Gains in unilateral hopping, jumping, standing on heels, and single leg standing (may occur particularly if steroids have been initiated)
- At the same time, compared to baseline function, young ambulatory boys may show temporary improvements of certain skills and increased velocity of stand from supine, 4-stair climb and 10-meter run/walk, due to maturation and growth.
- Loss of standing from the floor (eg, stand from supine) in those with most severe trajectory
- Loss of transition from lying supine to sit

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

This stage is characterized by a rapid decline in gross motor functions, upper limb function, and some pulmonary function parameters in the early second decade. There is marked progressive loss of muscle fiber in the proximal muscles, with growing weakness and the gradual loss of gross motor skills and ambulatory functions (including standing ability, stair climbing, and the ability to walk). Skeletal deformities may develop (ankle equinus contractures and iliotibial band contractures are the most common), and there is a risk of osteopenia and fractures. There is also a loss in height and increased weight gain in comparison with their normally functioning peer group. Sponsors should also be aware that during this period, individuals with more stable disease (in terms of stable functional performance) can be differentiated from those likely to experience gradual or rapid progression—as noted by the UC Davis model—based on scores or performance on certain prognostic outcome measures such as timed function tests, 6MWT, and NSAA.[40] For instance, a time to stand from supine of less than 5 seconds can differentiate between patients who are likely to show stability or improvement versus those who are likely to experience the onset of functional decline, greater than 10 second stand from supine predicts loss of standing ability, and greater than 10 second 10-meter run/walk predicts both loss of ambulation

within 2 years and onset of loss of upper limb function (see Box 1, UC Davis Duchenne Functional Milestones model below).

Key milestones during this stage:

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

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

As late ambulatory DMD children begin to experience a rapid decline in ambulatory endpoints, with the early onset of pulmonary decline and a slight loss of upper limb skills, there may be a brief transitional phase between or overlapping the late ambulatory and early nonambulatory stages. While the age range during which the phase may occur is wide, the period itself may be quite brief, on the order of months. As defined by the HERCULES model, this stage includes individuals with DMD who can no longer walk or run 10 meters but, critically, can still stand or transfer bearing their own, or most of their own, weight. At some point, patients can only stand briefly with assistance. While the HERCULES model (see Figure 2) defines this function as 100% weight bearing (the ability to stand independently), being able to stand with assistance and partially support one's weight is important to patients and caregivers due to the burden of unassisted transfers.[53]

Key milestones during this stage:

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

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

The age at transition to nonambulatory status is typically delayed by 3 to 3.5 years if daily corticosteroids are used.[40] Nonambulatory status has often been defined as the inability to walk 10 meters within 30 seconds. After boys can no longer walk, they require powered mobility. They also experience continued muscular deterioration throughout their upper and lower limbs. Skeletal deformities such as limb contractures can form due to static positioning of weak limbs (where the joint cannot be actively moved through the full range of motion against gravity) with significant fibrotic and fatty infiltration of muscle tissue and sarcomere shortening. Postural maintenance and sitting balance are initially intact but progressively lost. In patients not treated with steroids, spine deformity commonly becomes problematic. There is increasing loss of upper limb function (with decreasing ability to reach overhead, dress independently, and bring hand to mouth for feeding). Both the UC Davis Disease Progression Milestones and HERCULES model add granularity to this stage by distinguishing between those patients with progressive losses of upper limb function on the Brooke Scale or Performance of Upper Limb (PUL) entry items, and in the case of the HERCULES model, measures of pulmonary function predictive of nocturnal noninvasive ventilation and continuous daytime ventilation. The UC Davis Disease Progression model (see box 1) differentiates between those with a Brooke scale of 1 (PUL entry of 6), who, are at risk of losing full overhead reach, versus 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 of nonambulatory patients and the second most important noted by nonambulatory patients themselves.[54] Retention of HTMF is also an important criteria for the HERCULES staging system, which also proposes

distinctions based on FVC % predicted values associated with recommended nocturnal noninvasive ventilation (<50% FVC) and continuous day and night diurnal ventilation (< 30% FVC), values which are part of the DMD Care Considerations that are yet to be validated clinically. Over the course of the early nonambulatory stage, there is certainly continued decline in pulmonary function leading to the need for mechanical cough assistance (initially) and progressive risk of hypoventilation requiring noninvasive mechanical ventilation during night and subsequently both day and night. Shifting trunk position independently while seated is also an important function to patients for quality of life. Cardiomyopathy is evident by cardiac MRI in virtually all patients and in some patients by cardiac echo. 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, as measured by the Brooke scale or PUL entry criteria (see Figure 3)

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

Pulmonary function

- Loss of normal ability to cough/clear airway (need for mechanical cough assistance device)
- Need for nocturnal ventilatory assistance (with bilevel noninvasive ventilation device)

Late nonambulatory (*onset *15 to late third decade):
A key milestone that delineates the onset of the late ambulatory stage is the loss of unweighted HTMF. Almost all patients who have lost HTMF have FVC % predicted values below 50%.[55] In patients with cognitive challenges preventing the completion of pulmonary function testing, loss of HTMF is a proxy for a 50% threshold of FVC predicting need for noninvasive mechanical ventilation. The median age at loss of HTMF in DMD patients not treated with steroids was 15.4 years, whereas median age at loss of HTMF was 20.5 years in steroid-treated patients.[21] At this stage, core strength and upper limb strength weaken, making function and maintenance of good posture increasingly difficult. Individuals will require postural support of the trunk and head support from a seating system as well as power recline. Unweighted HTMF has been lost (Figure 2 and Box 1), and upper extremity function is severely limited to distal fine motor function and tabletop activities. According to a patient and caregiver survey, repositioning oneself in bed was the most important function for quality of life according to nonambulatory patients.[54] Controlling a wheelchair joystick and independent computer access are other functions that are critical quality-of-life concerns. Due to losses in chest wall and expiratory muscle function, virtually all patients require mechanical cough assistance and there is a high risk of nocturnal and daytime hypoventilation requiring noninvasive ventilation due to worsening diaphragm muscle function. There is risk for dysphagia and aspiration. Optimal nutritional management may require gastrostomy tube placement and enteral formula supplementation. Adequate phonation may become an issue late in the disease course. Older DMD patients have many unmet medical needs. Respiratory impairment and cardiomyopathy (heart failure and conduction abnormalities) are causes of morbidity and, eventually, mortality. It should be noted that adults with DMD are increasingly living into the late third to fourth decade with optimal care and we are gathering new experience with regard to their unique needs.

Key milestones during this stage:
Upper-limb function

- Loss of ability to place hands to tabletop
- Loss of trunk positioning in bed (loss of the ability to reposition oneself independently in bed)
- Loss of ability to use a computer/control wheelchair (distal hand function)
- Loss of ability to use 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. There is discordance between cardiac disease progression and skeletal muscle disease progression.[54]

In earlier guidance, the need for mechanical cough assistance and part or full-time ventilatory assistance as outlined in the DCC was based on peak cough flow (PCF) and forced vital capacity (FVC) thresholds (eg, FVC < 50% predicted, FVC < 30% predicted) that lacked precision.[56] Pulmonologists are increasingly defining true hypoventilation in individuals with DMD with other measures that include data from sleep studies (polysomnography) and in-home measures of hypoventilation (capnography) (see Outcome Measures section for more details).

Cardiac deterioration due to progressive cardiomyopathy may not be correlated with skeletal muscle deterioration.[44] With increased lifespan due to effective ventilation interventions, cardiomyopathy has become the leading cause of death among patients with DMD.[46,57] While symptoms are difficult to assess in nonambulatory patients who perform little physical activity, cardiac natural history is increasingly well defined by serial cardiac imaging, especially as MRI has become more widespread. Cardiac MRI is able to visualize fibrofatty replacement of the myocardium in the form of late gadolinium enhancement (LGE) and also provide reproducible measurement of cardiac dimensions and systolic function. Fibrofatty replacement of the myocardium is typically the earliest clinical 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 typically present in later stages of disease as fibrofatty replacement of the myocardium progresses and as systolic dysfunction develops. While arrhythmia burden generally parallels fibrofatty replacement of the myocardium and systolic function, there does appear to be a risk of atrial ectopy and arrhythmia, including atrial fibrillation, even in patients with preserved systolic function. Furthermore, 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 on page 80).

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.
- Progressive fibrofatty replacement of the myocardium is associated with worsening systolic function and culminates in the development of heart failure.

- Arrhythmias, including life threatening arrhythmias, develop as cardiomyopathy progresses.

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

Age-matched boys with DMD can progress at markedly different rates, which has created challenges for the interpretation of therapeutic trials. However, with more data from natural history studies, real world data, and the placebo arms from DMD treatment studies, the reasons for variability in outcomes have become clearer. Increasingly, sponsors can 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 example, baseline levels of function predict subsequent disease progression in DMD. Higher baseline function or stabilization of baseline function over the short-term, lead-in period of observation is usually associated with slower long-term decline.[36,58] Lower baseline function may be associated with rapid subsequent decline in ambulatory endpoints when patients have passed critical thresholds of strength and function. Among ambulatory boys, the course to loss of ambulation may be composed of distinct trajectory classes.[59] Sponsors should thus be aware that baseline measures of ambulatory capacity or other functional capacity 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 change in an endpoint over time.

The age at loss of clinically meaningful milestones (a proxy for disease severity) also predicts the age at loss of future milestones. For example, the age at loss of ambulation predicts the age at which subsequent loss of upper limb functions occurs and the age at which critical pulmonary milestones are reached.[60] 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.[40,58]

On the basis of such findings—using rigorous quantitative analysis of natural history, real-world data, and clinical trial data—prognostic models have been developed that better characterize disease progression and account for the heterogeneity of natural history progression that underpins many of the challenges in DMD drug development.[61] 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 could ideally include biomarkers such as quantitative dystrophin levels and skeletal muscle fat fraction as measured by MRI. See sections below for more on how these findings and models can help in the selection of optimal outcome measures, trial duration, and inclusion and exclusion criteria for enriched cohort selection and can all contribute to improved trial design.

Sponsors would be advised to avoid an imbalance in the ages of study participants, which can introduce substantial variability into a trial. It is critical that sponsors match control and treatment arms in clinical trials by both age and functional status. Sponsors are also advised to also take into account baseline functional performance—in relation to specific endpoints such as 6MWT, timed function tests, or NSAA—as baseline measures of key outcome measures will have an impact on the subsequent rate of progression over time.

Genetic predictors of disease progression

As noted in the diagnosis section, maintenance of the *DMD* gene transcriptional reading frame typically predicts dystrophinopathy phenotype: Frameshift mutations are predictive of a DMD phenotype, and in-frame mutations are predictive of milder BMD.[62] With some exceptions, this generally relates to the amount of residual dystrophin in muscle, with less severe phenotypes expressing more dystrophin in muscle. 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) may be associated with an altered 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.[63] 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.[63,64] With larger cohorts or longer follow-up, differences between subgroups may become 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).[65]

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 sources of heterogeneity listed here.

It is also worth noting that some mutations appear to cause more dystrophin-related abnormalities in nonskeletal muscle causing more pulmonary, cardiac, and neurocognitive impairment.[66,67] Participants with *DMD* mutations may be grouped by those only involving the region upstream of intron 44, considered Dp427 negative and Dp140/Dp71 positive (Group 1); those with *DMD* mutations involving 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 2); and participants with *DMD* mutations involving exon 63 and/or the region downstream of exon 63 were considered Dp427/Dp140/Dp71 negative (Group 3). Fifteen percent (15%) of DMD boys lacking only Dp427 had intellectual disability, compared with 25% of boys lacking Dp427 and Dp140 and 64% of boys lacking Dp427, Dp140 and Dp71.[68] Boys with DMD 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) in those lacking Dp140 and Dp71, with a clear cumulative effect of loss of isoforms.[69] 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 considerable, often exceeding the minimally clinically important difference (MCID) of approximately three points.[69-71] CNS manifestations such as autism, associated with certain brain isoforms of dystrophin, can also affect the acquisition of motor skills and motor milestones, and consequently can affect the measurement of milestones and participation in clinical trials.

Clinical trials of treatments that are not mutation-specific should collect appropriate samples for full genetic analysis. As noted in the Diagnostics chapter, some trial participants may need to be rescreened with a technique that provides a complete analysis of the *DMD* gene (see Diagnostics chapter).

Genetic modifiers

Genetic screening has identified polymorphisms in other genes that may have altered aspects of the response of muscle to dystrophin deficiency and/or drug treatment (eg, glucocorticoids). These genetic

modifiers may modify the onset, severity in terms of trajectory, or drug responsiveness of DMD patients and are instructive regarding key biochemical pathways involved in muscle damage, repair, or response to steroids. They are also important in increasing understanding of factors responsible for patient-to-patient variability and could eventually prove helpful in interpreting clinical trial data. Genetic modifier studies, as with most genetic-association studies in any human trait, typically require large numbers of patients studied using reliable and sensitive biochemical and/or clinical outcome measures. Differences in methods of characterizing or categorizing cohorts of patients, as well as ethnic differences in polymorphism allele frequencies, can lead to challenges in statistical analyses and reproducibility of genetic association studies. To date, at least six potential genetic modifiers have been identified:

- *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 roughly equivalent to the effect of steroid treatment, prolonging ambulation by as much as 2 years.[72]
- *Secreted phosphoprotein 1 (SPP1 or osteopontin) polymorphisms:* In the case of osteopontin, the genetic modifier may actually be modifying a patient's responses to corticosteroid management rather than affecting the disease itself directly.[73]
- *CD40 Exon Variants in the NF-κB and TGFβ Pathways:* The minor allele at rs1883832, in the 5'-untranslated region of CD40, has been associated with earlier LOA. This allele diminishes the expression of CD40, a co-stimulatory molecule for T cell polarization.[74]
- *TCTEX1D1 gene polymorphisms:* Variants in the TCTEX1 domain-containing 1 (TCTEX1D1) gene on chromosome 1 have been associated with age of ambulation loss. The minor alleles of two independent variants, known to affect TCTEX1D1 coding sequence and induce skipping of its exon 4, have been associated with earlier LOA.[75]
- *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 and 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.[76]
- *ß$_2$ adrenergic (ADRB2) receptor polymorphisms:* Patients with DMD expressing the Gly16 polymorphism have demonstrated systematically lower FVC% p values at any given age compared with patients expressing the Arg16 polymorphism.[77]

There may be other genetic modifiers yet to be identified. Sponsors should review the most current data on the subject to see whether screening for these genetic modifiers in their clinical trials is advisable for stratification or predefined sensitivity analyses to explain potential causes of variation in the outcomes of patients.

Differences in management that can affect the course of DMD

The standard of care received by patients with DMD may have significant implications on the design of trials in the population, depending upon the outcomes being 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 has largely been due to the use of glucocorticoids, management of spine deformity, pulmonary management, and cardiac management.

Steroid therapy: Corticosteroids delay the loss of ambulatory milestones, prolonging ambulation by about 3 to 3.5 years over time, and delay losses in upper-limb functioning so that young men can continue to raise their hand to their mouths and feed themselves until a later age.[40,60] Daily steroid treatment with prednisone or deflazacort is more effective than intermittent prednisone alternating 10 days on and 10 days off.[78] Steroids also affect pulmonary function—young men treated with steroids reach an older age before requiring mechanical cough assistance or noninvasive ventilation as defined by FVC parameters outlined in the DMD care considerations.[55,56] In addition, early treatment benefits often extrapolate to later stages of disease.[40] However, differences in patterns of steroid use—including whether the patient is on daily versus intermittent regimens, dosage, and time on treatment—may have variable effects on clinical progression and function.[79,80] The steroid used (prednisone/prednisolone versus deflazacort) may also affect outcomes, with deflazacort use associated with less functional decline in patients older than 8 years of age who are in the decline phase of the disease.[81-83] Since medical management of DMD with corticosteroids tends to be individualized, differences in efficacy and side effects between the steroid compounds and regimens may also result in differences in dose titration by clinicians or in patient/caregiver adherence.

Enrollment in the trials should either be restricted or stratified according to harmonized corticosteroid therapy. In trials of young patients 3 years of age and younger, there has been optional steroid management as an inclusionary factor. In patients aged 4 years and older, historically, 6 months of stable corticosteroid therapy regimen has been used as inclusion criteria. However, sponsors of clinical trials 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 not preclude allowing weight-based dose adjustments when needed.

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

Alternatives to glucocorticoid foundational standard-of-care therapy have been under evaluation in clinical trials.[84,85] Other agents being studied as adjunctive therapies to glucocorticoids may soon add to the underlying standard of care.

Other interventions: Other therapies such as night splinting, physical therapy, exercise, and the other standard-of-care interventions recommended by the DCC may affect functional performance.[86] For instance, the occurrence of contractures can also impact mobility and upper limb function, and approaches to prevent and manage contractures have been outlined in the DCC.[56] However, the efficacy of these approaches has yet to be clearly established in a well-powered study.[87]

There are even fewer data to characterize the effects of exercise, mobility approaches, nutrition, psychological and/or psychiatric care, noninvasive ventilation, or swallowing therapy on DMD progression; although one trial showed a benefit of recumbent cycle-assisted aerobic exercise in late ambulatory and early nonambulatory DMD.[88]

There are data, however, showing that some critical standard-of-care treatments may affect survival. For instance, spine deformity management, namely, timely spine surgery for curves > 30-40 degrees, has impacted survival.[89] Survival has been most impacted by pulmonary management.[90] Two recent studies have reported that lifespans in DMD can be lengthened substantially due to the implementation of noninvasive ventilation.[57,91] Consequently, a larger number of young men with DMD are living into their

20s and 30s, but often with significant disability. Glucocorticoid usage has also been associated with improved overall survival.[40]

Cardiac management in DMD, which includes prevention of progressive ventricular dysfunction with 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 had a positive effect on survival in young men with DMD-associated clinical cardiomyopathy, by reducing stress on the heart (afterload reduction)[92](see Cardiac section).

Standards of care should be observed at every center that is involved in these studies. Sponsors are advised to record any concurrent complementary therapy that study participants may be using. Some studies are attempting to monitor the family's adherence to physiotherapy, home stretching and splinting, in an effort to capture these variables for possible post-hoc analyses. Ideally, sponsors should control for as many of these factors as possible to reduce potential variability in disease course among participants in their clinical trials.

Finally, the financial costs of care can limit access to standard of care, and as boys/men with DMD progress, this financial burden increases.[93] Insurance and other third-party payers may cover some treatments and types of equipment but not others. In addition, there may be differences in lifelong care patterns that can accompany lower socioeconomic status. Sponsors may therefore consider how to provide support to participants from these communities, including the provision of access to standard-of-care therapies to maintain a consistently high standard of care among all trial participants.

C. Natural history across the spectrum of dystrophinopathy

While most DMD patients treated with steroids lose ambulation by the age of 16 years, there are other individuals with milder dystrophinopathy, including intermediate DMD, BMD, and women with dystrophin mutations who manifest symptoms.

BMD generally has later onset of symptoms and slower progression. BMD is characterized by wide interpatient variability in severity, with some patients having a clinical course similar to that observed for DMD, while other patients may develop mobility impairment and a myopathic gait in the late teens to early 20s, and another subset remain nearly, or in some cases completely, asymptomatic (with complaints consisting of mild muscle cramps and no overt weakness). The birth prevalence of BMD is about 1 in 20,000 males. The overall prevalence of BMD in proportion to all dystrophinopathy is likely greater than 25% of all dystrophinopathy due to the increased survival. If the cardiac effects of the disease are not present, the median age of death, worldwide can approach that of a natural lifespan, but there is great heterogeneity—with death from myocarditis sometimes seen in the teenage years. Finally, some female carriers of dystrophin mutations experience muscle degeneration similar to that in males with BMD (see below for more on clinical trials issues in BMD and other dystrophinopathies).

Ongoing natural history study needs

Given the relentless course of DMD and the challenges in conducting adequately powered studies in a rare disease, there is a need to establish adequate, 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. To be useful for natural history controls to augment placebo comparisons, the collection of natural history data must be of a certain rigor to satisfy FDA requirements.

Sponsors should refer to 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)

CONSIDERATIONS FOR OUTCOME MEASUREMENT SELECTION

I. 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, monitor clinical progression over time, and guide clinical care decision-making. These outcome measures can also be used in clinical trials in a number of ways. For instance, there are now data to support the use of certain outcome measures to identify and enrich for specific populations of participants at risk of progression during the course of DMD trials. Use of these outcome measures during participant screening and for stratification could reduce the risk of conducting an underpowered study that is unable to reach a clear conclusion about the effectiveness of a potential therapy.

Key Considerations in this section

- *This section describes outcome measure selection for staging disease, stratifying cohorts and for monitoring disease progression.*
- *Certain outcome measures can be used to identify specific populations of participants at risk of progression during the course of a DMD trial. Use of these outcome measures during participant screening and for stratification could reduce the risk of underpowering a study and not reaching a conclusive answer regarding the effectiveness of a potential therapy. It is possible for a study to have broad inclusion criteria, but, with stratification, enrich a group that the studies primary prespecified analysis is based on. There are more data now showing specific changes in outcome measures that may be clinically meaningful to patients and families, at different stages of disease. Performance measured by some tools are predictive of progression to disease milestones and thus may be useful as intermediate clinical endpoints.*
- *In addition to developmental and motor measures, the section reviews the use of pulmonary outcome measures, upper limb function measures, and activities monitored by digital technologies and wearable devices that can track the course of progression during the transitional through loss of ambulation and through the nonambulatory stages of DMD.*

Sponsors can also use these tools to measure the effect of treatment, selecting endpoints based on function in a variety of ways, including 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]; time required to perform the activity or activities) or as time-to-event for decline or loss of an ability. With a growing body of data showing that specific changes or thresholds on an outcome measure are predictive of the time to clinically meaningful events, or disease milestones, it may be possible to use these outcomes as intermediate clinical endpoints in a trial. This option may be preferable to using the timing of functional ability loss as the trial's primary endpoint. For young children in whom abilities are still developing, it may be appropriate to assess time to events in the positive sense (ie, the time to gain an activity or time to reach a certain developmental milestone).

Although existing outcome measures developed for clinical trials and/or clinical care in dystrophinopathies or related conditions may be appropriate for defining an endpoint in a clinical trial, FDA will also consider proposals for the use of novel outcome measures that are capable of measuring clinically meaningful effects in patients. FDA encourages sponsors to propose and, if necessary, develop new tools to measure primary and secondary endpoints that can validly and reliably assess patients with a wide spectrum of symptoms and disease stages. Sponsors should engage FDA early during the selection and/or development of efficacy endpoints.

There are outcome measures to monitor changes in clinically meaningful abilities in a number of functional domains that merit consideration. The sponsor should include an assessment of multiple efficacy endpoints, when feasible, to characterize the breadth of effects of novel therapies on dystrophin-related pathologies, including skeletal, respiratory, and cardiac muscle function, even if only one of these measurements is the primary endpoint. For instance, upper limb function as well as pulmonary and cardiac status can be measured before loss of ambulation, even in younger patients, depending on other inclusion criteria and the expected duration of the trial. It may also be useful to measure cardiac- or pulmonary-specific changes to demonstrate long-term benefit.

Efficacy endpoints that can measure change of function over a wide range of types and severity of deficits 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.

II. 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. More information is provided on each measure within the catalog of outcome measures in the appendix.

A. 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 III is a standardized functional measure assessing five domains of infant and young child development (cognitive, language, motor, adaptive, and social-emotional).[50-52] 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.[41] 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).[51,52]

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.

B. 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.[94,95] 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.[41,79,96-100] 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).[70]

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.[79] 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.[79] 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,[101-103] including recently, in a major trial as a primary endpoint.[78] Loss of the ability to stand has been shown to be predictive of time to LOA and time to 10% decline in ambulatory function.[104] Time to stand can be obtained reliably in younger DMD subjects[105] 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.[36] 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.[48,101-103] 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. 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.[101-103] It is easily obtained in the clinic and reliable in younger children.[105] 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.[104,106]

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).[35,104,107-109] 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.[110] In addition, recent modeling studies suggest ways to improve the 6MWT's predictive value.[111]

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.[112] 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.[113] 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

Myometry provides 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.[106] MMT appears less sensitive and reliable in comparison with quantitative muscle testing (QMT).[105] 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.[104,105] 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*).[114]

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.[36] 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.[115] 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.

More details on these and other measures listed in Box 2 that have been used to characterize the natural history of DMD and which could be used to monitor the effects of treatment in their development programs are included in the Catalog of Outcome Measures Appendix.

Box 2: Motor measures by stage of disease (see Catalog of Outcome Measures [*Under development: to be added in addendum*] for comments about clinical meaningfulness and specific considerations more on the use of each measure*)*

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)
- 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.

C. 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. In contrast to cardiac function measures, many of the pulmonary outcome measures correlate more closely with functional measures, such as upper limb functional measures. 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.

- **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 assist device. 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.[55] 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.[55,116] 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.[117] 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.[90] 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.[55]

- 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%.[55,58,118] 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.[119] 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.

D. 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.

E. 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.[120] 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.[121,122] These may include: *community functions measured passively by digital technology,* such as the ActiMyo, which provides a capacity measure of 95th percentile stride velocity (the measurement of which was recently qualified as a secondary endpoint by the European Medicines Agency);[123,124] *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;[125-127] 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.[128,129]

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,[130,131] 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.[132] 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).[133] 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.[134]

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*.[135]

F. 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*[6]
- *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*[136]
- *Core Patient-Reported Outcomes in Cancer Clinical Trials Guidance for Industry DRAFT GUIDANCE*[137]
- *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*[138]

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 [40,55] 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.[139-144] Others include the PROMIS (pediatric scales), NeuroQoL,[145] Individualized Neuromuscular Quality of Life (INQoL),[146] health state utility values such as the EQ-5D,[147,148] the Pittsburgh Sleep Quality Index,[149-152] 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[153]
- The PedsQL 4.0 DMD Module Scale[154]
- DMD Health Index (DMDHI)[Ref]
- DMD Caregiver-reported Health Index (younger age to 18 years of age)
- Egen Klassifikation 2 (EK2) scale[116,155-157]
- DMD Upper Limb PROM[158]
- DMD Quality of Life (DMD-QoL)[159,160]

In a review of instruments measuring caregiver quality of life conducted by consensus-based standards for the selection of health measurement instruments (COSMIN) group,[161] the best available instrument in the context of DMD was the PedsQL Family Impact Module.[162] 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.

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.

BIOMARKERS IN DUCHENNE MUSCULAR DYSTROPHY

I. General Comments

FDA shares the Duchenne and Becker communities' goal to develop biomarkers and surrogate endpoints that could rapidly provide meaningful data as a signal of drug function and thus information regarding whether there is a biological activity that could prove promising in terms of altering the disease course.

Key considerations within this section:

- *Dystrophin quantification has been used in the approval of several dystrophin-replacement therapies in DMD. There are a variety of quantification methods for assessing dystrophin; multiple methodologies could be required to properly reflect the expression and biodistribution of dystrophin.*
- *Sponsors should strive to minimize trauma for patients when including muscle biopsy and develop clear protocols for handling and preparing samples to reduce loss of valuable tissue.*
- *There is a significant body of evidence that MR measures are related to patient function, predictive of future changes in function, and suitable for use in both ambulatory and nonambulatory patients. Sponsors should consider including MR measures in trials to build evidence as potential surrogate endpoint.*
- *Circulating biomarkers may aid in characterizing disease progression and response to therapy, but further work is needed to link circulating biomarkers to specific mechanisms of action.*

Sponsors should be aware that biomarker development is an aspect of DMD and BMD that is continuously evolving. They should consider the inclusion of some of the biomarkers described in this section in their clinical development programs as endpoints to support an NDA for their lead product in development. They could also include biomarkers for predefined sensitivity analyses, which, by helping move the field forward towards a consensus on the utility of the biomarker, may reduce costs and speed the time required for the development of subsequent products.

This section describes a number of biomarkers that may have prognostic or predictive value in forecasting the patient's prognosis or likelihood to benefit from a particular treatment. More attention is given to pharmacodynamic (monitoring) biomarkers that provide an indication of response after treatment. By comparison to pretreatment values, a treatment-responsive/monitoring biomarker may, with sufficient data, have the potential to serve as a *surrogate endpoint biomarker* that could substitute for a clinical endpoint (for accelerated but not a full approval) by providing early and accurate prediction of both a clinical endpoint benefit (which may include clinical improvement or lack of improvement, or harm or lack of harm) and the effects of treatment on this biomarker.[114]

The designation of a surrogate endpoint biomarker requires agreement with regulatory authorities. A "holistic evaluation" of available data—including epidemiologic, therapeutic, pathophysiologic data, or some other scientific evidence—must demonstrate that a biomarker can predict changes in clinical endpoints and could be potentially used for an accelerated approval decision.[163]

Surrogate endpoint markers can also be the primary endpoint in "adequate and well-controlled studies." If there is a well-established relationship between the surrogate marker and clinical outcome, that trial can be used to provide evidence for conventional marketing approval. If on the other hand, there is not a well-established relationship between the surrogate marker and clinical outcome, but it is "reasonably likely" to predict a clinical outcome, then a positive effect on the surrogate endpoint could lead to an accelerated approval.

In DMD, biomarkers that faithfully report on both the health and amount of skeletal muscle may potentially be useful at different stages of the clinical trial process as prognostic, predictive, or pharmacodynamic biomarkers. The use of biomarkers in BMD and other dystrophinopathies in clinical trials is not as well established, but this section will attempt to reference what is known.

The biomarker section of this guidance is split into two subsections. This first addresses biomarkers found in muscle tissue. The biopsy-based biomarker used to date in most DMD trials—dystrophin—is widely accepted by experts in the field as the appropriate pharmacodynamic biomarker for therapies whose mechanism of action is directed toward its expression, as significant treatment-related expression may confirm the mechanism of action and be useful for selecting doses in subsequent trials. Although the primary molecular determinant of the DMD versus the BMD phenotype is whether or not a significant amount of dystrophin expression occurs, from 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.[65,164] Biopsy-based biomarkers may nonetheless be unattractive for use in large phase III studies due to the invasive collection method.

The second section looks at less invasive methodologies to measure changes in the muscle. Some of these quantify proteins, protein fragments, or genetic materials in the blood or urine; these are exploratory but worth greater investments due to the ease with which they can be measured. Some are imaging techniques that are much further along. In particular, the direct imaging of skeletal muscle using magnetic resonance imaging (MRI), or spectroscopy (MRS) has the potential to serve as an efficacy-response biomarker and surrogate endpoint.

Sponsors should be aware that scientific consensus regarding the utility of any of these exploratory biomarkers may have been reached since the time of issuance of this document and should discuss potential biomarkers with the FDA. In the meantime, however, evidence of an effect on an exploratory biomarker could provide supportive evidence for a claim of disease modification in an NDA. When combined with some other evidence suggestive of clinical benefit, sponsors could help establish the use of a biomarker as a surrogate endpoint (see *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).*

II. Quantification of Dystrophin as Biomarkers

A. General comments

Dystrophin organizes and stabilizes the sarcolemma to effectively distribute contractile forces and maintain myofiber structural integrity and function. Dystrophin is also a scaffolding protein necessary

for the proper localization (and thus 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 has already been discussed in the diagnosis chapter.

The accurate quantification of dystrophin in muscle tissue can provide important support for the clinical development of dystrophin-restorative therapies. Evidence of a significant effect on dystrophin levels, for instance, could provide proof of concept that a dystrophin-restorative therapy does in fact increase dystrophin production. We note that dystrophin quantification has served as a surrogate biomarker that led to accelerated approval of multiple antisense oligonucleoside drugs (AONs), including eteplirsen, golodirsen, casimersen, and viltolarsen.[165,166] However, subsequent controversy over the quantification methodology and validation needs to be carefully considered.[167]

B. Considerations related to muscle biopsies

To accurately measure either dystrophin or utrophin in skeletal muscle, significant attention must be paid to issues of specimen collection, including 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 methods of quantification proposed should address the sensitivity and reliability of the proposed assays. In addition to these technical issues, sponsors should address considerations regarding the ethics of biopsies in clinical studies, and in particular any rationale for repeated biopsies to assess changes in relative dystrophin levels from pretreatment muscle samples.

Ethical concerns of biopsies in children

Sponsors should be aware that there are ethical issues related to performing multiple muscle biopsies in patients with a degenerative neuromuscular disorder. In some cases, requiring a muscle biopsy may be a disincentive to participate in a trial. For instance, one recent study found that parents and patients were willing to have a biopsy for a dystrophin-restoring therapy in an open label trial but much less so in a placebo-controlled trial.[168] In another study, parents and patients reported that the requirement of a muscle biopsy was not a major disincentive to participate in the studies of GT products, however, it should be noted that the expectation of benefit from GT products has been high.[15] Biopsies also carry the risk of anesthesia, which is required for the pediatric population, and this risk is increased for protocols that require multiple biopsies.

Bearing in mind the invasiveness of the procedure, sponsors should consider whether biopsies are necessary when planning a clinical trial in order to minimize their use. If a biopsy is required, caregivers have stressed that it is very important that they receive the results for their child.[168] Therefore, it is critical that the biopsy provides a useful specimen and that an appropriate post-treatment interval has been chosen. Sponsors should have an SOP for obtaining, handling, preparing, and shipping biopsies (see below), and biopsies should not be performed at clinical sites where the specimens may be mishandled. If biopsies are required, sponsors should consider only selecting clinical trial sites that have performed and handled muscle biopsy before or designing trials in which the biopsies are only performed in such appropriate sites. Finally, sponsors of trials in which muscle biopsies are performed should commit to timely feedback about the biopsy analysis to the trial participants and to the 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.[169,170] 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.*[171]

- 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.

C. 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.[172,173] Finally, because of significant gender differences in dystrophin expression between males and females,[173] 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[173-177] 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.[174,177] 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.[173]

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.[174] 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.[173]

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,[174] 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.[164]

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.[172]

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.[178] This method can be modified to quantify mini or micro dystrophin following gene therapy.[179]

Benchmarking to immunoblot and immunostaining has been done in preclinical trials of exon skipping and has shown concordance between all methods.[180] 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: Dystrophin quantification method overview

Strengths	Limitations
Immunofluorescence microscopy	
● In-situ labeling (no extractions or transfers) ● Subcellular localization confirmation ● Tissue- and fiber-level information ● Excellent sensitivity to regional variation and small differences in expression ● Results can be cross-referenced with fiber morphometrics and tissue histopathology ● Opportunity to assess co-colocalization with multiple other markers ● Highly detailed quantitative information available	● No standard curve or absolute control for abundance – only relative quantification versus healthy muscle ● Moderate-high complexity of quantification methods ● Little consensus on standard staining and quantification approaches ● Signal quality depends on antibodies ● Potential for interference from non-specific staining or autofluorescence ● No information about total protein size
Western blotting (conventional)	
● Widespread acceptance as primary quantitative assay ● Simple and accessible quantification methods ● Opportunity to include a standard curve ● Protein size information ● Separation by size reduces interference from non-specific antibody staining	● No localization or tissue distribution information ● Poor sensitivity and reliability at low expression levels ● Signal quality depends on antibodies ● Requires protein extraction, which may be significantly affected by reagents or tissue pathology ● Requires protein transfer ● Difficult to identify or diagnose potential technical failures ● Potential for migration or transfer issues disproportionately affecting some samples
Capillary Western	
● Built-in quantification ● Opportunity to include a standard curve ● Relative protein size information* (*discordant from predicted size based on size standard) ● Separation by size reduces interference from non-specific antibody staining ● No transfer required ● Good sensitivity at low expression levels ● High throughput	● No localization or tissue distribution information ● Signal quality depends on antibodies ● Requires protein extraction, which may be significantly affected by reagents or tissue pathology ● Difficult to identify or diagnose potential technical failures ● Proprietary technology
Mass spectrometry	
● Provides absolute quantification ● Opportunity to include a standard curve or a control of known abundance ● Provides some information about isoform based on represented peptides ● No transfer required ● Good sensitivity at low expression levels	● No localization or tissue distribution information ● Requires protein extraction, which may be significantly affected by reagents or tissue pathology ● No information about total protein size ● Accessibility limitations (cost, difficulty, equipment and expertise availability)

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.[181] A range of mutations considered predictive of a severe DMD genotype may result in BMD, typically due to alteration of splicing of the *DMD* transcript to 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.[65]

It has been established that dystrophin levels correlate with the prognosis seen in female DMD carriers[182] expressing normal dystrophin,[183] and in many but not all male BMD patients expressing abnormal but at least partially functional dystrophin.[184] 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.

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

DMD is mostly caused by mutations in the *DMD* gene that lead to a reading frame shift and premature translation termination. Exon skipping approaches alter splicing of the dystrophin pre-mRNA, restoring the reading frame, in most cases by allowing translation of internally truncated, but functional dystrophin protein. Such exon skipping can be achieved with antisense oligonucleotides [AONs],[185] or AAV-encapsidated U7snRNAs expressing an RNA antisense sequence.[186-188]

A commonly used parameter to assess and compare the efficacy of various antisense molecules is the exon skipping percentage, which is defined as the percentage of transcripts in which the targeted exon is skipped relative to the total number of dystrophin transcripts (skipped plus 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. Hence, the measurement of exon skipping at the RNA level is an important assessment in verifying AONs' ability to successfully modify the appropriate gene target.

Due to the low abundance of dystrophin mRNA, the efficacy of AONs to induce exon skipping has predominantly been assessed at the transcriptional level using the semi-quantitative nested reverse-transcription polymerase chain reaction (RT-PCR) or quantitative PCR (qPCR) with differing protocols and amplification cycles. Another approach is digital droplet PCR (ddPCR), which should be considered as an alternative method that can allow absolute quantification of the various transcripts and may be more suitable for clinical trial samples.[189] RNA sequencing (RNA Seq) can also allow quantification of exon skipping, and has the additional advantage of allowing unbiased assessments of off-target skipping, providing additional evidence for the safety of exon skipping approaches.[190]

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.

III. Non-Biopsy Based Biomarkers

A. 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.

B. 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 magnetic resonance imaging (MRI) or spectroscopy (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.[191] 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.[192] 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.[193,194]

MR measures of fatty infiltration, which include both fat fraction and the transverse relaxation time of muscle tissue,[195] 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.[196] 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[193,195,197-200] and loss of HTMF.[193]

MR measures of fatty infiltration are sensitive to disease progression and therapeutic intervention in both DMD and BMD.[33,201-208] 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.[33,201,209,210] 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[211,212] or the transverse relaxation time (T_2) of muscle tissue or water.[201] Blood-oxygen-level-dependent (BOLD) imaging could be useful in the evaluation of therapies targeting nNOS;[213,214] and phosphorus-31 MRS may have utility for measuring therapies that aim to improve mitochondrial function.[215]

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[216-218] and correlated with function in DMD.[219-224] 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.

C. 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. 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. 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).[225]

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.[226] 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

A. 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 magnetic resonance spectroscopy (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.[192] Magnetic resonance spectroscopy (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 (^{1}H), carbon (^{13}C), and phosphorus (^{31}P). 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.[207,208,227-234] 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.[193,194,203,235-241] 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.[242-254] 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 T_1- 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.[248,255] 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 (T_2) time of muscle-measured multi-echo MRI (sometimes called bulk or global muscle T_2)[195] and to qualitative Mercuri grading.[256] Finally, the replacement of muscle with fat can be tracked by measuring the muscle area occupied by nonfatty tissue, sometimes called contractile area.[257-260] A number of studies have found moderate to strong correlations between muscle fat infiltration or its surrogates and measures of functional ability.[33,200,235,238,242,244,245,247,249-252,260-272] 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.[196] 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[195,198-200] and loss of HTMF.[193]

Considerable recent work has supported the responsiveness of MR measures of fatty infiltration to disease progression and therapeutic intervention in both DMD and BMD.[33,201-208,273] 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.[33,201,209,210]

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,[245,274] and has been directly linked with inflammatory markers (serum and tissue) in FSHD.[275] 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;[212,276] Na+ imaging also detects a decrease in inflammation with corticosteroid therapy.[211] Additionally, in quantitative T_2 (transverse relaxation time) weighted imaging, both MRI[277-282] and MRS[252,283] have been used to measure the T_2 of water, which is elevated in inflammation, muscle damage, and edema and decreased with corticosteroid treatment in DMD.[201]

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 T_2s of water molecules associated with collagen.

Cardiac MRI studies in DMD subjects have reported an age-related decrease in myocardial T_2 compared to controls[229,246] and an increase in myocardial T_2 heterogeneity.[284] Similar results have been observed in animal models with diabetic-induced cardiac fibrosis.[285,286] The decreased T_2 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 T_2 were seen in both calf and thigh skeletal muscles in boys with

DMD, but not healthy controls. This decrease in T_2 is typically masked in skeletal muscle imaging by the large amounts of fatty tissue deposition.[283] 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.[287]

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.[288] As such, it is well suited for imaging in young children[217,274] as well as older individuals. US imaging has been extensively applied to investigate cardiac abnormalities associated with DMD (see Cardiomyopathy section on page 80). Muscle atrophy and intramuscular fibrosis and fatty infiltration can be visualized using US of skeletal muscle.[289,290] US density analysis of skeletal muscle provides a sensitive method for distinguishing between healthy children and children with neuromuscular disorders.[291] 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.[220,221,223,292] Muscle ultrasound parameters are significantly correlated with age in DMD, reflecting increased muscle pathology with disease progression.[291,293] These measures are also sensitive to disease progression longitudinally in DMD.[217,218] 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). [217,219-223]

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.[217,294,295] Recently, the high sensitivity to disease progression of EIM has been demonstrated.[216] 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).[224]

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.[296] 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.[212,233,281,297-300] 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.[235,278,281]

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.[301] 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.

B. 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.[180] 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.[302] In designing clinical trials of utrophin upregulation therapies, sponsors should address considerations raised for dystrophin quantification, above.

C. 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,[303-306] RNAs (miRNA and mRNAs)[307-310] and proteins.[311-315]

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,[313] mass spectrometry-based proteomics methods,[316] and aptamer panels.[314,315] 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.[317-319] 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.[310,320,321] 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),[322] fibronectin,[323] muscle protein fragments in serum and urine, succinate in *mdx,* prostaglandin D2,[324] and 3-methyl-L-histidine.[325] 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.[312] 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.[305,326] Another recent study identified nine blood protein biomarkers related to muscle mass that correlated with disease milestones, functional tests, and respiratory capacity.[327]

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[328,329] and competing analogs.[329] 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 tropinin 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.[330]

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.[225] 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.

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 drug development programs are to demonstrate the effectiveness of a drug in treating or preventing a disease or condition, to assess the magnitude and frequency of that effect, and to assess the risks of the drug, thereby enabling a benefit-risk assessment and appropriate labeling.[34] One of the statutory requirements for drug marketing approval is "substantial evidence" that the drug will have its claimed effect.[114] 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.[331]

Key considerations in this section

- *The section contains key learnings from past trials: a chief of which is that age is not the best criteria for enrichment of patient trajectories, but rather baseline disease severity characteristics.*
- *The section describes key considerations in DMD trial design and analysis, including recommendations on concurrent therapy and duration in order to measure clinical benefit at different disease stages.*
- *The section considers the use of modelling, natural history data, real-world data, and prediction models to measure treatment effect in DMD trials for simulating treatment comparators. Innovative trial designs can also include delayed placebo (or run-in trials, in which natural history data are used in the run-in to the trial) and roll-over trials in order to make trials more efficient and reduce participants exposure to placebo. For instance, the DMD community has been working on a master protocol for a platform trial that can share placebo patients and reduce the proportion of individuals randomized to placebo.*
- *Finally, a brief discussion of clinical trial considerations in BMD and other dystrophinopathies is included.*

I. Key Learnings from Past DMD Trials

Sponsors developing drugs for rare disease often face unexpected challenges in the design, conduct, and evaluation of clinical trials that can make the collection of "substantial evidence" challenging. In addition to the logistical and statistical issues related to enrolling and conducting trials in small, dispersed, and heterogenous patient populations, problems may arise due to the limited natural history data characterizing the disease, limited experience working with often novel outcome measures or limited standardization of the measurement across different cohorts, and uncertainty regarding the selection of endpoints that reliably show a clinical effect within the specified duration of the trial. The history of drug development in DMD is illustrative of many of these challenges and may be instructive for the design and evaluation of trials in dystrophinopathies such as DMD and BMD moving forward.[332,333]

There can be tension between the community's goal to enroll a wide spectrum of individuals across disease stages and phenotypes, versus having inclusion and exclusion criteria focused on enrolling individuals more likely to experience disease progression based on a specific endpoint in the absence of treatment during a trial. Yet, in retrospect, a number of DMD trials had suboptimal inclusion/exclusion criteria with insufficient enrichment for patients with the optimal short-term trajectory, while missing/excluding some of those who may have shown a treatment effect.

In other cases, the trial duration may have been too short to demonstrate efficacy with the selected clinical endpoint, although there may have been biomarker evidence of a treatment effect, and perhaps

even intermediate clinical outcome measures suggestive of a treatment effect in post-hoc analyses. While not useful for regulatory approvals, findings from post-hoc analyses can be used to refine the selection of outcomes and optimal target population for subsequent studies. For instance, there are data now demonstrating that a change in near-term outcomes, such as changes on TFTs where time to stand from supine could be used in a predefined analysis as an intermediate clinical outcome predictive of down-stream progression to LOA (see Box 1).

One of the most significant lessons from earlier DMD trials is that age is not the best criteria for enrichment of patient trajectories, but rather baseline disease severity characteristics. Recognizing that, on average, loss of milestones occurs at or near certain ages, sponsors conducted trials that enrolled similarly aged boys which failed to demonstrate clinical efficacy because the participants were at different stages of disease (with placebo patients predicted to be stable and those allocated to active treatment predicted to decline), or treatment groups had different disease phenotypes and highly variable rates of progression. Registrational studies may have benefited from prognostic enrichment by enrolling participants likely to progress to an endpoint within the timeframe of the study factors as long as their condition is still medically addressable by a given treatment. The use of outcome measures to stage and stratify participants can also help sponsors evaluate the effect of treatment in different subgroups who may have different disease trajectories.

Recognizing the need to bring drugs to market efficiently, it is recommended that sponsors discuss with the FDA how their drug development package can best gain experience and document safety in the different populations affected by DMD and other dystrophinopathies, including what studies or programs might be put in place prior to marketing, as well as post-marketing commitments.

II. 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.[334] 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.)*

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

- 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. 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.[78]
- 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.

B. 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. For instance, recent clinical trials using dystrophin restoration strategies which produce low levels of dystrophin and peak levels of dystrophin after the first year 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, although they have also been used in trials of shorter duration.[78]

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.

III. 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. 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 (see: Demonstrating Substantial Evidence of Effectiveness for Human Drug and Biological Products: Draft Guidance for Industry. Rockville, Maryland; FDA: 2019. 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.[34] 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 an 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.[335]
- 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.

A. 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)

IV. 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.*

V. 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.[336] 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.

VI. 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.

VII. 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.[337-342] (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 [337]

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.

CARDIOMYOPATHY

I. 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.

Key considerations within this section:

- *DMD related cardiomyopathy is characterized by fibrofatty replacement of the myocardium, with an extended timeline of cardiac disease progression culminating in full thickness fatty replacement of the myocardium. This suggests maximum therapeutic benefit will be garnered only by developing trials focused on BOTH early and later stage disease. A singular focus on trials powered to examine late-stage disease in order to incorporate mortality outcomes may miss an important therapeutic window prior to irreversible, fatty replacement of the myocardium.*
- *Harmonization of diagnostic evaluation and therapeutics is integral to trial design but must be balanced with the need for inclusivity and access. Consensus recommendations regarding potential cardiac biomarkers and their consideration in trial design will not only facilitate effective trial design but would also provide a means to develop a more robust real-world data infrastructure. This infrastructure is <u>currently</u> needed to assess ongoing clinical trials and for future trials, both cardiac and noncardiac.*
- *The understanding of cardiac disease progression has evolved as longitudinal, granular cardiac data has emerged over the last decade. These data and the creation of multicenter networks have made cardiac clinical trials in DMD more feasible. Creation of a roadmap to assess effectiveness of cardiac therapies in DMD will further facilitate the timely development of therapies.*

II. Background

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.[343] 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.[40,344,345] 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.[346-350]

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.[351] Subclinical cardiac injury is evident through the development of troponin leak and via several cardiac imaging biomarkers.[352] 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.[353-355] 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.[356,357] 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.[352,358] The natural history of LGE and its relation to long-term outcomes is better understood.[356,357] 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.[356,359] 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.[360]

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.[360,361] 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.[346,362,363] The use of prophylactic therapy and the heterogeneous nature of the introduction of these therapies should be considered when assessing previously published cohort data.[364]

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.[365-367] 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.[368,369] 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.[370-372] 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.[349] 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.

III. DMD Cardiac Assessment, Trial Designs, Potential Outcome Measures

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.[359] 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.[351,373-375] 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.[376] 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.[346,363,377-379] 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.

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.[380] 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.[347,350] 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[39].

    a. 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.
    a. 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.[343,346,349,362,372,381,382] Sinus tachycardia, abnormal heart rate variability (HRV), and atrial ectopy are early manifestations of the disease.[382,383] Ventricular ectopy, including ventricular tachycardia, atrial fibrillation, and ventricular fibrillation are later manifestations of disease and generally occur as systolic function decreases.[370,371]

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 <u>any</u> DMD related trial.
    a. 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.[352,358] 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.[384] 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.[364]

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.[41,385] The use of additional medication classes including mineralocorticoid receptor antagonists (MRA) should be noted given preliminary evidence that they may affect disease progression.[363,377] 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.

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.[386,387]

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.[346,362,388] While steroids have become standard of care, the application of cardiac therapies remains highly variable.[364,381] 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.[389] 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.[390 50] 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.

IV. 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.

GENE THERAPY FOR DMD AND OTHER DYSTROPHINOPATHIES: APPROACHES, PATIENT-CENTERED CONSIDERATIONS, AND DEVELOPMENT PATHWAY

Key considerations within this section:

- *This section draws upon existing FDA guidance on gene therapy (GT) and considers how sponsors can apply it to the development of GT products for DMD.*
- *Technical challenges for the development of GT products that are unique to dystrophinopathies include the target tissues—both skeletal muscle and cardiac muscle—as well as the size and complexity of DMD gene that a GT would be designed to restore or correct.*
- *Sponsors should consider the implications of the immune responses and safety issues that currently limit the administration, and preclude re-administration, of some of the GT products furthest along in development.*
- *Priorities for preclinical studies include dose selection so that clinical trials start with a dose expected to have a therapeutic effect, as well as early evaluation of the effects of GT on the heart.*
- *While well-controlled placebo-controlled studies are recommended for GT products that are not expected to have large, self-evident effects, sponsors are encouraged to discuss novel trial designs with FDA that limit the time or necessity that a trial participant is on placebo.*
- *The section includes guidance on corticosteroid treatment prior to and during clinical trials, participant selection criteria and safety considerations including long-term monitoring of GT trial participants.*
- *Efficacy endpoints considerations are the same as in trials of non-GT product for DMD. Intermediate clinical endpoints and surrogate endpoints reasonably likely to lead to or predict clinical benefit could be the basis for a GT to be granted accelerated approval. Given the inability to repeat dosing at the present time, there should be some evidence suggestive of clinical benefit, whether through demonstration of high levels of expression of a functional transgene or demonstration of restored expression of the endogenous gene after gene editing, for the proteins produced by gene therapy to be considered a surrogate endpoint meeting the "reasonably likely" standard for accelerated approval. Evidence from other candidate surrogate endpoints (such as imaging) could support an application.*

I. 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.

II. 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] rather than the full protein.[391,392] 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 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.[393]

[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.

- 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.[393]

- Safety considerations and other limitations due to immune responses directed against the vector capsid, viral DNA and the expressed transgene product that limit administration to some individuals. 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.

III. 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[394] and Human Gene Therapy for Neurodegenerative Diseases[395] 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.

IV. 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 another chapter 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*,[396] and *Human Gene Therapy for Rare Diseases*[394]).

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*.[396]
    - 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.

V. 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.

A. 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.*[397]

- 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.[15] 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.

B. 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.[40,78] 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.[398]

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

C. 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.[399]

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.

D. 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.

Given that the cardiac impact of these therapies has not been modelled adequately in preclinical studies, it is essential that all studies of GT products evaluate the cardiac impact in patients and monitor for changes in heart function and health. Note that GT products that treat the skeletal muscle may have different effects or no effect on the heart. However, if a treatment improves skeletal muscle function, increased physical activity could place more load on the heart (please see Cardiomyopathy section).

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.

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*).[400] 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.

E. 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.

G. 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.

H. Expedited programs

As described in the guidance document *Expedited Programs for Serious Conditions—Drugs and Biologics*,[401] 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, 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 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 microdystrophin (once there is 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. It is also worth noting that insurers and other third-party payers may be more likely to provide reimbursement for a GT product with additional biomarker and outcome measure data.

At the time of writing, unlike most drugs, re-dosing is not possible for GT products. This means that a GT product is a one-time treatment, which changes the risk versus benefit calculus. Individuals with DMD may also only have one opportunity to benefit from a GT product using a specific vector. However, each product's 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 the complexity of gene therapy may not be appreciated or understood by the general public, it is essential to ensure prospective research participants fully understand the procedure, and the potential risks and benefits of this approach.

United States (US) Food and Drug Administration (FDA) regulations (21 CFR part 50) and the Federal Policy for the Protection of Human Subjects, which applies to federally-funded research and is usually referred to as the Common Rule, describe requirements for informed consent.

Both regulations discuss the required elements of informed consent (FDA 21 CFR 50.25(a), Common Rule 46.116) for any clinical biomedical research in the United States.

(a) *Basic elements of informed consent.* In seeking informed consent, the following information shall be provided to each subject:

(1) A statement that the study involves research, an explanation of the purposes of the research and the expected duration of the subject's participation, a description of the procedures to be followed, and identification of any procedures which are experimental

(2) A description of any reasonably foreseeable risks or discomforts to the subject

(3) A description of any benefits to the subject or to others which may reasonably be expected from the research

(4) A disclosure of appropriate alternative procedures or courses of treatment, if any, that might be advantageous to the subject

(5) A statement describing the extent, if any, to which confidentiality of records identifying the subject will be maintained and that notes the possibility that the FDA may inspect the records

(6) For research involving more than minimal risk, an explanation as to whether any compensation and any medical treatments are available if injury occurs and, if so, what they consist of, or where further information may be obtained

(7) An explanation of whom to contact for answers to pertinent questions about the research and research subjects' rights, and whom to contact in the event of a research-related injury to the subject

(8) A statement that participation is voluntary, that refusal to participate will involve no penalty or loss of benefits to which the subject is otherwise entitled, and that the subject may discontinue

participation at any time without penalty or loss of benefits to which the subject is otherwise entitled.

(b) *Additional elements of informed consent.* When appropriate, one or more of the following elements of information shall also be provided to each subject:

(1) A statement that the particular treatment or procedure may involve risks to the subject (or to the embryo or fetus, if the subject is or may become pregnant) which are currently unforeseeable.

(2) Anticipated circumstances under which the subject's participation may be terminated by the investigator without regard to the subject's consent.

(3) Any additional costs to the subject that may result from participation in the research.

(4) The consequences of a subject's decision to withdraw from the research and procedures for orderly termination of participation by the subject.

(5) A statement that significant new findings developed during the course of the research which may relate to the subject's willingness to continue participation will be provided to the subject.

(6) The approximate number of subjects involved in the study.

(c) When seeking informed consent for applicable clinical trials, as defined in 42 U.S.C. 282(j)(1)(A), the following statement shall be provided to each clinical trial subject in informed consent documents and processes. This will notify the clinical trial subject that clinical trial information has been or will be submitted for inclusion in the clinical trial registry databank under paragraph (j) of section 402 of the Public Health Service Act. The statement is: "A description of this clinical trial will be available on *http://www.ClinicalTrials.gov,* as required by U.S. Law. This web site will not include information that can identify you. At most, the Web site will include a summary of the results. You can search this web site at any time."

(d) The informed consent requirements in these regulations are not intended to preempt any applicable federal, state, or local laws which require additional information to be disclosed for informed consent to be legally effective.

(e) Nothing in these regulations is intended to limit the authority of a physician to provide emergency medical care to the extent the physician is permitted to do so under applicable federal, state, or local law.

[46 FR 8951, Jan. 27, 1981, as amended at 76 FR 270, Jan. 4, 2011]

A common complaint from prospective and enrolled research subjects about informed consent is the amount of medical jargon, 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), 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 an opportunity to discuss that information.
- Key Information (new section)
    - 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 participants 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)].

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 is being done, and what they will be asked to do. However, formal consent to enter the trial comes from the parent or guardian.

The investigator and the institutional review board (IRB) need to take into account the ages, maturity, and psychological state of the children when judging whether they are capable of giving assent to involvement in a proposed research activity.

Oral consent, using a script, may be appropriate for younger children (eg, 4-11 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 participants, 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 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 and their 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; getting the minimally effective or a nontherapeutic dose, uncertainty of durability of effects achieved; whether biopsies will be necessary and the possible aftereffects of the procedure; potential for long-term follow-up period that could last years, possible impact on participation in future clinical trials, or disqualification for other treatment options and death.

Post consent online

Clinical studies that are conducted or supported by a Common Rule federal agency are required to post one consent form used in the enrolling of participants to a publicly accessible federal website such as ClinicalTrials.gov or Regulations.gov. 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.

COMMUNITY IMPERATIVES

I. Benefit-Risk Preferences and Patient Experience Data

- Given the many unmet needs of patients with Duchenne and Becker, the community's preferences for treatments have been well documented and quantified. We have demonstrated a willingness to accept uncertainty and risk in exchange for the potential of slowing disease progression.

- Preference data have shown that patients and caregivers have similar preferences for benefit and risk across studies to date. The potential benefit of delaying the loss of function linked to quality of life and activities of daily living are prioritized over potential risks and uncertainties. Each loss of function over time is catastrophic to the lives of patients and caregivers.

- We encourage the FDA to continue to be responsive to patient and caregiver preferences for emerging therapies, recognizing some may be lifelong therapies for patients and that the benefit/risk assessments may differ over time or be dependent on treatment modality.

- We appreciate FDA's Center for Drug Evaluation and Research (CDER) exercising regulatory flexibility in Duchenne, granting several accelerated approvals to date, and for the willingness to directly engage with the Duchenne and Becker community over time. We ask for continued flexibility from CDER and anticipate that the Center for Biologics Evaluation and Research (CBER) will demonstrate similar regulatory flexibility to the extent possible within gene and/or cell therapy as potential therapies move through the development and review process. We ask that sponsors that have benefited from this regulatory flexibility fulfill their corresponding obligations – to both patients and regulators – in a timely and effective fashion.

- Sponsors need to collect patient experience data relevant to their development programs including patient reported outcomes, patient preference data, as well as other data that can demonstrate meaningfulness of change in clinical outcome measures. We request sponsors work directly with patient advocacy groups regarding data related to the types of patient experience they plan to collect or are currently collecting. We encourage co-development when possible and reporting back results to the advocacy groups and community at large.

- Many people with Duchenne and Becker, at all ages and stages of disease, want to participate in clinical trials and need access to therapies. We ask that the FDA support and encourage drug developers to shape their programs accordingly by leading with regulatory flexibility reflective of evolved benefit/risk profiles for patients of advancing disease. We ask that drug developers make use of this flexibility responsibly.

II. Diagnosis

- The community recognizes that treating earlier is likely to have a greater favorable impact on the course of disease. The community continues to be dedicated to reducing the diagnostic delay as we have new FDA approved treatments available for Duchenne.

- The community understands and has demonstrated that newborn screening is an effective way to eliminate the diagnostic odyssey in Duchenne, facilitating earlier, life-alerting health outcomes, and must be incorporated into the US newborn screening public health system. Sponsors should support current efforts to make this a reality.

- Access to genetic testing has increased since 2014 and must continue to be expanded to those with older genetic testing results as current therapies that are approved are for specific genetic mutations. Efforts must be made to expand testing for female carriers of Duchenne and Becker. Sponsors should work collaboratively with patient groups to increase access to testing.

- The community's understanding of Duchenne has continued to evolve, and Duchenne is no longer considered a disease that affects only boys and men. Sponsors should continue to expand clinical trial inclusion criteria to reflect our growing understanding of the patient population to include nonambulatory adults and affected girls and women.

III. Natural History and Trial Design

Data sharing:

- The community is grateful to individual patients and their families for their sacrifices and contributions to our growing understanding of natural history. We appreciate the efforts of FDA, drug sponsors, academics, and patient groups working in collaboration to improve disease-progression modeling. Both the Duchenne Regulatory Science Consortium (D-RSC), with the Critical Path Institute (C-Path), and the Collaborative Trajectory Analysis Project (cTAP) consortiums are modeling available data sets to inform drug development efforts and better characterize the natural history of disease. We strongly urge sponsors and academic institutions not currently involved in these efforts to join and contribute deidentified clinical trial and registry data to maximize the use of data for drug development, including making data available for regulatory filings and audits, as that is viewed as invaluable to patients and families, and to all efforts in drug development.

- The community appreciates that individuals of the same age with DMD may progress at different rates and is aware that this was one reason that some clinical trials that emphasized age as the key inclusion criteria failed to reach their primary endpoint during the course of the study. Analysis of natural history data from databases, registries, and placebo arms of clinical trials has shown that functional measures and timing of milestones are reasonably predictive of

the rate and timing of progression. The community believes that functional measures, stage of disease, and risk of progression should be reflected in the stratification criteria of trials.

- The community requests that both sponsors and academic institutions provide timely access to placebo arm data from clinical trials and natural history data sets to combine, aggregate, and analyze the data to better define comparative measures for clinical trial effect sizes, potential sources of heterogeneity in outcomes, and ideally to provide enough evidence for comparator arms of future trials to potentially lessen chance for placebo.

- The sharing of natural history data (including data from placebo arms) must never be delayed or blocked by sponsors, clinicians, or academic institutions.

- Sponsors must make clear during the informed consent process how patient-level data from the placebo arms of clinical trials will be used for research following the end of the study. Academic institutions must be held to the same data sharing standards as sponsors.

- In addition, with the evolution of Health Insurance Portability and Accountability Act (HIPAA) and general data protection rules (GDPR) in Europe, the community believes that consent form language must be harmonized to not only protect patients, but to remove barriers to data sharing and secondary uses of data. We need to maximize the utility of every single data point. In this disease, every data point is precious.

A. Becker and late-stage Duchenne:

- More efforts are needed to further enhance our understanding of disease progression in Becker and in late-stage Duchenne. Sponsors and academics currently involved in studies related to Becker and late-stage Duchenne must make clear their intention and timelines for the sharing of natural history data and placebo arm data with current data modeling efforts that are underway.

B. Participation in natural history studies:

- Families sacrifice time and even their ability to participate in clinical trials by being involved in natural history studies. Clear expectations must be set for those participating in natural history studies connected to clinical trials in regard to how participation impacts (or does not impact) potential inclusion in a clinical trial.

IV. Outcome Measures Selection

- While we work with the outcome measures of today, our community recognizes a need to continue to push for more sensitive, specific, patient-friendly outcome measures. Efforts are

underway to develop, validate, and potentially even qualify new measures. We request that the FDA support this effort by allowing for flexibility when drug developers include these measures as exploratory/secondary endpoints in trials, and we request that drug developers act on this flexibility for the benefit of the community as well as their own programs.

- The community understands that it is critical to incorporate data elements within clinical trials that reflect meaningful value to patients, providers, regulators, and payers. We urge sponsors to incorporate endpoints and outcome measures that reflect measurable and meaningful impacts to patients and caregivers, in collaboration with all relevant stakeholder groups.

- The community knows that what is meaningful may depend on the specific context: A difference in dosing or side effects may matter more at one age or disease stage than another, and what might seem like a small difference on an outcome measure may make a tremendous difference in the day-to-day life of a patient and their caregivers.

- Seemingly small changes in an outcome measure (eg, a 1- to 2-point change for a specific function on the NSAA or PUL) may actually reflect dramatic impacts in terms of quality of life and the ability to complete activities of daily living. And while Zolegensma in spinal muscular atrophy type 1 (SMA1) achieved a profound improvement including reversal of function loss, and prolonged event-free survival, in diseases such as Duchenne, where progression is over many years and each loss of function is a devastating event, stability of disease, or even a delay in the rate of progression, is a meaningful benefit for patients.

- The community wants to emphasize that in this disease, once a milestone of disease or a critical function is lost, it is unlikely to ever be recovered. For this reason, stability in disease is a benefit, and the community would prefer that clinical trials feature as endpoints, outcome measures that assess the degree to which one can perform an activity, rather than complete loss of function.

V. Biomarkers

- The community believes the collection of biomarkers is critical to advancing drug development and to understanding the impact of potential therapies. However, the community also believes that such samples—be they blood, urine, or muscle, and associated clinical data—must be made available to the broader research community following trials to advance our evolving understanding of Duchenne and Becker and benefit all research.

- Sponsors should monitor more than one biomarker as secondary efficacy endpoints and for safety monitoring, including the use of blood and urine. As new safety biomarkers have emerged through the Critical Path Institute (C-Path), we ask sponsors to include such measurements in trials.

- MRI/MRS outcomes have continued to evolve and have demonstrated a useful noninvasive way to measure both the health and amount of skeletal muscle. We ask that sponsors consider MRI within their studies or within a subset of patients in their trials and that MRI data is collected at baseline.

- For a treatment aimed at restoring dystrophin expression, the community continues to believe it is sufficient that showing an increase in dystrophin over baseline is reasonably likely to confer clinical benefit to a medically addressable population, though the size of the effect will depend on stage of disease. We encourage FDA to continue the use of this surrogate endpoint for accelerated approval.

- Biopsies must only be included in trials where the goal is to restore dystrophin expression or via a genetic therapy with a micro-dystrophin. Biopsies should be limited to two (baseline and at another time point) to the extent possible. The community asks the FDA and sponsors to consider the use of needle biopsies rather than open biopsies, given the risk of anesthesia and subsequent scarring, whenever possible as techniques and analysis have advanced since the first guidance was submitted.

VI. Trial Design

- Time is not on the side of patients and families. With each loss of function, patients and their families experience distinct and catastrophic loss that is linked to critical activities of daily living and increased burden caregivers.

- The community wants trials that are inclusive of people of all ages across the spectrum of disease—to whatever degree possible. It may be possible to do this by stratifying the study population into different subgroups, evaluating efficacy in a subgroup of those most at risk of progression to the trial's primary endpoints (increasing the power of the trial to reach a clear conclusion), and at the same time, evaluating safety and other exploratory data in patients at other disease stages and ages. This could help assure a broad label for products if they receive approval.

- The community was encouraged that FDA's complex innovative trial designs (CID) pilot program included a Duchenne study. The community asks the agency to make every effort to work with sponsors and the patient community to explore trial designs that reduce the number of patients on placebo in trials. This could include trials of different experimental therapies that share a placebo arm.

- Based on the progress made to date with modeling disease progression, the community feels strongly that disease characterization has matured and utilizing natural history external control data in trial design must be explored.

- The community requests that sponsors who are designing clinical trials explore with regulators the potential use of innovative trial designs (such as incorporating adaptive trial design and/or using historical control data to enrich placebo arm data).

- Increasingly, analysis from both the Duchenne Regulatory Science Consortium (D-RSC) with the CPATH and the Collaborative Trajectory Analysis Project (cTAP) supports a shift away from using age to define a trial population and constrain eligibility. We encourage the FDA and drug developers to adapt swiftly to this emerging awareness and better design eligibility criteria according to measures that better identify the stage of disease.

- Sponsors should work with clinics to avoid patients having to perform outcome measure tests repeatedly when visits are both care-related and study-related.

- The best responses may be seen with combination therapies that combine treatments that target muscle with those targeting the extracellular environment. The community urges both sponsors and FDA to design and launch combination therapy studies to develop a combination of therapies to address all the disease factors responsible for muscle degeneration and functional loss.

- The community feels strongly that participation in one trial should not make one ineligible for entry into a future clinical therapeutic trial after a reasonable period of washout. While we hope that in the future there may be contexts where a treatment may alter the clinical trajectory of a patient, even in such cases, there will still be a need for other treatments—either in combination or used sequentially. The field will need clinical data to guide the use of potential new therapies in pre-treated patients or patients on newly approved therapies. Sponsors should therefore work together with FDA and the community to provide a mechanism, including well-controlled trials for approved drugs, and open-label studies or individualized treatment INDs in other contexts, to gather safety data and clinical experience of their potential therapy in patients on or with exposure to other novel treatments.

VII. Cardiac

- Through numerous patient preference studies conducted to date, the community has demonstrated strong preferences for treatments targeting the heart. These studies have shown that for nonskeletal muscle treatment targets, patients and caregivers prioritize treatments that target symptoms related to cardiac and pulmonary function.

- Our understanding of disease progression has evolved as more data on cardiac disease progression has become available. The FDA's traditional feel-function-survive heart disease paradigm is not applicable to patients with Duchenne- and Becker-related cardiomyopathy. The FDA needs to work with pediatric cardiologists on identifying acceptable cardiac outcome measures for patients with dystrophinopathies.

- Regardless of treatment modality and treatment target, sponsors must monitor cardiac function during all clinical trials and accept recommendations from pediatric cardiologists regarding use of cardiac MRI (cMRI).

- The community is strongly in favor of exploring cardiac medications currently approved in other indication and encourages physicians to understand the threshold for these medications to be utilized.

- Sponsors designing trials specific to treating cardiac function in later stage patients must consider trial burden and include ways to decentralize trials to the extent possible.

- We encourage sponsors to look at cardiac trials for Duchenne patients with slower disease progression and those diagnosed with Becker.

- As the standard of care has evolved, it is critical that sponsors allow the addition of cardiac drugs after trials arms complete or when decrease in cardiac function is observed.

VIII. Gene Therapy

- The community remains optimistic regarding gene therapy. We ask that FDA clarify the current thinking on use of the accelerated approval pathway for gene therapies that clearly show evidence of addressing the underlying cause of disease in order to set clear expectations for both sponsors and patients.

- We encourage sponsors to include preference studies within their submission and request that FDA incorporate these data within the review process.

- The community continues to be frustrated by the lack of transparency from sponsors about plans and timing for follow-on studies with broader inclusion criteria that would include nonambulatory patients, adults, and those who are currently excluded based on mutation. While we recognize timelines are challenging to provide, *time* is the most precious commodity our community has.

- To the extent possible, sponsors focused on genetic therapies need to share patients' time-sensitive data that may have an impact on decisions relating to care with patients and treating physicians, upon request, as soon as possible without impacting the course/conduct of the trial.

- Sponsors must monitor cardiac function via blood markers and cMRI consistently across all gene therapy studies. Sponsors should collect baseline cMRI then again at 12 months in order to understand the effect of micro/minidystrophin on the heart.

- Sponsors must consider the dilemma for families in terms of “what’s next” after gene therapy to preserve and protect the potential impact. Sponsors and FDA need to be clear about long-term follow-up during the initial consent process and throughout the study in terms of the use of interventions as well as the possibility of participation in other studies.

- Sponsors are encouraged to share informed consent document drafts with advocacy partners for critical feedback. Families should be given appropriate support during the IC process and be provided with a clear picture of benefits, risks, and unknowns associated with gene therapies. Informed consent must be an ongoing process and discussed at each visit.

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