Applying the Accelerated Approval Pathway to AAV Gene Therapy for Treatment of Duchenne Muscular Dystrophy

A Pathway Development Consortium White Paper[a]

Executive Summary

Duchenne muscular dystrophy (Duchenne) is a serious, rare genetic disease, affecting primarily boys. It is characterized by progressive muscle degeneration that results in loss of function and early death due to respiratory or cardiac failure.[1] Limited treatment options are available, predominantly for small subsets of the patient population; thus, Duchenne is a disease with large unmet medical needs.

The adeno-associated virus (AAV) vector is the leading gene delivery system for treating inherited neuromuscular diseases.[2-3] The gene encoding the full-length dystrophin protein is too large to be accommodated into a single AAV vector.[4-5] Hence, gene therapy based on AAV delivery of shortened yet functional genes (microdystrophin genes) has emerged as a promising treatment.[6-7]

This white paper seeks to explain the rationale for use of the accelerated approval pathway to advance AAV gene therapy development for Duchenne patients. In addition, it identifies two surrogate endpoints reasonably likely to predict clinical benefit—muscle fat fraction (FF) obtained by magnetic resonance (MR) methods and microdystrophin—that could be evaluated in clinical trials to support accelerated approval of AAV gene therapies. Finally, it discusses some aspects that are important for approval of medical products generally (e.g., supportive evidence and benefit-risk considerations) and others that are unique to medical products pursuing accelerated approval (i.e., confirmatory data to verify and describe clinical benefit).

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Use of the Accelerated Approval Pathway in Duchenne

Duchenne Muscular Dystrophy and Related Therapies

Duchenne is a recessive, X-linked neuromuscular disorder caused by mutations in the dystrophin gene, which spans approximately 2.4 megabases and includes 79 exons.[1,8] These mutations lead to little or no dystrophin production (typically <3% of the normal quantity of dystrophin).[9] Dystrophin is a protein critical in physically stabilizing the membranes of muscle cells. Patients with Duchenne experience a near-total absence of full-length dystrophin production, resulting in progressive muscle degenerations that manifest primarily as muscle weakness impairing walking, other motor functions, breathing, and cardiac function,[10] with the most common cause of death being cardiorespiratory failure.[11] Although the pace of symptom progression is heterogenous in the Duchenne population, muscle weakness typically begins between ages 3 and 5 years, with loss of ambulation usually occurring by age 12. Duchenne predominantly affects males, with an estimated incidence of about 16 live male births per 100,000 in the U.S.[12] Rarely, females are also affected by Duchenne, with around 8% of female carriers having some degree of muscle weakness or cardiomyopathy.[13]

The US Food and Drug Administration (FDA) has approved five treatments for Duchenne: deflazacort, eteplirsen, golodirsen, viltolarsen, and casimersen. Deflazacort is a glucocorticoid that was granted traditional approval, while the rest are exon-skipping drug products that were granted accelerated approval based on a mean increase in internally truncated dystrophin[b] production in skeletal muscle (quantified using Western blot). The four exon-skipping products showed a mean change from baseline (% normal dystrophin) of 0.28%–5.3% (0.28% for eteplirsen, 0.92% for golodirsen, 5.3% for viltolarsen, and of 0.8% for casimersen).[14-17] These four latter therapies are indicated for treatment of only a small fraction of Duchenne patients, however, based on the specific genetic subtype studied. Specifically, the approved exon-skipping drugs—eteplirsen, golodirsen, viltolarsen, and casimersen—are indicated only for a combined 29% of all Duchenne patients (13%, 8%, 8%, and 8% of Duchenne patients, respectively – with both golodirsen and vitolarsen being approved for the same subpopulation with gene mutations amenable to exon 53 skipping).[18] The only approved therapeutic option for the remaining 71% of Duchenne patients is deflazacort, as these patients are not eligible for treatment with the approved exon-skipping drugs.

Several types of medical products are currently under development for Duchenne. One direct way of treating this disease would be to restore the expression of dystrophin. Gene therapy based on AAV-mediated delivery of microdystrophin[c] genes has emerged as a promising method, since the gene encoding the full-length dystrophin protein is too large to fit inside a single AAV vector.[7] Microdystrophin genes are designed to be small enough to fit into an AAV vector while retaining the key functionality of the full-length dystrophin protein by being rationally designed to include the most critical protein domains. AAV vectors can transduce cells that are not actively dividing, and they are minimally-integrating, nonpathogenic, and less immunogenic than gene therapies that use other delivery mechanisms.[2] Trials are now studying the safety and efficacy of systemically administered AAV vectors to deliver different forms of microdystrophins to slow or stabilize the loss of muscle function throughout the body.[19]

[b] In this document, the term “internally truncated dystrophin” refers to the de novo dystrophin proteins with intact N- and C- terminal regions, but missing internal regions, which the FDA has accepted as a surrogate endpoint in evaluation of exon-skipping drugs.

[c] Throughout this document we use the term “microdystrophin” to be consistent; the term mini-dystrophin has also been used in other settings or interchangeably.

FDA's Accelerated Approval Pathway

The accelerated approval provisions included in the FDA Safety and Innovation Act (FDASIA), amending section 506(c) of the Federal Food, Drug, and Cosmetic Act (FD&C Act), provide that FDA may grant accelerated approval to:

> "…a product for a serious or life-threatening disease or condition…upon a determination that the product has an effect on a surrogate endpoint that is reasonably likely to predict clinical benefit, or on a clinical endpoint that can be measured earlier than irreversible morbidity or mortality, that is reasonably likely to predict an effect on irreversible morbidity or mortality or other clinical benefit, taking into account the severity, rarity, or prevalence of the condition and the availability or lack of alternative treatments."[20]

FDA has issued final guidance on available programs to expedite the development of drugs and biologics for serious conditions (FDA's Expedited Guidance).[21] In addition to other programs, this guidance discusses the use of the accelerated approval pathway. FDA notes that when a product has received accelerated approval, "FDA has determined that an effect on the endpoint used to support approval—a surrogate endpoint or an intermediate clinical endpoint—is reasonably likely to predict clinical benefit." The guidance acknowledges the risks of the accelerated approval pathway, including that the product may ultimately not show a clinical benefit and that information may be lacking regarding rare or delayed adverse events because the data supporting accelerated approval often include fewer trials of shorter duration with fewer patients. For these reasons, there are specific qualifying criteria for the use of the accelerated approval pathway, namely for drugs "intended to treat a serious condition and that appear to provide a meaningful advantage over available therapy."[21]

Serious Condition

As stated in the FDA regulations in 21 CFR part 312, Subpart E,[22] the Agency has committed to facilitating and expediting the availability of new therapies to patients with serious conditions, especially when no satisfactory alternative therapies exist, while preserving appropriate standards for safety and effectiveness. The Subpart E regulations specifically recognize that patients and physicians "...are generally willing to accept greater risks or side effects from products that treat life-threatening and severely-debilitating illnesses..." than they would for less serious diseases.

In its Expedited Guidance, FDA provides a definition of a serious disease or condition as:

> ". . . associated with morbidity that has substantial impact on day-to-day functioning. Short-lived and self-limiting morbidity will usually not be sufficient, but the morbidity need not be irreversible if it is persistent or recurrent. Whether a disease or condition is serious is a matter of clinical judgment, based on its impact on such factors as survival, day-to-day functioning, or the likelihood that the disease, if left untreated, will progress from a less severe condition to a more serious one."[21]

Duchenne is clearly a serious condition consistent with FDA's definition. Therefore, a drug that is intended to have an effect on Duchenne or on a serious aspect of Duchenne, such as a direct effect on a serious manifestation or symptom, would be eligible for accelerated approval, if other criteria were met.

Meaningful Advantage Over Available Therapy

FDA's Expedited Guidance[21] clarifies the flexibility provided by section 506 of the FD&C Act, as amended by FDASIA, which requires FDA to ". . .tak[e] into account . . . the availability or lack of alternative treatments." Meaningful advantage over available therapies could include "...an alternative therapy with efficacy comparable to available therapy, but with a different mechanism of action, [that] could be of added clinical value in a disease setting in which a significant number of patients may respond differently to the new therapy." In addition, the guidance explains that there are "...situations where a drug could be shown to provide a meaningful advantage over available therapy, including some in which there may not be a demonstrated direct efficacy or safety advantage."[21]

The guidance defines the term "available therapy" as a therapy that "...is approved or licensed in the United States for the same indication being considered for the new drug[d] and is relevant to current U.S. standard of care (SOC) for the indication."[21] It also states that "...a drug would not be considered available therapy if the drug is granted accelerated approval based on a surrogate endpoint or an intermediate clinical endpoint and clinical benefit has not been verified by post-approval studies." Based on these definitions, Duchenne does have available therapy, but the exon-skipping approaches currently approved via the accelerated approval pathway would not be considered available therapy. As a result, Duchenne continues to have a significant unmet medical need.

FDA's Expedited Guidance defines the term "unmet medical need" as "...a condition whose treatment or diagnosis is not addressed adequately by available therapy."[21] Examples provided in the guidance of a new treatment that would address an unmet medical need include a product that:

- "Has an effect on a serious outcome of the condition that is not known to be influenced by available therapy (e.g., progressive disability or disease progression when the available therapy has shown an effect on symptoms, but has not shown an effect on progressive disability or disease progression)
- Has an improved effect on a serious outcome(s) of the condition compared with available therapy (e.g., superiority of the new drug to available therapy when either used alone or in combination with available therapy (i.e., as demonstrated in an add-on study))
- Has an effect on a serious outcome of the condition in patients who are unable to tolerate or failed to respond to available therapy
- Can be used effectively with other critical agents that cannot be combined with available therapy
- Provides efficacy comparable to those of available therapy, while (1) avoiding serious toxicity that occurs with available therapy, (2) avoiding less serious toxicity that is common and causes discontinuation of treatment of a serious condition, or (3) reducing the potential for harmful drug interactions

[d] There may be many approved therapies with varying relevance to how a serious disease is treated in the U.S., including therapies that are no longer used or are used rarely. Only in exceptional cases will a treatment that is not approved for the indicated use or is not FDA-regulated (e.g., surgery) be considered available therapy. In those cases, FDA may consider an unapproved or unlicensed therapy to constitute available therapy if the safety and effectiveness are supported by compelling evidence, including extensive evidence in the literature (e.g., certain well-established oncologic treatments).

- Provides safety and efficacy comparable to those of available therapy but has a documented benefit, such as improved compliance, that is expected to lead to an improvement in serious outcomes…"[21]

AAV gene therapies could provide a meaningful advantage over available therapy, consisting of the regular use of steroids. This includes deflazacort, a corticosteroid that reduces inflammation and activity of the immune system and that improves muscle strength in patients with Duchenne.[23] The ongoing use of steroids carries with it the risk of serious complications such as altered endocrine function, immunosuppression and an increased risk of infection, altered cardiovascular/renal function, and behavioral and mood disturbances.[24] Meaningful advantage over available therapy could be based on effects that target the underlying cause of Duchenne, effects that are superior or additive to those of steroid therapy, or providing treatment options to patients unable to tolerate or unresponsive to steroid therapies.

Endpoints

The final criterion for the use of the accelerated approval pathway is the use of an endpoint, either a surrogate or an intermediate clinical endpoint, that is reasonably likely to predict clinical benefit. FDA's Expedited Guidance states that "...[a]n application for accelerated approval should also include evidence that a proposed surrogate endpoint or an intermediate clinical endpoint is reasonably likely to predict the intended clinical benefit of a drug. Determining whether an endpoint is reasonably likely to predict clinical benefit is a matter of judgment that will depend on the biological plausibility of the relationship between the disease, the endpoint, and the desired effect and the empirical evidence to support that relationship."[21]

A surrogate endpoint used as the basis for accelerated approval is not one that has been validated to show clinical benefit. Validated surrogate endpoints are known to predict clinical benefit and can be used for traditional approval. FDA noted in its Final Rule on the Accelerated Approval Regulations that "...[w]hether a given endpoint is, in fact, reasonably likely to predict clinical benefit is inevitably a matter of judgment. FDA, using available internal and external expertise, will have to make informed judgments in each case presented, just as it does now. The agency acknowledges that there are well-recognized reasons for caution when surrogate endpoints are relied on... A sponsor must persuasively support the reasonableness of the proposed surrogate as a predictor and show how the benefits of treatment will outweigh the risks. Such presentations are likely to be persuasive only when the disease to be treated is particularly severe (so that considerable risk is acceptable) and/or when the surrogate endpoint is well supported. In addition, it will be the sponsor's clear obligation to resolve any doubts as to clinical value by carrying out definitive studies."[25]

This paper will describe the evidence demonstrating that two surrogate endpoints—muscle FF obtained by MR methods and microdystrophin—are each reasonably likely to predict clinical benefit for an AAV gene therapy in the treatment of Duchenne.

Current Outcome Measures

In 2018, FDA issued final guidance on the development of drugs for the treatment of Duchenne and related dystrophinopathies.[26] The guidance notes that "FDA has no defined set of required or recommended clinical outcome measures for studies in dystrophinopathies…" and suggests that existing

or novel outcome measures[e] that can measure clinically meaningful effects may be appropriate. Much work has been done, and more is ongoing, around identifying endpoints that can show meaningful clinical improvements in patients with Duchenne.

North Star Ambulatory Assessment

The North Star Ambulatory Assessment (NSAA) is a test that evaluates 17 activities, each graded on a scale of 0–2, to assess functional motor abilities in ambulatory Duchenne patients. The subjective test is used clinically to evaluate disease progression, within studies documenting natural history, and as part of interventional clinical trials designed to demonstrate efficacy.[27-32] The activities included in the NSAA are those thought to be critical to preserving ambulation, those thought to be most clinically relevant, and those that reflect disease burden.[33-34] When administered with proper training, the NSAA has been shown to be reliable, reproducible, valid, and accessible in various settings.[31,33,35-37] Its use has been validated in patients over age 5, and it can also be used in patients who are 4 years old.[38] A revised version of the NSAA suitable for boys between the ages of 3 and 5 years has been developed and tested, and results suggest that it can "...assess early functional changes and obtain information on how young [Duchenne] boys acquire new abilities with increasing age and how this correlates with their peers."[39] In addition, NSAA has shown consistency across multiple placebo arms, natural history, and real-world data sources in terms of 48-week change after accounting for known prognostic factors.[40] As a part of the postapproval requirements for the accelerated approval of eteplirsen, NSAA is the primary endpoint for the confirmatory trial to verify and describe the predicted clinical benefit.[41]

6-Minute Walk Test

The 6-Minute Walk Test (6MWT) is used to assess the submaximal level of functional capacity by measuring self-paced walking distance, as a reflection of the exertion required for activities of daily living.[42] The subjective test is a global evaluation that incorporates "...all the systems involved during exercise, including the pulmonary and cardiovascular systems, systemic circulation, peripheral circulation, blood, neuromuscular units, and muscle metabolism."[42] Although the 6MWT was not specifically developed for use in patients with Duchenne, it has been adapted and shown to be reliable, valid, and reproducible and can be used to monitor disease progression and as an endpoint in natural history studies and clinical trials for patients with Duchenne.[28-29,31,43-58] In addition, 6MWT has shown consistency across multiple clinical trial placebo arms and natural history data sources in terms of 48-week change.[59] In a regulatory context, the 6MWT is included in the labeling of eteplirsen,[60] is the primary endpoint for the confirmatory trial to verify and describe the predicted clinical benefit for golodirsen[61] and casimersen,[62] and was the basis for EMA's conditional approval of ataluren.[53,63]

Fat Fraction

This section describes the evidence that muscle FF obtained by MR methods meets the criteria of a surrogate endpoint reasonably likely to predict clinical benefit (i.e., improved motor function) in patients with Duchenne. This section describes what FF is, the biological plausibility of its relationship to Duchenne, how it is measured, and the data supporting its use as a basis for accelerated approval.

[e] One example is stride velocity 95th centile measured at the ankle (SV95C). The European Medicines Agency's Committee for Medicinal Products for Human Use (CHMP) has adopted this measure as an acceptable secondary endpoint in pivotal or exploratory drug trials for regulatory purposes, when measured by a valid and suitable wearable device, as an indicator of maximal performance for ambulant Duchenne patients ≥5 years old.

Overview

Muscle FF provides an objective quantitative measure of the extent of muscle replacement by fat in patients with muscular dystrophies, including Duchenne.[64] Muscle FF has been measured and monitored using both magnetic resonance spectroscopy (MRS) and a quantitative imaging approach, referred to as chemical shift-encoded (CSE) or Dixon imaging. MR-measured muscle FF is a sensitive, objective marker for disease progression and surrogate endpoint for studies of patients with Duchenne.[65-66]

Magnetic Resonance Measurements

Muscle FF measured by Dixon imaging is highly correlated with results of MRS over the disease spectrum.[67] Both methods have been shown to be reliable and reproducible across sites.[68-69]

FF measures in patients with Duchenne have shown high intersite and interobserver reliability and repeatability when implementing robust quality assurance procedures. Such measures include rigorous training of each MR operator in standardized procedures, standardization of sequence parameters and spectroscopy voxel or scan location, centralized quality control inspection of the data, monitoring of MR system performance, and centralized data management and data processing.[68,70-71] Similarly, investigators of a multicenter study using standardized MRI and MRS methods across centers to examine lower extremity skeletal muscles in ambulatory children with Duchenne have shown exceptional correlation in quantitative MRI and MRS results both within and between centers.[68]

Clinical Outcome Predicted by Fat Fraction

The evidence outlined in this paper supports that changes in fat fraction of muscle is a surrogate endpoint reasonably likely to predict changes in motor function that reflect a clinical benefit for patients with Duchenne. As noted elsewhere in this paper, postapproval studies will verify and describe the predicted clinical benefit.

Biological Plausibility

Increases in FF of muscles in Duchenne patients reflect key characteristics of disease pathology. The absence of dystrophin leads to the progressive wasting of skeletal muscles that is the predominant pathology in Duchenne.[72] In patients with Duchenne, impaired muscle regeneration leads to degenerating muscle fibers being progressively replaced by fat and fibrotic tissue, resulting in loss of functional muscle tissue.[1,65]

As detailed in studies discussed later, robust evidence suggests that a treatment that slows the increase in FF is reasonably likely to predict clinical benefit and that the predictive value relates to both current functional status and future clinical outcomes. Treatment with corticosteroids, known to be effective in delaying functional decline, results in a meaningful difference in FF.[66,73] This has also been observed with promising experimental treatments.[74-75] Altogether, this evidence leads to the conclusion that a change in FF predicts a change in functional measures.

Rationale for Use as Surrogate Endpoint

In addition to the biological plausibility described above, the use of FF as a surrogate endpoint provides a variety of valuable benefits. It is more sensitive than clinical measures when evaluating disease progression, therefore allowing for reduced trial sample sizes,[65,76] a critically important element for studies in rare diseases.

Unlike some of the functional endpoints currently used in trials, the use of an objective biomarker such as FF eliminates the impact of motivational factors that can influence performance and other biases (e.g., observer or physician observations). Risk of bias is a challenge FDA has identified in guidance regarding the use of these endpoints.[26] As an objectively measured endpoint, FF reduces external factors that can impact the results of clinical outcome assessments.[77-78] Meanwhile, its strong correlation with functional measures provides validity.

The use of this surrogate endpoint also allows for a reduction in study duration. It can predict changes in function (e.g., ambulatory function and loss of ambulation), which allows for optimized clinical trial design.[71,79-82] Moreover, impact from treatment occurs rapidly; studies have shown effects of corticosteroid therapy on FF within 3–6 months.[83]

Finally, FF measurement is a noninvasive approach that can provide critical longitudinal insight into muscle histopathology without the need for repeated muscle biopsies. Biopsies, in addition to being invasive procedures with limits on the amounts of tissue that can be collected and how frequently they can be repeated, can be unreliable due to small sample volumes and the heterogenous nature of fatty replacement and Duchenne histopathology in general.[65] In addition to other advantages mentioned, imaging measures can cover a much larger region of muscle than can biopsies, increasing the likelihood of a reliable result.

Relationship of Fat Fraction to Motor Function

Natural History Studies in Duchenne

Below is a brief overview of the natural history studies in Duchenne, including results derived from placebo arms in interventional studies.

Leg muscle FF in Duchenne patients is higher than that in healthy individuals

Patients with Duchenne have significantly higher FF levels in leg muscles than do age-matched healthy individuals, across age groups.[84-90] For example, vastus lateralis (VL) FF is higher in Duchenne boys compared with healthy boys:

- 27.3% in Duchenne vs. 13.7% in healthy controls (Duchenne patients n=42, mean age 9.9)[91]
- 25.6% in Duchenne vs. 2.3% in health controls (Duchenne patients n=58, mean age 7.5)[92]
- 18.8% in Duchenne vs. 11% in healthy controls (Duchenne patients n=13, mean age 8.8)[93]
- 18.7% in Duchenne vs. 2.4% in health controls (Duchenne patients n=104, median age 8.6)[73]
- 17.4% in Duchenne vs. 4.3% in healthy controls (Duchenne patients n=21, mean age 9.5)[94]

Variation in muscle FF across muscles in Duchenne patients

Cross-sectional studies have shown considerable heterogeneity in muscle FF across leg muscles in patients with Duchenne, consistent with clinical features of disease progression. In general, proximal muscles such as the gluteal and thigh muscles display higher FF values than do the lower leg muscles.[90,95] For example, one study in 20 Duchenne patients ages 5–15 years (mean age 8.6) reported the highest FF in the gluteus maximus at 46.3%, compared with the lowest FF found in gracilis muscle, at 2.7%.[96] In another study of 13 patients ages 6–17 years (mean age 8.9), FF values within the thigh were highest in the gluteus maximus at 52.2% and lowest in the sartorius at 18.5%.[97] A study of 19 patients ages 6–14 years (mean age 10.5) showed that the lateral gastrocnemius muscle had the highest FF at 28.4%, and the posterior tibialis muscle had the lowest FF at 8.2%.[98]

FF increases over time

Muscle FF increases with age in Duchenne patients.[85,93,96-97,99] While most studies have compared baseline FF with the FF value at 1 year, at least one showed that in a group of Duchenne boys ages 5–12.9 years, even a 3-month change in FF was statistically significant.[66]

The rate of the age-related increase in FF appears to vary across different muscles. For example, in the *ImagingDMD* study, the rate of age-related increase (from age 5 to age 16) in VL FF was about twice that in soleus FF based on a cohort of 160 patients.[81]

Some studies have observed or estimated the increase of FF over time; below are several examples:

- An increase of 8.8 ± 11.2%/year for VL FF and 3.1 ± 4.4%/year for soleus FF in 23 patients (mean age ~6 years)[100]
- An increase of 7 ± 7% for VL FF and 3 ± 4% for soleus FF in 12 months in 109 Duchenne patients ages 5–12.9 years (mean age 8.7)[66]
- An increase of 6.9%/year for VL FF in 9 patients ages 4–13 years (mean age 8.6)[101]
- An increase of 5%/year in the FF of major leg muscle groups, including quadriceps femoris and hamstrings, in 20 patients ages 5–23 years (mean age 11.2)[102]

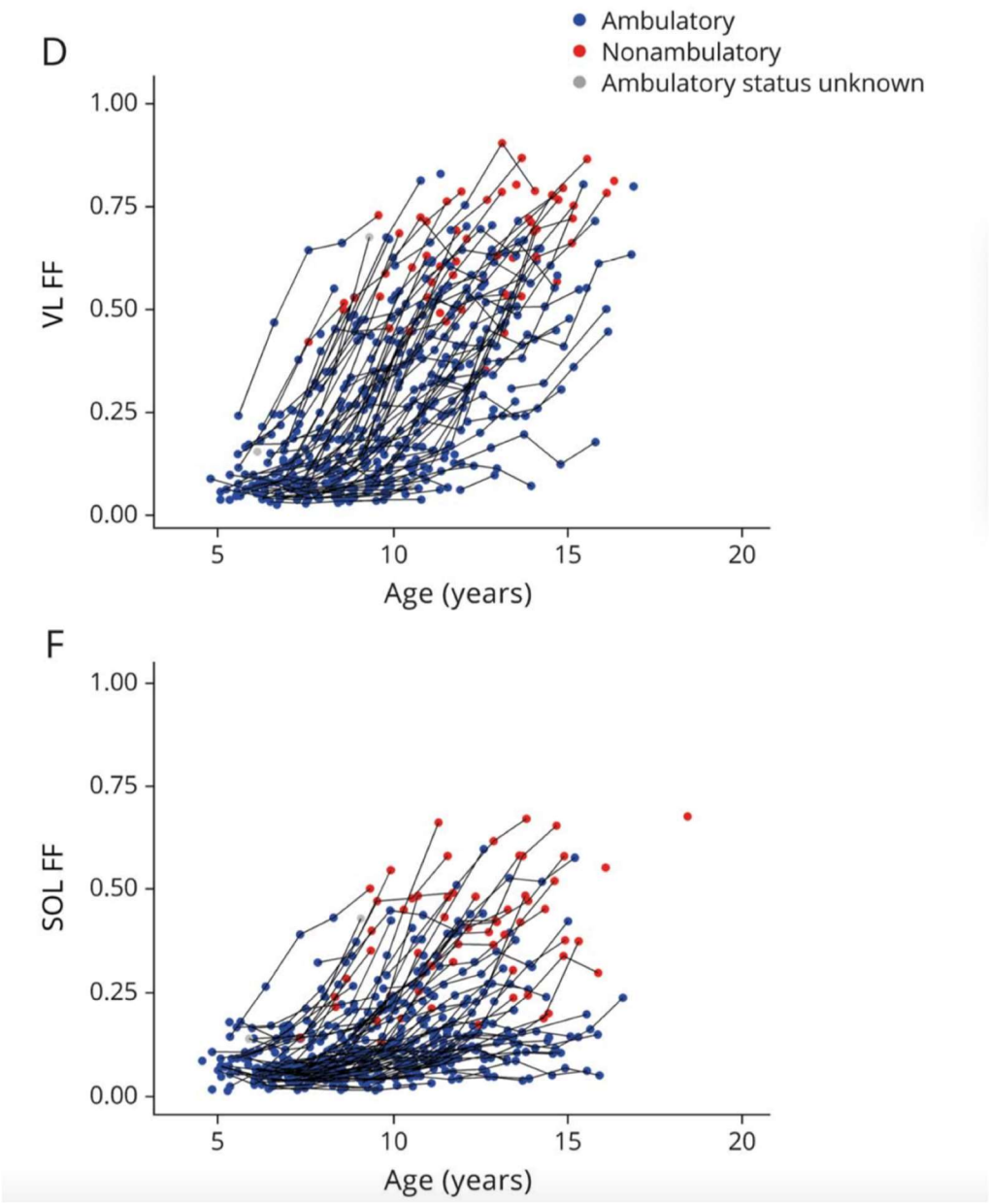
Characterizing the increase in FF over time

Several studies have examined the age-related increase in FF in patients with Duchenne.[71,73,79] For example, a study of 104 Duchenne boys ages 4.2–16.9 years (median age 8.6) showed that the increase in FF follows a sigmoid curve; i.e., FF increased slowly early in the disease when the patients were young, accelerated in preadolescent years, and progressed slowly in later stages of disease.[71,73,79-80,95,103]

A study of 20 Duchenne patients ages 5–23 years (mean age 14.9) reported that ambulant individuals <7 years old had an annual increase of 3.2% in thigh muscle FF, while those >7 years of age (also ambulant) had annual increases of 9.1%.[76]

A longitudinal study over 48 months in 160 patients showed that the average rate of progression over 12 months depends on the baseline FF. Individuals with very low FF (VL FF <0.10) tended to have small increases in FF over the next 12 months (VL FF change <0.05). The largest increases were noted in individuals with a VL FF between 0.10 and 0.50 (mean annual change 0.10) (Figure 1).[81]

Figure 1. Progression of MR-Measured fat fraction (FF) in the vastus lateralis (VL) and soleus (SOL) muscles with increasing age. Reprinted from Barnard et al.[81]



Modeling approaches have been used to characterize the changes in leg muscle FF over time at both the individual and population levels. A nonlinear mixed-effect model based on a normal cumulative distribution function was used to capture the time dependence of muscle FF change in the soleus and VL of 100 Duchenne patients. Disease progression modeling showed that the average age at which half the muscle was replaced by fat occurred 4.8 years earlier in the VL than in the soleus, reflecting more rapid disease progression in VL. The muscle FF disease progression model also showed the effect of chronic corticosteroid treatment on the muscle FF disease trajectory.[73]

Cross-sectional relationship between FF and functional outcomes

Numerous studies have reported an inverse correlation between baseline leg muscle FF and ambulatory functional outcomes commonly used in clinical trials in Duchenne, as discussed below. The strongest correlations are found with FF measures in rapidly progressing proximal muscles.

NSAA

A study of 120 Duchenne patients ages 6–15 showed a correlation coefficient of -0.57 between baseline thigh muscle FF and NSAA score.[104]

In a study of 23 Duchenne patients with a mean age ~6 years, the annualized rate of change in the NSAA score (-4.37 ± 9.29/year) corresponded to the annualized rates of change in VL FF and soleus FF of 8.8 ± 11.2% and 3.1 ± 4.4%, respectively.[100]

6MWT

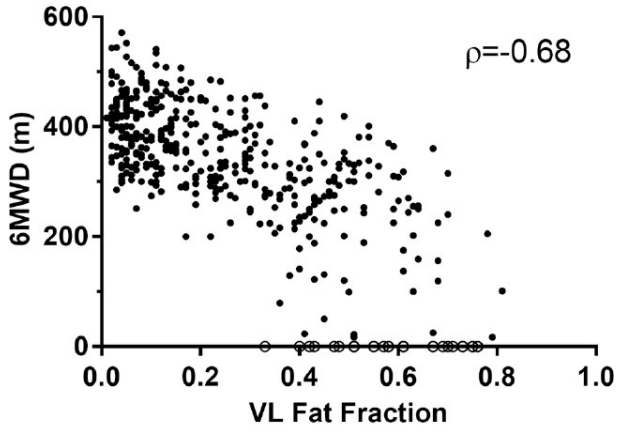
Several studies have reported an inverse correlation between baseline FF and 6MWT. For example, the *ImagingDMD* study found that in 136 Duchenne patients ages 4–14 years, the correlation coefficient between muscle FF and 6MWT was -0.68 for VL FF and -0.59 for soleus FF.[82]

Two studies showed an inverse correlation between thigh muscle FF and 6MWT: a study of 120 Duchenne patients ages 6–15 noted a correlation coefficient of -0.57,[104] and a study of 47 patients ages 6.5–10.8 years (mean age 8.2) reported a correlation coefficient of -0.423.[105]

In a study of 13 Duchenne boys ages 6–14 years (mean age 8.8), the 6MWT was strongly and inversely correlated with FF in all thigh muscles; subjects with ≤20% VL FF walked >400 m, and conversely, those with >20% VL FF walked <400 m.[93]

As shown in Figure 2 below, 6MWT distance decreases progressively with increasing FF, until a certain FF threshold is reached, after which distances drop drastically.[82]

Figure 2. Correlation between fat fraction in the vastus lateralis (VL) and the 6-minute walk test distance (6MWD). Reprinted from Barnard et al.[82]



Loss of ambulation

Sentinel events such as loss of ambulation are important milestones in the progression of Duchenne and are used as endpoints in clinical trials. The *ImagingDMD* study showed that in 136 patients ages 4–14 years, the loss of the ability to perform functional skills was strongly associated with FF in the leg

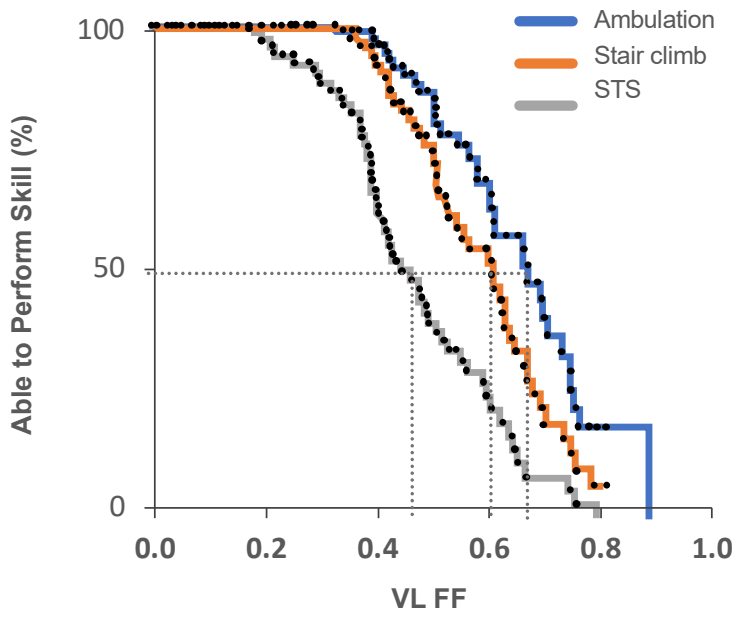
muscles, particularly the VL muscle. Once VL FF was ≥60%, over 50% of participants were nonambulatory.[82]

Similarly, Fischmann et al. reported that loss of ambulation was associated with a mean FF of >50% for the quadriceps, hamstrings, and adductors combined.[102]

Predictive association between FF and future function

Baseline FF levels can serve as a useful prognostic biomarker (e.g., in 12–24 months) as well as loss of ambulation. For example, the *ImagingDMD* study showed that the probability of functional stability or improvement over the next 12 months was >50% in individuals with very low baseline VL FF (<10%), while the probability of declining or losing function over the same period was highest above an FF of 40%.[81] In addition, those with a baseline VL FF <20% were likely to retain the ability to walk, climb stairs, and rise from the floor over the next 12 and 24 months, whereas those with a baseline VL FF >30% were more likely to lose functional ability over 24 months, with >50% of them losing the ability to walk (Figure 3).[81]

Figure 3. Kaplan-Meier plot for loss of 3 functional skills (supine-to-standing [STS], stair climbing, and ambulation) in relation to vastus lateralis (VL) fat fraction (FF). Reprinted from Barnard et al.[81]



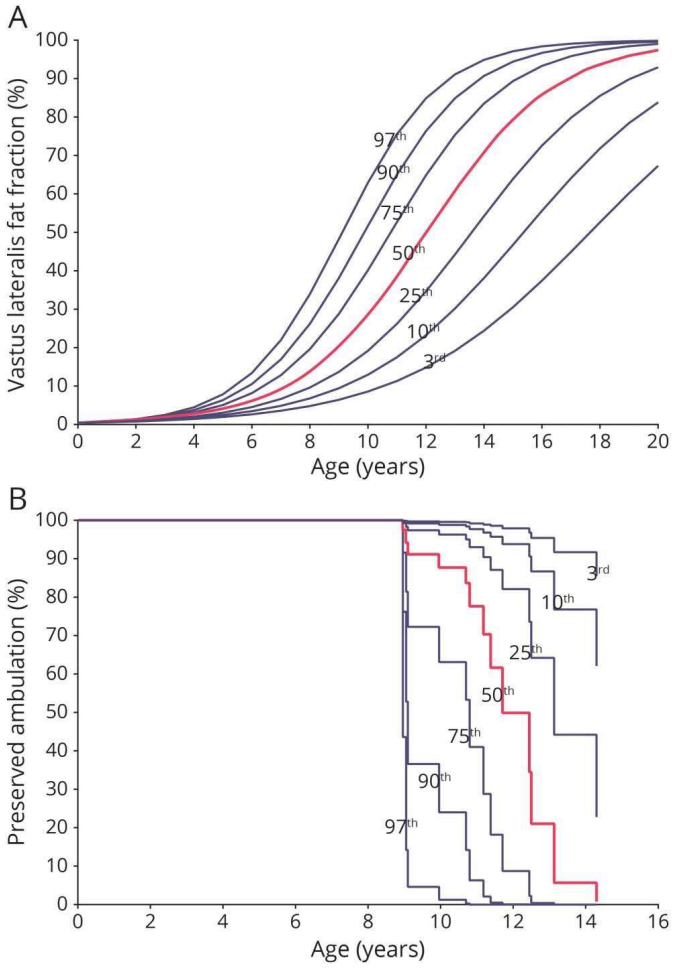
A study of 38 Duchenne patients (mean age 9.2 and 11.2 years across two cohorts) showed that a higher FF at any age increases the risk of losing ambulation, indicating that a higher FF is a risk factor independent of the age of the patient. A hazard ratio analysis showed that a 10% higher VL FF at any age corresponded to a 4.11-fold increase in the instantaneous risk of loss of ambulation.[71]

A study of 20 Duchenne patients (mean age 11.2 years) found that a 50% cutoff for FF (in the quadriceps and hamstrings) predicted loss of ambulation with a sensitivity of 100% and a specificity of 91%.[102]

The magnitude of 12-month change in VL FF has also found to be associated with the likelihood of functional improvement, stability, decline, or loss of ability. More than half of individuals with negligible or small changes in VL FF (change ≤2%) in one study either remained stable or had improved functional test performance over 12 months; in contrast, nearly 90% of those with increases in VL FF >15% declined in function or lost function.[81]

Finally, the *ImagingDMD* study showed that VL muscle FF predicts loss of ambulatory function. A Kaplan-Meier curve for loss of functional skills as a function of VL FF illustrates the range of values over which loss of function is most likely. The odds of losing ambulation within 12 months increased >10 times with a 20% increase in VL FF. Of note, FF provides an added risk over age to lose ambulation. This can be appreciated in Figure 4, which shows that depending on which percentile of FF the patient is, the risk of losing ambulation at any age is increased.[71]

Figure 4. Growth charts and survival curves based on patient data of the relationship between level vastus lateralis fat fraction (A) and preserved ambulation (B) with increasing age. Reprinted from Naarding et al.[71]



Other functional outcomes

Timed functional tests

These tests often include supine-to-standing (STS) time, 10-meter or 30-feet walk/run, and four-stair climbing time. The *ImagingDMD* study found that of the Duchenne patients who remained ambulatory with a VL FF ≥60%, STS time averaged >12 sec, stair climb time averaged >9 sec, and 10-meter walk/run time averaged >11 sec, illustrating significantly diminished function.[82]

Several studies also have reported positive correlations between FF of leg muscles (including VL and peroneal muscles) and time needed to run 10 meters or 30 feet.[87,91,96,104] In addition, a study of 120 Duchenne patients ages 6–15 showed correlation coefficients of 0.53 and 0.60 between thigh muscle FF and STS and four-stair climbing times, respectively.[104]

Motor function scores

The Motor Function Measurement (MFM) is a quantitative scale that measures motor function abilities in persons with neuromuscular disease. It comprises 32 items that assess a range of abilities across three functional domains: standing and transfers, axial and proximal motor function, and distal motor function.[105]

In a study of 20 Duchenne patients ages 5–23 years (mean age 14.9), a strong inverse correlation was found between MFM scores and thigh muscle FF values at baseline and 1-year follow-up, and between the annual changes in MFM and FF.[76]

Two studies reported strong inverse correlations between the D1 component of MFM (standing position and transfers) and the FF of leg muscles (quadriceps and hamstrings) in a study of 20 patients (mean age 11.2 years)[102] and thigh muscle FF in a study of 47 patients ages 6.5–10.8 years (mean age 8.2 years).[106]

In addition, functional grade (as measured by the Brooke scale) was found to be strongly inversely correlated with FF measured across several leg muscles in 9 patients ages 4–13 years (mean age 8.6).[101]

Arm FF in Duchenne

Upper extremity (e.g., forearm, deltoid, and biceps) FF is higher in patients with Duchenne than in healthy participants, and it increases with age.[101,107-110] One study also reported a steeper slope of FF increase in the arms of Duchenne patients after loss of ambulation.[109]

In addition, FF levels in the upper extremities inversely correlate with function. For example, two studies (n=119 and n=22 patients) showed that FF measures in the deltoid and biceps brachii were strongly correlated with the Brooke Upper Extremity scale and the total performance of upper limb (PUL)[110] and with PUL, grip, and pinch.[108]

Two studies (n=25 and n=40 patients) reported inverse correlations between FF in the upper limbs (e.g., flexor muscles of the forearm) and functional outcomes such as grip, pinch, and MFM total score.[80,111]

A study of 20 patients that investigated the relation between hand-to-mouth movement and elbow flexor FF reported a hazard ratio that corresponds to a 3.13-fold increase in the instantaneous risk of loss of hand-to-mouth movement in patients with a 10-percentage point higher elbow flexor FF at any age.[103] Thus, similar to the data shown for the VL, upper extremity muscle FF can predict future function.

Finally, a study of 15 nonambulant Duchenne boys observed a progressive increase in forearm FF over 12 months, reaching significance from 6 months on, accompanied by a significant loss in pinch strength at 6 months and a loss of upper limb function and grip force over 12 months.[107]

Summary of Duchenne natural history data and implications for trial design

- Muscle FF increases with disease progression in patients with Duchenne, with the most rapid rates initially of progression in proximal leg muscles.
- Muscle FF levels in patients with Duchenne are inversely related to muscle function and can predict changes in future function and clinical milestones.
- For clinical trials designed to investigate product efficacy in the treatment of Duchenne, it is scientifically justifiable to recruit patients with a range of muscle FF that corresponds to the accelerated phase of disease progression.
- Compared with functional tests such as 6MWT, FF is objective and more sensitive in detecting disease progression. Thus, the use of FF may reduce the number of participants needed to detect stabilization of disease progression. For example, 13 subjects per group would be needed to detect a 1-year difference in VL FF with 80% power. In comparison, 68 subjects per group would be needed to detect a difference in 6MWT. The difference is most striking in the age group of 7.0–8.9 years: n=9 needed for VL FF vs. n=83 for 6MWT.[66] Others note that thigh muscle FF is the most sensitive and powerful marker of Duchenne disease progression, with a sample size of 4 at 1-year follow-up, followed by the D1 domain of MFM (standing and transfer function) with a sample size of 12.[112]
- Stage of disease and the therapeutic being investigated will influence selection of the muscles that should be evaluated. For example, in younger boys, muscles with fast progression rates such as the VL may be more preferable. In older patients, more slowly progressing muscles may be preferable. Further, although much work has focused on lower extremity muscles in Duchenne, monitoring disease progression in upper extremity muscles may be of greater interest in studies of patients who are late-ambulatory or nonambulatory, as these muscles are still crucial to many basic functions (e.g., eating, hygiene, using a computer, or writing) and thus have important consequences for quality of life. Investigators of a multicenter study have shown progressive involvement of upper extremity muscles (both the deltoid and biceps brachii) in Duchenne and the feasibility of measuring FF to help track disease progression over a wide range of ages.[110] However, successfully measuring arm muscles across multiple sites may present challenges.

Clinical Data

Multiple microdystrophin gene therapy candidates are being evaluated in Phase 1, 2, and 3 clinical studies for systemic treatment of Duchenne. Although the first patients in these studies were dosed within the last few years, encouraging results have emerged that support the potential benefit of treatment, especially compared with the natural history of the disease. As is common in Duchenne trials, the primary endpoints for each of these studies have focused on either the production of microdystrophin, as the primary mechanism of action of a microdystrophin gene therapy, or assessments of motor function. Microdystrophin protein levels have been shown to increase in a generally dose-dependent manner in dose-ranging studies, and motor function has been shown to be either stable or improved across longer-term assessments.

Among the more classical functional endpoints included in the studies, the evaluation of FF has been either listed as a secondary endpoint or reported in preliminary reports by a number of sponsors. Pfizer

was one of the first companies to present 1-year data from their Phase 1b study of PF-06939926, an AAV9 microdystrophin, which Pfizer terms mini-dystrophin. In addition to dose-dependent microdystrophin production, FF levels in treated patients were also decreased when compared with baseline, in contrast to the functional declines observed in an external control cohort.[74] These data support the observed trends in improved motor function, especially as patients were in an age range that would be expected to show both declines in function and increases in FF over a 1-year duration. Sarepta Therapeutics, developing the AAVrh74 microdystrophin candidate SRP-9001, has also presented and published data on their Phase 1/2 study. At the single dose level evaluated, treated patients had better motor function and lower FF levels in leg muscles at the 1-year timepoint compared with natural history.[75] Although these results were not compared with baseline assessments and patients were generally younger, the low levels of FF observed at the posttreatment timepoint supports the overall improvements in motor function and muscle pathophysiology described as a potential result of treatment. Additional sponsors, such as Solid Biosciences, have included the assessment of skeletal muscle MRI in their study protocols but have not yet shared data. The older ages of patients included in such studies may provide important information related to the expected changes in FF levels with treatment over time and to differences observed as a result of age. In addition to the early-stage studies, Phase 3 studies are also underway. These could provide additional information in blinded, placebo-controlled settings about the differentiation of these therapies compared with well-matched control subjects.

Prior to the evaluation of microdystrophin gene therapies, additional therapeutic approaches were evaluated for Duchenne that, while failing to meet their primary endpoints, provided data on the natural progression of FF within this population and utility of its assessment in clinical trials. Pfizer's domagrozumab, a monoclonal antibody therapy designed to inhibit myostatin, included FF as a secondary endpoint along with multiple additional MR assessments. Recent publication of the results from the placebo cohort in this study showed that efficient protocols were established and maintained across sites around the globe, and an inverse correlation between changes in FF and motor function was noted. The authors concluded that the results supported the use of MRI as a biomarker of disease progression for Duchenne.[104] Similarly, a study by Catabasis of the NF-κB inhibitor edasalonexent showed that MRS-based measurements of FF in lower limbs of control patients showed increases consistent with natural history, and corresponding decreases in motor function.[100] A study of corticosteroid therapy in Duchenne found that over 1 year, corticosteroid-naive boys (n=6, mean age 6.4) had greater increases in FF in both the VL and soleus muscles compared with boys receiving corticosteroid treatment (n=9, mean age 6.2). In addition, boys receiving corticosteroid treatment showed greater knee extensor muscle strength and better performance on the 10-meter walk, supine to standing, and stair-climbing tests compared with corticosteroid-naive boys; however, ankle plantar flexor peak torque and the average 6MWT did not differ between the two groups.[83]

Together, these results provide evidence that FF could be used effectively across sites through establishment of defined protocols and centralized analyses, and they support the results of natural history studies in both open-label and blinded, placebo-controlled clinical trials in characterizing increases in FF that inversely correlate with functional declines.

Conclusions for Appropriate Use of the Surrogate Endpoint Fat Fraction

The use of FF as a reasonably likely surrogate endpoint is supported by the evidence provided above. The data suggest that FF could be used as a surrogate endpoint to support accelerated approval for patients with Duchenne. Specifically, clinical trials could be designed to:

- enroll and recruit patients with a range of muscle FF that corresponds to accelerated phase of the disease progression;
- assess FF levels in appropriate muscles in patients with Duchenne for disease progression; and
- show an effect of a therapeutic on FF (e.g., change in trajectory or stabilization of disease progression).

Currently, most data have expanded our understanding of changes in the upper leg over 1 year, specifically in the VL. Additional data are being collected that can broaden the use of FF for assessment during other points of disease burden for patients with Duchenne. For example, this could include using other muscles (e.g., the soleus and muscles of the upper extremities) that are impacted later in the course of disease. In addition, the *ImagingDMD* program at the University of Florida is developing a modeling-based clinical trial simulation (CTS) tool that focuses on MRS-based muscle FF and functional outcome measures in Duchenne. In partnership with the Critical Path Institute's Duchenne Regulatory Science Consortium, this drug development tool is being evaluated via the FDA's Fit-for-Purpose initiative. When complete, this CTS tool will be made publicly available and will enhance efforts by sponsors and investigators to improve patient selection and optimize clinical trial design in Duchenne.

Microdystrophin

Overview

As mentioned previously, it is not possible to administer the full-length dystrophin gene in an AAV vector to treat patients with Duchenne because the size of its coding sequence (about 11.5 kb) greatly exceeds the approximately 5-kb AAV packaging capacity. This problem was solved with development of microdystrophin genes constructs <4 kb, created to be shortened but functional versions of the full-length dystrophin.[19] These constructs include genetic sequences that have been curated to include coding for elements identified as most critical from a full-length dystrophin. Various gene therapy candidates aim to improve the Duchenne phenotype by delivering vectors expressing microdystrophin complementary DNA (cDNA), which will in turn produce a microdystrophin protein that will function similarly to full-length dystrophin by linking the subsarcolemmal cytoskeleton with the extracellular matrix and recruiting primary members of the dystrophin-associated protein complex (DAPC) to stabilize the muscle. The rationale is that production of microdystrophin will result in improved muscle function, just as production of internally truncated dystrophin from the four products granted accelerated approval by FDA for patients with Duchenne was considered reasonably likely to predict clinical benefit. For the purpose of this white paper, unless otherwise noted the terms "dystrophin" and "microdystrophin" refer to the proteins, not the gene or construct (e.g., microdystrophin construct, microdystrophin cDNA).

Clinical Outcome Predicted by Microdystrophin

The evidence outlined in this paper supports the use of expression of microdystrophin in muscle as a surrogate endpoint reasonably likely to predict changes of muscle function that reflect a clinical benefit for patients with Duchenne. As noted elsewhere in this paper, postapproval studies will verify and describe the predicted clinical benefit.

Biological Plausibility

As FDA has repeatedly asserted, "...[t]he role of dystrophin is well-characterized in the pathophysiology of DMD."[f] Dystrophin is a critical muscle protein that is reduced or absent in patients with Duchenne. Dystrophin's critical role is linking the subsarcolemmal actin cytoskeleton to the extracellular matrix via the DAPC.[113-114] This link helps reduce muscle stress generated during contraction, while its absence leads to muscle cell injury, subsequent degeneration, and a gradual loss of muscle cells.[115]

Microdystrophin gene constructs produce versions that are shorter than the full-length dystrophin normally produced endogenously, yet they remain functional. The expressed microdystrophin is considered therapeutic when the expression is durable and the protein is appropriately membrane-localized, recruits members of the DAPC, and stabilizes or increases muscle force generation, resulting in increased muscle strength and prevention of muscle cell necrosis.[116-119]

Protein size alone does not always correlate with the clinical phenotype in muscular dystrophies. In fact, some individuals with shorter dystrophin (due to large deletions involving multiple exons) have milder diseases compared with those with longer dystrophin.[120] In addition, disease severity of dystrophinopathy is determined not by the size of the gene but rather by the quantity, as well as the quality, of the dystrophin produced. Specifically, patients with severe Duchenne have <5% of the normal quantity of dystrophin, whereas patients with dystrophin levels between 5% and 10% of normal, regardless of protein size, have an intermediate phenotype [mild Duchenne or severe Becker Muscular Dystrophy (Becker)]. In contrast, patients with mild to moderate Becker phenotype usually have protein levels above 20%.[121]

Regulatory History of Shortened Dystrophin as an Endpoint for Accelerated Approval

FDA has accepted a statistically significant increase in a shortened version of dystrophin as a surrogate endpoint supporting accelerated approval of four products thus far:

- Exondys 51 (eteplirsen), Sept 2016
- Vyondys 53 (golodirsen), Dec 2019
- Viltepso (viltolarsen), Aug 2020
- Amondys 45 (casimersen), Feb 2021

For each of these four accelerated approvals, FDA relied upon demonstration of a small increase in de novo (internally truncated) dystrophin protein in skeletal muscle. Although there was public disagreement about whether the magnitude of expression was meaningful, the consensus was that increased dystrophin production in skeletal muscles was reasonably likely to predict clinical benefit and therefore was an acceptable surrogate endpoint for accelerated approval. Thus, the production of a shortened version of dystrophin resulting from treatment is established as an FDA-accepted surrogate endpoint to support accelerated approval of a product intended to treat Duchenne.

Rationale for Use as a Surrogate Endpoint

In addition to the regulatory precedent and biological plausibility described above, the use of microdystrophin as a surrogate endpoint provides a variety of valuable benefits. Unlike functional endpoints currently used in trials, microdystrophin—as an objectively measured endpoint—reduces

[f] FDA has repeatedly noted this in its summary reviews for the four Duchenne products granted accelerated approval, which rely on increased production of an internally truncated version of dystrophin protein.

external factors that can impact the results of clinical outcome assessments, a challenge FDA has identified in guidance regarding the use of these endpoints.[26,77-78] Additionally, the use of this surrogate endpoint allows a reduction in study duration. Clinical studies have reported that protein expression has been seen as early as 2 months after treatment with increased expression at 12 months, supported also by previous preclinical work.[118,122-126]

In addition, because all microdystrophin constructs used in clinical evaluation have been designed to produce controlled and consistent protein expression across muscle cells, the similarities between microdystrophins are closer than the variations induced by mutations in exon-skipped internally truncated dystrophin. For example, the eteplirsen approval was based on six different Duchene mutations amenable to exon-51–skipping therapy, although additional mutations are known to exist. FDA noted that there may be some differences in functionality of the protein produced from exon-51–skipped transcripts, and that the different internally truncated dystrophins produced in patients with different mutations could also confound interpretation of possible effects on clinical course based on differences in dystrophin levels.[127-128] As an example, while exon skipping typically removes a single exon from the transcript, each spectrin-like repeat (SR) in the dystrophin rod domain is encoded by about two exons. Thus, in many cases when an exon is skipped, the encoded protein carries only part of one of the SRs, which can impact the stability of the resulting internally-truncated protein.[117,129] More stable proteins result from maintaining properly phased SRs, which has become a gold-standard design strategy to produce the synthetic microdystrophins.[117]

Rational Design of Functional Microdystrophin

The rational design and development of functional microdystrophins has been based on naturally occurring mutations in BMD patients and repeated studies in Duchenne animal models, in which nonfunctional microdystrophin constructs are readily distinguished from functional versions through durability of expression and improved functional outcomes.[116-118,130]

Preclinical Studies

Published studies have consistently shown that restoring dystrophin in muscles improves muscle morphology, strength, and resistance to contraction-induced injury. This was initially shown in 1993 by Cox et al., who expressed a full-length dystrophin in striated muscles of transgenic *mdx* mice.[131] In treated mice, expression levels up to 50 times normal restored DAPC localization, muscle histology and strength without any associated toxicity. This proof-of-concept study revealed that gene therapy had the potential to treat patients with Duchenne.

Similar results were obtained in 1993 and 1995 by different groups. First, minimized dystrophin was delivered by adenovirus-mediated transfer to *mdx* mice intramuscularly. In this study, expression of the truncated protein protected the fibers efficiently against the muscle degradation process.[132] Then two groups generated additional transgenic *mdx* mice expressing either full-length or Becker-like dystrophins, the latter of which were based on a patient with a 1 MB deletion (of exons 17–48) and who remained ambulatory until his death in his late 70s.[120] Very mild phenotypes have been associated with deletion of exons 13–48, an even larger deletion.[133] In these studies, dystrophic pathology was eliminated in muscles that expressed ≥20% of normal levels of full-length dystrophin, and higher levels of uniform expression did not further increase strength beyond normal.[134-135] The Becker-like dystrophins did not restore quite as much strength as the full-length dystrophin did, although they completely halted ongoing necrosis and regeneration and led to largely normal muscle morphology.[136]

These results provided the basis to develop even smaller microdystrophins with normal SR repeat phasing that could be administered using AAV vectors.

The first studies of functional microdystrophins small enough to be carried by AAV vectors were published starting in 2000. These microdystrophins are encoded by cDNAs smaller than ~4 kb.[117,137] In one study, three different microdystrophins were expressed in *mdx* mice by intramuscular injection of AAV vectors. All three of these vectors produced microdystrophins, although in different amounts, and improved muscle morphology. Since delivery was localized, no measurements of strength were performed.[137] In the second study, a variety of full-length dystrophins and microdystrophins were compared in transgenic *mdx* mice, and three different microdystrophins were further analyzed after intramuscular injection into a single muscle of *mdx* mice.[117] As in earlier studies, the larger dystrophins were often, but not always, better at restoring normal strength, although all showed a significant benefit.[117] With microdystrophins, maximally functional activity came from designs that preserved SR phasing and that favored the use of subdomains normally adjacent to each other in the native protein. These smaller proteins also retained the major protein-interaction domains within dystrophin and were assessed in multiple muscles and at various ages of the dystrophic mice. The critical elements identified in these studies are all incorporated in the design of the microdystrophins currently in clinical development. Finally, an earlier study tested IM injection of vectors carrying between 1 and 3 SR domains, but these constructs had minimal functional benefit leading to the conclusion that a minimum of 4 SRs is needed for significant functional benefit.[117,137-139] Together these various studies highlighted both the striking functionality of small dystrophins and that expression up to normal levels improved or eliminated dystrophy. In addition, these studies were the basis for identifying critical domains needed for functional microdystrophin constructs.

Subsequent studies by many groups moved towards testing systemic delivery of AAV vectors expressing various microdystrophin in striated muscles of *mdx* mice, and later, the canine model of Duchenne. These studies showed the ability of microdystrophin in adult mammals to prevent and reverse pathology and increase strength.[140] The degree of phenotypic improvement was also shown to depend on the dose of AAV vector delivered (and hence the amount of microdystrophin produced). Early systemic AAV delivery studies revealed the ability to deliver microdystrophin gene constructs to all striated muscles in a dose-dependent manner.[123,141] Lower doses led to dystrophin expression in a mosaic pattern, which partially improved morphology and strength, whereas higher doses led to more uniform levels of dystrophin and a more complete rescue of the dystrophic phenotype in mice.[123,142] Phenotypic reversal was also observed in old *mdx* mice (up to 2 years old).[118,143-145] Microdystrophin has been similarly effective in canine models of Duchenne.[122,125,146-149]

Critical elements of functional microdystrophin

The ability to express functional microdystrophins from coding sequences small enough to be carried by AAV vectors is based on 1) studies of deletions that removed various domains from dystrophin and their effects on functionality of the protein, as discussed under Biological Plausibility, and 2) the knowledge gained from natural deletions occurring in Becker muscular dystrophy patients.[150] The critical functional elements of dystrophin are described below:

1. <u>N-terminal actin-binding domain</u>
   Truncations of the N-terminal actin-binding domain (N-ABD) have not resulted in favorable outcomes; consequently, the entire actin-binding domain (ABD) is retained in all microdystrophins constructs. The primary reason is that such truncations greatly reduce the

stability of dystrophin.[151-156] Dystrophin also has a central ABD,[157-159] and as long as the N-ABD is present and functional, the central ABD can be deleted.[117,151,157,160-161]

2. Cysteine-rich/beta-dystroglycan–binding domain
   The beta-dystroglycan–binding domain (Dbd) is composed of a WW domain within hinge 4 and the cysteine-rich domain.[150,162-165] Essentially all mutations or deletions in this Dbd have been observed to inactivate dystrophin completely; hence this region is present in all functional microdystrophins constructs.[129,166-169]
3. Rod domain with hinge 1, hinge 4, and at least four SRs
   Shortened dystrophins lacking all SRs are nonfunctional.[117] Hinge 1, hinge 4 and at least four SRs are needed to prevent dystrophy and increase strength in *mdx* mice.[117-118,130,137-139,170-171] All microdystrophin and mini-dystrophin constructs in clinical testing have either four or five SRs.
4. Choice and relative order of SRs
   The various microdystrophin constructs in clinical testing with AAV vectors carry either four or five SRs.[117-118,130,137,170,172-173] Since full-length dystrophin has 24 SRs, many combinations of SRs can be used to generate a protein expressed by a coding sequence small enough for packaging into AAV vectors.[174] Most microdystrophin constructs published to date have retained the first and last SRs, as these blend the central rod domain into the adjacent ABD and Dbd domains and are presumed to have a unique structure. Together these studies indicate that several combinations of four or five SRs can generate microdystrophins that are stable and that support normal muscle function.[118]

Relationship of Microdystrophin to Muscle Function

Several clinical studies have quantified microdystrophin in muscle biopsies from patients who received AAV-microdystrophin therapy. The expressed microdystrophin was appropriately membrane-localized and recruited members of the DAPC, demonstrating the clinical proof of concept for delivering functional microdystrophin. For example, interim results were presented from an ongoing Phase 1/2 study of a single infusion of SGT-001 (an investigational AAV-microdystrophin gene therapy) at a dose of 5×10^{13} or 2×10^{14} vg/kg. In three patients receiving the higher dose, biopsies of skeletal muscle taken 3 months later showed widespread distribution of microdystrophin-positive muscle fibers with colocalization of neuronal nitric oxide synthase (nNOS) and β-sarcoglycan.[175] Long-term biopsy data collected from these three patients, taken 2 years, 1.5 years, and 1 year after dosing, indicate evidence of durable and widespread expression of the microdystrophin.[176] In addition, the NSAA score was stable with minimal change and the 6MWT distances were maintained at 1.5 years after treatment, suggesting clinical benefit compared with trajectories typically observed in natural history cohorts. Clinical data available at 24 months following infusion revealed continued patient benefit in maintaining motor function, as assessed by 6MWT and NSAA, when compared to natural history declines.[177]

One-year data from a Phase 1b study of an investigational AAV9 mini-dystrophin gene therapy (PF-06939926) showed that for the three patients in the low-dose cohort (1×10^{14} vg/kg), the mean proportion of muscle fibers expressing dystrophin was 28.5% at 2 months and 21.2% at 12 months after dosing.[74] For the 6 patients in the high-dose cohort (3×10^{14} vg/kg), these measures were between 48.4% and 50.6% (n=3), respectively. The patients also showed a functional improvement from baseline NSAA scores after 1 year compared with an external control group: an increase of 1 point for the study patients (n=19) vs. a median loss of 4 points for the external control group (n=66; $p<0.005$).[122]

Finally, in a Phase 1/2a study, four Duchenne patients received a single dose of 2×10^{14} vg/kg recombinant AAVrh74-microdystrophin gene therapy. Transduction was confirmed in all patients,

indicating successful delivery to skeletal muscle. At 12 weeks after treatment, 81.2% of muscle fibers expressed microdystrophin with a mean intensity of 96% at the sarcolemma. Western blot showed a mean expression of 74.3% without fat or fibrosis adjustment and 95.8% with adjustment. Microdystrophin expression also resulted in an increase in β-sacroglycan, a critical component of the DAPC, suggesting that microdystrophin can promote restoration and reconstitution of the DAPC. In addition, functional outcomes (e.g., NSAA score) were improved in these patients up to 1 year after treatment.[126]

Quantity and Distribution of Microdystrophin

Several factors must be considered when assessing microdystrophin expression, including quantity produced, distribution to skeletal muscles throughout the body, and distribution amongst muscle cells within a given muscle type. The exact quantity of dystrophin or microdystrophin required to improve clinical phenotype is not known. Levels of dystrophin in healthy humans vary widely, with differences of 3- to 5-fold observed across sampled levels.[178-179] Additionally, some deletion mutations, such as exons 3–7 or 3-9 deletion, can lead to a low-level expression of shortened dystrophin with a milder phenotype, implying that any increase in internally truncated dystrophin could be beneficial.[180-183] Animal studies have also shown improvements in muscle function when dystrophin levels were increased from low levels.[184] While not curative, these improvements might affect patient quality of life and stabilize disease progression, both of which would be clinical benefits for patients living with Duchenne.

In addition to the total quantity of expressed dystrophin or microdystrophin, the importance of the distribution of such expression is not fully understood. For example, the clinical benefit of 10% of fibers positive (on tissue section) with 50% total dystrophin (in whole muscle lysate) versus 50% fibers positive (on tissue section) with 10% total dystrophin (in whole muscle lysate) is not known. Studies in transgenic *mdx* mice expressing full-length dystrophin or microdystrophins, or in *mdx* mice injected with an AAV-microdystrophin vector, have revealed that some protection from dystrophy occurs even if expression is nonuniform, i.e., mosaic.[117,134,136,185] However, these studies also reveal that uniform expression is more protective than mosaic expression. Further, low-level expression of microdystrophin in a uniform pattern tends to be more protective against necrosis than high-level mosaic expression.[134,184,186-190]

Another consideration is body-wide distribution. In nonclinical studies, multiple tissues can be evaluated to interrogate distribution of expression. AAV-microdystrophin gene therapy treatment in animal models has been shown to result in robust body-wide protein expression. It is not feasible or ethical to perform biopsies on multiple muscles to clinically assess systematic expression in patients with Duchenne. Preclinical data have shown the presence of microdystrophin in skeletal and cardiac muscles after administration of AAV microdystrophin gene therapy in both dystrophic dogs and mice, confirming body-wide expression. Data from dystrophic dogs show protein expression in both skeletal and cardiac muscle.[125,148-149,] Data from dystrophic mouse models reinforce the findings of widespread protein expression seen in dystrophic dogs.[123,137,139,141,144-145,173, 191] Similarly, skeletal and cardiac expression was seen with administration of AAV reporter gene in a dystrophic dog model.[125]

Biopsy and Measurement Methods

A muscle biopsy is required to quantify dystrophin and microdystrophin levels in Duchenne patients. These biopsy and assay methods have been used not only in clinical practice but also as the basis for approval for treatments for Duchenne. The techniques for obtaining, freezing, and handling samples to ensure that the tissue is appropriate for analysis have been well documented.[192-193] Techniques have improved in recent years, limiting the amount of tissue needed to reduce the burden on patients.

Multiple techniques, beyond the scope of this paper, have been developed to characterize and quantify dystrophin and microdystrophin. These include Western blot (WB), capillary-based WB, mass spectrometry (MS), and immunofluorescence staining (IF), and they each provide critical yet limited information about the presence of dystrophin or the transduced microdystrophin. Collectively, they can provide complementary evidence of the appropriate localization of microdystrophin (IF), protein integrity and relative quantitation (WB, capillary-based WB), and absolute amount (MS).[179,194-198] All validated techniques have demonstrated dystrophin or microdystrophin expression, dose response, and sarcolemmal localization.

Conclusions for Appropriate Use of the Surrogate Endpoint Microdystrophin

The use of microdystrophin as a reasonably likely surrogate endpoint is supported by the evidence provided above. The use of microdystrophin is based on the biological relationship and the primary defect of patients with Duchenne, which is the lack of dystrophin. Microdystrophin is rationally designed to incorporate critical elements of a full-length dystrophin protein so that it is functional, correctly localized to the muscle cell membrane, recruiting members of the DAPC, and stabilizing the muscle. Based on preclinical and clinical data, microdystrophin can be evaluated as a surrogate endpoint as early as 2 months after dosing to support accelerated approval for patients with Duchenne.

Supportive Evidence for Accelerated Approval

In 2019, FDA issued a draft guidance, Demonstrating Substantial Evidence of Effectiveness for Human Drug and Biological Products (Substantial Evidence Guidance).[199] In the *Federal Register* notice accompanying this guidance, FDA acknowledged that "FDA must reach the conclusion that there is substantial evidence of effectiveness to approve a drug; however, the degree of certainty supporting such a conclusion may differ, depending on clinical circumstances (e.g., severity and rarity of the disease and unmet medical need)."[200]

In the case of accelerated approval, there must be evidence supporting a determination that a surrogate endpoint is reasonably likely to predict clinical benefit, as well as substantial evidence of effectiveness based on a treatment effect on that surrogate endpoint. But as the preamble to the final rule on accelerated approval notes, reliance on a surrogate endpoint "...almost always introduces some uncertainty into the risk/benefit assessment, because clinical benefit is not measured directly and the quantitative relation of the effect on the surrogate to the clinical effect is rarely known."[201]

This section addresses how additional data, here referred to as supportive evidence, can provide support to allow a conclusion of substantial evidence of effectiveness. FDA has stated that it is a matter of judgment whether an endpoint is reasonably likely to predict clinical benefit. This judgment considers factors including the biological plausibility of the relationship between the disease, the endpoint, and the desired effect and the empirical evidence to support that relationship. Similarly, supportive evidence can leverage other measures (e.g., biomarkers) that can provide further confidence that the treatment effect seen with a reasonably likely surrogate endpoint is substantial evidence of effectiveness. For example, supportive evidence can include objective measures or other endpoints that are not necessarily clinical benefits included in clinical trials as secondary or exploratory endpoints. These endpoints do not have to be prespecified or statistically significant, as they are intended to provide more confidence surrounding the accelerated approval.

While this paper does not discuss specific endpoints that could be considered for supportive evidence to include in a BLA, resources are available. In 2014, Parent Project Muscular Dystrophy (PPMD) issued a guidance to assist sponsors in developing medical products for the treatment of Duchenne.[202] In 2018, FDA issued a guidance for developing treatments for Duchenne and other related dystrophinopathies.[26] Both of these documents discuss endpoints that could be useful in demonstrating effectiveness in different stages of disease and could also be considered supportive evidence. In addition, PPMD has an ongoing project to update its 2014 guidance to better reflect emerging science related to disease progression and clinical development, including AAV gene therapy candidates for patients with Duchenne.[203] When completed, this updated guidance will provide an additional resource for sponsors to better identify potential endpoints that could provide supportive evidence as part of a BLA submission.

Postapproval Studies to Verify and Describe Clinical Benefit

All products granted accelerated approval are subject to certain requirements, which may include postapproval studies[g] to verify and describe clinical benefit.[204-206] The importance of postapproval studies being conducted with due diligence, as required by the law and regulations,[205-209] is paramount to the success of the accelerated approval program. Sponsors must ensure that the required postapproval studies are well designed and completed in a timely manner. These trials are intended to verify and describe the clinical benefit of the product or indication, particularly important in areas such as gene therapy, where exposure is by definition long-term.

FDA's Expedited Guidance discusses various approaches that can be used to verify and describe clinical benefit in postapproval studies. Most often, these trials should enroll the same patient population as the preapproval studies and evaluate an endpoint that directly measures clinical benefit. In Duchenne, proposed surrogate endpoints such as microdystrophin and FF obtained using MR methods could be used to support accelerated approval, and measurements obtained later in the same population during postapproval studies could be used to verify and describe the anticipated clinical benefit. It is possible to use the same trial that supported accelerated approval to verify and describe clinical benefit, which may also allow this part of the trial to be nearly complete at the time of the accelerated approval. However, the protocol and statistical analysis plan should clearly account for an analysis of the surrogate endpoint data being used to support the accelerated approval, with continuation of the trial to obtain data on the clinical endpoint that will be the basis for verifying the clinical benefit.

When this is not possible (e.g., due to commercial availability after accelerated approval, which has, in some cases, made enrollment challenging), a different but related population may be used, although this may impact the indication and population ultimately granted traditional approval. This could include patients at a different stage of disease process. For example, if the original population studied in the trial that resulted in accelerated approval was ambulatory, further studies to verify and describe the anticipated clinical benefit could enroll nonambulatory patients.

[g]In this paper we use the term "postapproval studies" for those required after a product has been approved under the accelerated approval pathway, to verify and describe the predicted effect on irreversible morbidity or mortality or other clinical benefit. This terminology is consistent with 21 U.S. § 356(c). We note, however, that other terminology is used in FDA's regulations at 21 CFR 314.50 and 21 CFR 601.41 ("postmarketing studies" and "postmarketing clinical study") and in FDA's 2014 Guidance for Industry Expedited Programs for Serious Conditions – Drugs and Biologics ("postapproval confirmatory trials", "confirmatory trials", "postmarketing confirmatory trials", "postmarketing studies or trials", and "postapproval studies or trials").

If a gene therapy is designated as Regenerative Medicine Advanced Therapy (RMAT), postapproval requirements may be met by "...postapproval monitoring of all patients treated with such therapy prior to approval of the therapy, ..." [210] among other methods, such as the use of real-world evidence (RWE) not explicitly available to other products.

As noted, postapproval studies must be well-designed and completed in a timely manner. FDA's Expedited Guidance states that the FDA and the sponsor should agree on the design and conduct of any postapproval studies before approval, that the protocol should be developed as early as possible, and that timelines for the trial should be specified, including (for example) enrollment and trial completion. In addition, FDA states that sponsors that are planning to seek accelerated approval before submission of the marketing application should have postapproval studies underway at the time the marketing application is submitted.

Gene therapies approved using the accelerated approval pathway have the opportunity to leverage long-term follow-up (LTFU) studies—required to identify and mitigate long-term risks associated with the therapy—to also verify and describe the predicted clinical benefit. As discussed in FDA's guidance on this topic, the "...LTFU protocol for gene therapy trials is primarily designed to capture delayed adverse events in study subjects as well as to understand the persistence of the GT product. As a sponsor, you may consider designing the LTFU protocol to assess the long term clinical efficacy, and durability of your product."[211] This approach will allow efficient assessment of patients for both safety and efficacy and help ensure timely completion of postapproval studies required under accelerated approval.

Both the law and regulations allow expedited withdrawal of approval if an applicant fails to conduct a required postapproval study with due diligence, the postapproval study fails to verify the predicted clinical benefit, or other evidence shows that the product is not safe or effective under the conditions of use.[208-209,212] Withdrawal of an accelerated approval may be initiated by the Agency[208-209,212] or by the applicant.[213-214] Indeed, the integrity of and confidence in the accelerated approval program rests on the assurance of not only timely completion of postapproval studies but also the prompt withdrawal from the market if clinical benefit is not confirmed. Therefore, applicants must embrace these responsibilities and conduct adequate and well-controlled postapproval studies with due diligence, and request withdrawal of the accelerated approval immediately if they become aware that the postapproval study has failed to verify the predicted clinical benefit or when they become aware of other evidence showing that the product is not safe or effective under the approved conditions of use.

Benefit-Risk Framework Considerations

All FDA approvals of marketing applications for new drugs and biologics, including accelerated approvals, are based on a benefit-risk assessment. This assessment considers the evidence of safety and effectiveness submitted in an NDA or BLA, as well as factors such as the nature and severity of the condition intended to be treated or prevented, available therapies for the condition (and benefits and risks associated with them), and potential risk-management tools that might help ensure that benefits outweigh a product's risks. When potential serious safety risks are identified or expected to exist, a product's benefits and risks must well characterized, and FDA must determine that the benefits to the indicated population are likely to outweigh these risks. FDA employs a Benefit-Risk Framework to identify and assess the important issues and considerations of the requisite benefit-risk assessment (Figure 5).[215]

Figure 5. FDA's Benefit-Risk Framework for New Drug and Biologic Review[215]

Dimension	Evidence and Uncertainties	Conclusions and Reasons
Analysis of Condition		
Current Treatment Options		
Benefit		
Risk and Risk Management		
Conclusions Regarding Benefit-Risk		

AAV gene therapy has some unique factors that should inform the benefit-risk determination. For example, due to naturally occurring anti-AAV antibodies and those developed after gene therapy administration, repeat dosing of gene therapies is currently thought not to be possible. Thus, AAV gene therapy is anticipated to be a one-time administration.[216] Although this is an advantage for patient compliance, it makes durability an important issue. It also makes selection of gene therapy treatments critical for patients.

Below we discuss each dimension of FDA's Benefit-Risk Framework. We have included excerpts from this framework for the approval of casimersen (the most recently approved product for patients with Duchenne)[17] to highlight some of the considerations factored into FDA's benefit-risk assessment.

Analysis of Condition (Figure 6)

A detailed analysis of Duchenne has been addressed previously in this paper. It is a serious disease that leads to progressively worsening symptoms and, ultimately, untimely death.

Figure 6. Analysis of Condition Section from Casimersen Approval

Dimension	Evidence and Uncertainties	Conclusions and Reasons
Analysis of Condition	• DMD is a rare progressive X-linked neuromuscular disorder caused by mutations in the dystrophin gene. Lack of dystrophin results in degeneration of muscle fibers, inflammation, and ultimately replacement of muscle by fibrotic and adipose tissue. • The disease causes progressive and profound muscle weakness and degeneration. Muscle weakness typically begins between ages 3 to 5 years, with loss of ambulation usually occurring by 12 years of age. Death typically occurs before age 30 years, generally from respiratory and/or cardiac muscle involvement. The disease prevalence is estimated to be 1.4 per 10,000 males ages 5 to 24 years.	DMD is a serious and life-threatening disease. The loss of muscle strength in DMD is progressive, leading to loss of ambulation in the teens, followed by decline in respiratory and cardiac function, resulting in death typically in the third decade.

Current Treatment Options (Figure 7)

Discussion of current treatment options for Duchenne also has been presented previously in this paper. We underscore here that current treatment options for Duchenne are limited, and a very strong unmet medical need exists for disease-modifying treatments that are well tolerated and that can slow or stop disease progression.

Figure 7. Current Treatment Options Section from Casimersen Approval

Current Treatment Options	• Emflaza (deflazacort) is a glucocorticoid approved for treatment of Duchenne muscular dystrophy (DMD) in patients 2 years of age and older. • Exondys 51 is approved for the treatment of Duchenne muscular dystrophy (DMD) in patients who have a confirmed mutation of the DMD gene that is amenable to exon 51 skipping. • Vyondys 53 and Viltepso are approved for the treatment of Duchenne muscular dystrophy (DMD) in patients who have a confirmed mutation of the DMD gene that is amenable to exon 53 skipping.	Deflazacort, the only drug with full approval for the treatment of DMD, has a modest response rate, and there is evidence that a substantial proportion of DMD patients are not using steroids, in part because of their safety profile. There are no therapies targeted to mutations amenable to exon-45 skipping.

FDA's Benefit-Risk Framework for any new treatment for Duchenne would also include casimersen's approval in its analysis of current treatment options.

Benefit (Figure 8)

FDA's analysis of this dimension will of course be product-specific.

Figure 8. Benefit Section from Casimersen Approval

Benefit	• Truncated dystrophin quantification by western blot showed a mean change in dystrophin levels from 0.9% of normal at Baseline to 1.7% of normal at Week 48 in the casimersen group, compared to a mean change from 0.5% of normal at Baseline to 0.8% of normal at Week 48 in the placebo group. The casimersen group had a statistically significantly greater increase in dystrophin protein levels from Baseline to Week 48 compared to the placebo group (mean difference of 0.594%; $p = 0.004$). • Exon 45 skipping was confirmed by measurement and sequence verification of exon 45 skipped mRNA. The casimersen group had a statistically significantly greater increase in percent exon-skipping from Baseline to Week 48 than the placebo group (mean difference of 1.599; $p < 0.001$).	The applicant has demonstrated a small, but statistically significant increase in de novo (truncated) dystrophin protein with casimersen compared to placebo in DMD patients with a genetic mutation amenable to exon 45 skipping. Although there remains uncertainty regarding the level of dystrophin that would be likely to confer clinical benefit, the increase in dystrophin levels demonstrated for casimersen is similar in size to that established for eteplirsen and golodirsen, drugs that received accelerated approval based on a conclusion by CDER that the increase in dystrophin level was reasonably likely to predict clinical benefit.

As noted, the use of the accelerated approval pathway does result in greater "...uncertainty into the risk/benefit assessment, because clinical benefit is not measured directly and the quantitative relation of the effect on the surrogate to the clinical effect is rarely known."[25] However, FDA recognizes that regulatory flexibility is warranted in certain cases, including for serious diseases with unmet medical need such as Duchenne.[199] In the draft guidance on Demonstrating Substantial Evidence of Effectiveness for Human Drug and Biological Products, FDA discussed areas where flexibility is available, noting that there are many "...characteristics of the evidence supporting effectiveness that can vary (notably, trial designs, trial endpoints, statistical methodology), and evidence that varies in such ways potentially can provide substantial evidence of effectiveness but because of these characteristics may provide greater or lesser certainty."[199] The Agency holds a longstanding position of flexibility around types of data and evidence that can meet the substantial evidence requirement, and although any approval must be supported by a finding of substantial evidence of effectiveness, as FDA has explained, "...the degree of certainty supporting such a conclusion may differ, depending on clinical circumstances (e.g., severity and rarity of the disease and unmet medical need)".[199] In particular, with regard to life-threatening and severely debilitating diseases, FDA has explained that its Subpart E regulations "...call for FDA to exercise

its broad scientific judgment in applying the evidentiary approval standards to drugs for life-threatening and severely debilitating diseases, especially where there is no satisfactory alternative therapy."[199]

Risk and Risk Management (Figure 9)

Again, FDA's analysis of this dimension must be product-specific.

Figure 9. Risk and Risk Management Section from Casimersen Approval

Risk and Risk Management	• At the time of the NDA submission, there were 76 patients exposed to casimersen, with 59 patients with >48 weeks of exposure, and 19 patients with >120 weeks of exposure. • The most common adverse reactions (incidence ≥20% and 5% higher than placebo) were upper respiratory tract infections, cough, pyrexia, headache, arthralgia and oropharyngeal pain. • Renal toxicity was the primary toxicity observed in nonclinical studies. No serious renal adverse reactions or events of acute renal toxicity were observed in the clinical studies. Compared to patients on placebo, more patients who received casimersen had increases in urine protein > 1+. The potential for kidney toxicity will be described in the Warnings and Precautions section of labeling. • There is also a risk of infection and other complications related to the indwelling catheters that may be used to administer casimersen, but this risk is not specific to casimersen. • There is inadequate data to assess the potential for QT prolongation and immunogenicity.	Overall, the most frequent adverse events observed with casimersen were mild; none caused substantial or permanent harm to patients. Upper respiratory tract infections, cough, pyrexia, headache are the most common adverse events. Nonclinical studies indicate a potential for renal toxicity in humans, but no serious renal adverse reaction have been observed in clinical studies. The seriousness of the indication, along with the unmet medical need, make the risk for serious renal toxicity acceptable. It will be important to inform patients and prescribers about the risk, and a warning regarding the potential for kidney toxicity will be included in labeling. Additional pharmacovigilance for kidney toxicity will also be requested. Because of limitations due to the small number of patients exposed and duration of exposure in the clinical trials, it is likely that adverse reactions not identified to date, or of a magnitude not observed to date, will occur in the postmarketing setting. Risk management can be achieved through clear product labeling and routine postmarketing surveillance, plus additional pharmacovigilance for kidney toxicity. The applicant will be required to assess the immunogenicity of casimersen, and evaluate the potential for QT prolongation as post-marketing requirements.

FDA has acknowledged that "...what is a feasible and sufficient safety assessment is a matter of scientific and regulatory judgment based on the particular challenges posed by each drug and disease, including patients' tolerance for risk in the setting of unmet medical need."[217] With respect to rare diseases, the FDA has explained that "...[t]he goal of safety evaluation during drug development is to characterize the drug's safety profile in a reasonable number of patients over a reasonable duration of time, consistent with the intended use of the drug."[217] For rare diseases, however, "reasonable" requires "...consideration of feasibility challenges posed by the limited number of patients with the disease."[217] In its guidance on Duchenne Muscular Dystrophy and Related Dystrophinopathies, FDA noted that "Drugs shown to provide an important benefit will generally need less safety data to provide adequate assurance that benefits outweigh risks."[26] FDA has also explicitly said that when considering the benefit-risk framework and making regulatory decisions regarding drugs and biologics for dystrophinopathies, it will "...consider patient and caregiver tolerance for risk and the serious and life-threatening nature of these conditions. For example, patients may be willing to tolerate substantial risk of harm if a drug might delay loss of important abilities such as ambulation. However, tolerance for risk may vary among individuals and be affected by disease stage and severity; FDA would consider this heterogeneity in regulatory decisions."[26]

Patients and their caregivers are generally willing to tolerate risks with gene therapies to treat Duchenne. In 2018, PPMD and RTI International conducted a stated-preference survey to assess patient and caregiver treatment preferences for potential emerging gene therapies. The study included measuring "maximum acceptable risk (MAR) of mortality" in exchange for a noncurative benefit for a finite duration.[218] The study found a high tolerance for the risk of mortality for treatments that were noncurative, and risk tolerance increased with disease progression. The study also showed that patients and caregivers have similar preferences for benefits and risks.[218]

Although AAV vectors are known to be less immunogenic than gene therapies that use other delivery mechanisms, there are other potential risks to consider. A Cellular, Tissue and Gene Therapies Advisory Committee (CTGTAC) Meeting held in September 2021 took a closer look at some of the specific safety concerns around AAV vector-based gene therapy products.[219] Several potential concerns and ways to mitigate these potential risks were discussed, in addition to ways to better understand whether these issues are actual risks to patients. Specific topics raised in the CTGTAC's discussions that are potentially relevant to AAV vector-based gene therapy products for Duchenne that use the systemic route of administration included adverse events such as dorsal root ganglion (DRG) and peripheral nerve toxicities, thrombotic microangiopathies (TMAs), and hepatotoxicities.[220]

It is important to better understand the clinical relevance of the toxicities that have been noted, especially in cases where they have been observed only in animal models. For example, the CTGTAC noted that questions remain regarding the mechanisms of DRG toxicity and their relevance to humans. Even if they are clinically relevant, the severity of the risk must be balanced with the risk of not providing access to a therapeutic option that is potentially life sustaining or lifesaving to patients with Duchenne. In considering the risk of potential DRG toxicity—which may result in numbness of the hands, for instance—its relative weight would need to be compared with the known risk of a child with Duchenne's condition worsening and leading to death in the absence of treatment. When inputting the potential DRG toxicity risk into the Benefit-Risk Framework, the data collected by PPMD regarding risk tolerance in the Duchenne community (referenced above) suggest this is a risk many patients and caregivers would be willing to accept in exchange for therapeutic benefit from a noncurative gene therapy.

There is experience from approved AAV gene therapies that supports the safety of AAV vector-based gene therapy products and the ability to mitigate risks discovered post approval. At the CTGTAC meeting, it was shared that four patients with spinal muscular atrophy (SMA) out of the 1,400 who had received Zolgensma® to date experienced TMA, a rare disorder that can cause low platelet counts, organ damage, other serious issues, and, in some cases, death.[219,221] These findings led to a revision of Zolgensma's label.[222] The meeting attendees discussed mitigation steps, such as screening for TMA early, and potential treatments for the conditions that could be implemented.[223] In fact, current AAV gene therapies for Duchenne have reported a few cases of thrombocytopenia associated with TMA-like complement activation in clinical trials. To mitigate this risk, product labeling—including appropriate monitoring recommendations—should be developed for approved products.

Similarly, hepatotoxicities observed in SMA, X-linked myotubular myopathy, and hemophilia were also discussed. This hepatotoxicity often presents as liver enzyme elevation. Corticosteroids have been used to dampen AAV-mediated hepatotoxicities.[218] The Zolgensma label includes both instructions on administration of systemic corticosteroids as well as a Boxed Warning to alert prescribers to the possibility of acute serious liver injury and elevated aminotransferases.[222] Thus, this risk can also be mitigated through product labeling and monitoring.

Overall, in its discussion, the CTGTAC noted[223] that benefit-risk assessments of AAV gene therapy products should be made on a case-by-case basis, taking into account elements such as alternative available treatments, the patient's age at the time of gene therapy, pre-existing conditions, and route of administration.

Finally, more recently, four companies with clinical trials for Duchenne AAV gene therapies shared that there may be a class effect causing serious adverse events. These events, characterized by muscle weakness (some with cardiac involvement), shared a similar presentation and duration across the various therapies in clinical development. Through analysis of relevant case information, the companies hypothesize that these events are related to a T-cell–mediated immune response to the transgene protein. This collaborative effort has resulted in identification of risk factors and development of strategies to mitigate the risks.[224]

References

1. Duan D, Goemans N, Takeda S, Mercuri E, Aartsma-Rus A. Duchenne muscular dystrophy. Nat Rev Dis Primers. 2021;7(1):13.
2. Wang D, Tai PWL, Gao G. Adeno-associated virus vector as a platform for gene therapy delivery. Nat Rev Drug Discov. 2019;18(5):358-378.
3. Li C, Samulski RJ. Engineering adeno-associated virus vectors for gene therapy. Nat Rev Genet. 2020;21(4):255-272.
4. Lostal W, Kodippili K, Yue Y, Duan D. Full-length dystrophin reconstitution with adeno-associated viral vectors. Hum Gene Ther. 2014;25(6):552-562.
5. Koo T, Popplewell L, Athanasopoulos T, Dickson G. Triple trans-splicing adeno-associated virus vectors capable of transferring the coding sequence for full-length dystrophin protein into dystrophic mice. Hum Gene Ther. 2014;25(2):98-108.
6. Scott JM, Li S, Harper SQ, Welikson R, Bourque D, DelloRusso C, Hauschka SD, Chamberlain JS. Viral vectors for gene transfer of micro-, mini-, or full-length dystrophin. Neuromuscular Disorders: NMD. 2002;12 Suppl 1:S23-9.
7. Duan D. Systemic AAV Micro-dystrophin Gene Therapy for Duchenne Muscular Dystrophy. Mol Ther. 2018;26(10):2337-2356.
8. Kunkel LM. 2004 William Allan Award address. Cloning of the DMD gene. Am J Hum Genet. 2005;76(2):205-214. doi:10.1086/428143.
9. Hoffman EP. Causes of clinical variability in Duchenne and Becker muscular dystrophies and implications for exon skipping therapies. Acta Myol. 2020;39(4):179-186.
10. National Institute for Neurological Disorders and Stroke. Muscular Dystrophy: Hope Through Research. August 2013. Available at: https://www.ninds.nih.gov/Disorders/Patient-Caregiver-Education/Hope-Through-Research/Muscular-Dystrophy-Hope-Through-Research
11. Van Ruiten HJ, Marini Bettolo C, Cheetham T, Eagle M, Lochmuller H, Straub V, Bushby K, Guglieri M. Why are some patients with Duchenne muscular dystrophy dying young: An analysis of causes of death in North East England. Eur J Paediatr Neurol. 2016;20(6):904-909.
12. Darras BT, Urion DK, Ghosh PS. Dystrophinopathies. GeneReviews®. Updated Apr 26, 2018. Available at: http://www.ncbi.nlm.nih.gov/books/NBK1119/
13. Apkon S, Kinnett K, Cripe L, Duan D, Jackson JL, Kornegay JN, Mah ML, Nelson SF, Rao V, Scavina M, Wong BL, Flanigan KM. Parent Project Muscular Dystrophy Females with Dystrophinopathy Conference, Orlando, Florida June 26 - June 27, 2019. J Neuromuscul Dis. 2021;8(2):315-322. doi: 10.3233/JND-200555. PMID: 33361607.
14. FDA Center for Drug Evaluation and Research. Summary Review Application Number: 206488. Available at: https://www.accessdata.fda.gov/drugsatfda_docs/nda/2016/206488_summary%20review_Redacted.pdf
15. FDA Center for Drug Evaluation and Research. Summary Review Application Number: 211970. Available at: https://www.accessdata.fda.gov/drugsatfda_docs/nda/2019/211970Orig1s000SumR.pdf
16. FDA Center for Drug Evaluation and Research. Summary Review Application Number: 212154. Available at: https://www.accessdata.fda.gov/drugsatfda_docs/nda/2020/212154Orig1s000SumR.pdf
17. FDA Center for Drug Evaluation and Research. Summary Review Application Number: 213026. Available at: https://www.accessdata.fda.gov/drugsatfda_docs/nda/2021/213026Orig1s000SumR.pdf
18. Figueiredo M. Exon Skipping Dominates DMD Treatment, But What's on Horizon. Muscular Dystrophy News Today, February 25, 2021, available at: https://musculardystrophynews.com/2021/02/25/exon-skipping-dominates-duchenne-gene-therapy-horizon/
19. Duan D. Micro-dystrophin gene therapy goes systemic in Duchenne muscular dystrophy patients. Hum Gene Ther. 2018;29(7):733-736.
20. 21 U.S.C. § 506(c)(1)(A) of the FD&C Act.
21. FDA Guidance for Industry: Expedited Programs for Serious Conditions – Drugs and Biologics. May 2014. Available at: https://www.fda.gov/media/86377/download
22. 21 CFR 312 Subpart E (Drugs Intended to Treat Life-threatening and Severely-debilitating Illnesses).
23. FDA news release: FDA approves drug to treat Duchenne muscular dystrophy. February 9, 2017, available at: https://www.fda.gov/news-events/press-announcements/fda-approves-drug-treat-duchenne-muscular-

dystrophy
24. Emflaza (deflazacort) Prescribing Information. June 2021. Available at: https://www.accessdata.fda.gov/drugsatfda_docs/label/2021/208684s005,208685s005lbl.pdf
25. FDA. Final Rule, New Drug, Antibiotic, and Biological Drug Product Regulations; Accelerated Approval (57 FR 58942, 58944, December 11, 1992).
26. FDA Guidance for Industry: Duchenne Muscular Dystrophy and Related Dystrophinopathies: Developing Drugs for Treatment. February 2018. Available at: https://www.fda.gov/media/92233/download
27. Mazzone E, Vasco G, Sormani MP, Torrente Y, Berardinelli A, Messina S, D'Amico A, Doglio L, Politano L, Cavallaro F, Frosini S, Bello L, Bonfiglio S, Zucchini E, De Sanctis R, Scutifero M, Bianco F, Rossi F, Motta MC, Sacco A, Donati MA, Mongini T, Pini A, Battini R, Pegoraro E, Pane M, Gasperini S, Previtali S, Napolitano S, Martinelli D, Bruno C, Vita G, Comi G, Bertini E, Mercuri E Functional changes in Duchenne muscular dystrophy: a 12-month longitudinal cohort study. Neurology. 2011;77(3):250-256.
28. Mazzone ES, Pane M, Sormani MP, Scalise R, Berardinelli A, Messina S, Torrente Y, D'Amico A, Doglio L, Viggiano E, D'Ambrosio P, Cavallaro F, Frosini S, Bello L, Bonfiglio S, De Sanctis R, Rolle E, Bianco F, Magri F, Rossi F, Vasco G, Vita G, Motta MC, Donati MA, Sacchini M, Mongini T, Pini A, Battini R, Pegoraro E, Previtali S, Napolitano S, Bruno C, Politano L, Comi GP, Bertini E, Mercuri E. 24 month longitudinal data in ambulant boys with Duchenne muscular dystrophy. PLoS One. 2013;8(1): e52512. Erratum in: PLoS One. 2013;8(11).
29. McDonald CM, Henricson EK, Abresch RT, Han JJ, Escolar DM, Florence JM, Duong T, Arrieta A, Clemens PR, Hoffman EP, Cnaan A; CINRG Investigators. The cooperative international neuromuscular research group Duchenne natural history study-a longitudinal investigation in the era of glucocorticoid therapy: design of protocol and the methods used. Muscle Nerve. 2013;48(1):32-54.
30. Ricotti V, Ridout DA, Scott E, Quinlivan R, Robb SA, Manzur AY, Muntoni F; NorthStar Clinical Network. Long-term benefits and adverse effects of intermittent versus daily glucocorticoids in boys with Duchenne muscular dystrophy. J Neurol Neurosurg Psychiatry. 2013;84:698-705.
31. Nelson LL, Iannaccone ST. Clinical outcome assessments in Duchenne muscular dystrophy and spinal muscular atrophy: past, present and future. Neuromuscul Disord. 2021;31(10):1028-1037.
32. Miller NF, Alfano LN, Iammarino MA, Connolly AM, Moore-Clingenpeel M, Powers BR, Tsao CY, Waldrop MA, Flanigan KM, Mendell JR, Lowes LP. Natural history of steroid-treated young boys with Duchenne muscular dystrophy using the NSAA, 100 m, and timed functional tests. Pediatr Neurol. 2020;113:15-20.
33. Mazzone ES, Messina S, Vasco G, Main M, Eagle M, D'Amico A, Doglio L, Politano L, Cavallaro F, Frosini S, Bello L, Magri F, Corlatti A, Zucchini E, Brancalion B, Rossi F, Ferretti M, Motta MG, Cecio MR, Berardinelli A, Alfieri P, Mongini T, Pini A, Astrea G, Battini R, Comi G, Pegoraro E, Morandi L, Pane M, Angelini C, Bruno C, Villanova M, Vita G, Donati MA, Bertini E, Mercuri E. Reliability of the North Star Ambulatory Assessment in a multicentric setting. Neuromuscul Disord. 2009;19(7):458-461.
34. Scott E, Eagle M, Mayhew A, Freeman J, Main M, Sheehan J, Manzur A, Muntoni F; North Star Clinical Network for Paediatric Neuromuscular Disease. Development of a functional assessment scale for ambulatory boys with Duchenne muscular dystrophy. Physiother Res Int. 2013;17:101-109.
35. Mazzone E, Martinelli D, Berardinelli A, Messina S, D'Amico A, Vasco G, Main M, Doglio L, Politano L, Cavallaro F, Frosini S, Bello L, Carlesi A, Bonetti AM, Zucchini E, De Sanctis R, Scutifero M, Bianco F, Rossi F, Motta MC, Sacco A, Donati MA, Mongini T, Pini A, Battini R, Pegoraro E, Pane M, Pasquini E, Bruno C, Vita G, de Waure C, Bertini E, Mercuri E. North Star Ambulatory Assessment, 6-minute walk test and timed items in ambulant boys with Duchenne muscular dystrophy. Neuromuscul Disord. 2010;20(11):712-716.
36. Mayhew A, Cano S, Scott E, Eagle M, Bushby K, Muntoni F; North star clinical network for paediatric neuromuscular disease. Moving towards meaningful measurement: Rasch analysis of the North star ambulatory assessment in Duchenne muscular dystrophy. Dev Med Child Neurol. 2011;53(6);535-542.
37. Mayhew AG, Cano SJ, Scott E, Eagle M, Bushby K, Manzur A, Muntoni F; North Star Clinical Network for Neuromuscular Disease. Detecting meaningful change using the North star ambulatory assessment in Duchenne muscular dystrophy. Dev Med Child Neurol. 2013;55(11):1046-1052.
38. De Sanctis R, Pane M, Sivo S, Ricotti V, Baranello G, Frosini S, Mazzone E, Bianco F, Fanelli L, Main M, Corlatti A, D'Amico A, Colia G, Scalise R, Palermo C, Alfonsi C, Tritto G, Romeo DM, Graziano A, Battini R, Morandi L, Bertini E, Muntoni F, Mercuri E. Suitability of North Star Ambulatory Assessment in young boys with Duchenne muscular dystrophy. Neuromuscul Disord. 2015;25:14-18.
39. Mercuri E, Coratti G, Messina S, Ricotti V, Baranello G, D'Amico A, Pera MC, Albamonte E, Sivo S, Mazzone

ES, Arnoldi MT, Fanelli L, De Sanctis R, Romeo DM, Vita GL, Battini R, Bertini E, Muntoni F, Pane M. Revised North Star Ambulatory Assessment for young boys with Duchenne muscular dystrophy. PLoS ONE. 2016;11(8):e0160195.

40. Muntoni F, Signorovitch J, Sajeev G, Goemans N, Wong B, Tian C, Mercuri E, Done N, Wong H, Moss J, Yao Z, Ward SJ, Manzur A, Servais L, Niks EH, Straub V, de Groot IJ, McDonald C, North Star Clinical Network, PRO-DMD-01 Study, The Association Française contre les Myopathies (AFM), The DMD Italian Group, and The Collaborative Trajectory Analysis Project (cTAP). Real-world and natural history data for drug evaluation in Duchenne muscular dystrophy: suitability of the North Star Ambulatory Assessment for comparisons with external controls. Neuromuscul Disord. 2022;32(4):271-283.
41. FDA Center for Drug Evaluation and Research. Approval Package Application Number: 206488. Available at: https://www.accessdata.fda.gov/drugsatfda_docs/nda/2016/206488Orig1s000Approv.pdf
42. ATS Committee on Proficiency Standards for Clinical Pulmonary Function Laboratories. ATS statement: guidelines for the six-minute walk test. Am J Respir Crit Care Med. 2002;166(1):111-117.
43. McDonald CM, Henricson EK, Han JJ, Abresch RT, Nicorici A, Elfring GL, Atkinson L, Reha A, Hirawat S, Miller LL. 6-minute walk test as a new outcome measure in Duchenne muscular dystrophy. Muscle Nerve. 2010;41:500-510.
44. McDonald CM, Henricson EK, Abresch RT, Florence J, Eagle M, Gappmaier E, Glanzman AM; PTC124-GD-007-DMD Study Group, Spiegel R, Barth J, Elfring G, Reha A, Peltz SW. The 6-minute walk test and other clinical endpoints in Duchenne muscular dystrophy: reliability, concurrent validity, and minimal clinically important differences from a multicenter study. Muscle Nerve. 2013;48(3):357-368.
45. McDonald CM, Henricson EK, Han JJ, Abresch RT, Nicorici A, Atkinson L, Elfring GL, Reha A, Miller LL. The 6-minute walk test in Duchenne/Becker muscular dystrophy: longitudinal observations. Muscle Nerve. 2010;42(6):966-974.
46. Henricson E, Abresch R, Han JJ, Nicorici A, Goude Keller E, de Bie E, McDonald CM. The 6-minute walk test and person-reported outcomes in boys with Duchenne muscular dystrophy and typically developing controls: longitudinal comparisons and clinically-meaningful changes over one year. PLoS Curr. 2013;5:ecurrents.md.9e17658b007eb79fcd6f723089f79e06.
47. Mazzone M, Bianco F, Main M, van den Hauwe M, Ash M, de Vries R, Fagoaga Mata J, Stein S, De Sanctis R, D'Amico A, Palermo C, Fanelli L, Scoto MC, Mayhew A, Eagle M, Vigo M, Febrer A, Korinthenberg R, de Visser M, Bushby K, Muntoni F, Goemans N, Sormani MP, Bertini E, Pane M, Mercuri E. Six minute walk test in type III spinal muscular atrophy: a 12 month longitudinal study. Neuromuscul Disord. 2013;23(8):624-628.
48. Pane M, Mazzone ES, Sivo S, Sormani MP, Messina S, D'Amico A, Carlesi A, Vita G, Fanelli L, Berardinelli A, Torrente Y, Lanzillotta V, Viggiano E, D Ambrosio P, Cavallaro F, Frosini S, Barp A, Bonfiglio S, Scalise R, De Sanctis R, Rolle E, Graziano A, Magri F, Palermo C, Rossi F, Donati MA, Sacchini M, Arnoldi MT, Baranello G, Mongini T, Pini A, Battini R, Pegoraro E, Previtali S, Bruno C, Politano L, Comi GP, Bertini E, Mercuri E. Long term natural history data in ambulant boys with Duchenne muscular dystrophy: 36-month changes. PLoS One. 2014;9(10):e108205. Erratum in: PLoS One. 2015;10(3):e0121882. Erratum in: PLoS One. 2015;10(12):e0144079.
49. Mercuri E, Signorovitch JE, Swallow E, DMD Italian Group; Trajectory Analysis Project (cTAP).Categorizing natural history trajectories of ambulatory function measured by the 6-minute walk distance in patients with Duchenne muscular dystrophy. Neuromuscul Disord. 2016;26(9):576-583.
50. Arora H, Willcocks RJ, Lott DJ, Harrington AT, Senesac CR, Zilke KL, Daniels MJ, Xu D, Tennekoon GI, Finanger EL, Russman BS, Finkel RS, Triplett WT, Byrne BJ, Walter GA, Sweeney HL, Vandenborne K. Longitudinal timed function tests in Duchenne muscular dystrophy: imaging DMD cohort natural history. Muscle Nerve. 2018;58(5):631-638.
51. Mendell JR, Rodino-Klapac LR, Sahenk Z, Roush K, Bird L, Lowes LP, Alfano L, Gomez AM, Lewis S, Kota J, Malik V, Shontz K, Walker CM, Flanigan KM, Corridore M, Kean JR, Allen HD, Shilling C, Melia KR, Sazani P, Saoud JB, Kaye EM; Eteplirsen Study Group. Eteplirsen for the treatment of Duchenne muscular dystrophy. Ann Neurol. 2013;74(5):637-647.
52. Mendell JR, Goemans N, Lowes LP, Alfano LN, Berry K, Shao J, Kaye EM, Mercuri E; Eteplirsen Study Group and Telethon Foundation DMD Italian Network. Longitudinal effect of eteplirsen versus historical control on ambulation in Duchenne muscular dystrophy. Ann Neurol. 2016;79(2):257-271.
53. McDonald CM, Campbell C, Torricelli RE; Clinical Evaluator Training Group; ACT DMD Study Group. Ataluren

in patients with nonsense mutation Duchenne muscular dystrophy (ACT DMD): a multicentre, randomised, double-blind, placebo-controlled, phase 3 trial. Lancet. 2017;390(10101):1489-1498.
54. Victor RG, Sweeney HL, Finkel R, McDonald CM, Byrne B, Eagle M, Goemans N, Vandenborne K, Dubrovsky AL, Topaloglu H, Miceli MC, Furlong P, Landry J, Elashoff R, Cox D; Tadalafil DMD Study Group. A phase 3 randomized placebo-controlled trial of tadalafil for Duchenne muscular dystrophy. Neurology. 2017;89(17):1811-1820.
55. Shieh PB, Mcintosh J, Jin F, Souza M, Elfring G, Narayanan S, Trifillis P, Peltz SW, Mcdonald CM, Darras BT; the ACT DMD Study Group. Deflazacort versus prednisone/prednisolone for maintaining motor function and delaying loss of ambulation: a post HOC analysis from the ACT DMD trial. Muscle Nerve. 2018;58(5):639-645.
56. Goemans N, Mercuri E, Belousova E, Komaki H, Dubrovsky A, McDonald CM, Kraus JE, Lourbakos A, Lin Z, Campion G, Wang SX, Campbell C; DEMAND III study group. A randomized placebo-controlled phase 3 trial of an antisense oligonucleotide, drisapersen, in Duchenne muscular dystrophy. Neuromuscul Disord. 2018;28(1):4-15.
57. Alfano LN, Charleston JS, Connolly AM, Cripe L, Donoghue C, Dracker R, Dworzak J, Eliopoulos H, Frank DE, Lewis S, Lucas K, Lynch J, Milici AJ, Flynt A, Naughton E, Rodino-Klapac LR, Sahenk Z, Schnell FJ, Young GD, Mendell JR, Lowes LP. Long-term treatment with eteplirsen in nonambulatory patients with Duchenne muscular dystrophy. Medicine (Baltimore). 2019;98(26):e15858.
58. McDonald CM, Henricson EK, Abresch RT, Florence JM, Eagle M, Gappmaier E, Glanzman AM; PTC124-GD-007-DMD Study Group, Spiegel R, Barth J, Elfring G, Reha A, Peltz S. The 6-minute walk test and other endpoints in Duchenne muscular dystrophy: longitudinal natural history observations over 48 weeks from a multicenter study. Muscle Nerve. 2013;48(3):343-356.
59. Goemans N, Signorovitch J, Sajeev G, Yao Z, Gordish-Dressman H, McDonald CM, Vandenborne K, Miller D, Ward SJ, Mercuri E, investigators from PRO-DMD-01 Study, CINRG DNHS, ImagingDMD, and DMD Italian Group, et al. Suitability of external controls for drug evaluation in Duchenne muscular dystrophy. Neurology. 2020;95(10):e1381-e1391.
60. FDA Center for Drug Evaluation and Research. Clinical Outcome Assessment (COA) Compendium. June 2021. Available at: https://www.fda.gov/media/130138/download
61. FDA Center for Drug Evaluation and Research. Approval Package Application Number: 211970. Available at: https://www.accessdata.fda.gov/drugsatfda_docs/nda/2019/211970Orig1s000Approv.pdf
62. FDA Center for Drug Evaluation and Research. Accelerated Approval Letter Application Number: 213026. Available at:https://www.accessdata.fda.gov/drugsatfda_docs/appletter/2021/213026Orig1s000ltr.pdf
63. European Medicines Agency. Translarna (ataluren). Available at: https://www.ema.europa.eu/en/medicines/human/EPAR/translarna
64. Bray TJ, Chouhan MD, Punwani S, Bainbridge A, Hall-Craggs MA. Fat fraction mapping using magnetic resonance imaging: insight into pathophysiology. Br J Radiol. 2018;91(1089):20170344.
65. Burakiewicz J, Sinclair CDJ, Fischer D, Walter GA, Kan HE, Hollingsworth KG. Quantifying fat replacement of muscle by quantitative MRI in muscular dystrophy. J Neurol. 2017;264(10):2053-2067.
66. Willcocks RJ, Rooney WD, Triplett WT, Forbes SC, Lott DJ, Senesac CR, Daniels MJ, Wang DJ, Harrington AT, Tennekoon GI, Russman BS, Finanger EL, Byrne BJ, Finkel RS, Walter GA, Sweeney HL, Vandenborne K. Multicenter prospective longitudinal study of magnetic resonance biomarkers in a large Duchenne muscular dystrophy cohort. Ann Neurol. 2016;79(4):535-547.
67. Triplett WT, Baligand C, Forbes SC, Willcocks RJ, Lott DJ, DeVos S, Pollaro J, Rooney WD, Sweeney HL, Bönnemann CG, Wang DJ, Vandenborne K, Walter GA. Chemical shift-based MRI to measure fat fractions in dystrophic skeletal muscle. Magn Reson Med. 2014;72(1):8-19.
68. Forbes SC, Walter GA, Rooney WD, Wang DJ, DeVos S, Pollaro J, Triplett W, Lott DJ, Willcocks RJ, Senesac C, Daniels MJ, Byrne BJ, Russman B, Finkel RS, Meyer JS, Sweeney HL, Vandenborne K, et al. Skeletal muscles of ambulant children with Duchenne muscular dystrophy: validation of multicenter study of evaluation with MR imaging and MR spectroscopy. Radiology. 2013;269:198–207.
69. Schlaffke L, Rehmann R, Rohm M, Otto LAM, de Luca A, Burakiewicz J, Baligand C, Monte J, den Harder C, Hooijmans MT, Nederveen A, Schlaeger S, Weidlich D, Karampinos DC, Stouge A, Vaeggemose M, D'Angelo MG, Arrigoni F, Kan HE, Froeling M. Multi-center evaluation of stability and reproducibility of quantitative MRI measures in healthy calf muscles. NMR Biomed. 2019;32(9):e4119.

70. Hooijmans MT, Niks EH, Burakiewicz J, Anastasopoulos C, van den Berg SI, van Zwet E, Webb AG, Verschuuren JJGM, Kan HE. Non-uniform muscle fat replacement along the proximodistal axis in Duchenne muscular dystrophy. Neuromuscul Disord. 2017;27:458–464.
71. Naarding KJ, Reyngoudt H, van Zwet EW, Hooijmans MT, Tian C, Rybalsky I, Shellenbarger KC, Le Louër J, Wong BL, Carlier PG, Kan HE, Niks EH. MRI vastus lateralis fat fraction predicts loss of ambulation in Duchenne muscular dystrophy. Neurology. 2020;94(13):e1386-e1394.
72. Straub V, Mercuri E; DMD outcome measure study group. Report on the Workshop: Meaningful outcome measures for Duchenne muscular dystrophy, London, UK, 30-31 January 2017. Neuromuscul Disord. 2018;28(8):690-701.
73. Rooney WD, Berlow YA, Triplett WT, Forbes SC, Willcocks RJ, Wang DJ, Arpan I, Arora H, Senesac C, Lott DJ, Tennekoon G, Finkel R, Russman BS, Finanger EL, Chakraborty S, O'Brien E, Moloney B, Barnard A, Sweeney HL, Daniels MJ, Walter GA, Vandenborne K. Modeling disease trajectory in Duchenne muscular dystrophy. Neurology. 2020;94(15):e1622-e1633.
74. Pfizer press release: Pfizer's New Phase 1b Results of Gene Therapy in Ambulatory Boys with Duchenne Muscular Dystrophy (DMD) Support Advancement into Pivotal Phase 3 Study. May 15, 2020, available at: https://www.pfizer.com/news/press-release/press-release-detail/pfizers-new-phase-1b-results-gene-therapy-ambulatory-boy
75. Willcocks RJ, Forbes SC, Walter GA, Sweeney L, Rodino-Klapac LR, Mendell JR, Vandenborne K. Assessment of rAAVrh.74.MHCK7.micro-dystrophin gene therapy using magnetic resonance imaging in children with Duchenne muscular dystrophy. JAMA Netw Open. 2021;4(1):e2031851.
76. Bonati U, Hafner P, Schädelin S, Schmid M, Naduvilekoot Devasia A, Schroeder J, Zuesli S, Pohlman U, Neuhaus C, Klein A, Sinnreich M, Haas T, Gloor M, Bieri O, Fischmann A, Fischer D. Quantitative muscle MRI: A powerful surrogate outcome measure in Duchenne muscular dystrophy. Neuromuscul Disord. 2015;25(9):679-685.
77. Alfano LN, Lowes LP, Berry KM, Yin H, Dvorchik I, Flanigan KM, Cripe L, Mendell JR.T.P.1: Pilot study evaluating motivation on the performance of timed walking in boys with Duchenne muscular dystrophy. Neuromuscular Disord. 2014;24(9-10):860.
78. Servais L, Camino E, Clement A, McDonald C, M, Lukawy J, Lowes L, P, Eggenspieler D, Cerreta F, Strijbos P. First regulatory qualification of a novel digital endpoint in Duchenne muscular dystrophy: A multi-stakeholder perspective on the impact for patients and for drug development in neuromuscular diseases. Digit Biomark. 2021;5:183-190.
79. Li W, Zheng Y, Zhang W, Wang Z, Xiao J, Yuan Y, et al. Progression and variation of fatty infiltration of the thigh muscles in Duchenne muscular dystrophy, a muscle magnetic resonance imaging study. Neuromuscul Disord. 2015;25(5):375-380.
80. Lilien C, Reyngoudt H, Seferian AM, Gidaro T, Annoussamy M, Chê V, Decostre V, Ledoux I, Le Louër J, Guemas E, Muntoni F, Hogrel JY, Carlier PG, Servais L; PreU7 Study Group. Upper limb disease evolution in exon 53 skipping eligible patients with Duchenne muscular dystrophy. Ann Clin Transl Neurol. 2021;8(10):1938-1950.
81. Barnard AM, Willcocks RJ, Triplett WT, Forbes SC, Daniels MJ, Chakraborty S, Lott DJ, Senesac CR, Finanger EL, Harrington AT, Tennekoon G, Arora H, Wang DJ, Sweeney HL, Rooney WD, Walter GA, Vandenborne K. MR biomarkers predict clinical function in Duchenne muscular dystrophy. Neurology. 2020;94(9):e897-e909.
82. Barnard AM, Willcocks RJ, Finanger EL, Daniels MJ, Triplett WT, Rooney WD, Lott DJ, Forbes SC, Wang DJ, Senesac CR, Harrington AT, Finkel RS, Russman BS, Byrne BJ, Tennekoon GI, Walter GA, Sweeney HL, Vandenborne K. Skeletal muscle magnetic resonance biomarkers correlate with function and sentinel events in Duchenne muscular dystrophy. PLoS One. 2018;13(3):e0194283.
83. Arpan I, Willcocks RJ, Forbes SC, Finkel RS, Lott DJ, Rooney WD, Triplett WT, Senesac CR, Daniels MJ, Byrne BJ, Finanger EL, Russman BS, Wang DJ, Tennekoon GI, Walter GA, Sweeney HL, Vandenborne K. Examination of effects of corticosteroids on skeletal muscles of boys with DMD using MRI and MRS. Neurology. 2014;83(11):974-980.
84. Batra A, Lott DJ, Willcocks R, Forbes SC, Triplett W, Dastgir J, Yun P, Reghan Foley A, Bönnemann CG, Vandenborne K, Walter GA. Lower extremity muscle involvement in the intermediate and Bethlem myopathy forms of COL6-related dystrophy and Duchenne muscular dystrophy: A cross-sectional study. J Neuromuscul Dis. 2020;7(4):407-417.

85. Hooijmans MT, Doorenweerd N, Baligand C, Verschuuren JJGM, Ronen I, Niks EH, Webb AG, Kan HE. Spatially localized phosphorous metabolism of skeletal muscle in Duchenne muscular dystrophy patients: 24-month follow-up. PLoS One. 2017;12(8):e0182086.
86. Forbes SC, Willcocks RJ, Triplett WT, Rooney WD, Lott DJ, Wang DJ, Pollaro J, Senesac CR, Daniels MJ, Finkel RS, Russman BS, Byrne BJ, Finanger EL, Tennekoon GI, Walter GA, Sweeney HL, Vandenborne K. Magnetic resonance imaging and spectroscopy assessment of lower extremity skeletal muscles in boys with Duchenne muscular dystrophy: a multicenter cross sectional study. PLoS One. 2014;9(9):e106435.
87. Lott DJ, Forbes SC, Mathur S, Germain SA, Senesac CR, Lee Sweeney H, Walter GA, Vandenborne K. Assessment of intramuscular lipid and metabolites of the lower leg using magnetic resonance spectroscopy in boys with Duchenne muscular dystrophy. Neuromuscul Disord. 2014;24(7):574-582.
88. Mankodi A, Azzabou N, Bulea T, Reyngoudt H, Shimellis H, Ren Y, Kim E, Fischbeck KH, Carlier PG. Skeletal muscle water T2 as a biomarker of disease status and exercise effects in patients with Duchenne muscular dystrophy. Neuromuscul Disord. 2017;27(8):705-714.
89. Torriani M, Townsend E, Thomas BJ, Bredella MA, Ghomi RH, Tseng BS. Lower leg muscle involvement in Duchenne muscular dystrophy: an MR imaging and spectroscopy study. Skeletal Radiol. 2012;41(4):437-445.
90. Wokke BH, van den Bergen JC, Versluis MJ, Niks EH, Milles J, Webb AG, van Zwet EW, Aartsma-Rus A, Verschuuren JJ, Kan HE. Quantitative MRI and strength measurements in the assessment of muscle quality in Duchenne muscular dystrophy. Neuromuscul Disord. 2014;24(5):409-416.
91. Kim HK, Serai S, Lindquist D, Merrow AC, Horn PS, Kim DH, Wong BL. Quantitative skeletal muscle MRI: Part 2, MR spectroscopy and T2 relaxation time mapping-comparison between boys with Duchenne muscular dystrophy and healthy boys. AJR Am J Roentgenol. 2015;205(2):W216-W223.
92. Li Z, Zeng H, Han C, Sun L, Fang D, Yu H, Quan X. Effectiveness of high-speed T2-corrected multiecho MR spectroscopic method for quantifying thigh muscle fat content in boys with Duchenne muscular dystrophy. AJR Am J Roentgenol. 2019 Mar 12:1-7.
93. Mankodi A, Bishop CA, Auh S, Newbould RD, Fischbeck KH, Janiczek RL. Quantifying disease activity in fatty-infiltrated skeletal muscle by IDEAL-CPMG in Duchenne muscular dystrophy. Neuromuscul Disord. 2016;26(10):650-658.
94. Hooijmans MT, Damon BM, Froeling M, Versluis MJ, Burakiewicz J, Verschuuren JJ, Niks EH, Webb AG, Kan HE. Evaluation of skeletal muscle DTI in patients with Duchenne muscular dystrophy. NMR Biomed. 2015;28(11):1589-1597.
95. Veeger TTJ, van Zwet EW, Al Mohamad D, Naarding KJ, van de Velde NM, Hooijmans MT, Webb AG, Niks EH, de Groot JH, Kan HE. Muscle architecture is associated with muscle fat replacement in Duchenne and Becker muscular dystrophies. Muscle Nerve. 2021;64(5):576-584.
96. Gaeta M, Messina S, Mileto A, Vita GL, Ascenti G, Vinci S, Bottari A, Vita G, Settineri N, Bruschetta D, Racchiusa S, Minutoli F. Muscle fat-fraction and mapping in Duchenne muscular dystrophy: evaluation of disease distribution and correlation with clinical assessments. Preliminary experience. Skeletal Radiol. 2012;41(8):955-961.
97. Ponrartana S, Ramos-Platt L, Wren TA, Hu HH, Perkins TG, Chia JM, Gilsanz V. Effectiveness of diffusion tensor imaging in assessing disease severity in Duchenne muscular dystrophy: preliminary study. Pediatr Radiol. 2015;45(4):582-589.
98. Wokke BH, van den Bergen JC, Hooijmans MT, Verschuuren JJ, Niks EH, Kan HE. T2 relaxation times are increased in Skeletal muscle of DMD but not BMD patients. Muscle Nerve. 2016;53(1):38-43.
99. Reyngoudt H, Marty B, Boisserie JM, Le Louër J, Koumako C, Baudin PY, Wong B, Stojkovic T, Béhin A, Gidaro T, Allenbach Y, Benveniste O, Servais L, Carlier PG. Global versus individual muscle segmentation to assess quantitative MRI-based fat fraction changes in neuromuscular diseases. Eur Radiol. 2021;31(6):4264-4276.
100. Finkel RS, Finanger E, Vandenborne K, Sweeney HL, Tennekoon G, Shieh PB, Willcocks R, Walter G, Rooney WD, Forbes SC, Triplett WT, Yum SW, Mancini M, MacDougall J, Fretzen A, Bista P, Nichols A, Donovan JM. Disease-modifying effects of edasalonexent, an NF-κB inhibitor, in young boys with Duchenne muscular dystrophy: Results of the MoveDMD phase 2 and open label extension trial. Neuromuscul Disord. 2021;31(5):385-396.
101. Wren TA, Bluml S, Tseng-Ong L, Gilsanz V. Three-point technique of fat quantification of muscle tissue as a marker of disease progression in Duchenne muscular dystrophy: preliminary study. AJR Am J Roentgenol. 2008;190(1):W8-W12.

102. Fischmann A, Hafner P, Gloor M, Schmid M, Klein A, Pohlman U, et al. Quantitative MRI and loss of free ambulation in Duchenne muscular dystrophy. J Neurol. 2013;260:969–974.
103. Naarding KJ, van der Holst M, van Zwet EW, van de Velde NM, de Groot IJM, Verschuuren JJGM, Kan HE, Niks EH. Association of elbow flexor MRI fat fraction with loss of hand-to-mouth movement in patients with Duchenne muscular dystrophy. Neurology. 2021;97(17):e1737-e1742.
104. Sherlock SP, Zhang Y, Binks M, Marraffino S. Quantitative muscle MRI biomarkers in Duchenne muscular dystrophy: cross-sectional correlations with age and functional tests. Biomark Med. 2021;15(10):761-773.
105. Bérard C, Payan C, Hodgkinson I, Fermanian J; MFM Collaborative Study Group. A motor function measure for neuromuscular diseases. Construction and validation study. Neuromuscul Disord. 2005;15(7):463-470.
106. Schmidt S, Hafner P, Klein A, Rubino-Nacht D, Gocheva V, Schroeder J, Naduvilekoot Devasia A, Zuesli S, Bernert G, Laugel V, Bloetzer C, Steinlin M, Capone A, Gloor M, Tobler P, Haas T, Bieri O, Zumbrunn T, Fischer D, Bonati U. Timed function tests, motor function measure, and quantitative thigh muscle MRI in ambulant children with Duchenne muscular dystrophy: A cross-sectional analysis. Neuromuscul Disord. 2018;28(1):16-23.
107. Ricotti V, Evans MR, Sinclair CD, Butler JW, Ridout DA, Hogrel JY, Emira A, Morrow JM, Reilly MM, Hanna MG, Janiczek RL, Matthews PM, Yousry TA, Muntoni F, Thornton JS. Upper limb evaluation in Duchenne muscular dystrophy: Fat-water quantification by MRI, muscle force and function define endpoints for clinical trials. PLoS One. 2016;11(9):e0162542.
108. Willcocks RJ, Triplett WT, Forbes SC, Arora H, Senesac CR, Lott DJ, Nicholson TR, Rooney WD, Walter GA, Vandenborne K. Magnetic resonance imaging of the proximal upper extremity musculature in boys with Duchenne muscular dystrophy. J Neurol. 2017;264(1):64-71.
109. Wary C, Azzabou N, Giraudeau C, Le Louër J, Montus M, Voit T, Servais L, Carlier P. Quantitative NMRI and NMRS identify augmented disease progression after loss of ambulation in forearms of boys with Duchenne muscular dystrophy. NMR Biomed. 2015;28(9):1150-1162.
110. Forbes SC, Arora H, Willcocks RJ, Triplett WT, Rooney WD, Barnard AM, Alabasi U, Wang DJ, Lott DJ, Senesac CR, Harrington AT, Finanger EL, Tennekoon GI, Brandsema J, Daniels MJ, Sweeney HL, Walter GA, Vandenborne K. Upper and lower extremities in Duchenne muscular dystrophy evaluated with quantitative MRI and proton MR spectroscopy in a multicenter cohort. Radiology. 2020;295(3):616-625.
111. Hogrel JY, Wary C, Moraux A, Azzabou N, Decostre V, Ollivier G, Canal A, Lilien C, Ledoux I, Annoussamy M, Reguiba N, Gidaro T, Le Moing AG, Cardas R, Voit T, Carlier PG, Servais L. Longitudinal functional and NMR assessment of upper limbs in Duchenne muscular dystrophy. Neurology. 2016;86(11):1022-1030.
112. Nagy S, Schädelin S, Hafner P, Bonati U, Scherrer D, Ebi S, Schmidt S, Orsini AL, Bieri O, Fischer D. Longitudinal reliability of outcome measures in patients with Duchenne muscular dystrophy. Muscle Nerve. 2020;61(1):63-68.
113. Ervasti JM. Dystrophin, its interactions with other proteins, and implications for muscular dystrophy. Biochim Biophys Acta. 2007 Feb;1772(2):108-17. doi: 10.1016/j.bbadis.2006.05.010. Epub 2006 Jun 7. PMID: 16829057..
114. Ervasti JM, Campbell KP. A role for the dystrophin-glycoprotein complex as a transmembrane linker between laminin and actin. J Cell Biol. 1993;122:809-823.
115. Gumerson JD, Michele DE. The dystrophin-glycoprotein complex in the prevention of muscle damage. J Biomed Biotechnol. 2011;2011:210797. doi: 10.1155/2011/210797. Epub 2011 Oct 5. PMID: 22007139; PMCID: PMC3189583.
116. Banks GB, Gregorevic P, Allen JM, Finn EE, Chamberlain JS. Functional capacity of dystrophins carrying deletions in the N-terminal actin-binding domain. Hum Mol Genet. 2007;16:2105-2113.
117. Harper SQ, Hauser MA, DelloRusso C, Duan D, Crawford RW, Phelps SF, Harper HA, Robinson AS, Engelhardt JF, Brooks SV, Chamberlain JS. Modular flexibility of dystrophin: Implications for gene therapy of Duchenne muscular dystrophy. Nat Med. 2002;8:253-261.
118. Ramos JN, Hollinger K, Bengtsson NE, Allen JM, Hauschka SD, Chamberlain JS. Development of novel micro-dystrophins with enhanced functionality. Mol Ther. 2019;27:623-635.
119. Wasala NB, Lai Y, Shin JH, Zhao J, Yue Y, Duan D. Genomic removal of a therapeutic mini-dystrophin gene from adult mice elicits a Duchenne muscular dystrophy-like phenotype. Hum Mol Genet. 2016 Jul 1;25(13):2633-2644. doi: 10.1093/hmg/ddw123. Epub 2016 Apr 22. PMID: 27106099; PMCID: PMC5181634.
120. England SB, Nicholson LV, Johnson MA, Forrest SM, Love DR, Zubrzycka-Gaarn EE, Bulman DE, Harris JB,

Davies KE. Very mild muscular dystrophy associated with the deletion of 46% of dystrophin. Nature. 1990;343:180-182.
121. Darras BT. Muscular dystrophies. In: Samuels MA, Feske SK, eds. Office Practice of Neurology (Second Edition). Churchill Livingstone, 2003:684-697, https://doi.org/10.1016/B0-44-306557-8/50109-X. (https://www.sciencedirect.com/science/article/pii/B044306557850109X)
122. Butterfield RJ. One year data from ambulatory boys in a phase 1b, open-label study of fordistrogene movaparvovec (PF-06939926) for Duchenne muscular dystrophy. Presented as part of Muscular Dystrophy Association Clinical and Scientific Conference; March 2022; Nashville, TN.
123. Gregorevic P, Allen JM, Minami E, Blankinship MJ, Haraguchi M, Meuse L, Finn E, Adams ME, Froehner SC, Murry CE, Chamberlain JS. rAAV6-microdystrophin preserves muscle function and extends lifespan in severely dystrophic mice. Nat Med. 2006;12:787-789.
124. Shin JH, Pan X, Hakim CH, Yang HT, Yue Y, Zhang K, Terjung RL, Duan D. Microdystrophin ameliorates muscular dystrophy in the canine model of Duchenne muscular dystrophy. Mol Ther. 2013;21:750-757.
125. Yue Y, Pan X, Hakim CH, Kodippili K, Zhang K, Shin JH, Yang HT, McDonald T, Duan D. Safe and bodywide muscle transduction in young adult Duchenne muscular dystrophy dogs with adeno-associated virus. Hum Mol Genet. 2015;24:5880-5890.
126. Mendell JR, Sahenk Z, Lehman K, Nease C, Lowes LP, Miller NF, Iammarino MA, Alfano LN, Nicholl A, Al-Zaidy S, Lewis S, Church K, Shell R, Cripe LH, Potter RA, Griffin DA, Pozsgai E, Dugar A, Hogan M, Rodino-Klapac LR. Assessment of systemic delivery of rAAVrh74.MHCK7.micro-dystrophin in children with Duchenne muscular dystrophy: A nonrandomized controlled trial. JAMA Neurol. 2020;77(9):1122–1131.
127. FDA Center for Drug Evaluation and Research. Clinical Pharmacology and Biopharmaceutics Review Application Number: 206488. Available at: https://www.accessdata.fda.gov/drugsatfda_docs/nda/2016/206488Orig1s000ClinPharmR.pdf
128. FDA Center for Drug Evaluation and Research. Medical Review Application Number: 206488. Available at: https://www.accessdata.fda.gov/drugsatfda_docs/nda/2016/206488Orig1s000MedR.pdf
129. Koenig M, Beggs AH, Moyer M, Scherpf S, Heindrich K, Bettecken T, Meng G, Muller CR, Lindlof M, Kaariainen H, de la Chapellet A, Kiuru A, Savontaus ML, Gilgenkrantz H, Récan D, Chelly J, Kaplan JC, Covone AE, Archidiacono N, Romeo G, Liechti-Gailati S, Schneider V, Braga S, Moser H, Darras BT, Murphy P, Francke U, Chen JD, Morgan G, Denton M, Greenberg CR, Wrogemann K, Blonden LA, van Paassen MB, van Ommen GJ, Kunkel LM. The molecular basis for Duchenne versus Becker muscular dystrophy: correlation of severity with type of deletion. Am J Hum Genet. 1989;45(4):498-506.
130. Lai Y, Thomas GD, Yue Y, Yang HT, Li D, Long C, Judge L, Bostick B, Chamberlain JS, Terjung RL, Duan D. Dystrophins carrying spectrin-like repeats 16 and 17 anchor nNOS to the sarcolemma and enhance exercise performance in a mouse model of muscular dystrophy. J Clin Invest. 2009;119:624-635.
131. Cox GA, Cole NM, Matsumura K, Phelps SF, Hauschka SD, Campbell KP, Faulkner JA, Chamberlain JS. Overexpression of dystrophin in transgenic mdx mice eliminates dystrophic symptoms without toxicity [see comments]. Nature. 1993;364:725-729.
132. Vincent N, Ragot T, Gilgenkrantz H, Couton D, Chafey P, Grégoire A, Briand P, Kaplan J, Kahn A, Perricaudet Ml. Long–term correction of mouse dystrophic degeneration by adenovirus–mediated transfer of a minidystrophin gene. Nat Genet. 1993; 5: 130–134.
133. Passos-Bueno MR, Vainzof M, Marie SK, Zatz M. Half the dystrophin gene is apparently enough for a mild clinical course: confirmation of its potential use for gene therapy. Hum Mol Genet. 1994;3:919-922.
134. Phelps SF, Hauser MA, Cole NM, Rafael JA, Hinkle RT, Faulkner JA, Chamberlain JS. Expression of full-length and truncated dystrophin mini-genes in transgenic mdx mice. Hum Mol Genet. 1995;4:1251-1258.
135. Wells DJ, Wells KE, Asante EA, Turner G, Sunada Y, Campbell KP, Walsh FS, Dickson G. Expression of human full-length and minidystrophin in transgenic mdx mice: implications for gene therapy of Duchenne muscular dystrophy. Hum Mol Genet. 1995;4:1245-1250.
136. Chamberlain JS. Dystrophin levels required for correction of Duchenne muscular dystrophy. Basic Appl Myol. 1997;7:251-255.
137. Wang B, Li J, Xiao X. Adeno-associated virus vector carrying human minidystrophin genes effectively ameliorates muscular dystrophy in mdx mouse model. Proc Natl Acad Sci U S A. 2000;97:13714-13719.
138. Yuasa K, Miyagoe Y, Yamamoto K, Nabeshima Y, Dickson G, Takeda S. Effective restoration of dystrophin-associated proteins in vivo by adenovirus-mediated transfer of truncated dystrophin cDNAs. FEBS Lett.

1998;425:329-336.
139. Yoshimura M, Sakamoto M, Ikemoto M, Mochizuki Y, Yuasa K, Miyagoe-Suzuki Y, Takeda S. AAV vector-mediated microdystrophin expression in a relatively small percentage of mdx myofibers improved the mdx phenotype. Mol Ther. 2004;10(5):821-828.
140. Wasala NB, Yue Y, Hu B, et al. Life-long outcomes of systemic AAV micro-dystrophin gene therapy in a murine Duchenne muscular dystrophy model. Hum Gene Ther. 2023. In-press.
141. Gregorevic P, Blankinship MJ, Allen JM, Crawford RW, Meuse L, Miller DG, Russell DW, Chamberlain JS. Systemic delivery of genes to striated muscles using adeno-associated viral vectors. Nat Med. 2004;10:828-834.
142. Wang B, Li J, Fu FH, Xiao X. Systemic human minidystrophin gene transfer improves functions and life span of dystrophin and dystrophin/utrophin-deficient mice. J Orthop Res. 2009;27:421-426.
143. Bostick B, Shin JH, Yue Y, Duan D. AAV-microdystrophin therapy improves cardiac performance in aged female mdx mice. Mol Ther. 2011;19:1826-1832.
144. Gregorevic P, Blankinship MJ, Allen JM, Chamberlain JS. Systemic microdystrophin gene delivery improves skeletal muscle structure and function in old dystrophic mdx mice. Mol Ther. 2008;16:657-664.
145. Bostick B, Shin JH, Yue Y, Wasala NB, Lai Y, Duan D. AAV micro-dystrophin gene therapy alleviates stress-induced cardiac death but not myocardial fibrosis in>21-m-old mdx mice, an end-stage model of Duchenne muscular dystrophy cardiomyopathy. J Mol Cell Cardiol. 2012;53(2):217-222.
146. Hakim CH, Kodippili K, Jenkins G, Yang HT, Pan X, Lessa TB, Leach SB, Emter C, Yue Y, Zhang K, Duan SX, Yao G, Schneider JS, Yang NN, Chamberlain JS, Duan D. AAV micro-dystrophin therapy ameliorates muscular dystrophy in young adult Duchenne muscular dystrophy dogs for up to thirty months following injection. Mol Ther 2018;26 (5S1):5 (abstract).
147. Kornegay JN, Bogan JR, Bogan DJ, Childers MK, Li J, Nghiem P, Detwiler DA, Larsen CA, Grange RW, Bhavaraju-Sanka RK, Tou S, Keene BP, Howard JF, Jr., Wang J, Fan Z, Schatzberg SJ, Styner MA, Flanigan KM, Xiao X, Hoffman EP. Canine models of Duchenne muscular dystrophy and their use in therapeutic strategies. Mamm Genome. 2012;23:85-108.
148. Kornegay JN, Li J, Bogan JR, Bogan DJ, Chen C, Zheng H, Wang B, Qiao C, Howard JF, Jr., Xiao X. Widespread muscle expression of an AAV9 human mini-dystrophin vector after intravenous injection in neonatal dystrophin-deficient dogs. Mol Ther. 2010;18:1501-1508.
149. Le Guiner C, Servais L, Montus M, Larcher T, Fraysse B, Moullec S, Allais M, François V, Dutilleul M, Malerba A, Koo T, Thibaut JL, Matot B, Devaux M, Le Duff J, Deschamps JY, Barthelemy I, Blot S, Testault I, Wahbi K, Ederhy S, Martin S, Veron P, Georger C, Athanasopoulos T, Masurier C, Mingozzi F, Carlier P, Gjata B, Hogrel JY, Adjali O, Mavilio F, Voit T, Moullier P, Dickson G. Long-term microdystrophin gene therapy is effective in a canine model of Duchenne muscular dystrophy. Nat Commun. 2017;8:16105.
150. Abmayr S, Chamberlain J. The structure and function of dystrophin. In: Winder SJ, ed. The Molecular Mechanisms of Muscular Dystrophies. Georgetown: Landes Biosciences, 2006:14-34.
151. Amann KJ, Renley BA, Ervasti JM. A cluster of basic repeats in the dystrophin rod domain binds F-actin through an electrostatic interaction. J Biol Chem. 1998;273:28419-28423.
152. Beggs AH, Hoffman EP, Snyder JR, Arahata K, Specht L, Shapiro F, Angelini C, Sugita H, Kunkel LM. Exploring the molecular basis for variability among patients with Becker muscular dystrophy: dystrophin gene and protein studies. Am J Hum Genet. 1991;49:54-67.
153. Corrado K, Mills PL, Chamberlain JS. Deletion analysis of the dystrophin-actin binding domain. FEBS Lett. 1994;344:255-260.
154. Corrado K, Rafael JA, Mills PL, Cole NM, Faulkner JA, Wang K, Chamberlain JS. Transgenic mdx mice expressing dystrophin with a deletion in the actin-binding domain display a "mild Becker" phenotype. J Cell Biol. 1996;134:873-884.
155. Levine BA, Moir AJ, Patchell VB, Perry SV. Binding sites involved in the interaction of actin with the N-terminal region of dystrophin. FEBS Lett. 1992;298:44-48.
156. Norwood FL, Sutherland-Smith AJ, Keep NH, Kendrick-Jones J. The structure of the N-terminal actin-binding domain of human dystrophin and how mutations in this domain may cause Duchenne or Becker muscular dystrophy. Structure Fold Des. 2000;8:481-491.
157. Rybakova IN, Amann KJ, Ervasti JM. A new model for the interaction of dystrophin with F-actin. J Cell Biol. 1996;135:661-672.

158. Rybakova IN, Ervasti JM. Dystrophin-glycoprotein complex is monomeric and stabilizes actin filaments in vitro through a lateral association. J Biol Chem. 1997;272:28771-28778.
159. Ervasti JM, Rybakova IN, Amann KJ. A multiple site, side binding model for the interaction of dystrophin with F-actin. Soc Gen Physiol Ser. 1997;52:31-44.
160. Rybakova IN, Patel JR, Ervasti JM. The dystrophin complex forms a mechanically strong link between the sarcolemma and costameric actin. J Cell Biol. 2000;150:1209-1214.
161. Warner LE, DelloRusso C, Crawford RW, Rybakova IN, Patel JR, Ervasti JM, Chamberlain JS. Expression of Dp260 in muscle tethers the actin cytoskeleton to the dystrophin-glycoprotein complex and partially prevents dystrophy. Hum Mol Genet. 2002;11:1095-1105.
162. Huang X, Poy F, Zhang R, Joachimiak A, Sudol M, Eck MJ. Structure of a WW domain containing fragment of dystrophin in complex with beta-dystroglycan. Nat Struct Biol. 2000;7:634-638.
163. Jung D, Yang B, Meyer J, Chamberlain JS, Campbell KP. Identification and characterization of the dystrophin anchoring site on beta-dystroglycan. J Biol Chem. 1995;270:27305-27310.
164. Rentschler S, Linn H, Deininger K, Bedford MT, Espanel X, Sudol M. The WW domain of dystrophin requires EF-hands region to interact with beta-dystroglycan. J Biol Chem. 1999;380:431-442.
165. Suzuki A, Yoshida M, Yamamoto H, Ozawa E. Glycoprotein-binding site of dystrophin is confined to the cysteine- rich domain and the first half of the carboxy-terminal domain. FEBS Lett. 1992;308:154-160.
166. Bies RD, Caskey CT, Fenwick R. An intact cysteine-rich domain is required for dystrophin function. J Clin Invest. 1992;90:666-672.
167. Ishikawa-Sakurai M, Yoshida M, Imamura M, Davies KE, Ozawa E. ZZ domain is essentially required for the physiological binding of dystrophin and utrophin to {beta}-dystroglycan. Hum Mol Genet. 2004;13:693-702.
168. Rafael JA, Cox GA, Corrado K, Jung D, Campbell KP, Chamberlain JS. Forced expression of dystrophin deletion constructs reveals structure-function correlations. J Cell Biol. 1996;134:93-102.
169. Sakuraba H, Hori S, Ohtani S, Hanaka S, Abe T, Shimmoto M, Suzuki Y. A case of Duchenne muscular dystrophy with truncated dystrophin. Significance of a cysteine-rich domain for functional expression of dystrophin protein. Brain Dev. 1993;15:222-225.
170. Sakamoto M, Yuasa K, Yoshimura M, Yokota T, Ikemoto T, Suzuki M, Dickson G, Miyagoe-Suzuki Y, Takeda S. Micro-dystrophin cDNA ameliorates dystrophic phenotypes when introduced into mdx mice as a transgene. Biochem Biophys Res Commun. 2002;293:1265-1272.
171. Wasala LP, Watkins T, Wasala N, Burke M, Yue Y, Lai Y, Yao G, Duan D. The implication of hinge 1 and hinge 4 in micro-dystrophin gene therapy for Duchenne muscular dystrophy. Hum Gene Ther. 2022 Oct 31. doi: 10.1089/hum.2022.180. Epub ahead of print. PMID: 36310439.
172. Banks GB, Judge LM, Allen JM, Chamberlain JS. The polyproline site in hinge 2 influences the functional capacity of truncated dystrophins. PLoS Genet. 2010 May 20;6(5):e1000958. doi: 10.1371/journal.pgen.1000958. PMID: 20502633; PMCID: PMC2873924.
173. Hakim CH, Wasala NB, Pan X, Kodippili K, Yue Y, Zhang K, Yao G, Haffner B, Duan SX, Ramos J, Schneider JS, Yang NN, Chamberlain JS, Duan D. A Five-Repeat Micro-Dystrophin Gene Ameliorated Dystrophic Phenotype in the Severe DBA/2J-mdx Model of Duchenne Muscular Dystrophy. Mol Ther Methods Clin Dev. 2017 Jul 27;6:216-230. doi: 10.1016/j.omtm.2017.06.006. PMID: 28932757; PMCID: PMC5596503.
174. Ebner J, Pan X, Yue Y, Sideromenos S, Marksteiner J, Koenig X, Hilber K, Duan D. Microdystrophin Therapy Rescues Impaired Na Currents in Cardiac Purkinje Fibers From Dystrophin-Deficient Mdx Mice. Circ Arrhythm Electrophysiol. 2022 Aug;15(8):e011161. doi: 10.1161/CIRCEP.122.011161. Epub 2022 Aug 2. PMID: 35917466; PMCID: PMC9396648.
175. Solid Biosciences. Solid Biosciences Reports Efficacy and Safety Data from the Ongoing IGNITE DMD Clinical Trial and Resumption of Patient Dosing in the 2E14 vg/kg Cohort. March 15, 2021. Available at: https://www.solidbio.com/about/media/press-releases/solid-biosciences-reporssts-efficacy-and-safety-data-from-the-ongoing-ignite-dmd-clinical-trial-and-resumption-of-patient-dosing-in-the-2e14-vg-kg-cohort.
176. Solid Biosciences. Solid Biosciences Reports 1.5-Year Data from Patients in the Ongoing IGNITE DMD Phase I/II Clinical Trial of SGT-001. September 27, 2021. Available at: https://www.solidbio.com/about/media/press-releases/solid-biosciences-reports-1-5-year-data-from-patients-in-the-ongoing-ignite-dmd-phase-i-ii-clinical-trial-of-sgt-001.
177. Solid Biosciences. Solid Biosciences Reports Fourth Quarter and Full-Year 2021 Financial Results and 2-Year Efficacy and Safety Data from the Ongoing Phase I/II IGNITE DMD Clinical Trial of SGT-001. March 14, 2022.

Available from: https://www.solidbio.com/about/media/press-releases/solid-biosciences-reports-fourth-quarter-and-full-year-2021-financial-results-and-2-year-efficacy-and-safety-data-from-the-ongoing-phase-i-ii-ignite-dmd-clinical-trial-of-sgt-001 [Last accessed 10/5/22].

178. Koeks Z, Janson AA, Beekman C, Signorelli M, van Duyvenvoorde HA, van den Bergen JC, Hooijmans MT, Alleman I, Hegeman IM, Verschuuren JJGM, van Deutekom JC, Spitali P, Datson NA, Niks EH. Low dystrophin variability between muscles and stable expression over time in Becker muscular dystrophy using capillary Western immunoassay. Sci Rep. 2021;11(1):5952.
179. Beekman C, Janson AA, Baghat A, van Deutekom JC, Datson NA. Use of capillary Western immunoassay (Wes) for quantification of dystrophin levels in skeletal muscle of healthy controls and individuals with Becker and Duchenne muscular dystrophy. PLoS One. 2018;13(4):e0195850.
180. Nakamura A, Fueki N, Shiba N, Motoki H, Miyazaki D, Nishizawa H, Echigoya Y, Yokota T, Aoki Y, Takeda S. Deletion of exons 3-9 encompassing a mutational hot spot in the DMD gene presents an asymptomatic phenotype, indicating a target region for multiexon skipping therapy. J Hum Genet. 2016;61(7):663-667.
181. Winnard AV, Mendell JR, Prior TW, Florence J, Burghes AH. Frameshift deletions of exons 3-7 and revertant fibers in Duchenne muscular dystrophy: mechanisms of dystrophin production. Am J Hum Genet. 1995;56(1):158-166.
182. Muntoni F, Gobbi P, Sewry C, Sherratt T, Taylor J, Sandhu SK, Abbs S, Roberts R, Hodgson SV, Bobrow M. Deletions in the 5' region of dystrophin and resulting phenotypes. J Med Genet. 1994;31(11):843-847.
183. Bello L, Morgenroth LP, Gordish-Dressman H, Hoffman EP, McDonald CM, Cirak S; CINRG investigators. DMD genotypes and loss of ambulation in the CINRG Duchenne Natural History Study. Neurology. 2016;87(4):401-409.
184. van Putten M, Hulsker M, Nadarajah VD, van Heiningen SH, van Huizen E, van Iterson M, Admiraal P, Messemaker T, den Dunnen JT, 't Hoen PA, Aartsma-Rus A. The effects of low levels of dystrophin on mouse muscle function and pathology. PLoS One. 2012;7(2):e31937.
185. Rafael JA, Sunada Y, Cole NM, Campbell KP, Faulkner JA, Chamberlain JS. Prevention of dystrophic pathology in mdx mice by a truncated dystrophin isoform. Hum Mol Genet. 1994;3:1725-1733.
186. van Putten M, van der Pijl EM, Hulsker M, Verhaart IE, Nadarajah VD, van der Weerd L, Aartsma-Rus A. Low dystrophin levels in heart can delay heart failure in mdx mice. J Mol Cell Cardiol. 2014;69:17-23.
187. Li D, Yue Y, Duan D. Preservation of muscle force in Mdx3cv mice correlates with low-level expression of a near full-length dystrophin protein. Am J Pathol. 2008 May;172(5):1332-41. Doi: 10.2353/ajpath.2008.071042. Epub 2008 Apr 1. PMID: 18385524; PMCID: PMC2329842.
188. Li D, Yue Y, Duan D. Marginal level dystrophin expression improves clinical outcome in a strain of dystrophin/utrophin double knockout mice. PLoS One. 2010 Dec 20;5(12):e15286. doi: 10.1371/journal.pone.0015286. PMID: 21187970; PMCID: PMC3004926.
189. de Feraudy Y, Ben Yaou R, Wahbi K, Stalens C, Stantzou A, Laugel V, Desguerre I; FILNEMUS Network; Servais L, Leturcq F, Amthor H. Very Low Residual Dystrophin Quantity Is Associated with Milder Dystrophinopathy. Ann Neurol. 2021 Feb;89(2):280-292. doi: 10.1002/ana.25951. Epub 2020 Nov 24. PMID: 33159473; PMCID: PMC7894170.
190. Wasala NB, Yue Y, Vance J, Duan D. Uniform low-level dystrophin expression in the heart partially preserved cardiac function in an aged mouse model of Duchenne cardiomyopathy. J Mol Cell Cardiol. 2017;102:45-52.
191. Wang Z, Zhu T, Qiao C, Zhou L, Wang B, Zhang J, Chen C, Li J, Xiao X. Adeno-associated virus serotype 8 efficiently delivers genes to muscle and heart. Nat Biotechnol. 2005;23(3):321-328.
192. Lee CC, Hoang A, Segovia D, Herbst A, Barthelemy F, Gibbs E, Crosbie R, Nelson SF, Miceli C, Wanagat J. Enhanced methods for needle biopsy and cryopreservation of skeletal muscle in older adults. J Cytol Histol. 2020;11(2):10.37421/jch.2020.11.553.
193. Meng H, Janssen PM, Grange RW, Yang L, Beggs AH, Swanson LC, Cossette SA, Frase A, Childers MK, Granzier H, Gussoni E, Lawlor MW. Tissue triage and freezing for models of skeletal muscle disease. J Vis Exp. 2014;(89):51586.
194. Vetter TA, Nicolau S, Bradley AJ, Frair EC, Flanigan KM. Automated immunofluorescence analysis for sensitive and precise dystrophin quantification in muscle biopsies. Neuropathol Appl Neurobiol. 2022;48(3):e12785.
195. Farrokhi V, Walsh J, Palandra J, Brodfuehrer J, Caiazzo T, Owens J, Binks M, Neelakantan S, Yong F, Dua P, Le Guiner C, Neubert H. Dystrophin and mini-dystrophin quantification by mass spectrometry in skeletal

muscle for gene therapy development in Duchenne muscular dystrophy. Gene Ther. 2021:10.1038/s41434-021-00300-7.
196. Torelli S, Scaglioni D, Sardone V, Ellis MJ, Domingos J, Jones A, Feng L, Chambers D, Eastwood DM, Leturcq F, Yaou RB, Urtizberea A, Sabouraud P, Barnerias C, Stojkovic T, Ricci E, Beuvin M, Bonne G, Sewry CA, Willis T, Kulshrestha R, Tasca G, Phadke R, Morgan JE, Muntoni F. High-throughput digital image analysis reveals distinct patterns of dystrophin expression in dystrophinopathy patients. J Neuropathol Exp Neurol. 2021;80(10):955-965.
197. Aartsma-Rus A, Morgan J, Lonkar P, Neubert H, Owens J, Binks M, Montolio M, Phadke R, Datson N, Van Deutekom J, Morris GE, Rao VA, Hoffman EP, Muntoni F, Arechavala-Gomeza V; Workshop Participants. Report of a TREAT-NMD/World Duchenne Organisation Meeting on dystrophin quantification methodology. J Neuromuscul Dis. 2019;6(1):147-159.
198. Schnell FJ, Frank DE, Fletcher S, Johnsen RD, Wilton SD. Challenges of interpreting dystrophin content by Western blot. US Neurology. 2019;15(1):40–46.
199. FDA Draft Guidance for Industry: Demonstrating Substantial Evidence of Effectiveness for Human Drug and Biological Products. December 2019. Available at: https://www.fda.gov/regulatory-information/search-fda-guidance-documents/demonstrating-substantial-evidence-effectiveness-human-drug-and-biological-products
200. Federal Register. 84 FR 70196 (December 20, 2019).
201. FDA. Final Rule, New Drug, Antibiotic, and Biological Drug Product Regulations; Accelerated Approval (57 FR 58942, 58944 (December 11, 1992).
202. Parent Project Muscular Dystrophy web page, Guidance for Industry: Duchenne Muscular Dystrophy Developing Drugs for Treatment over the Spectrum of Disease. Available at: https://www.parentprojectmd.org/wp-content/uploads/2021/07/2014_Community_Guidance.pdf
203. Parent Project Muscular Dystrophy news: PPMD Begins Process to Update Community-Led Duchenne Guidance for FDA. July 22, 2021, available at: https://www.parentprojectmd.org/ppmd-begins-process-to-update-community-led-duchenne-guidance-for-fda/
204. 21 U.S.C. § 356(c)(2)(A)
205. 21 CFR 314.510
206. 21 CFR 601.41
207. 21 U.S.C. § 356(c)(3)(A)
208. 21 CFR 314.530
209. 21 CFR 601.43
210. 21 U.S.C. § 356(g)(7)
211. FDA Guidance for Industry: Long Term Follow-Up After Administration of Human Gene Therapy Products. January 2020. Available at: https://www.fda.gov/media/113768/download
212. 21 U.S.C. § 356(c)(3)
213. 21 CFR 314.150(d)
214. 21 CFR 601.5(a)
215. FDA Draft Guidance for Industry: Benefit-Risk Assessment for New Drug and Biological Products. September 2021. Available at: https://www.fda.gov/media/152544/download
216. Louis Jeune V, Joergensen JA, Hajjar RJ, Weber T. Pre-existing anti-adeno-associated virus antibodies as a challenge in AAV gene therapy. Hum Gene Ther Methods. 2013;24(2):59-67.
217. FDA Draft Guidance for Industry: Rare Diseases: Common Issues in Drug Development. January 2019. Available at: https://www.fda.gov/media/119757/download
218. Peay HL, Fischer R, Mange B, Paquin RS, Smith EC, Sadosky A, Russo L, Ricotti V, Rensch C, Morris C, Martin AS, Ganot A, Beaverson K, Mansfield C. Patients' and caregivers' maximum acceptable risk of death for non-curative gene therapy to treat Duchenne muscular dystrophy. Mol Genet Genomic Med. 2021;9(5):e1664.
219. FDA. Cellular, Tissue, and Gene Therapies Advisory Committee (CTGTAC) September 2-3, 2021 Meeting Announcement. Available at: https://www.fda.gov/advisory-committees/advisory-committee-calendar/cellular-tissue-and-gene-therapies-advisory-committee-september-2-3-2021-meeting-announcement
220. FDA. CTGTAC. Briefing document for the meeting on September 2-3, 2021. Available at:

https://www.fda.gov/media/151599/download
221. Chand D. Clinical findings of thrombotic microangiopathy (TMA). Presented as part of CTGTAC meeting on September 2-3, 2021. Available at: https://www.fda.gov/media/151999/download
222. Zolgensma (onasemnogene abeparvovec-xioi) Prescribing Information. October 2021. Available at: https://www.fda.gov/media/126109/download
223. FDA. CTGTAC. Summary Minutes of the CTGTAC meeting on September 2-3, 2021. Available at https://www.fda.gov/media/154397/download
224. Carsten B, Belluscio B, Braun S, Morris C, Singh T, Muntoni F. A collaborative analysis by clinical trial sponsors and academic experts of anti-transgene SAEs in studies of gene therapy for DMD. Poster Presented at MDA Clinical & Scientific Conference; March 13-16, 2022; Nashville, TN. Available at: https://mdaconference.org/node/1557