Is broader collaboration the critical key to better biomarkers in Duchenne Muscular Dystrophy?

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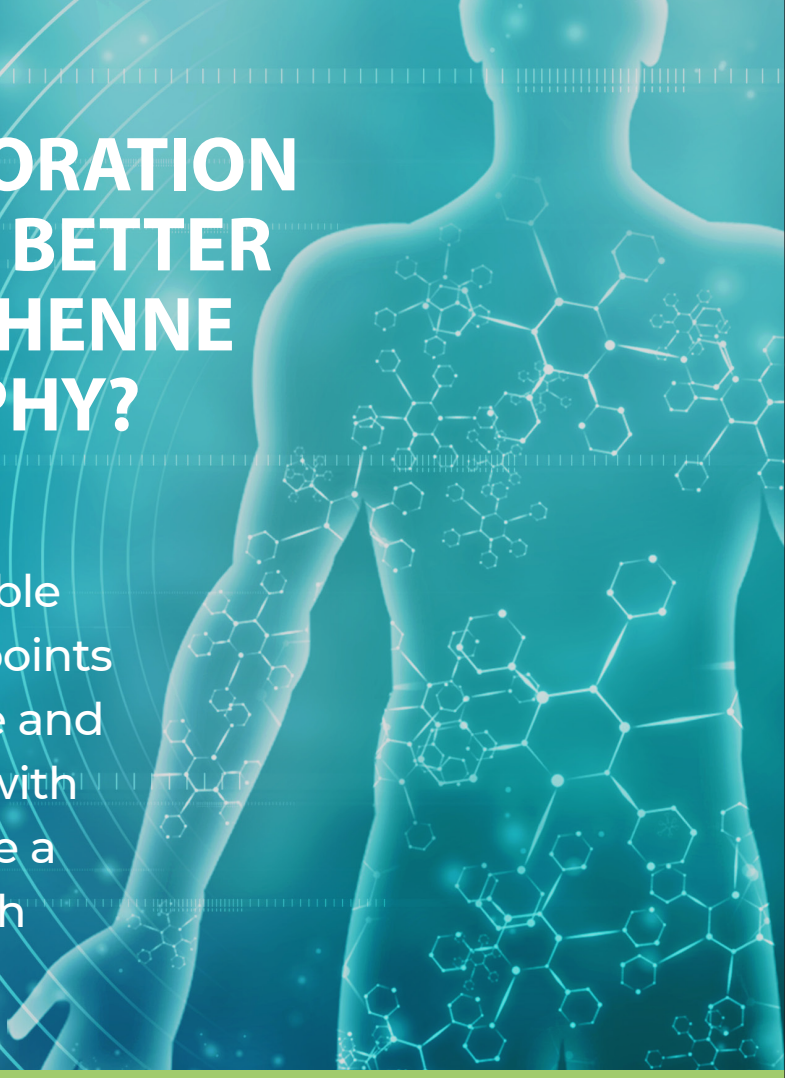
IS BROADER COLLABORATION THE CRITICAL KEY TO BETTER BIOMARKERS IN DUCHENNE MUSCULAR DYSTROPHY?



A toolkit of robust and reproducible biomarkers and innovative end points remains crucial for finding a cure and better therapies for those living with DMD today. Could the solution be a consortia approach to accomplish what no one can do alone?

This thought paper by VISION-DMD, supported by an Expert Advisory Panel, offers a summary of the current challenges and knowledge gaps in the use of robust biomarkers for optimizing clinical trial outcomes. It highlights the urgent need for a central and coordinated approach to support their identification, adoption and qualification to accelerate future research breakthroughs for DMD.

WHAT IS DUCHENNE MUSCULAR DYSTROPHY (DMD)

DMD is a progressive and severe, rare genetic disease affecting approximately 1 in 3,500 to 5,000 male births and very rarely girls.

The disease is devastating: untreated, boys become progressively weaker during childhood, losing independent ambulation at an average age of nine years, and death often occurs by early adulthood due to cardio-respiratory failure.

A severe type of muscular dystrophy, DMD is caused by a genetic inability to create dystrophin, a protein that protects skeletal and heart muscle from injury caused by normal contraction and relaxation. The disorder is caused by an X chromosome mutation.

While DMD currently remains incurable, long-term use of corticosteroids is widely accepted as standard of care.

Despite many therapeutic advances over the past 30 years, there is still no cure for DMD. However:

- Studies are deepening our understanding of the mechanisms of the disease.
- Drugs that aim to restore the missing dystrophin protein – or treat Duchenne symptoms by protecting muscles, reducing fibrosis or inflammation – are in clinical development.
- Potential for further progress exists through the use of innovative MRI techniques, gene therapies and the use of precision sensors.

But there's a long way to go.

Most crucially, there is a need focus on the most promising options and create community consensus around the disease's potential biggest game-changers – biomarkers for specific contexts of use in drug development and clinical trial design – fragmentation of approaches remains an important hurdle to progress.

Multiple activities are advancing work on proposed or potential biomarkers targeting DMD and related neuro muscular diseases (NMDs) and candidate technologies. These include international academic and industry-led collaborations and clinical development programmes.

But this research is fragmented globally, hampering and slowing the selection and validation of the most promising biomarker approaches.

In the future, with improved coordination of biomarker utilisation, however, barriers could be removed to allow research to focus on all participants' shared objective

That is a better understanding and targeting of the causes of DMD and potential treatment lines so that we can stop the disease in its tracks.

Biomarkers in brief

Biomarkers are a measurable indicator of a biological condition.

In the regulatory framework, the term 'biomarkers' encompasses a broad range of novel methodologies, including:

- **Biomarkers (prognostic/diagnostic and predictive)**
- **Clinical outcome assessments (PRO, ClinRO, ObsRO)**
- **Imaging markers**
- **Symptom scales**
- **Animal Models**
- **Statistical Methods**

Biomarker acceptance in the EU and US is growing. Figures show that across all indications, over half of newly approved drugs in the US and EU between 2015 and 2019 were supported by biomarker data during at least one of the development stages (at 69% for the EMA and 59% for the FDA).

Could the goal of agreeing better biomarker guidelines be reached faster by aligning people and resources?

By enabling:

- The Duchenne community (scientists, clinicians, patient organisations, regulators and industry) to engage more productively to advance activities towards identifying knowledge gaps and standardising approaches to the use and further research of biomarkers?
- The data that supports the technical and clinical validation of existing biomarkers and those in development to be shared within a single, publicly available dataset to compare, contrast and combine results from different studies?
- The establishment and funding of a collaborative mechanism to spearhead and catalyse the development of potent biomarkers towards the level of evidence needed for use in trials or for regulatory qualification?

> Generally, companies are developing biomarkers and tools in the context of a single drug development. What is missing and may facilitate developments in the NMD area is more collaboration and more data sharing between companies, academics and patient organisations.

Pavel Balabanov - Head of Therapies for Neurological and Psychiatric Disorders at European Medicines Agency

THE TRANSFORMATIVE ROLE OF BIOMARKERS IN CLINICAL TRIAL SUCCESS

Genetic and biochemical research over the years has characterized the cause, pathophysiology and development of DMD, providing potential therapeutic targets and biomarkers.

Promising results using biomarkers have shown that under the right conditions, their integration into evidence-based medicine could transform drug discovery, development and approval processes for the disease.

The availability of qualified biomarkers would not only benefit DMD patient management. It would also speed up the evaluation of medicinal products.

But whatever the therapeutic area, distinguishing between a potential biomarker and a reliable biomarker that can be universally used to guide important clinical and commercial decisions is far from easy.

Biomarkers in Duchenne

These can be divided into two main groups:

- **Diagnostic biomarkers, used to identify the disease:** established as serum creatine kinase (CK) activity or genetic mutations in the DMD gene
- **Monitoring biomarkers:** that can be assessed repeatedly over time.

These are split into:

- **Prognostic biomarkers:** that identify likelihood of a clinical event or disease progression
- **Predictive biomarkers:** these can be used to gain information about the effect of a therapeutic intervention .

These comprise:

- **Safety biomarkers** (now well established)
- **Pharmacodynamic biomarkers** (often drug specific such as the use of dystrophin for dystrophin upregulating drugs).
- **Stratification biomarkers** are starting to be used for identifying patients who are likely to respond or not respond to a type of drug or intervention allowing stratification (selection) of patient groups for treatments

Ideally biomarkers should be safe and easy to measure, cost efficient, changeable with treatment and consistent across gender and ethnic groups.

Biomarkers as cells, genetic variations, miRNAs, proteins, lipids and/or metabolites indicative of disease severity, progression and treatment response have the potential to improve development and approval of therapies, clinical management of DMD and patients' life quality.

Biomarkers may be used during drug development to show that a drug is hitting its target, and to aid in dose selection.

Those associated with patients' performance that indicate clinical benefit, such as surrogate endpoints, which show smaller inter-individual variation, would also enable smaller and better controlled studies to be performed, hastening the evaluation of medicinal products.

However, their validation and clinical uptake for DMD is complex, explains Cristina Al-Khalili Szigyarto, Senior Researcher at the Royal Institute of Technology (KTH), School of Biotechnology, Stockholm, Sweden.

Defining context of use means addressing fundamental questions.

Why are we using this biomarker? Is this for monitoring disease progression, for predicting populations of patients likely to progress in a certain way, for demonstrating a drug action? What do we want to show with the biomarker? Why is the biomarker fit for this purpose?

"The process is dependent on how well the context of use is defined based on clinical utility as well as the feasibility in translating such markers to the patient, based on available resources," says Cristina Al-Khalili Szigyarto.

Blurry biomarker boundaries

Historically, discovery, validation and qualification of biomarkers has been considered a linear process, but in reality, she says, this is not the case.

"Biomarkers discovered analysing one patient cohort can fail in subsequent validation steps due to the lack of reproducibility when analysing another cohort," she adds. "This a major challenge in particular when analysing small cohorts within rare disorders."

At KTH, her team is now designing strategies that address these challenges, aiming at conforming the results in different cohorts, sample types and with different methods.

Biomarkers may be able to be used for multiple contexts of use. A biomarker that could be useful for monitoring disease progression could also be useful as a surrogate endpoint.

However, for regulators to accept a biomarker as a surrogate requires significantly more data supporting its connection to clinical outcomes than for its use as a monitoring biomarker that might inform inclusion criteria or dosing.

A coordinated approach may be the best way to advance this process faster. Biomarkers could be developed initially for some contexts of use, while further data is gathered to support more complex contexts in the future.

Regulatory qualification of biomarkers

Further expertise may be facilitated by the Critical Path Institute (C-Path), a non-profit, public-private partnership with the FDA that has experience in qualification of biomarkers and standardisation of data.

A collaboration between C-Path and Parent Project Muscular Dystrophy has formed the Duchenne Regulatory Science Consortium (D-RSC). This is specifically to develop tools to accelerate therapy development for DMD, primarily to develop a clinical trial simulation tool.

This will allow informed development of future clinical trial protocols and provide an evidence base to support patient stratification decisions for trial inclusion, selection of specific clinical study endpoints, and data analysis strategies.

"The objectives of qualification are

> Collaboration is urgently needed for retrospective validation of potential prognostic biomarkers by pooling all available data, identifying who can bring this forward to develop a package for assessment by regulators to make progress.

Pietro Spitali
Assistant Professor at Leiden University Medical Center and Duchenne expert

to make drug development tools (DDTs) publicly available to be used for a specific context of use in drug development, streamline drug development, making it easier for regulatory applications by the drug developer and facilitate integration of qualified DDTs in regulatory review and to provide a framework for scientific collaboration," explains Jane Larkindale, former Executive Director, D-RSC, C-Path.

C-Path is well positioned to seek regulatory endorsement for tools developed by the consortium or associated activities from both the US Food and Drug Administration (FDA) and the European Medicines Agency (EMA).

Potential to support international biomarker validation efforts also exists via cTAP, a US-based global coalition of some of the most prominent people and organizations within each different group of stakeholders in the Duchenne field, with a strong industry drive.

"Due to the broad collaboration of cTAP partners, a rich database of invaluable longitudinal data has been developed … Consistency across data sources needs to be determined and comparison of performance," says Susan Ward, Founder and Executive Director, cTAP-Duchenne.

Using the cTAP platform, researchers have for the first time categorized the heterogeneity of natural history progression that underpins many of the challenges in Duchenne drug development and developed prognostic models that more than double the power of the study.

This and other breakthroughs by cTAP collaborators have the potential to enhance clinical trial design and analysis, identify prognostic factors, and guide biomarker research.

But success is still some way off.

"A link between biomarkers and clinical benefit is the key goal to show if a drug is working," says Pietro Spitali, Assistant Professor at Leiden University Medical Center and Duchenne expert.

Thus far, he says: "There are currently no biomarkers for Duchenne that can do this."

Without biomarker robustness, trials will fail

Up to now, the field of DMD biomarker development has not reached its translational potential. The reasons for this are numerous and go beyond the intervention. They include:

- **high (intra- and inter-) patient variability in performance**
- **noisy outcome measures**
- **small patient populations**
- **good vs bad responders**

The patient community perspective

For the Duchenne patient community, biomarkers that have clinically meaningful outcome measures are a priority.

Robust biomarkers, they know, could lead to shorter and less invasive trials, more reliable outcomes and a surrogate endpoint.

"After 20 years of clinical trials in Duchenne, not a single biomarker has been qualified, although there are very few in any indication," says Elizabeth Vroom, Co-Founder of the World Duchenne Organisation.

"That is why patient foundations have recognised the need for a central platform to allow patients to share their samples and data to benefit other trials and research."

To do this, the Duchenne Parent Project has built a data platform where patients' individual data is now stored in data lockers .

Noisy outcomes

The failure of the potentially promising DMD therapy, Kyndrisa (Drisapersen), is an example of a Phase 3 trial failure caused by low signal to noise ratio (Goemans et al. 2018).

Matrix Metalloproteinase-9 (MMP-9) serum levels were quantified as an efficacy biomarker because it increased over time in natural history cohorts and was thought to be connected to fibrosis (Nadarajah et al. 2011). However, when the Phase 3 results were evaluated and compared with the placebo arm there was no difference for MMP-9 serum levels.

The treatment did reduce serum CK and serum lactate dehydrogenase (LDH) compared to the placebo arm (Goemans et al. 2018), which may be interpreted as reduced muscle damage due to treatment.

But for the MMP-9 the relevance of the biomarker was unclear.

VISION-DMD and the importance of biomarker context of use

A new drug looks set to offer improved quality of life for young boys with DMD by potentially offering an alternative to high-dose glucocorticoids that have significant side effects.

The VISION-DMD program is developing vamorolone as a first-in-class steroidal anti-inflammatory drug for DMD and other chronic inflammatory states.

Innovations in clinical trial design include integration of biomarkers as objective measures of safety and efficacy.

This phase 2a study clearly states the proposed context of use and objectives for the biomarkers investigated: secondary outcomes for pharmacodynamic safety, exploratory outcomes for drug mechanism of action and exploratory outcomes for expanded pharmacodynamic safety.

So far, the results are promising. As steroids can cause adrenal suppression the study investigated morning cortisol. There was no clear dose effect but at the highest concentration (0.06mg/kg/day), adrenal suppression was seen, but not in the lower doses.

The bone turnover biomarkers also had changes in the high dose cohorts, which were not seen in the lower doses.

A Phase 2b trial [NCT03439670] has recently finished. The Treatment Period 1 study (24 weeks) met the primary endpoint of superiority in change of time to stand from supine positioning to standing (TTSTAND) velocity with vamorolone 6 mg/kg/day versus placebo (p=0.002) with a treatment difference of 0.06 [95% CI:0.02–0.10] rises/second from baseline. The study also demonstrated superiority of both vamorolone dose levels (2 and 6 mg/kg/day) versus placebo across multiple secondary endpoints. Results of Treatment period 2 (24 weeks) are expected Dec 2021.

Should top-line results from this trial be positive, they are expected to support an application to the FDA/EMA requesting the approval of vamorolone for DMD in early 2022.

FOUR POTENTIAL BIOMARKERS AND TECHN

1. NMR IMAGING AND SPECTROSCOPY

Therapeutic trials have seen a systematic use of imaging as an outcome measure and intramuscular fat fraction (FF) has been used to predict a positive effect of treatment.

Intramuscular fat fraction (FF) is a robust indicator of the extent of muscle destruction using MR imaging. It has shown remarkable sensitivity of disease progression with a threshold demonstrated to be less than 1% and a high discriminant power.

Using the standard response mean as an indicator and evaluator of the discriminate power, it has often achieved a higher value than clinical and function evaluation.

However, as an integrative biomarker, FF is thought to have limitations in predicting therapeutic response, so there is a real need for biomarkers of disease activity.

One of the main goals of Pierre Carlier's lab at the Institut de Myologie is to move from qualitative and semi-quantitative evaluation to truly quantitative imaging measurements, which are essential to ensure the clinical relevance of high-technology imaging procedures.

Potential other areas of interest also include detecting membrane phospholipid turnover, ionic homeostasis disturbances and sarcolemma leakiness.

cTAP intelligence has highlighted that qualification of FF MRI is only a high priority for very few companies. But the majority of companies in DMD would contribute to qualifying FF MRI as a 'validated' biomarker, and support action to advance MRI to a regulator-approved qualified biomarker

"Therapeutic trials have seen a systematic use of imaging as an outcome measure and FF has been used to predict a positive effect of treatment."
Pierre Carlier, Director of the Institut de Myologie's NMR Laboratory, France

2. GENETIC TESTS AS DISEASE BIOMARKERS

The dystrophin gene is a large and complex gene with 79 exons covering 2.2 Mb on the locus.

Mutations in this gene cause Duchenne and Becker muscular Dystrophy (DMD and BMD) and other milder phenotypes.

Our current knowledge of the relationship between genotype and phenotype in Duchenne is however still incomplete.

More work needs to be done to understand how the DMD gene works and is regulated to allow correlation between the gene mutations and phenotype.

The use of genetic tests as potential disease biomarkers in DMD has been highlighted by research that shows disease severity can vary across muscle, cardiac and cognitive functions.

A comprehensive understanding of the molecular pathways and hub genes involved is now needed to discover new therapeutic targets, explore the underlying mechanism, and to identify biomarkers for prognostic evaluation.

"Performing detailed genetic characterization of the DMD locus may reveal important information and serve as a disease severity genetic biomarker. We envisage that deep genetic characterization (by CGH or WGS) should be used in the future to clarify phenotype-genotype correlations or deep phenotyping evaluated in clinical trials."
Alessandra Ferlini Md, Phd, University Of Ferrara, Italy University College London, UK

3. PHARMACODYNAMIC (PD)BIOMARKERS

ReveraGen' VISION-DMD programme has used PD biomarkers in the early phase open label trials of vamorolone to demonstrate the proof of concept mechanism of action of efficacy and anti-inflammatory effects of the drug.

Biomarkers have also been used as secondary outcomes to show reduction in safety concerns associated with glucocorticoids.

Innovative biomarkers were also employed to argue against placebo effect and demonstrate proof-of-concept mechanism of action in open label Phase 2a studies.

They have been used to benchmark safety concerns against glucocorticoids to move into the next phase trial.

PD biomarkers may be used to clinically de-risk future trials of vamorolone in other indications, and support trials in older and younger aged children with Duchenne to supplement PK/ safety data where outcome measures are more challenging.

"In novel trial designs and innovation in paediatrics and rare diseases, PD biomarkers could potentially be used to demonstrate a Pharmacokinetic (PK)/PD relationship and support extrapolation of efficacy between patient groups."
Laurie Conklin, Former Director of Regulatory Affairs, ReveraGen Biopharma, US

4. DIGITAL MOTOR BIOMARKERS

There is a great demand for accurate non-invasive measures to better define the natural history of disease progression or treatment efficacy in DMD and to facilitate the inclusion of a large range of participants in DMD clinical trials.

SYSNAV has developed a digital biomarker which measures movements through different outcome, including a recently qualified 95% stride velocity. The SYSNAV digital outcome platform consists of sensors, IT systems and processes for clinical trials (e.g. training, compliance moniroting, logistics). Stride velocity 95th centile (SV95C) is the first wearable acquired digital endpoint to receive qualification from the European Medicines Agency (EMA) to quantify the ambulation ability of ambulant DMD patients aged ≥5 years in drug therapeutic studies and it is also currently under review for FDA qualification.

Such markers could be a non-invasive way of evaluating muscle health, limiting the need for muscle biopsies, decreasing the variability of assessment and addressing outcomes early, which would help reduce trial length or size.

With 95% stride velocity SYSNAV were able to control variability to demonstrate the effect of the disease or the positive effects of a drug after just six months.

"This new outcome brings many benefits allowing shorter trials with 8x fewer patients. It is important for pharmaceutical companies as they can develop drugs faster, important for regulators as they have more robust evidence to judge the efficacy of drugs on the parameters that are relevant to them, and it is also important to patients as this will bring better access to treatment, and is not as intrusive as some tests. In 2020, thanks to patient feedback and advice from healthcare professionals, a new version, called SYDE, was born. SYDE has the same precision but is smaller, lighter and more comfortable."

– **Damien Eggenspieler, Healthcare Program Director, Sysnav, France.**

TO ACHIEVE LIFE-CHANGING RESULTS REQUIRES BOLDNESS.

Because, as Pietro Spitali of Leiden University Medical Center expresses it so clearly: "There are many individual groups working on their own biomarkers who understand the need for qualification but no one is taking the lead.

"This has created a vacuum between what is being done, the range of evidence that is being collected and the next steps in moving this forward."

A selection of the strongest biomarker candidates is now needed whose evidence base can be advanced towards supporting their use and validation with regulators.

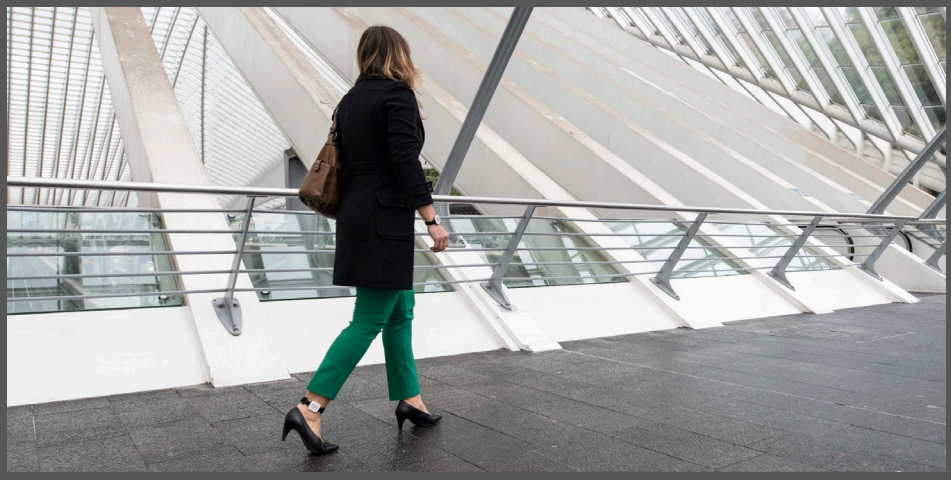
Ample capabilities exist towards advancing this goal thanks to the expertise, good practice and initiatives of forward-thinking organisations.

These include: C-Path, the Duchenne Regulatory Science Consortium (D-RSC), cTAP and Parent Project Muscular Dystrophy (PPMD)in the US, and the World Duchenne Organisation (WDO)and EURO-NMD European Reference Network in the EU.

The DMD community overall is well placed to engage the required support from patient organisations, clinicians, industry and other stakeholders in the US, EU and elsewhere.

While this thought paper does not propose any one solution, it is intended to stimulate discussion within the Duchenne community and to foster collaboration.

The new wearable SYDE monitor from SYSNAV



We believe a suggested way forward might look like this:

Where are we now?

Action: Creation of a landscape analysis of biomarkers in development for Duchenne and the existing data sets and samples that support their technical and clinical validation shared within a single, publicly available dataset for analysis.

Where do we want to be?

Goal: Qualification of one or more DMD biomarkers as an end goal.

How will we get there?

Action: Preparation of a business plan for developing a consortium approach to further developing appropriate biomarkers to the next stage of validation and/or qualification if the data supports it.

What is our route map?

- **Step 1:** Development of criteria for prioritising biomarker candidates and identification of missing data with respect to their technical and clinical validation.
- **Step 2:** Establishment of standard operating procedures, protocols or data standards to combine datasets and fill gaps.
- **Step 3:** Identification of lead candidates that may be ready to be considered for qualification by regulatory bodies based on the data.
- **Step 4:** Generation of protocols/analysis plans to further develop the biomarkers towards the level of evidence needed for use in trials and/or qualification.

"The key to achieving the qualification of a biomarker for DMD is more transparency and information sharing."

Cristina Al-Khalili Szigyarto
is a senior researcher at the Royal Institute of Technology (KTH), School of Biotechnology, Stockholm, Sweden

"It's important that we really know the correct place and value of biomarkers in the drug development process."

Elizabeth Vroom
Co-founder of the World Duchenne Organisation

To find out more, we invite you to read VISION-DMD's full workshop report here
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More information about VISION-DMD can be found here:
https://vision-dmd.info

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