VISION-DMD

Future clinical trial design and innovative end points (Biomarkers and Imaging) for DMD and other Neuromuscular diseases workshop report 2019

The VISION-DMD project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement number 667078.

CERATIUM
HELPING RESEARCH HAPPEN

The workshop was held on 29th November 2018 at Motol University Hospital Prague and was a co-run event by the VISION-DMD project and the European Reference Network for Neuromuscular Disease EURO-NMD.

The first part of the workshop summarised the state of the art with presentations from leading experts from industry, academia, regulators, patient foundations and the clinical specialists and was attended by over 50 participants. Following the presentations an expert panel discussion with invited participants addressed the current landscape and future aims and objectives. A follow-up discussion (January 2019, Leiden), future planning and summary of biomarker data collated so far are also described.

Authors: Christina Olsen, Ritchie Head, Jana Knerova, Jane Larkindale, Susan Ward, Pietro Spitali and Hermien Kan supported by our event chairperson Jana Haberlova, the presenters from the Prague Workshop, and attendees from the Prague Working Group and Leiden meetings. This report must not be used as a resource for commercial purposes or further research without prior permission of the VISION DMD consortium. Via corresponding author Ritchie Head (ritchie.head@ceratium.eu).

This report is a reflection of the stakeholder workshop on Future clinical trial design and innovative end points (Biomarkers and Imaging) for DMD and other Neuromuscular diseases workshop, which took place in Prague, 29th November 2018, and the follow-up Action Plan meeting on Biomarker assessment towards standardised approaches and validation held in Leiden 15th Jan 2019.

Contents

1. Executive Summary

To add once body of report is agreed

2. Presentations Biomarker Workshop Prague Nov 2018

INTRODUCTION TO THE WORKSHOP. *JANA HABERLOVA, FNM AND RITCHIE HEAD, CERATIUM LTD*

The workshop was organised by the VISION-DMD project in collaboration with the ERN EURO-NMD to utilise the capacity of the European experts already attending the ERN annual meeting. The workshop aim was to make progress in the identification and validation of biomarkers for NMD by bringing together researchers, clinicians, patient organisations, regulators and industry to address the barriers, and identify the knowledge gaps.

VISION-DMD is an EC funded Horizon 2020 project delivering Phase 2 clinical trials of Vamorolone for DMD but also examines other novel elements such as the use of Venture Philanthropy in drug development in rare disease, innovative MRI techniques and other biomarkers and the use of mobile health bands. The EURO-NMD ERN is an EC initiative to develop a European Research Network of experts in Neuromuscular Diseases (NMD) to share and develop best practice and clinical guidelines to improve the diagnosis and care of NMD rare disease patients in the EU.

The workshop aims are to highlight the state of the art, engage with ERN participants, identify and prioritise knowledge gaps, and find potential routes forward by building momentum with existing platforms and key players. Key outcomes will be a roadmap of the next steps, a policy document for the EC ad stakeholders, a list of initiatives to engage with, champions identified to lead on key actions, roles and responsibilities allocated, and funding sources considered.

STATE OF THE ART: USE OF BIOMARKERS IN CLINICAL STUDIES AND THEIR LIMITATIONS. *PIETRO SPITALI, LEIDEN UMC NETHERLANDS*

There are many diagnostic biomarkers used in Duchenne, e.g. serum creatine kinase (CK) activity, or genetic mutations in the DMD gene, and it is generally accepted that there is no further need for diagnostic biomarker development. Current developments focus on monitoring biomarkers that can be repeatedly measured and change with the course of the disease. Prognostic biomarkers identify the likelihood of a clinical event or disease progression, while drug response predictive biomarkers give information about the effect of a therapeutic intervention. Safety biomarkers are already well established, and pharmacodynamic/response biomarkers while often specific to an intervention or type of intervention (e.g. dystrophin for dystrophin upregulating drugs), they may be more general – e.g. most diabetes drugs normalize blood sugar levels. The ultimate goal would be to develop biomarkers as a surrogate endpoint which could anticipate clinical benefit. In practise many clinical trials fail, but it is often not known if the failure is due to the intervention or whether failure is down to the design and evaluation of the trial. The power of the clinical studies, the influence of high intra and inter patient variability in performance, and imperfect outcome measures, can all be a factor in

trial failure. Noisy outcome measures reduce the power of trials, so the signal to noise ratio can be low. As an example, the Phase 3 Drisapersen (Kyndrisa) study had a pre specified 90% power, but this was in fact shown to be only 53%, with the study underpowered it is difficult to know if the drug failed or was failed by the trial design. Rare diseases also by nature have small patient populations and there can be good and bad responders in any patient population. There will be differences in disease progression and may be differences in response. The Drisapersen phase 3 study had 186 DMD patients. MMP-9 serum levels were quantified as an efficacy biomarker due to the belief that MMP-9 was connected to fibrosis as it increased over time in natural history cohorts. At the baseline level of the Phase 3 trial there was elevated MMP-9 levels compared with healthy controls. In two natural history cohorts from Leiden and Newcastle, MMP-9 serum level increased over time. In the Phase 1 study in 12 patients there was a decline in MMP-9 levels which was thought to be a response to the treatment and could be used to predict response. When the Phase 3 results were evaluated and compared with the placebo arm there was no difference between them for MMP-9 serum levels. The treatment did reduce serum CK and serum lactate dehydrogenase (LDH) compared to the placebo arm which may be interpreted as reduced muscle damage due to treatmenthowever for MMP-9 the relevance of the biomarker was unclear.

The "context of use" describes a precise scenario under which data supports the use of the biomarker in drug development, including which populations of patients it applies to. If the biomarker is qualified by the regulators, it will be qualified only for this specific scenario unless or until data supports broader use. The phase 2a vamorolone study (VISION-DMD) clearly states the proposed context of use for the biomarkers investigated: secondary outcomes for pharmacodynamic safety, exploratory outcomes for drug mechanism of action and exploratory outcomes for expanded pharmacodynamic safety. These are clear objectives for the biomarkers, and they are supported by a body of literature. The Phase 2a vamorolone trial was a 2-week study with 4 dose groups of 12 DMD boys per group. Vamorolone claims to have the benefits of steroids with fewer side effects. 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. As this was small trial more evidence from further studies will be required to make a strong claim and work is ongoing.

A link between biomarkers and clinical benefit is the key goal to show if a drug is working and the related clinical improvement. There are currently no biomarkers for Duchenne that can do this. Looking at retrospective data, it is well established that patients with Duchenne lose their ability to walk and patients treated with steroids walk longer than those not treated. Work was undertaken by several research groups on a survival model to predict age of loss of ambulation. In the study 149 patients were investigated and sampled annually, 97 entered the study as non-ambulant, 37 were ambulant at the last study visit, and 15 lost ambulation during the study. A time to event analysis was undertaken and blood samples tested for markers able to improve the prediction on top of age. Three markers were found that could improve this prediction. MDH2, KRT10 and DES.

In conclusion, there is an urgent need for monitoring biomarkers, and while pharmacodynamic biomarkers are needed to show target engagement, they are drug specific and should be undertaken by industry as opposed to academics. It is vital that the context of use is defined for a biomarker and prediction of clinical benefit is the main objective to accelerate approval of a drug.

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.

BIOMARKERS FOR DMD AND OTHER NEUROMUSCULAR DISEASES: HOW DOES THE EMA SUPPORT THEIR DEVELOPMENT? *PAVEL BALABANOV, HEAD OF CENTRAL NERVOUS SYSTEM AND OPHTHALMOLOGY (AD INTERIM) EUROPEAN MEDICINES AGENCY. NETHERLANDS*

The EMA supports and stimulates progress in the field of biomarkers in NMD, as this will provide additional tools for regulators to make decisions. In order to make this progress reality, regulators need to work in close collaboration with the community and ensure mutual awareness and sufficient platforms for a continuous dialogue are available. In the regulatory framework the term "biomarkers" often refers to a broad range of novel methodologies including Biomarkers (prognostic/diagnostic and predictive), Clinical Outcome Assessments (PRO, ClinRO, ObsRO), Imaging Markers, Symptom Scales, Animal Models and Statistical Methods. In the past, when companies or consortia have approached regulators to discuss biomarkers, the main issues for discussion have been the expectations of the regulators for the different levels of available data for validation, in relation to the intended context of use of the novel methodology.

The validation of biomarkers generally concerns tools intended to be used in the following context : biomarkers for preclinical development, clinical development and drug utilisation. Most discussions with applicants are around validating a surrogate endpoint to be used in clinical trials, instead of currently used scales or relevant other measures, serving as endpoints. In the field of Duchenne and Neuromuscular disease this is a task not easily achieved. The difficulty partially arises from the fact that due to the specifics of the diseases involved, and the requirement that there is a clear demonstration of a relationship between the biomarker and a meaningful outcome, registered with the traditional tools, the level of data needed to consider something validated becomes very high. The above is especially true when we are discussing the validation of a biomarker to replace a traditional clinical endpoint, where the level of evidence needed to validate the biomarker requires a clear relationship to be confirmed between the data supporting the biomarker's relationship with clinically relevant outcome, in the specific context of use. 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. With joint efforts from all stakeholders a validation could be made, not just to provide data for the development of a single drug product but one relevant throughout the spectrum of a disease. Therefore, bringing together all stakeholders for cooperation and data sharing into discussion with regulators is important.

The biomarker qualification process at EMA is governed by the Scientific Advice Working Party (SAWP), who serve as the primary scientific assessment group, allowing extensive networking within the Agency and its collaborators from the EU member states, and the Committee for Medicinal Products for Human Use (CHMP). Peer review, discussion and adoption of final responses (Advice Letter or Qualification Opinion) is undertaken by the CHMP plenary. The EMA encourage early contact with participants about potential intended validation applications and the scientific level of the available data, so that these scientific bodies have the opportunity for an early dialogue with the applicants, informing and facilitating the next steps. The key message is "come early and talk to us".

The official route to have a tool/biomarker validated by the EMA for a specified context of use is a process called 'Qualification of Novel Methodologies'. This is a regulatory assessment process the result of which will be either a *qualification opinion* (if enough data are provided) or a *qualification advice* which is completely confidential and will provide the guidance on what is needed to go forward. The procedure allows for a full exchange with the involved experts, providing sufficient detail on the expected requirements for validation. If the novel methodology is shown to be promising, but there is insufficient data for a qualification opinion, there is a public measure called a Letter of Support from the EMA providing a high-level summary, context of use, available data and on-going and future investigations. The aim is to encourage data sharing and facilitate studies for eventual qualification. If the data provided is sufficient to qualify the biomarker, the EMA will give a qualification opinion, and provide a detailed publicly available document. This is always a positive opinion, there are no negative opinions in this procedure. This procedure does require a payment of fees but there are fee exemptions available. Anyone can apply to this process and the EMA are open to discuss the costs of time and resource to find the best way forward.

BIOMARKERS IN DMD – A PATIENT PERSPECTIVE. *ELIZABETH VROOM, DUCHENNE PARENT PROJECT. NETHERLANDS*

Patient groups have been working on biomarkers for Duchenne for some time and organised several meetings on this topic. It's important that we really know the correct place and value of biomarkers in the drug development process. Biomarkers in Duchenne are important to parents as the hope is that they will lead to shorter trials, more reliable outcomes, a surrogate endpoint and potentially extrapolation to other groups, so trials are less burdensome for patients. However, parents are sensitive to the "hype" produced by drug companies and it is important to families hoping for "drugs that work", that biomarkers are used in the right context.

Patient organisations have key concerns about biomarkers. Communication about the role of biomarkers is crucial for families. A biomarker may give a hint that a drug is working, but that does not mean the drug is useful for the community or will be approved by regulatory bodies. The knowledge of the whole community about the role of biomarkers in the regulatory process is a concern, as the people working in this field may think every biomarker has an important role, but this is not always the case. Another concern is the handling, optimal use and informed consent of samples, especially biopsies. Transparency is important so that everybody knows what is being done and information is shared, specifically with patient groups. There are serious concerns about the use of muscle biopsies in placebo-controlled trials. Patients only want biopsies when they are really useful. Children in a placebo-controlled trial might have up to 5 biopsies but be in the placebo group, this high burden seems unfair to the patient community. Probably the place for biopsies is a phase 2 trial and not in placebo-controlled phase 3 trials. Options for a less invasive biomarker collection should be considered.

It is important that before samples are taken, patients and families know the regulatory authorities have agreed with the proposed methods of analysis, and that they have easy access to their data at the end of the trial. It is vital for everybody that we improve knowledge about the role and choice of biomarkers, and use appropriate wording and accurate information, when communicating the role of biomarkers.

A key requirement is to relate biomarkers to clinically meaningful outcome measures and PROMS (patient reported outcome measures). As an example, bone turnover biomarkers are often discussed,

but how do they translate to clinic? How do they correspond with fewer fractures in children? The focus needs to be on how much they must change to be clinically meaningful.

After twenty years of clinical trials in Duchenne not a single biomarker has been qualified, although there are very few in any indication. 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. The Duchenne Parent Project have built a data platform where patients individual data is stored in ***data lockers***. These lockers can store their data on different healthcare outcomes and potentially clinical trial information when possible, for reuse to the benefit of the whole community.

There is a real need to include patients and families into the decision-making process about the choice of the biomarkers and clinical trial design in general. It is the children who give their blood, muscle, sweat, tears and access to every part of their body and life participating in trials, they should not be regarded as a number or resource to be used, but as little boys with lives, hopes and dreams.

VALIDATION OF BIOMARKERS FOR CLINICAL TRIALS. *CHRISTINA AL-KHALILI SZIGYARTO. KUNGLIGA TEKNISKA HOGSKOLAN (KTH) SWEDEN.*

A review of publications from the last seven years, shows that the increasing number of publications on discovery of molecular biomarkers has not resulted in a similar increase in the number of qualified biomarkers. Validation and clinical uptake of biomarkers is far more complex than initially expected and often associated with several challenges. 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. An important aspect is to include the perspectives of all major stakeholders e.g. patients, clinicians, researchers, geneticists, pharma companies, etc., in such efforts, to ensure integration of relevant information, requirements and standards early in the process. Successful translation of biomarkers to clinical use, relies both on the specificity and sensitivity of what it is supposed to measure e.g. disease progression as well as the analytical validation, i.e. what the methodology can actually deliver in terms of reproducibility and accuracy. Furthermore, biomarkers need to add significant clinical benefit in contrast to already existing markers to be integrated into clinicians working environment.

Historically, discovery, validation and qualification of biomarkers has been considered a linear process, but in reality, 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. This a major challenge in particular when analysing small cohorts within rare disorders. Although not considered to make a major scientific impact, confirmatory studies are necessary to ensure that true biomarker candidates are prioritised. Another challenge for biomarker validation within rare disorders is to have the right samples suitable for the planed study, meaning samples of good quality, representative for the disease phenotype, and with consistent clinical information. Missing information regarding the patient or the sample may mean that the data is not usable and has to be discarded. Furthermore, strategies used for biomarker discovery often rely on methods that are also associated with drawbacks, e.g. large coefficient of variation or poor limit of detection. Ideally if a biomarker is successfully identified using one method, other independent methods are required to ensure reliability. Biomarkers are more likely to be successfully translated to clinical use if they are thoroughly characterised with respect to inter- and intra-patient variability, to ensure reliable interpretation in a clinical setup. Currently there is very little focus on characterising biomarkers and much more work is needed. Numerous biomarkers discovered, still lack clinically acceptable assays, assays that still remain to be developed.

KTH has designed strategies that address these challenges aiming at conforming the results in different cohorts, sample types and with different methods. Molecular biomarkers discovered are analysed in both serum and plasma collected at geographically dispersed hospitals using antibody-based methods. Only biomarkers detectable in both sample types are considered for further validation as they represent more stable and reliable targets not affected by sample preparation procedures. To compensate for the small cohort size and limited number of samples inter-cohort comparison is used to exclude biomarkers influenced by other factors that the disease e.g. sample handling or biased patient recruitment. Orthogonal validation, has been designed to prove the value of the biomarkers. Two independent analytical methods, one immune-based and one mass spectrometry-based are used to quantify the biomarkers. The orthogonal validation can confirm the results by different methods also in blinded studies.

Proteomics analysis of blood suggest large variation between subjects, thus illustrating the need for a personalised medicine approach, i.e. using the patient as their own reference. Currently little information is available regarding which molecular components are present in blood and at which concentrations. Thus, normal levels and individual variation of biomarker abundance has to be characterised. Large individual variation of patients is problematic, resulting in the context of use not always being valid, for example, a biomarker may change depending on the age of the patient.

Biomarker validation must be performed through a decision-making process, that continuously assesses the results in relation to the predefined context of use. Ideally validation of several biomarkers is performed in parallel moving from multiplex to single plex enabling failures to be discarded throughout the process. The key to achieving the qualification of a biomarker for Duchenne muscular dystrophy is more transparency and information sharing. To achieve qualification and clinical uptake interlinking and transparency is required, so early stage experimental data has to be available for discussion and put in a larger context e.g. through a scientific advisory board that includes a broad representation of stakeholders.

MONITORING DISEASE ACTIVITY IN DMD PATIENTS WITH NMR IMAGING AND SPECTROSCOPY. *PIERRE CARLIER INSTITUTE OF MYOLOGY. FRANCE*

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.

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. At an individual level the progression of the FF in Duchenne patients also reflects the extreme phenotypic variability of the disease and results in a large scattering of the FF trajectories. Projects coordinated at Leiden, have attempted to apply trajectories to patients based on FF data, with variable results. In the context of precision medicine with FF, the difficultly is determining whether there is positive effect of intervention in a severe phenotype or failure in a moderate phenotype. More work is needed to refine the trajectories, and more data is needed for a patient estimation of trajectory, based on age, muscle, steroid, exon mutation, functional tests and the gene modifiers, specifically, SPP1 and LTBP4 that have been shown to have a strong impact on the

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

Water T2 (wT2) measures water mobility in the tissues. It is a non-specific marker, even exercise can increase wT2, but it does reflect the intensity of the underlying mechanism and is quite sensitive. In natural history studies it has shown to have a predictive value, and in addition wT2 correlates well with increases in FF and muscle transformation rate. The difficulty for its use in Duchenne is that steroids induce a rapid decrease in wT2 which can make it difficult to assess an intervention (treatment of DMD with Corticosteroids is part of the current standard of care in many countries).

This results in a need for other biomarkers of disease activity particularly for muscular dystrophy with the objective of monitoring more closely the functional consequences of the absence of dystrophin and ultimately the impact of a therapeutic gene expression. Potential areas of interest include detecting membrane phospholipid turnover, ionic homeostasis disturbances and sarcolemma leakiness.

Membrane phospholipid turnover. When sensing membrane phospholipids turnover, Phosphodiesterase's (PDEs) as a biomarker of disease activity have been investigated in Becker and DMD. PDEs are elevated very early on in the disease, well before any fat infiltration can be detected and they remain elevated throughout the course of the disease. It is assumed they reflect the membrane turnover in the dystrophic muscle due to the successive waves of fibre necrosis and regeneration, and if so, dystrophin expression should stop these processes and normalisation of the PDE should be the signature of it. This has been successfully shown in experiments with GRMD dogs.

***Ionic homeostasis disturbance*.** It is well established that the absence of dystrophin causes ionic homeostasis disturbances. The intracellular pH of a dystrophic muscle is abnormally alkaline which can be demonstrated by phosphorous spectroscopy, although with some uncertainty because of possible contamination by extracellular volume. Another intracellular pH measure, photon spectroscopy of carnosine, has potential to be used as a functional marker of dystrophin expression.

Although MR is not able to measure calcium, it is possible to measure magnesium which has mostly opposite properties to calcium through small shifts in (Adenosine triphosphate) ATP resonances. Due to competition between magnesium and proton for the ATP, the intracellular pH must also be determined. In all muscle investigated the free intramuscular concentration of magnesium is decreased in the Duchenne patients leading to a hypothesis that dystrophin expression will result in normalisation of these levels.

Improvements in technology now allow for imaging of total sodium content, as well as intracellular weighted sodium maps with acquisition time compatible with clinical research. The lower leg of a young Duchenne patient, at a time when there is minimal fat infiltration and the wT2 is in normal range, the intracellular weight of sodium single intensity is systematically abnormal, suggesting that with intracellular sodium imaging we might have an earlier and more sensitive marker than wT2.

Measurements of levels of protons, magnesium and sodium may prove to be biomarkers of muscle damage/regeneration. Normalisation of these measurements could be indicative of a therapeutic effect in NMDs.

***Sarcolemma leakiness*.** To determine sarcolemma leakiness it is possible to measure the degree of restriction of water diffusion in the tissue. Diffusion is the statistical distance a molecule travels over a period of time. With a longer diffusion time the apparent diffusion coefficient will be impacted. If a molecule must cross a semi permeable membrane the diffusion coefficient will be less, and even more

restricted if the molecule is in an impermeable environment. The diffusion will appear more restricted if the cell size is smaller but less restricted if there is an increase in membrane permeability. This has been observed in the dystrophic muscle of an MDX mouse, which also has a smaller muscle fibre size than WT mouse. Results indicate diffusion is less restricted in the MDX mouse and by using the restricted diffusion measurement we can address the degree and sensitivity of the membrane permeability in the dystrophic muscle. This can also be done to some extent using wT2 decay in tissue by potentially revealing tissue compartments. In a clinical setting, using the muscle of control subjects, two compartments can be reliably identified i.e. two resonances, one at a long T2 which is the vascular space, and a predominant one at medium T2 which is the intracellular space. In Duchenne patients the T2 spectra are profoundly abnormal, the long T2 component is much higher indicating an expansion of the extracellular space, and the medium T2 is often split. An extensive simulation has indicated this is due to an increase in sarcolemma permeability, directly monitoring membrane leakiness. While it has been long recognised that phosphorous spectroscopy can identify a number of metabolic but also functional anomalies in the dystrophic muscle, we now have another series of biomarkers that allow us to assess membrane leakiness, either directly or through its consequences on the intracellular ionic composition. Three of these are proton MMR, meaning in theory they can be implemented in modern MR scanners.

In parallel there is a need to develop faster acquisition, as time is a major constraint in a clinical examination, especially in children. One example of this is fast partial matrix acquisition which combines compressed sensing with MR fingerprinting to identify and quantify multiple parameters. The end result can continuously map the FF map and T1 map over a whole segment in less than two minutes.

To detect and quantify fibrosis to show chronic degenerative changes in the diseased muscle is the ideal in Duchenne, but this is not currently available. Replacement of striated skeletal muscle by connective tissue, mainly collagen, is a major factor of degenerative change that affect muscles chronically damaged by disease. One solution may be optimising ultrashort echo-time (UTE) imaging, but it remains to be determined if this approach can quantify an increase in collagen fraction

In summary, fat fraction in its several variants are powerful biomarkers to evaluate the extent, severity and progression of chronic degenerative changes but have limited predictive value. Fast responding instant biomarkers of disease activity targeting tissue organisation, myocyte energy metabolism, membrane permeability and ionic regulation are important, while a key challenge is the evaluation of fibrosis.

SETTING UP A QUANTITATIVE IMAGING PROTOCOL FOR YOUNG DMD PATIENTS: PERSONAL EXPERIENCE, OBSTACLES AND SOLUTIONS. *KIEREN HOLLINGSWORTH, NEWCASTLE UNIVERSITY UK*

This work was undertaken as part of the VISION-DMD project. The initial mission statement was to have exploratory MR endpoints in the Phase 2b vamorolone studies using a network of 25-30 sites in Europe and Australia on boys aged 4-7 years old. The protocol was designed to be short, transferrable, simple and applicable to 1.5 and 3.0 Tesla scanners. The protocol included just two measures, the first measure looked at the muscle fat fraction which is expected to increase with disease progression, i.e. with a positive intervention (e.g. vamorolone) the fat fraction in the muscle should not increase further. This is done using a quantitative Dixon type measurement quantifying the water and fat

content of the muscle. The second measure is a muscle T2 relaxation time which reflects inflammation and odema change within muscle. Without intervention, the muscle water relaxation (T2) is expected to be elevated relative to boys without DMD. With a positive intervention it is expected that the muscle water relaxation (T2) parameter could decrease during the study. As the age range of the subjects was very young, some potentially only four years old, and it was not possible to sedate or anesthetise subjects, the scan time was kept as short as possible. Key to child participation is preparation. Children need to know what to expect in terms of noise and environment, and limiting boredom as movement is insupportable for MR. To address this, scans had very specific anatomical coverage with target muscles, because if the anatomical coverage of the scan is limited, the acquisition time can be reduced, and compliance problems minimised. The total scan time for this protocol is 10 minutes, so even with time to settle the child into position in the scanner, the total patient cooperation time can be reduced to around 20 minutes for cooperative children. Based on the age range of the subjects the target muscle was the vastus lateralis muscle, with a secondary reading on the biceps femoris long head in the left upper leg. This requires just one positioning of the child in the scanner, removing the need to reposition the child for imaging of upper and lower leg. Immobilization of the legs is key to obtaining good images. Keeping the scan to just one leg makes the protocol more applicable, as some sites have scanner hardware that only accurately scans one leg at a time. To ensure quality assurance, manuals for training and acquisition were created to provide to sites. Scanners were to be qualified with a phantom object scanned at every child visit., The specification of the positioning of the child was provided along with information on how the data would be coded for transmission to the central analysis site. Radiographers and operators also were required to be qualified using video training and scanning of phantom objects.

MRI site selection is key to a successful study. The sites were asked to complete a questionnaire about the technical details and availability of their scanners, the staff, and the previous experience of the site. The study required a designated MRI leader and ability to provide training for up to three trained operatives. In the original design of the Phase 2b study, MRI was integral to the study and nearly all trial sites returned the completed MRI questionnaires. At month 14 of the project, a decision was made to make the MRI an optional ancillary study, so sites could opt out of participating in the MRI study or sites without MRI capability could be selected for the main study. This resulted in the MRI ancillary study being unable to meet the minimum power requirements. The review of the questionnaires sent out to sites demonstrates the capacity to use MRI in quantitative trials in DMD. Questionnaires were sent to 42 sites in Europe, North America, Australia, Israel and Turkey and only 2 sites did not respond. 35 sites of the 40 that responded had suitable equipment and staffing to participate. In Europe all 26 sites returned questionnaires and of these, 21 sites were assessed as suitable to participate. Globally, of the 35 sites found to be suitable, 26 sites had 3.0T scanners and 9 (European) sites were using 1.5T. 34 out of 35 scanners found to be suitable were made by Siemens and Philips. 2 sites (all in Europe) were excluded because they could not guarantee that consistent trained operators would run the scans and 3 sites (all in Europe) were excluded because the scanner was incapable of running a 16-echo T2 sequence without a costly upgrade. 26 of the 35 of the sites assessed as suitable had experience of clinical trials involving muscle imaging, 24 had experience of T2 acquisition and around 17 sites had experience of fat fraction measurement.

Key recommendations for future MRI studies in DMD are to get involvement of expert input from the design stage and to consider the use networks of sites already active such as the Imaging-DMD network or CRIS. The site selection and contracting process must have MRI assessment as an integral part and the number of MRI sites should be minimised wherever possible.

VISION DMD INNOVATIVE BIOMARKERS: AN INDUSTRY CASE STUDY - PHARMACODYNAMIC BIOMARKERS IN EARLY PHASE TRIALS OF VAMOROLONE. *LAURIE CONKLIN REVERAGEN BIOPHARMA USA*

Vamorolone is a modified steroid, with a single modification of the 9,11 bond. It has a double bond whereas prednisone has a carbonyl group. This modification has shown an improved safety profile in preclinical studies while retaining anti-inflammatory properties. Other properties of vamolorone that may be beneficial to Duchenne patients are that it is an MR antagonist (similar to eplerenone) and membrane stability counteracting dystrophin deficiency. Vamorolone binds the glucocorticoid receptor and inhibits NF-kB, which is the anti-inflammatory mechanism of action. It does not affect the glucocorticoid response element presumably due to lack of dimerization. The challenge is to reproduce preclinical results in clinical trials, so biomarkers are being used to demonstrate proof of concept that vamorolone is showing an anti-inflammatory response in patients and reducing side effects. It is important to define context of use by addressing: Why are we using this biomarker? What we want to show with the biomarker, and why the biomarker is fit for this purpose?

ReveraGen used biomarkers in the early phase open label trials 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. Pharmacodynamic (PD) biomarkers are also being used to de-risk trials of vamorolone in other indications, and support extrapolation to other patient groups.

Results from the Phase 2a vamorolone study in Duchenne have been published. This was a 2 week on, 2 week off wide dose ranging study from 0.25mg/kg/day up to 6mg/kg/day vamorolone. Patients progressed to a 6 month extension study on the same dose, and then opted into a 24 month long term extension study with dose escalation to either 2 or 6 mg/kg/day.

Dose response PD biomarkers were used to demonstrate proof of concept of efficacy. Steroid responsive biomarkers in patients with Duchenne were identified. Serum samples were taken from Duchenne patients pre-treated with steroids and Somascan technology was used to identify steroid responsive protein serum biomarkers. This data was then compared to patients with inflammatory bowel disease, pre and post treatment with steroids to see if the same steroid responsive biomarkers were responsive in that population. Seven steroid responsive inflammatory proteins were identified in both diseases and these were pre specified in the Phase 2a clinical study. Serum creatine kinase (CK) was also investigated as it is an indication of muscle membrane stability. The Phase 2a extension trial was an open label 6-month study which also included clinical outcome measures of efficacy. The primary outcome measure was Time To Stand and a number of secondary outcome measures were included. However as the co-efficient of variance is high with some of these measures, more objective markers of drug affect are needed. Time to stand and 6-minute walk test both showed a dose response in the highest dose groups.

Drug safety biomarkers are important for assessing side effects associated with glucocorticoids in early phase trials. Clinical safety as shown by adverse events and serious adverse events showed no dose

limiting safety concerns in adult volunteers or Duchenne patients. Safety biomarkers are important in order to benchmark vamorolone to prednisone. A Binghamton University survey of Duchenne parents with children on prednisone or deflazacort looked at which side effects are of most concern. Loss of bone density, weight gain, stunting of growth, delayed puberty and suppressed immunity were the most concerning side effects for families. The vamorolone trials are investigating bone turnover biomarkers for bone formation and bone resorption, in addition to fasting insulin, fasting glucose and first morning cortisol. Results so far have shown no decrease in bone formation (osteocalcin), no increase in fasting glucose or changes in HbA1c. There are some changes in Insulin at the highest dose (6mg/kg/day) and some incidence of adrenal suppression (based on morning cortisol measurement) at the higher doses. In the current Phase 2b vamorolone study, an Adrenocorticotropic hormone (ACTH) stimulation test is used to provide a more accurate representation of adrenal insufficiency. Evaluation of the incidence of spine fractures will help correlate biomarker changes with clinical outcomes. The Phase 2a data de-risks and supports vamorolone 2.0 and 6.0 mg/kg advancing to Phase 2b blinded, placebo and active controlled trial enrolling 120 boys.

In novel trial designs and innovation in paediatrics and rare diseases, Pharmacodynamic (PD) biomarkers could potentially be used to demonstrate a Pharmacokinetic (PK)/PD relationship and support extrapolation of efficacy between patient groups. Many steroid responsive biomarkers are also steroid responsive in other inflammatory diseases. ReveraGen as a drug development company is interested in running small, short term proof of concept trials to demonstrate the drug is working similarly in these other indications.

ReveraGen have used innovative biomarkers in the vamorolone program 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 and used this information 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.

GENETIC TESTS AS DISEASE BIOMARKERS IN DMD. *ALESSANDRA FERLINI MD, PHD, UNIVERSITY OF FERRARA, ITALY UNIVERSITY COLLEGE LONDON, UK*

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. Majority of mutations are copy number variations (CNVs) accounting for 75% approximately of all mutation types in dystrophinopathies while small mutations occur in 25% of patients. A combination of standard methods and next generation sequencing can pick up easily and accurately all these mutation types. *DMD* gene shows splicing isoforms transcribed from 7 different specific promoters, three of them driving full length isoforms and consequently proteins.

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 reading frame rule explains the vast majority (over 90% of patients) of mutation type effects. Indeed, mutations (all types) that maintain the reading frame (in-frame) generally result in shorter but partly functional dystrophin and are associated to a milder phenotype like Becker Muscular Dystrophy (BMD). Mutations (any type) that disrupt the reading frame (out of frame) may result in premature

truncated proteins. If the reading frame is disrupted, translation is incomplete and the C-terminus of the protein cannot be produced, so the protein will not be functional resulting in very low levels of functional dystrophin. To some extent, though with controversial reports, nonsense mediated decay of the mRNA may occur contributing to low translation capacity of the *DMD* messenger. In this context the size of deletions or duplications that lead to out-of-frame mutations do not affect the clinical phenotype. However, there are exceptions to the reading frame rule: For example, (i) out-of-frame mutation occurring at the 50 region of the gene are often associated with BMD phenotype. This is due to the presence of alternative translation initiation sites in exon 6 that activate alternative translation; (ii) nonsense mutations causing BMD phenotype where exon recognition sequences might be involved causing exon skipping events; (iii) In-frame deletions exist causing DMD phenotype).

To further complicate the genotype-phenotype correlation, different sub phenotypes can also occur in DMD but also BMD. Age at loss of ambulation and cardiac involvement and cognitive disturbances are examples of variable expression in terms of both disease severity and variable phenotype. There are some hypothesis to explain these variable phenomics in DMD (as deletions preserving the transcription of the shorter 3' isoforms that might be implicated in intellectual disabilities, or the lost os some crucial exons (at the 5' of the gene or in the mid road region) that have been described as associated with dilated cardiomyopathy. Pane et al. 2014 described as some deletions are associated with a better performance of DMD boys in the 6-minute walk test over 12 months. This experiment showed that boys eligible for exon 44 skipping exhibited better baseline values and less drastic changes over the 12 month period and performed better at this test compared to the subgroup eligible for skipping exon 45 and 53. Research by Kaspar et al. (2009) studying cardiac involvement that is present in around 70% of BMD cases showed that an early onset of severe dilatated cardiopathy (DCM) in BMD patients is generally caused by a mutation at the 5'prime end of the dystrophin gene, including the muscle (M) promoter of the M isoform, while later development of DCM is linked to deletions in exons 45 –55.

There is also evidence of a link between mutation location and cognitive deficit in DMD boys. Deletions of exon 52 were associated to cognitive impairment (Rapaport et al. 1991), while deletions of the second half of the gene were more frequently associated with lower IQ (Bushby et al. 1995) and the loss of dystrophin isoforms Dp140 and Dp71 have been reported to have the most impact on IQ (Moizard et al. 1998). Generally, a strong association exists between cognitive defect and cumulative loss of the 3 prime dystrophin isoforms in the brain (Taylor et al. 2010) with a crucial role of the Dp140 (Chamova et al. 2013). The relationship between the isoforms and the cognitive profile is further supported by the higher incidence of neurodevelopmental disorders in patients missing Dp140 compared to patients missing only Dp427. Ricotti et al. (2016) reported an increased risk of neurocognitive phenotype linked to mutations towards the 3'end of the dystrophin gene (that includes the short 3' isoforms Dp140 and Dp71). Similarly, attention deficit hyperactivity disorder (ADHD) seems to be associated to the 3 prime end mutations around exon 44 (Pane et al. 2012). However, patients with point mutations are also frequently affected by cognitive involvement and ADHD - even more so than those with deletions and duplications. So far, there is no explanation for this in terms of genotype-phenotype correlation.

In summary, we can observe an overlap between the genotype and related phenotype. While dilatated cardiomyopathy is mainly confined to the 5´ end of the gene (Kaspar et al. 2009), the motor function deficit (Pane et al. 2014), and cognitive involvement as well as neurodevelopmental disorders are commonly caused by deletions in a region at the 3' end of the gene consisting mainly of the exon 44 (Rapaport et al. 1991, Bushby et al. 1995, Moizard et al. 1998). Therefore, more

attention should be paid to this region to investigate further the genotype-phenotype relationship in DMD.

In conclusion, exceptions to the reading frame rule occur, in relation to DMD mutations, in regions involved in isoform expression regulation or in specific protein domains. Dual DMD mutations may also occur and cause a n additive effect in disease diagnostics. Thus, 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.

THE USE AND DEVELOPMENT OF DIGITAL MOTOR BIOMARKER IN CLINICAL TRIALS. *DAMIEN EGGENSPIELER SYSNAV. ACTIMYO FRANCE*

SYSNAV has developed a digital biomarker which measures movements through different outcome, including the recently qualified 95% stride velocity. Drugs are aimed at improving the condition of patients and so motor function is relevant to patients as how they walk and interact is important. Current techniques to assess muscle function use old outcome measures such as the 6MWT or stair climbing. The SYSNAV digital outcome platform consists of sensors, IT systems and training for clinical trials. This has received a qualification from the EMA and is under evaluation by the FDA. www.ema.europa.eu/en/documents/scientific-guideline/qualification-opinion-stride-velocity-95th-centile-secondary-endpoint-duchenne-muscular-dystrophy_en.pdf.

Precision sensors are worn on the wrist and ankles of DMD patients throughout the day to measure the 3D trajectory of upper and lower limbs. This provides parameters for the patient's motor function, and how they evolve over time. Qualification is a long process in order to quantify variability and sensitivity to change. The SYSNAV device measures the movements of patients, which can translate either into an activity measure, either to a movement description during clinical trials and can see variation of the activity depending on the time of day, the day of the week and the activity the subject is undertaking. The difficulty in developing this motor outcome is translating the activity into one single point that makes sense and is relevant for the drug effect and for the patient. SYSNAV had extensive discussions and interaction with the EMA on what single outcome could be validated, and essentially what mattered to patients. Three key questions needed to be addressed. 1) Can we control variability, this includes variability with all the parameters, such as time and day etc, and what parameters affect measurements, can we quantify factors that can impact these and minimise that impact? 2) How does it compare with the current gold standard outcome measures such as the 6MWT? 3) Understanding what the sensitivity to change is?

Comparing 95% stride velocity to 6MWT was an important parameter for regulators. From natural history data, using the 6MWT after 1 year you would expect to measure a decrease, so patients do not score as well. When you check the data, variability is high, meaning a large number of patients are required to reduce variability. With 95% stride velocity SYSNAV were able to control this variability so that we can see the effect of the disease or the positive effects of a drug after just 6 months. This new outcome brings many benefits allowing shorter trials with 8 times 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.

A good biomarker needs to have 3 components, the recording device itself, the digital outcome that is calculated and the clinical trial services. The acquisition device must be precise, comply with the regulations for data security, confidentiality and integrity and have positive patient use. This means good compliance and patient participation. The research must show understanding of the disease, longitudinal and transversal proof of relevance of outcome and its sensitivity to change. Clinical trial services require the necessity of training, and process including compliance, IT structure, QMS and data acquisition.

The 95% stride velocity is the first outcome to have received a qualification from the EMA. Briefly, it represents the 5% most rapid strides of a patient is natural environment. It can be technically collected by any device that presents the EMA defined level of precision. Data have been acquired so far using the Actimyo® device, which is a magneto inertial sensors that basically capture any movement of the limb where it is placed, the ankles or the ankle + the wrist for ambulant, the wrist and the wheelchair for non-ambulant, which allows in addition to 95% stride velocity to clinical trial sponsors access to other upper and lower body movements measurements. SYSNAV is not the data owner so sharing of the data depends on the willingness of the clinical trial sponsor. SYSNAV have a policy of publishing as much as possible and undertaking discussion with the EMA for better understanding of the disease and the outcome. To pursue the qualification process and to reach the qualification as a primary outcome, more data is required and SYSNAV are building this database with this aim.

3. *Expert panel group discussion – Landscape review and Future aims Prague Nov 2018*

Following the presentations session, an **expert panel group** chaired by ***Pietro Spitali*** and ***Hermien Kan Leiden UMC,*** discussed the roadmap of next steps for biomarker qualification for DMD, identifying existing platforms already making progress in biomarker validation and champions to lead on key actions.

3.1 Expert Panel Discussion List of Attendees

Alessandra Ferlini	University of Ferrara	Italy
Andrea Sarasin	Sarepta	Switzerland
Cristina Al-Khalili Szigyarto	KTH Royal Institute of Technology	Sweden
Christina Olsen	Ceratium Ltd	UK
Dimitrios Athanisaou	UPPMD (WDO)	Greece
Damien Eggenspieler	SYSNAV	France
Elizabeth Vroom	UPPMD (WDO)	Netherlands
Erik Niks	Leiden UMC	Netherlands
Fleur Chandler	Duchenne UK	UK
Giorgio Tasca	Fondazione Policlinico Universitario 'A Gemelli	Italy
Hermien Kan	Leiden UMC	Netherlands
Jana Haberlova	Motol University Hospital	Czechia
Jorde Diaz Manero	Hospital de la Santa Creu i Sant Pau. Barcelona	Spain
Kieren Hollingsworth	Newcastle University	UK
Laurent Servais	Institute of Myology	France
Laurie Conklin	ReveraGen Biopharma	USA
Pierre Carlier	Institute of Myology	France

Pietro Spitali	Leiden UMC	Netherlands
Ritchie Head	Ceratium Ltd	UK
Sara Cazzaniga	Italfarmaco	Italy

Attending by Video Conference

Pavel Balabanov	EMA	Netherlands
Joanne Donovan	Catabasis	USA
Jane Larkindale	Critical Path Institute	USA
Susan Ward	Collaborative Trajectory Analysis Project	USA

3.2 Part 1: Landscape review cTAP and D-RSC presentations

Pietro introduced presentations from two US based initiatives with the potential to support international biomarker validation efforts.

Susan Ward, Collaborative Trajectory Analysis project (cTAP http://ctap-duchenne.org/) USA. cTAP are interested in supporting work on biomarkers but are not actively pursuing qualifying biomarkers. cTAP is a precompetitive initiative, multi registry multi trial project funded by industry, which represents stakeholders in drug development in Duchenne, focusing on helping drug companies to design trials that take advantage and learn from natural history. Due to the broad collaboration of cTAP partners, a rich database of invaluable longitudinal data has been developed. To date c-TAP have delivered tools for drug developers in Characterisation, Prediction, Simulation and External Control foundations. cTAP have several published papers and more are in development.

Many of the cTAP drug company members are already using biomarkers in their trials, as primary endpoints, primarily dystrophin levels, and also some groups using MRI % fat fraction. Many more biomarkers as used as exploratory endpoints (markers of muscle damage, inflammation, fibrosis etc.).

cTAP's main interest in biomarkers is in trial design and interpretation, and the relationship between function and the biomarker cTAP companies are most interested in. Consistency across data sources needs to be determined and comparison of performance. cTAP support the objectives of the workshop but do not have a role in biomarker qualification.

Jane Larkindale, D-RSC Critical Path Institute, USA (https://c-path.org/programs/d-rsc/***).*** The Duchenne Regulatory Science Consortium (D-RSC) was formed to develop tools to accelerate therapy development for Duchenne Muscular Dystrophy and is part of C-Path, a public private partnership bringing together scientific consortia of industry, academia, and government for sharing of data/expertise aiming for official regulatory endorsement of novel methodologies and drug development tools. D-RSC has been established for 3 years and initial objectives achieved include the development of a data sharing platform for Duchenne clinical data, and development and publication of a CDISC therapeutic area standard for DMD. An ongoing objective is the development of a clinical trial enrichment platform for DMD.

C-PATH has a lot of experience in biomarker qualification and exists to develop drug development tools, take them through the regulatory pathways (EMA and FDA) and get them approved by the community and regulators to accelerate better clinical trials.

There are many elements to consider in order to qualify a biomarker. The drug development need needs to be described, in this case, monitoring biomarkers to establish how Duchenne progresses over time and how that might be changed by a drug treatment. Then the context of use needs to be defined, to show how the biomarker will be used in a drug development context. The potential benefits and

risks need to be considered and the level of evidence must be determined to support the context of use.

Considerations for biomarker qualification include:

- Biological rationale for use of the biomarker.
- Characterization of how the biomarker changes relative to clinical outcomes and treatments. (Type of data and study); Comparison to current standards
- Reproducibility of data (need for test dataset and confirmatory dataset)
- Use of appropriate, pre-specified statistical methods to demonstrate the hypothesized relationships for the COU. This is the type of work cTAP and D-RSC could do
- Assay performance (accuracy, precision, analytical sensitivity, sample stability, analytical specificity, reportable range, reference interval, cut offs etc).

C-Path has undertaken qualification of biomarkers in other disease areas. One example is a program to qualify skeletal muscle damage biomarkers as non-clinical safety biomarkers. The initial step was to produce a clinical statement of need for these biomarkers. CK activity is a helpful biomarker but is not sufficient, as small increases in CK are difficult to interpret and variable. AST and ALT are not considered injury biomarkers, so there was a clear need to develop biomarkers that would demonstrate that a drug was not causing skeletal muscle damage. To determine the context of use a 'use' statement is produced. The use statement for this example is *'Plasma/serum measurement of skeletal troponin I fast-twitch (Type II) (TNNI2), myosin light chain 3 (MYL3), fatty-acid binding protein 3 (FABP3), and creatine kinase muscle type (CKM), in conjunction with aspartate transaminase (AST) and total creatine kinase (CK) enzymatic activity can sensitively and specifically diagnose and monitor skeletal muscle (SKM) degeneration/necrosis'.* In addition to the use statement, the conditions for qualified use are also detailed describing how the biomarkers will be measured. The link to the data package for this example is https://c-path.org/wp-content/uploads/2015/04/pstc-skmwg-nonclinical-summarydatapackage.pdf.

One of the critical pieces of data for this qualification was comparing the novel biomarkers with the existing biomarkers. The EMA and FDA have awarded Letters of Support for further study towards clinical qualification of these biomarkers, which is a good way for the regulators show they agree with what has been done to date. Skeletal muscle damage biomarkers are halfway towards obtaining the level of data required and working towards qualification.

C-Path have a lot of experience in the FDA and EMA pathways for biomarker qualification. Qualification is a formal process of review and acceptance. The objectives of qualification are to make drug development tools 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.

D-RSC have not undertaken biomarker development so far but are interested in starting a biomarker working group. D-RSC has experience of writing regulatory packages, undertaking the statistical analysis, creating models to support biomarker development and are keen to work with the DMD community on biomarker qualification.

3.3 Part 2: Future aims discussion

Pietro Spitali explained how there are many individual groups working on their own biomarkers who understand the need for qualification, but no one is taking the lead in the Duchenne community which 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. This activity needs to be inclusive and avoid replication, so it is important to identify how we can facilitate this, engage with key stakeholders and make progress.

Fleur Chandler from Duchenne UK asked about the disease modelling D-RSC is undertaking predicting the disease for use by Health Technology Assessment and FDA and the use of biomarkers for prediction and progression. Jane Larkindale explained that it depends on the biomarker but there is evidence that some biomarkers, particularly imaging biomarkers could potentially be used to predict disease progression, and this will require modelling which C-Path has experience of and could undertake for the right biomarker and the right data.

Joanne Donovan, Catabasis, asked for clarification of how high the bar was for developing biomarkers for qualification, and if the Duchenne community was in a position to achieve this. Jane Larkindale agreed that the bar is set high for qualification, but it is dependent on context of use, if you are trying to qualify a biomarker as a surrogate endpoint, the bar is very high and for Duchenne a long way off. However, biomarkers used to predict which patients will progress faster or predict which patients will reach a particular endpoint within a set time period, is achievable. C-PATH have used this strategy before, getting biomarkers qualified for simple context of use, then building additional evidence over time to support more difficult context of use, although the reality is a surrogate endpoint for Duchenne is a long way off.

Dimitrios Athanasiou from WDO aksed if there were potentialissues with the transfer of data following the introduction of the EU GDPR. Jane Larkindale responded that C-Path has a lot of European data and are fully GDPR compliant but continue to review GDPR issues to ensure they can continue to receive data from Europe. The only concern would be if an investigator had not correctly obtained informed consent, e.g. if the wording is not correctly written to allow sharing of data. Joanne Donovan asked from a company perspective if there was key language that needed to be included in a standard informed consent. Jane Larkindale explained how the Alzheimer's consortium had published a paper two years ago describing what should be included on a good informed consent to allow data sharing and it is planning to produce an updated common informed consent addressing GDPR, which will hopefully be published soon.

Pietro Spitali explained that the purpose of the workshop was to reach a consensus on the prospective biomarkers that could be taken forward for qualification. The table below was presented as a starting point for the discussion with some suggested biomarkers to open initial discussion. This table will be developed, added to and circulated following the workshop.

MRI Fat fraction was discussed to determine if there is consensus on the use of fat fraction, what is still needed, whether there is agreement on how it is quantified, and if there is a regulatory opinion. Hermien Kan explained that MRI can be very dependent on the institution, and in her experience the success of MRI on children relies on ensuring dedicated expert MRI operatives, the availability of streamed television, and a protocol is no longer than 30 minutes. Krista Vandenborne, PI of ImagingDMD, clarified that both MR spectroscopy and imaging in fat fraction has been done in a large group of patients including young children, and believed most of the components listed on the table are in place. The most challenging issue is to show 'change upon treatment', it is the measure and the not so much the measurement used that matters, so in the case of fat fraction it could be measured by spectroscopy or imaging. Jane Larkindale agreed. It doesn't matter which device you use as long as

it reaches the specification set by the qualification standard. Hermien Kan explained there are publications and evidence available, but no one has gathered this information and approached regulatory bodies with this information as a package, so the goal is to identify different biomarkers and identify who can take these forward. Laurent Servais from the Institute of Myology highlighted the problem that people do not do anything for free without a good reason. The other issue is that the EMA validate an outcome not a device, so a company may make the effort and expect financial recompense but lose out to another device manufacturer. Another consideration is the need for academics to achieve publications. It is important to identify the final outcome and who will benefit from it. Pierre Carlier from the Institute of Myology added that a growing issue is that it is getting harder to get finance for research and the research is generally exploited by people making money.

Pietro Spitali summarised that most of the trials have been on children aged 4-9 years old, and a lot of the MR evidence to show accumulation of fat fraction is building up from 9-10 years onwards. Erik Niks thought the disadvantage of this measurement was that it shows the end stage of the pathology. He questioned if you do not know how this pathology arises, is it rationale to measure the end state of a disease that you want to target earlier in the pathology? Krista Vandeborne summarised the data that is available. They have published data looking at the relationship between MR and a number of time functional tests. There is also a paper about to be submitted that shows the ability of MR to predict loss of ambulation and other functional measures. They have strong data for both fat fraction and MRI T2 (heavily influenced by fat fraction) on the ability to predict clinical changes. Imaging DMD have had a conference call with the FDA to discuss the process of taking this forward to qualification but have realised the complexity and the need for support from the entire community. Imaging DMD is invested in this topic area and think MR can be valuable and are keen to have a role and contribute, including sharing the data to make this happen. Imaging DMD would not be able to do this alone and following internal discussions would be open to working with other partners including C-PATH or other entities but believe a whole community effort is needed to do this.

Fleur Chandler queried on the use of loss of ambulation as an endpoint as from the work of Duchenne UK (DUK) a more meaningful measure is the loss of ability to weight bear, and DUK are interested how these markers will be linked to really relevant endpoints. This could be how the patient community could be involved to highlight what is meaningful in terms of change in the disease. Hermien Kan thought most fat fraction measures were measured against loss of ambulation as this was considered the most relevant endpoint, but if there is another relevant endpoint and the data is available, it could be linked using disease modelling. It is important that outcomes that are meaningful to patients are identified for this process.

Cristina Al-Khalili from KTH suggested that context of use was added to the table, as different stakeholders may have different perspectives. Laurent Servais suggested mechanism of action should be defined beforehand as it is important to keep it simple for regulators. Hermien Kan confirmed that as Jane Larkindale explained earlier it is important to start with a simple context of use then gather more evidence to expand it.

Dimitrios Athanasiou was concerned that a patient may have a stable or minimising fat fraction with no clinical benefit, so we need to show that without fat infiltration the muscle functions. Erik Niks thought it would be challenging as we need abiomarker to depict the course of the disease in a shorter timeframe than a clinical trial, and it should be about the disease and not reflect the pathology. Any drug slowing disease progression should have an impact on fat fraction. Regulators will not approve MRI, they will approve fat fraction infiltration, so there is a need to explain very precisely what is expected. An example was provided by Pietro Spitali. In exon skipping you should

see dystrophin, but this doesn't necessarily link to improvements in loss of ambulation. Academic groups can't take up the task to validate all possible targets for all possible drugs in development. It was suggested that a patient with 70% fat fraction in the leg will not be walking. Any drug should show a benefit to ambulation, so to prolong the ambulation phase fat fraction should not go beyond 70%. While we should not try to oversimplify, it is clear that 70% fat fraction results in 100% loss of ambulation.

Pierre Carlier explained while we need to make things understandable, a simple model will be incorrect. Fat fraction is typically an over-simplification and there is no consensus on the use of fat fraction. Muscle fatty transformation rate may be a better measure when trying to appreciate the impact of a treatment. From the Dixon data you can also extract the prototype mass index which may be a better target if can show an increase in the prototype mass index, combined with a strength measurement.

Joanne Donovan highlighted the variability from patient to patient. In trial design, looking at the trajectory of the change in an individual patient before treatment and on treatment, may be a more sensitive way of looking at the variability in many of these biomarkers from patient to patient. The problem is there is no gold standard and all the clinical markers show variability in measurements done several weeks apart. Because the clinical markers are so variable it is harder to qualify biomarkers that may be more precise in disease progression. It's hard to believe that increasing fat fraction could ever reverse so why is this not the gold standard for disease progression?

Pavel Balabanov explained that the field needed to use a combination of biomarkers for the specific context of use. Used alone they could be utilised for lower levels of context of use, but the issue with singling out one single measurement that will be valid to monitor disease progression across the whole spectrum is that we can not be sure this is the only measurement that describes it. Even if you are 100% certain about the measurement e.g. fat fraction, and all the methodology is done the same way, can you be certain that the functional performance of the two boys you are comparing is exactly the same? This was raised earlier when discussing the potential difference in remaining muscle and can be so significant that they will differ substantially in their functioning. Using the heart as an example, we could start thinking about a combination of cardiac flow and volume parameters in combination with a specific pattern, type and level of fat fraction, and any scar tissue that best characterises the functioning. The field needs to consider the combination of two or more measures that can be combined in a general complex or panel of biomarkers to reflect better progression and potentially the effects of drugs with different mechanisms of action.

Hermien Kan believed the table presented was too simple, but this was intended a starting point. Pietro thanked everyone for attending and highlighted that alone we cannot bring this forward and efforts need to be centralised to make this successful.

4. Post workshop progress: Action Plan meeting Leiden Jan 2019

Biomarker assessment towards standardised approaches and validation.

Attendees: Pietro Spitali, Hermien Kan (Leiden University Medical Centre); Jane Larkindale (Critical Path Institute), Susan J. Ward (cTAP), Eric Camino (PPMD), Christina Olsen, Ritchie Head (Ceratium/VISION- DMD).

Purpose: To develop a roadmap and action plan to advance development of biomarkers for various contexts of use in Duchenne (and related NMDs) research. Specifically aiming to,

- review biomarkers in development for Duchenne and related NMDs with respect to their proposed contexts of use, and the amount of data that supports their use with respect to both technical and clinical validation;
- establish what further evidence needs to be developed for the most promising biomarkers to be used as validated endpoints for needed contexts of use;
- propose a cost-effective route forwards towards acceptance by regulators of the most useful biomarkers with regulators.

Background: Multiple activities are advancing work on proposed or potential biomarkers targeting DMD and related NMDs, including international academic and industry led collaborations and clinical development programmes, but these activities are fragmented. Expertise and datasets are spread across academic and industry groups internationally, hampering the selection and validation of the most promising approaches. To support ongoing efforts to overcome this situation the VISION-DMD project organised an event in Prague (Nov. 2018) to bring together experts with a view to developing a roadmap that would identify routes to bring the community together to advance biomarker research. The goal is to develop a roadmap by which the most promising biomarkers for specific contexts of use needed in DMD drug development may be identified. Once a list of proposed biomarkers and their contexts of use have been identified, gaps in the data supporting the use of such biomarkers will be determined, and a pathway to gathering such missing data will be identified. The group will work towards development of more standardised global techniques and operating procedures in biomarker studies to ensure the ability to compare, contrast and combine results from different studies If the data is supportive, the group would consider seeking regulatory acceptance of the biomarkers. This builds on previous work and ongoing activities mentioned below**.**

Key Deliverables: A roadmap outlining key steps to help the community engage more productively to advance activities towards encouraging standardized approaches to the use and further research of biomarkers.

Technical deliverables:

1) Landscape analysis of biomarkers in development for Duchenne and the existing data that supports their technical and clinical validation; datasets and samples that may be shared with the community.

2) 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.

3) Integration of key data into a single, publicly available dataset for analysis.

4) Development of protocols/analysis plans to further develop the biomarkers towards the level of evidence needed for use in trials and/or qualification.

5) Possibly qualification of one or more biomarkers. [if supported]

Approach: The intention is to establish working groups of experts to review the "biomarkers" (biological; imaging; and wearables) from the Prague workshop and previous events (e.g. European Neuromuscular Centre workshop 2017; MYO-MRI COST Action BM1304; etc), the literature and on-going studies. The next step is to develop a plan for the next steps in identifying the most promising biomarkers and their ideal contexts of use in drug development. The context of use describes the way that the biomarker may be used in drug development (e.g. in monitoring disease progression, in predicting populations of patients likely to progress in a certain way, in demonstrating a drug action). Biomarkers may be able to be used for multiple contexts of use – a biomarker that may be useful for monitoring disease progression may also be useful as a surrogate endpoint. However, for regulators to accept the 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. As such, biomarkers may be developed initially for some contexts of use while further data is gathered to support more complex contexts in the future.

The data that exists to support each potential biomarker will be assessed. The Working Groups will then select those biomarkers that seem to be most promising based on technical and clinical validation data to date, and which could be used for valuable "contexts of use" in drug development (e.g. monitoring or pharmacodynamic biomarkers). It has been proposed in Prague to utilise existing initiatives with the skills and expertise to advance the envisaged work. For example:

- The Critical Path Institute (C-Path), a nonprofit public-private partnership has experience in qualification of biomarkers and standardization of data. A collaboration between the Critical Path Institute and Parent Project Muscular Dystrophy has formed the Duchenne Regulatory Science Consortium (D-RSC) specifically to develop tools to accelerate therapy development for Duchenne Muscular Dystrophy, 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. C-Path are 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).
- The Collaborative Trajectory Analysis Project (cTAP) is active in the collation and analysis of widely distributed and fragmented datasets. This offers an alternative approach analysing data *in situ* that is complementary to developing an overarching database. The cTAP team advocate data analysis approaches that interrogate data *in situ,* effectively *visiting data* and undertaking analysis using appropriate data analysis tools and methods.

C-Path/D-RSC would be positioned to help a qualification effort (or other effort towards regulatory acceptance of biomarkers).A number of individuals and organisations such as US based cTAP (Susan Ward), and patient representatives World Duchenne Organisation (WDO) and Parent Project Muscular Dystrophy (PPMD) in the EU and US respectively are well positioned alongside the academic communities to help interrogate datasets and engage the required support from the DMD community including patient organisations, clinicians, industry and other stakeholders. Specific actions to be addressed in the short term are:

4.1 LANDSCAPING: A review of the current state of the art related to fluid biomarker/imaging/wearables/other biomarker activities. This will identify the main groups involved and the type of data held to support various types of biomarker use. This will build on previous work

and the Prague expert presentations and discussion. The identification and location of existing "community" databases and other robust datasets supporting biomarkers needs mapping – where is the data / who is the responsible data manager / what type of data has been collected? Hermien Kan started to develop a table/infographic to summarise landscape (see 4.8). This will be circulated for expert input or populated through targeted webinars. An example first draft table for MRI FF is shown below, not yet complete.
The focus will be on DMD but there is value in considering related diseases for some biomarkers. For example, in the use of muscle ultrasound there is data that cuts across diseases as well as in one disease area, and it is likely fluid biomarkers have cross talk as well.

4.2 BIOMARKER SHORTLISTING: Develop criteria for prioritising biomarker candidates that have data supporting their potential for community adoption for specific contexts of use, and identify what data is missing with respect to technical and clinical validation for such potential biomarkers. Establish standard operating procedures, protocols or data standards needed in order to combine datasets and fill gaps. Identify lead candidates that may be ready to be considered for qualification by regulatory bodies based on the data. These candidates should be agreed upon by key opinion leaders/expert panel. Selection of first biomarkers for consideration towards validation for DMD. The selection criteria for expert panels also needs to be agreed.

4.3 ACCESS TO DATA, CONTEXT OF USE AND ANALYSIS: The mapped data sets need to be assessed for accessibility - ownership and open access and further practical issues type, quality, transfer, archiving and any legal issues related to access and use (e.g. GDPR compliance). Recommendations for future collection of data in order to facilitate easy data sharing (e.g. language for consent documents, use of standardized protocols and data standards) should be developed. This should include guidelines on how data should be provided to the Critical Path Institute, data useagreements and the purpose/usage rights to ensure the community delivers robust and accessible datasets for predictive and prognostic markers.

The proposed database needs designing to be broadly accessible and scientific guidance is required to determine what data goes into the database and how it is mapped and interpreted. Gaps in current data will also need to be identified and plans implemented to acquire this missing data. Although activities will not be limited to C-Path, the project will need scientific guidance at the mapping stage and a clear goal in mind, so that the correct data is brought in and aggregated in meaningful way, and steps to gather additional data are in place.

The recent World Duchenne Organisation/VISION-DMD Data sharing for Duchenne meeting (Amsterdam, April 2019) highlighted the EC open data requirements and 'FAIR Guiding Principles for scientific data management and stewardship' that need to be adopted to support the findability, accessibility, interoperability and reuse of digital assets. This is now helping to drive machine-interpretability as data become more complex and provides a complementary or alternative approach to data warehousing activities, which accumulate increasing amounts of data, cTAP are well positioned to support these activities related to DMD biomarkers. There is also a danger of warehousing leading to silos with restricted access in some cases. This would need to be avoided since the community will create most value out of the fragmented data through sharing with others and allowing data to move around or be shared by data visiting. Advances in Big Data analytics are

expanding possibilities to *visit data,* so for some datasets it may be better or easier to foster secure engagement than be rigid about physical location of data or centralisation of data. The challenge with this is that if it can be visited, but not transferred it cannot be mapped, integrated, combined or shared with regulators meaning that it cannot be used for qualification or endorsement.

A data management and analysis plan needs to be developed. Decision making processes in selection and use of data need refining to encourage exchange and greater efficiency in use. This requires standardized data collation and accessibility initiatives. Although standardization of how data is represented (C-Path input) is important, another urgent issue is the standardization of data collection. iDMD has standardized MRI measurements and metrics in 4 centres in the US, and similar standardization has been achieved, or is ongoing, in a substantial number of centres in the EU. But there is also a large body of valuable MRI data in the EU that contributes to scientific insights despite using metrics that differ from those iDMD has settled on. How best to handle this situation is beyond the scope of the current document, but there is a need for a resolution or work around.

4.4 BIOMARKER VALIDATION AND USE PLANS: depending on the assessment of what data is needed, key opinion leaders and biomarker experts should recommend what data is needed in order to complete technical and clinical validation of a biomarker or if data supports its exploratory use in trials at this time. Combining the available community expertise and Critical Path's capability should be encouraged to build synergies.

A distinction needs to be made between **technical validation**: demonstration that the assay does what it is supposed to do; **clinical validation**: demonstration that the assay matches with the relevant clinical change for the *context of use*; and **qualification**: which is the regulatory pathway to seek endorsement for a biomarker. Different biomarkers are required for various contexts of use, and levels of evidence will vary with the context of use. A case study example is C-Path's imaging biomarker for polycystic kidney disease. This was first qualified as a prognostic biomarker, but as more evidence has been developed, there is now talk of using it as a surrogate endpoint (https://www.fda.gov/Drugs/DevelopmentApprovalProcess/DrugDevelopmentToolsQualificationProgram/ucm458492.htm).

A selection of the strongest biomarker candidates is now needed, and plans for any required work towards improving the evidence base for a defined *context of use,* to confirm the strengths of the candidate biomarkers, and to support validation needs in discussion with regulators.

4.5 DEVELOPING CLINICAL TRIAL PLANNING AND ANALYSIS TOOLS:

Initiatives such as C-Path and cTAP are utilising data and analytics to build smarter trials and providing an important link to drug develop programmes and trial designers. Both provide useful existing platforms to accelerate future activities.

There are a range of non-MRI biomarkers in DMD clinical trials excluding drug-specific and safety biomarkers, companies are now developing and/or already using drug-independent markers of pathophysiology

- fibrosis
- inflammation
- vastly improved quantification of dystrophin
- muscle-type specific markers of degradation

.

The usefulness of such approaches needs to be reviewed and routes to share assays, lessons and

> Example: MRI FF. cTAP is addressing critical issues to deliver smarter trials, for example lowering unaccounted for variance when analysing the results of the trial. To achieve this reduction in variance work is focussed on two areas:
>
> 1. reducing assessment-to-assessment variability including both the technical repeatability of measurements, and the patient-driven contribution;
> 2. improving prognostic power – which can be used to
>   a. define I/Es that enrich recruitment of target disease progression trajectories
>   b. adjust analysis of trial results to account for differences in randomization between cohorts
>
> The potential advantages of FF MRI to contribute to these issues includes:
>
> i. presumably a low variance between repeated assessments of the same sample compared to clinical endpoints;
> ii. MRI is assumed to be less subject to patient mood, motivation, physical state etc (though I haven't seen this demonstrated)
> iii. is assumed to be less influenced by patient mood, motivation, physical state etc
> iv. MRI is a more precise/more accurate prediction of future change based upon baseline measurement;
> v. The potential to increase prognostic power when used in combination with clinical prognostic factors.

benefits identified. This work could happen on an international level through Working Groups. This approach will drive sharing of good practice and advance thinking in the field. An EC COST action or patient foundations could potentially provide funding to facilitate this.

The Working Group activities should address questions including:

- How ongoing and new initiatives can help design efficient and effective clinical trials, e.g. by selection of appropriate populations of patients, selection of informative monitoring biomarkers within a population, use in developing adaptive trials designs etc?
- How does the community develop smarter trials?
- How are biomarkers being used by industry today?
- How can lessons learned be captured to improve on-going and future uses of biomarkers?
- What core activities are needed to improve the biomarker knowledgebase and design future models.

3.6 BUSINESS PLAN FOR FUNDING: A business plan and funding strategy should be developed. Financing will be critical to ensure work progresses efficiently with a good coordination team. Potential sources of funding include:

- Patient groups are already very supportive of the academic and drug development communities and would provide significant added value regarding data access and use;
- Industry has a deep interest in biomarker development and validation, which could be undertaken in the same way as the Hercules project, apatient group-industry joint initiative on Health Technology Assessment addressing bottlenecks. But although interest in biomarker qualification is broad, funding it is often technology specific. Funding required for the biomarker work this document addresses should not be trial-specific although some funding may well be tied to timelines by companies if their needs can be met,
- EC/NIH grants: the EC have a strong international focus. A COST Action project would be an ideal vehicle to bring the community together for knowledge transfer through working groups and training, however this would not provide research or innovation funding. Ceratium could write up the RoadMap report using a COST action template - this would provide a VISION DMD project deliverable and a strong basis for a project application. In order to bring data together and allowing it to be part of an integrated dataset there needs to be personnel involved at the centres, a COST action would not pay for these personnel. Ideally both are required, a COST action to develop the network and share good practice, and extra funding for personnel on the ground. Sources could include: H2020/Horizon Europe (Cofund); Telethon; Venture Philanthropy; EC/NIH IRDiRC; Industry co-investors.

Follow-up discussions identified industry does have interest in FF MRI, and other technical approaches including molecular biomarkers, and wearable approaches to monitoring function. Specifically:

- several molecular biomarkers and a number of different approaches to wearables are of high interest, these are viewed as exploratory, and are either currently being, or planned to be assessed as “exploratory endpoints’ in clinical trials
- marked improvements in quantification of dystrophin are expected to become an industry standard. The originating company is not interested in driving this to qualified biomarker regulatory status, however several companies would support an external initiative to do so;
- cTAP intelligence 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.

3.7 NEXT STEPS: To address fragmentation of efforts and agree an approach to collating data, analysis and developing the evidence base to support the identification, adoption and the use of robust biomarkers across the Duchenne academic and industry communities requires an action plan. Initial steps:

1) **Prague and Leiden meeting write-ups circulated to participants**
    a. Follow-up with speakers and panel members though webinars for input on their priorities, strategy and establish interest in Working Group participation
    b. Publication plan for “white paper” and target journal reviewed
2) **Discussion on roles and responsibilities to implement the agreed approach**

a. Project plan produced and management team identified
b. Stakeholder Group initial outreach
c. Working Groups established
d. Webinars or Working Group meetings on biomarkers (i) Biologicals/Fluid; (ii) Imaging; (iii) wearables – to identify strongest candidates and begin to identify data resources
e. Revise planning based on funding available to build and finance an expert team: including academics and industry scientists with expertise in biomarker development, outcome measure development and disease modelling; patient community representatives, Key Opinion Leaders, regulators and regulatory science experts; data scientists who understand and map data, develop datasets, and analyse, visualise and interpret the datasets including pharmacometric modellers; experts in designing protocols to support new biomarkers, gather data and develop evidence based models to develop selected biomarkers.

3) Funding plans developed

a. Short term seed money
b. Longer term project funding (e.g. COST/EC/NIH/Patient Groups)

4) Task list

a. Industry use of biomarkers – current practice and future plans (SW)
b. Updated review of biomarker landscape
    i. EIM, Muscle Ultrasound drafted by JL – circulate to community
    ii. Fluid biomarker datasets updated draft needed (lead to be decided)
    iii. Wearables/e-health updated draft needed (lead to be decided)
c. Funding options review (RH/CO)
d. Expert Working Groups panel short list for invitations

5. Biomarker information tables

A summary of DMD biomarkers and associated data was produced by Jane Larkindale

5.1 Dystrophin
5.2 EIM – Electrical Impedance Myography
5.3 Quantitative Muscle Ultrasound
5.4 Fluid Biomarkers
5.5 Cardiac Fluid Biomarkers
5.6 Cardiac MRI
5.7 Cardiac Echocardiography
5.8 MRI FF (produced by H Kan)

Summary of DMD Biomarkers

5.1 Dystrophin

Consortium /Institution	Biomarker	Authors	Journal	Year	N	Correlation	Longitudinal data?	Interval
Flagship Bioscience	Dystrophin levels as a PD biomarker	Aeffner F, Faelan C, Moore SA1, Moody A, Black JC, Charleston JS, Frank DE, Dworzak J, Piper JK, Ranjitkar M, Wilson K, Kanaly S, Rudmann DG, Lange H, Young GD, Milici AJ.	Arch Pathol Lab Med. 2019 Feb;143(2):197-205. doi: 10.5858/arpa.2017-0536-OA. Epub 2018 Aug 31.	2019	Assay validation	N/A	N/A	N/A
Biomarin	ProteinSimple capillary immunoassay (Wes) method as a PD biomarker	Beekman C, Janson AA, Baghat A, van Deutekom JC, Datson NA.	PLoS One. 2018 Apr 11;13(4):e0195850. doi: 10.1371/journal.pone.0195850. eCollection 2018.	2018	Assay validation, 17 DMD, 25 BMD, 31 controls			
CINRG	Mass spec assay for dystrophin as a PD biomarker	Brown KJ, Marathi R, Fiorillo AA, Ciccimaro EF, Sharma S, Rowlands DS, Rayavarapu S, Nagaraju K, Hoffman EP, Hathout Y.	J Bioanal Biomed. 2012 Dec 18;Suppl 7. pii: 001	2012	Assay validation			
Nationwide Children's hospital	Dystrophin immunofluorescence quantification as a PD biomarker	Taylor LE, Kaminoh YJ, Rodesch CK, Flanigan KM.	Neuropathol Appl Neurobiol. 2012 Oct;38(6):591-601. doi: 10.1111/j.1365-2990.2012.01250.x.	2012	Assay development			

5.2 EIM – Electrical Impedance Myography

Consortium /Institution	Biomarker	Authors	Journal	Year	N	Correlation	Longitudinal data?	Interval
Beth Israel Deaconess Medical Center and others	Muscle composition by EIM as a monitoring or PD biomarker	Roy B Darras BT, Zaidman CM, Wu JS, Kapur K, Rutkove SB.	Clin Neurophysiol. 2019 Feb 12;130(4):515-520. doi: 10.1016/j.clinph.2019.01.018. [Epub ahead of print]	2019	36 DMD and 29 healthy boys between ages 5 and 13 years	Quantitative ultrasound and EIM	yes	Baseline, 6 and 12 mo.
Beth Israel Deaconess Medical Center and others	Muscle composition by EIM as a monitoring or PD biomarker,	Kapur K, Sanchez B, Pacheck A, Darras B, Rutkove SB, Selukar R.	IEEE Trans Biomed Eng. 2018 Nov 1. doi: 10.1109/TBME.2018.2879227. [Epub ahead of print]	2018	16 boys DMD, 12 controls	EIM - functional mixed-effects model using a state-space approach to describe the response trajectories	yes	2 years
Beth Israel Deaconess Medical Center and others	Muscle composition by EIM as a monitoring or PD biomarker,	Rutkove SB, Kapur K, Zaidman CM, Wu JS, Pasternak A, Madabusi L, Yim S, Pacheck A, Szelag H, Harrington T, Darras BT.	Ann Neurol. 2017 May;81(5):622-632. doi: 10.1002/ana.24874. Epub 2017 May 4.	2017	36 DMD and 29 controls	Compared to functional tests, steroid initiation	yes	2 years
DART_EIM Evaluators consortium	Muscle composition by EIM as a monitoring or PD biomarker,	Zaidman CM, Wang LL, Connolly AM Florence J, Wong BL, Parsons JA, Apkon S, Goyal N, Williams E, Escolar D, Rutkove SB, Bohorquez JL; DART-EIM Clinical Evaluators Consortium.	Muscle Nerve. 2015 Oct;52(4):592-7. doi: 10.1002/mus.24611. Epub 2015 Jul 24.	2015	61 DMD and 31 controls	6-minute walk distance (6MWD), North Star Ambulatory Assessment (NSAA), timed functional tests (TFTs), and strength (hand-held dynamometry).	No	
Beth Israel Deaconess	Muscle composition by EIM combined with quantitative muscle ultrasound as a	Shklyar I, Pasternak A, Kapur K, Darras BT, Rutkove SB.	Pediatr Neurol. 2015 Feb;52(2):202-5. doi:	2015	31 DMD, 26 controls	6-minute walk test, the North Star Ambulatory	No	

Consortium /Institution	Biomarker	Authors	Journal	Year	N	Correlation	Longitudinal data?	Interval
Medical Center and others	monitoring or PD biomarker,		10.1016/j.pediatrneurol.2014.09.014. Epub 2014 Oct 7.			Assessment, and handheld dynamometry.		
Beth Israel Deaconess Medical Center and others	Muscle composition by EIM combined with quantitative muscle ultrasound as a monitoring or PD biomarker,	Schwartz S, Geisbush TR, Mijailovic A, Pasternak A Darras BT, Rutkove SB.	Clin Neurophysiol. 2015 Jan;126(1):202-8. doi: 10.1016/j.clinph.2014.05.007. Epub 2014 May 17.	2015	28DMD, 24 healthy	Optimizing vs 6 MWD	No.	
Beth Israel Deaconess Medical Center and others	Muscle composition by EIM combined with quantitative muscle ultrasound as a monitoring or PD biomarker,	Geisbush TR, Visyak N, Madabusi L, Rutkove SB, Darras BT.	Clin Neurophysiol. 2015 Sep;126(9):1790-6. doi: 10.1016/j.clinph.2014.11.017. Epub 2014 Nov 28.	2015	22 healthy, 14 DMD	Inter-rater reliability, test-re-test	No	
Beth Israel Deaconess Medical Center	EIM compared to quant ultrasound	Rutkove SB, Geisbush TR, Mijailovic A, Shklyar I, Pasternak A, Visyak N, Wu JS, Zaidman C, Darras BT	Pediatr Neurol. 2014 Jul;51(1):88-92. doi: 10.1016/j.pediatrneurol.2014.02.015. Epub 2014 Feb 28.	2014	24 controls, 24 DMD	Comparing EIM and qUltrasound, age and NSAA	No	
Beth Israel Deaconess Medical Center	EIM compared to quant ultrasound	Rutkove SB, Darras BT.	J Phys Conf Ser. 2013;434(1). pii: 012069	2013	14 DMD 13 ontrols	qUS vs EIM, 6-minute walk test, timed tests and strength measurements. NSAA	No	

5.3 Quantitative Muscle Ultrasound

Consortium /Institution	Biomarker	Primary author	Journal	Year	N	Correlation	Longitudinal data?	Interval
Beth Israel Deaconess Medical Center and others	Muscle composition by ultrasound as a monitoring or PD biomarker	Roy B, Darras BT, Zaidman CM, Wu JS, Kapur K, Rutkove SB.	Clin Neurophysiol. 2019 Feb 12;130(4):515-520. doi: 10.1016/j.clinph.2019.01.018. [Epub ahead of print]	2019	36 DMD and 29 healthy boys between ages 5 and 13 years	Quantitative ultrasound and EIM	yes	Baseline, 6 and 12 mo.
Wash U, Beth Israel Deaconess Medical Center and others	Muscle composition by ultrasound as a monitoring or PD biomarker	Zaidman CM, Wu JS Kapur K, Pasternak A, Madabusi L, Yim S, Pacheck A, Szelag H, Harrington T, Darras BT, Rutkove SB.	Ann Neurol. 2017 May;81(5):633-640. doi: 10.1002/ana.24904. Epub 2017 May 4.	2017	36 DMD 28 controls.	Quantitative ultrasound [gray scale level (GSL), measured from the ultrasound image, and quantitative backscatter analysis (QBA)]vs functional assessments [6MWD and supine stand] in different muscles	No	
Emory	Quantitative EMG as a measure of muscle composition as a monitoring or PD biomarker	Verma SLin J Travers C McCracken C Shah D	Muscle Nerve. 2017 Dec;56(6):1168-1171. doi: 10.1002/mus.25678. Epub 2017 May 24.	2017	18	Q electromyography didn't change with time over 6 mo.	Every 6 months for 14 month	
MIT	Quantitative EMG as a measure of muscle composition as a monitoring or PD biomarker	Koppaka S, Shklyar I, Rutkove SB, Darras BT, Anthony BW, Zaidman CM, Wu JS.	J Ultrasound Med. 2016 Sep;35(9):1889-97. doi: 10.7863/ultra.15.04065. Epub 2016 Jul 14.	2016	19 DMD, 21 age matched controls	Quantitative US imaging using edge detection can distinguish patients with DMD from healthy controls	No	
Beth Israel Deaconess	Muscle composition by EIM combined with quantitative muscle ultrasound as a	Shklyar I, Pasternak A, Kapur K, Darras BT, Rutkove SB.	Pediatr Neurol. 2015 Feb;52(2):202-5. doi:	2015	31 DMD, 26 controls	6-minute walk test, the North Star Ambulatory Assessment, and handheld dynamometry.	No	

Consortium /Institution	Biomarker	Primary author	Journal	Year	N	Correlation	Longitudinal data?	Interval
Medical Center and others	monitoring or PD biomarker,		10.1016/j.pediatrneurol.2014.09.014. Epub 2014 Oct 7.					
Beth Israel Deaconess Medical Center	Monitoring with quant muscle ultrasound	Shklyar I, Geisbush TR, Mijialovic AS, Pasternak A, Darras BT, Wu JS, Rutkove SB, Zaidman CM	Muscle Nerve. 2015 Feb;51(2):207-13. doi: 10.1002/mus.24296. Epub 2014 Dec 23.	2015	25 DMD, 25 control	Comparing [quantitative backscatter analysis (QBA)] or by measuring these backscattered amplitudes after compression into grayscale levels (GSL) obtained from the images.	No	
Wash U	Monitoring with quant muscle ultrasound	Zaidman CM, Malkus EC, Connolly AM.	Muscle Nerve. 2015 Sep;52(3):334-8. doi: 10.1002/mus.24609.	2015	5 young DMD and 5 controls	QUS decreased as function was gaine in young boys	yes	17-29 mo.
Beth Israel Deaconess Medical Center	EIM compared to quant ultrasound	Rutkove SB, Geisbush TR, Mijailovic A, Shklyar I, Pasternak A, Visyak N, Wu JS, Zaidman C, Darras BT	Pediatr Neurol. 2014 Jul;51(1):88-92. doi: 10.1016/j.pediatrneurol.2014.02.015. Epub 2014 Feb 28.	2014	24 controls, 24 DMD	Comparing EIm and qUltrasound, age and NSAA	No	

5.4 Fluid Biomarkers

Consortium /Institution	Biomarker	Primary author	Journal	Year	N	Correlation	Longitudinal data?	Interval
Mass General others	mRNA splicing patterns in urine as a monitoring or PD biomarker	Antoury L, Hu N, Balaj L, Das S, Georghiou S, Darras B, Clark T, Breakefield XO, Wheeler TM.	Nat Commun. 2018 Sep 25;9(1):3906. doi: 10.1038/s41467-018-06206-0.	2018	12 DMD and 3 BMD as comparison to DM1	Comparison to DM1	no	
Kobe Gakuin university	Urinary titin as a diagnostic biomarker	Matsuo M, Shirakawa T, Awano H, Nishio H.	Clin Chim Acta. 2018 Nov;486:110-114. doi: 10.1016/j.cca.2018.07.041. Epub 2018 Jul 24.	2018	3 DMD, 100 unaffected		no	
Kobe Gakuin university	Urinary titin as a diagnostic biomarker	Awano H, Matsumoto M, Nagai M, Shirakawa T, Maruyama N, Iijima K, Nabeshima YI, Matsuo M.	Clin Chim Acta. 2018 Jan;476:111-116. doi: 10.1016/j.cca.2017.11.024. Epub 2017 Nov 23.	2018	145 samples from 113 patients.	DMD vs BMD, change with age, CK	no	
Diagnostic &Research Reagents Division, Immuno-biological Laboratories Co., Ltd. 1	Urinary titin as a diagnostic biomarker	Maruyama N, Asai T, Abe C, Inada A, Kawauchi T, Miyashita K, Maeda M Matsuo M, Nabeshima YI.	Sci Rep. 2016 Dec 19;6:39375. doi: 10.1038/srep39375	2016	Assay validation			
Genethon	Urinary titin as a diagnostic biomarker	Rouillon J, Zocevic A, Leger T, Garcia C, Camadro JM, Udd B, Wong B, Servais L, Voit T, Svinartchouk F.	Neuromuscul Disord. 2014 Jul;24(7):563-73. doi: 10.1016/j.nmd.2014.03.012. Epub 2014 Apr 13.	2014	Proteomic profiling to ID titin	104 DMD and controls	No	

Consortium /Institution	Biomarker	Primary author	Journal	Year	N	Correlation	Longitudinal data?	Interval
National Center Hospital, Tokyo	Prostaglandin metabolites in urine as a monitoring or PD biomarker	Takeshita E, Komaki H, Tachimori H, Miyoshi K, Yamamiya I, Shimizu-Motohashi Y, Ishiyama A, Saito T, Nakagawa E, Sugai K, Sasaki M.	Brain Dev. 2018 Nov;40(10):918-925. doi: 10.1016/j.braindev.2018.06.012. Epub 2018 Jul 10.	2018	61 DMD 35 control	Ambulant and non-ambulant, steroid and not	No	
Kobe university	Prostaglandin D2 metabolites in urine as a monitoring or PD biomarker	Nakagawa T, Takeuchi A, Kakiuchi R, Lee T, Yagi M, Awano H, Iijima K, Takeshima Y, Urade Y, Matsuo M.	Clin Chim Acta. 2013 Aug 23;423:10-4. doi: 10.1016/j.cca.2013.03.031. Epub 2013 Apr 19.	2013	79 control, 191 DMD	age	No	
Brazil	CD49d expression levels in blood-derived T-cell subsets as a monitoring biomarker	Savino W, Pinto-Mariz F, Mouly V.	Methods Mol Biol. 2018;1687:219-227. doi: 10.1007/978-1-4939-7374-3_16.	2018	Assay validation	Assay validation		
Brazil	CD49d expression levels in blood-derived T-cell subsets as a monitoring biomarker	Pinto-Mariz F, Rodrigues Carvalho L, Prufer De Queiroz Campos Araujo A, De Mello W, Gonçalves Ribeiro M, Cunha Mdo C, Cabello PH, Riederer I, Negroni E, Desguerre I, Veras M, Yada E, Allenbach Y, Benveniste O, Voit T, Mouly V, Silva-Barbosa SD, Butler-Browne G, Savino W.	Skelet Muscle. 2015 Dec 10;5:45. doi: 10.1186/s13395-015-0066-2. eCollection 2015	2015	75 DMD	Correlation with disease progression	No	

Consortium /Institution	Biomarker	Primary author	Journal	Year	N	Correlation	Longitudinal data?	Interval
Oxford and others	RNA signatures as monitoring or PD markers in serum and muscle	Coenen-Stass AML, Sork H, Gatto S, Godfrey C, Bhomra A, Krjutškov K, Hart JR, Westholm JO, O'Donovan L, Roos A, Lochmüller H, Puri PL, El Andaloussi S, Wood MJA, Roberts TC.	Mol Ther Nucleic Acids. 2018 Dec 7;13:1-15. doi: 10.1016/j.omtn.2018.08.005. Epub 2018 Aug 17.	2018	mice			
Reveragen	Serum protein biomarkers of inflammation and metabolism as PD biomarkers; [also safety biomarkers] – Secondary outcomes for pharmacodynamic safety (insulin resistance, adrenal suppression, bone turnover); 2. Exploratory outcomes for drug mechanism of action (inflammation biomarkers); 3. Exploratory outcomes for expanded pharmacodynamic safety.	Conklin LS, Damsker JM, Hoffman EP, Jusko WJ, Mavroudis PD, Schwartz BD, Mengle-Gaw LJ, Smith EC, Mah JK, Guglieri M, Nevo Y, Kuntz N, McDonald CM, Tulinius M, Ryan MM, Webster R, Castro D, Finkel RS, Smith AL, Morgenroth LP, Arrieta A, Shimony M, Jaros M, Shale P, McCall JM, Hathout Y, Nagaraju K, van den Anker J, Ward LM, Ahmet A, Cornish MR, Clemens PR	Pharmacol Res. 2018 Oct;136:140-150. doi: 10.1016/j.phrs.2018.09.007. Epub 2018 Sep 13.	2018	48 patients	Looked at CK, somascan of proteins, insulin resistance (fasting glucose and insulin), adrenal suppression (first-in-morning cortisol), and changes in bone turnover (osteocalcin and procollagen type 1 pro-peptide [P1NP] [bone formation], and C-terminal telopeptide [CTX] [bone resorption]). Plus additional exploratory proteins	no	2 weeks on drug
CINRG	Serum PD biomarkers of inflammatory / steroid response	Hathout Y, Conklin LS, Seol H, Gordish-Dressman H, Brown KJ, Morgenroth LP, Nagaraju K, Heier CR, Damsker JM, van den Anker JN, Henricson E, Clemens PR,	Sci Rep. 2016 Aug 17;6:31727. doi: 10.1038/srep31727.	2016	9 GC-naïve DMD patients, 5 GC-treated DMD patients, and 4 untreated healthy controls, both	Comappred DMD, IBM. Mainly proteomics.	yes	4 months

Consortium /Institution	Biomarker	Primary author	Journal	Year	N	Correlation	Longitudinal data?	Interval
		Mah JK, McDonald C, Hoffman EP.			before and after steroid initiation			
Leiden et al	Serum protein biomarkers as PD/monitoring biomarkers	Spitali P, Hettne K, Tsonaka R, Charrout M, van den Bergen J, Koeks Z, Kan HE, Hooijmans MT, Roos A, Straub V, Muntoni F, Al-Khalili-Szigyarto C, Koel-Simmelink MJA, Teunissen CE, Lochmüller H, Niks EH, Aartsma-Rus A	J Cachexia Sarcopenia Muscle. 2018 Aug;9(4):715-726. doi: 10.1002/jcsm.12304. Epub 2018 Apr 16.	2018	15 patients, 9 controls	31P MRS, functional tests, muscle strength, muscle mass	yes	4.4 years
University of Trento	Serum protein biomarkers as PD/monitoring biomarkers	Parolo S, Marchetti L, Lauria M, Misselbeck K, Scott-Boyer MP, Caberlotto L, Priami C.	PLoS One. 2018 Mar 12;13(3):e0194225. doi: 10.1371/journal.pone.0194225. eCollection 2018.	2018	42 patients, 28 controls	Patient vs. control	no	
CINRG	Serum protein biomarkers as PD/monitoring biomarkers	Boca SM, Nishida M, Harris M, Rao S, Cheema AK, Gill K, Seol H, Morgenroth LP, Henricson E, McDonald C, Mah JK, Clemens PR, Hoffman EP, Hathout Y, Madhavan S.	PLoS One. 2016 Apr 15;11(4):e0153461. doi: 10.1371/journal.pone.0153461. eCollection 2016.	2016	51 DMD and 22 controls	age	no	

Consortium /Institution	Biomarker	Primary author	Journal	Year	N	Correlation	Longitudinal data?	Interval
Pfizer et al	Serum concentrations of skeletal troponin I (sTnI), myosin light chain 3 (Myl3), fatty acid binding protein 3 (FABP3) and muscle-type creatine kinase (CKM) proteins as Monitoring/PD biomarkers	Burch PM, Pogoryelova O, Goldstein R, Bennett D, Guglieri M, Straub V, Bushby K, Lochmüller H, Morris C.	J Neuromuscul Dis. 2015 Sep 2;2(3):241-255.	2015	74 DMD, 38 BMD, 49 LGMD, 32 controsl	patient age, ambulatory status, cardiac function and treatment status	No	
Leiden et al	Serum concentrations of proteins as monitoring/PD	Oonk S, Spitali P, Hiller M, Switzar L, Dalebout H, Calissano M, Lochmüller H, Aartsma-Rus A, 't Hoen PA, van der Burgt YE.	Proteomics Clin Appl. 2016 Mar;10(3):290-9. doi: 10.1002/prca.201500044. Epub 2016 Jan 8.	2016	?			
CINRG	Serum concentrations of proteins as monitoring/PD	Hathout Y, Brody E, Clemens PR, Cripe L, DeLisle RK, Furlong P, Gordish-Dressman H, Hache L, Henricson E, Hoffman EP, Kobayashi YM, Lorts A, Mah JK, McDonald C, Mehler B, Nelson S, Nikrad M, Singer B, Steele F, Sterling D, Sweeney HL, Williams S, Gold L.	Proc Natl Acad Sci U S A. 2015 Jun 9;112(23):7153-8. doi: 10.1073/pnas.1507719112. Epub 2015 May 26.	2015	cohort 1 (The Parent Project Muscular Dystrophy-Cincinnati Children's Hospital Medical Center), 42 patients with DMD and 28 age-matched normal volunteers; and cohort 2 (The Cooperative International Neuromuscular Research Group, Duchenne N	Control vs DMD	No	

Consortium /Institution	Biomarker	Primary author	Journal	Year	N	Correlation	Longitudinal data?	Interval
					atural History Study), 51 patients with DMD and 17 age-matched normal volunteers.			
Stockholm and Leiden	ID of serum biomarkers for MDs	Ayoglu B, Chaouch A, Lochmüller H, Politano L, Bertini E, Spitali P, Hiller M, Niks EH, Gualandi F, Pontén F, Bushby K, Aartsma-Rus A, Schwartz E, Le Priol Y, Straub V, Uhlén M, Cirak S, 't Hoen PA, Muntoni F, Ferlini A, Schwenk JM, Nilsson P, Al-Khalili Szigyarto C.	EMBO Mol Med. 2014 Jul;6(7):918-36. doi: 10.15252/emmm.201303724.	2014	260 serum and plasma samples	MD vs controls	No	
Leiden	Fibronectin as a monitoring biomarker	Cynthia Martin F, Hiller M, Spitali P, Oonk S, Dalebout H, Palmblad M, Chaouch A, Guglieri M, Straub V, Lochmüller H, Niks EH, Verschuuren JJ, Aartsma-Rus A, Deelder AM, van der Burgt YE, 't Hoen PA.	Proteomics Clin Appl. 2014 Apr;8(3-4):269-78. doi: 10.1002/prca.201300072. Epub 2014 Mar 11.	2014	8 DMD patients, 38 milder Becker muscular dystrophy patients, 33 patients with other neuromuscular disorders, and 15 age-matched adult and child controls.	Vs other MD and control	Longitudinal samples from 22 DMD patients followed up	6 months up to 4 years.
Leiden et al	Serum MMP9 protein as a monitoring biomarker [were looking as a predictive marker of exon skipping response]	Lourbakos A, Yau N, de Bruijn P1, Hiller M, Kozaczynska K, Jean-Baptiste R, Reza M, Wolterbeek R, Koeks Z, Ayoglu B, de Klerk D, Campion G, Zaharieva I, Nadarajah VD, Nilsson P, Al-Khalili Szigyarto C, Muntoni F, Lochmüller H, Verschuuren JJ,	Sci Rep. 2017 Dec 20;7(1):17888. doi: 10.1038/s41598-017-17982-y.	2017	68 longitudinal samples belonging to 66 patients. We further studied 1536 samples obtained from 3 independent clinical trials with	CK, 6MWD – no real correlation	yes	

Consortium /Institution	Biomarker	Primary author	Journal	Year	N	Correlation	Longitudinal data?	Interval
		Goemans N, Tulinius M, Niks EH, de Kimpe S, Aartsma-Rus A, 't Hoen PAC, Spitali P.			drisapersen, an antisense oligonucleotide targeting exon 51: an open label study including 12 patients; a phase 3 randomized, double blind, placebo controlled study involving 186 patients; an open label extension study performed after the phase 3.			
Leiden et al	Serum MMP9 protein as a monitoring biomarker	Nadarajah VD, van Putten M, Chaouch A, Garrood P, Straub V, Lochmüller H, Ginjaar HB, Aartsma-Rus AM, van Ommen GJ, den Dunnen JT, 't Hoen PA.	Neuromuscul Disord. 2011 Aug;21(8):569-78. doi: 10.1016/j.nmd.2011.05.011. Epub 2011 Jul 2.	2011	63 DMD	matrix metalloproteinase-9 (MMP-9), tissue inhibitors of metalloproteinase-1 (TIMP-1) and osteopontin (OPN) w compared to age	some	4 years
Sun Yat-sen University	Serum creatinine as a diagnostic biomarker BMD vs DMD	Wang L, Chen M, He R, Sun Y, Yang J, Xiao L, Cao J, Zhang H, Zhang C.	Front Neurol. 2017 May 8;8:196. doi: 10.3389/fneur.2017.00196. eCollection 2017.	2017	68 patients with dystrophinopathy	Motor function and clinical phenotype	no	

Consortium /Institution	Biomarker	Primary author	Journal	Year	N	Correlation	Longitudinal data?	Interval
Sun Yat-sen University	Serum creatinine as a diagnostic biomarker BMD vs DMD	Zhang H, Zhu Y, Sun Y, Liang Y, Li Y, Zhang Y, Deng L, Wen X, Zhang C.	Dis Markers. 2015;2015:141856. doi: 10.1155/2015/141856. Epub 2015 Mar 17	2015	212 patients	Vignos	No	
Leiden et al	Serum metabolites as PD/monitoring biomarkers	Spitali P, Hettne K, Tsonaka R, Sabir E, Seyer A, Hemerik JB, Goeman JJ, Picillo E, Ergoli M, Politano L, Aartsma-Rus A.	J Cell Mol Med. 2018 Apr;22(4):2442-2448. doi: 10.1111/jcmm.13543. Epub 2018 Feb 14.	2018	30 DMD, plus other MDs.	Vs. other MDs, also vs.6MWD and NSAA	no	
Center for the Biomedical Research on Rare Diseases (CIBERER)	miR-30c and miR-181a in serum as monitoring/PD biomarkers	Llano-Diez M, Ortez CI, Gay JA, Álvarez-Cabado L, Jou C, Medina J, Nascimento A, Jimenez-Mallebrera C	Neuromuscul Disord. 2017 Jan;27(1):15-23. doi: 10.1016/j.nmd.2016.11.003. Epub 2016 Nov 11.	2017	21 DMD, 7 BMD, 22 controls	Age, steroid, motor function	No	
University College London	MiR-1, miR-133a,b and miR-206 in serum as monitoring/PD biomarkers	Zaharieva IT, Calissano M, Scoto M, Preston M, Cirak S, Feng L, Collins J, Kole R, Guglieri M, Straub V, Bushby K, Ferlini A, Morgan JE, Muntoni F.	PLoS One. 2013 Nov 25;8(11):e80263. doi: 10.1371/journal.pone.0080263. eCollection 2013.	2013	CMD vs DMD and BMD, sixteen DMD patients were non-ambulant with mean age of 14 years (age range between 10 and 17 years) and 28 DMD were ambulant with mean age of 8.2 years (age range 4 to 13 years). All	NSAA, ambulation, FVC, age, with and without eteplirsen	12 weeks on eteplirsen	

Consortium /Institution	Biomarker	Primary author	Journal	Year	N	Correlation	Longitudinal data?	Interval
					BMD patients (n = 5) were ambulant with mean age of 13.6 (age range between 9 and 18 years).			
Various Northstar sites	Downregulation of miRNA-29, -23 and -21 in urine as monitoring / PD markers	Catapano F, Domingos J1, Perry M, Ricotti V, Phillips L, Servais L, Seferian A, Groot I, Krom YD, Niks EH, Verschuuren JJ, Straub V, Voit T, Morgan J, Muntoni F	Epigenomics. 2018 Jul;10(7):875-889. doi: 10.2217/epi-2018-0022. Epub 2018 Mar 22.	2018	control (n = 20), Ambulant (n = 31) and nonambulant(n = 23) DMD patients.	miR-29c-3p, miR-23b-3p and miR-21-5p are promising novel noninvasive biomarkers for DMD, and miR-29c-3p levels are differentially affected by different steroid regimens, supporting the antifibrotic effect of steroid therapy	No	
Chinese groups	Serum miR-206 and other muscle-specific microRNA as diagnostic biomarkers	Hu J, Kong M, Ye Y, Hong S, Cheng L, Jiang L.	J Neurochem. 2014 Jun;129(5):877-83. doi: 10.1111/jnc.12662. Epub 2014 Feb 12	2014	39 DMD	Correlation with CK, muscle function, etc.	No	
Chinese groups	Serum miR-206 and other muscle-specific microRNA as diagnostic biomarkers	Li X, Li Y, Zhao L, Zhang D, Yao X, Zhang H, Wang YC, Wang XY, Xia H, Yan J, Ying H.	Mol Ther Nucleic Acids. 2014 Jul 22;3:e177. doi: 10.1038/mtna.2014.29	2014	healthy (*n* = 23), Becker (*n* = 15), and Duchenne (*n* = 52) children, aged from 1 to 14 years old	age	No	

5.5 Cardiac Fluid Biomarkers

Consortium /Institution	Biomarker	Primary author	Journal	Year	N	Correlation	Longitudinal data?	Interval
Vanderbilt and others	MMP7 as a prognostic or monitoring biomarker for DMD cardiac dysfunction and myocardial fibrosis,	Soslow JH, Xu M, Slaughter JC, Crum K, Chew JD, Burnette WB, Su YR, Tomasek K, Parra DA, Markham LW.	J Card Fail. 2019 Feb 11. pii: S1071-9164(19)30157-5. doi: 10.1016/j.cardfail.2019.02.006. [Epub ahead of print]	2019	42	Cardiac MRI for function and late gadolinium enhancement (LGE) Serum analyzed for MMP 1, 2, 3, 7, 9, 10 and TIMPs 1-4. MMP1, MMP7, and MMP10	No	
CINRG	Interleukin 1 Receptor-Like 1 Protein (ST2) as a prognostic biomarker for cardiomyopathy	Anderson J, Seol H, Gordish-Dressman H, Hathout Y, Spurney CF; CINRG Investigators.	Pediatr Cardiol. 2017 Dec;38(8):1606-1612. doi: 10.1007/s00246-017-1703-9. Epub 2017 Aug 18	2017	24 DMD, 6 controls	Echo: ejection and shortening fraction	No	
Stuttgart, Tubingen et al	Up-regulation of circulating miRNAs miR-222, miR-26a and miR-378a-5p indicates the presence of myocardial scars in MD patients. Plasma miR-222 reflecting structural - but not functional - cardiac alterations in MD patients.	Becker S, Florian A, Patrascu A, Rösch S, Waltenberger J, Sechtem U, Schwab M, Schaeffeler E, Yilmaz A.	J Cardiovasc Magn Reson. 2016 May 6;18(1):25. doi: 10.1186/s12968-016-0244-3.	2016	63 DMD, 26 controls	miRNA from serum vs. cMRI	No	

5.6 Cardiac MRI

Consortium /Institution	Biomarker	Primary author	Journal	Year	N	Correlation	Longitudinal data?	Interval
Vanderbilt and others	Cardiac fibrosis as a monitoring biomarker for DMD cardiomyopathy	Soslow JH, Xu M, Slaughter JC, Crum K, Chew JD, Burnette WB, Su YR, Tomasek K, Parra DA, Markham LW.	J Card Fail. 2019 Feb 11. pii: S1071-9164(19)30157-5. doi: 10.1016/j.cardfail.2019.02.006. [Epub ahead of print]	2019	42	Cardiac MRI for function and late gadolinium enhancement (LGE) Serum analyzed for MMP 1, 2, 3, 7, 9, 10 and TIMPs 1-4. MMP1, MMP7, and MMP10	No	
Stutgart,Tubingen et al	Up-regulation of circulating miRNAs miR-222, miR-26a and miR-378a-5p indicates the presence of myocardial scars in MD patients. Plasma miR-222 reflecting structural - but not functional - cardiac alterations in MD patients.	Becker S, Florian A, Patrascu A, Rösch S, Waltenberger J, Sechtem U, Schwab M, Schaeffeler E, Yilmaz A.	J Cardiovasc Magn Reson. 2016 May 6;18(1):25. doi: 10.1186/s12968-016-0244-3.	2016	63 DMD, 26 controls	miRNA from serum vs. cMRI	No	
Nationwide Children's	Cardiac strain as a monitoring and prognostic biomarker for cardiac progression	Hor KN, Kissoon N, Mazur W, Gupta R, Ittenbach RF, Al-Khalidi HR, Cripe LH, Raman SV, Puchalski MD, Gottliebson WM, Benson DW.	Pediatr Cardiol. 2015 Jan;36(1):111-9. doi: 10.1007/s00246-014-0972-9. Epub 2014 Aug 2.	2015	?			

5.7 Cardiac Echocardiography

Consortium /Institution	Biomarker	Primary author	Journal	Year	N	Correlation	Longitudinal data?	Interval
INSERM Montpellier	LV strain as a prognostic biomarker of DMD cardiomyopathy	Amedro P, Vincenti M, De La Villeon G, Lavastre K, Barrea C, Guillaumont S, Bredy C, Gamon L, Meli AC, Cazorla O, Fauconnier J, Meyer P, Rivier F, Adda J, Mura T, Lacampagne A.	J Am Soc Echocardiogr. 2019 Jan 21. pii: S0894-7317(18)30608-4. doi: 10.1016/j.echo.2018.10.017. [Epub ahead of print]	2019	108, 36 with early stage DMD	Speckle-tracking echocardiographic (STE) imaging of LV strain with conventional Echo	No	
CINRG	Cardiac strain as a monitoring and prognostic biomarker for cardiac progression	Spurney CF, McCaffrey FM, Cnaan A, Morgenroth LP, Ghelani SJ, Gordish-Dressman H, Arrieta A, Connolly AM, Lotze TE, McDonald CM, Leshner RT, Clemens PR.	J Am Soc Echocardiogr. 2015 Aug;28(8):999-1008. doi: 10.1016/j.echo.2015.03.003. Epub 2015 Apr 21.	2015	48 DMD	LV strain, EF etc.	No	

5.8 MRI FF

Consortium /Institutions	Primary author	Journal	Year	field strength	2p Dixon	3p Dixon	6p dixon	MRS	population	n	Correlations/article topic	Longitudinal data?	Interval
ImagingDMD	Arpan et al	Neurology	2014	3				x	DMD	15	corticosteroid use, strenght, function	yes	3 mo, 6 mo, 1 year
IoM Paris	Azzabou et al	JMRI	2014	3		x			multiple	many	validation of three exp T2	No	
imagingDMD	Barnard et al	PlosOne	2018	3		x		x	DMD	136	Function	yes	48 months
Basel	Bonati et al	Neuromusc Disord	2015	3	x				DMD	20	motor function	yes	1 year
Basel	Bonati et al	Muscle nerve	2015	3	x				BMD	3		yes	1 year
IoM Paris	Carlier et al	J Inher Metab dis	2015	3		x			Pompe	23	enzyme replacement therapy	yes	1 year
Univ of Basel Hospital	Fischmann et al	J Neurol	2012	1,5	x				OPMD	5	function	yes	13 months
Basel	Fischmann et al	J Neurol	2013	3	x				DMD	20	function	No	
Basel	Fischmann et al	J Neurol	2011	1,5	x				OPMD		function	No	
ImagingDMD	Forbes et al	Plos One	2014	3				x	DMD	123		No	
ImagingDMD	Forbes et al	Radiology	2013	3				x	DMD	30	reproducibility	No	
Messina	Gaeta et al	Skelet Radiol	2012	1,5	x				DMD	20	function	No	
Basel	Gloor et al	JRMI	2011	1,5	x				OPMD	8	various fat imaging techniques	No	

Consortium /Institutions	Primary author	Journal	Year	field strength	2p Dixon	3p Dixon	6p dixon	MRS	population	n	Correlations/article topic	Longitudinal data?	Interval
	Hiba et al	JRMI	2012	1,5		?			DM1	19		No	
IoM Paris	Hogrel et al	Neurology	2016	3		x			DMD				
Newcastle	Hollingsworth et al	Magn Res Med	2014	3		x			BMD	8	acceleration	No	
Leiden Úniversity Medical Center	Hooijmans et al	NMR Biomed	2015	3		x			DMD	24	DTI	No	
Leiden Úniversity Medical Center	Hooijmans et al	Neurmuscular disorders	2017	3		x			DMD	22	proximodistal	No	
Leiden Úniversity Medical Center	Hooijmans et al	NMR Biomed	2017	3		x			DMD	18	31P	No	
Leiden Úniversity Medical Center	Hooijmans et al	PlosOne	2017	3		x			DMD	18	31P	yes	
Duke university	Horvath et al	Muscle Nerve	2015	3	x				Pompe	7	function	No	
	Kim et al	AJNR	2015					x	DMD				
Copenhagen	Lokken et al	Annals of Neurology	2016	3		x			BMD, LGMD2I	14 and 12	strength	No	
ImagingDMD	Lott et al	Neuromusuclar disorders	2014					x	DMD	25			
Newcastle	Loughran et al	Radiology	2015	3		x			BMD	8	R2* and acceleration	No	
IoM Paris	Marty et al	NMR in Biomed	2016	3		x	x		mixed		EPG		

Consortium /Institutions	Primary author	Journal	Year	field strength	2p Dixon	3p Dixon	6p dixon	MRS	population	n	Correlations/article topic	Longitudinal data?	Interval
NIH/GSK	Mankodi et al		2016	3		x			DMD	13	IDEAL-CPMG	yes	
UCL/MRC centre for Neurmuscl disease	Morrow et al	Lancet Neurology	2015	3		x			CMT1A, IBM	20 and 20		yes	1 year
Basel	Nagy et al	J Vis Exp	2019	3					DMD	47	function		
Los Angeles	Ponrarta et al		2015	3			x		DMD	13			
UCL	Ricotti et al	PlosOne	2016	3		x			DMD	15		yes	3 months, 6 months, 1 year
ImagingDMD	Triplett et al	Magn Res Med	2013	3		x		x	DMD, COL6	71	correlation of MRI and MRS	No	
Leiden Úniversity Medical Center	van den Bergen et al	JNNP	2014	3		x			BMD	9	dystrophin levels	No	
IoM Paris	Wary et al	NMR in Biomed	2015			x			DMD	24 (9 longit)	Dixon in the arm, ambulation	yes	~1 year
ImagingDMD	Willcocks et al	Annals of Neurology	2016	3		x		x	DMD	109	function	yes	3 months, 6 months, 1 year
Newcastle, Copenhagen, Paris, London	Willis et al	Plos One	2013	3	x	x			LGMD2I	32	function, semi quantitative	yes	1 year

Consortium /Institutions	Primary author	Journal	Year	field strength	2p Dixon	3p Dixon	6p dixon	MRS	population	n	Correlations/article topic	Longitudinal data?	Interval
Newcastle, Copenhagen, Paris, London	Willis et al	Plos One	2014	3	x	x			LGMD2I	38	function	No	
Leiden Úniversity Medical Center	Wokke et al	NMR in Biomed	2014	3, 7		x			BMD	25	function, 31P MRS	No	
Leiden Úniversity Medical Center	Wokke et al	Neuromusc disord	2014	3		x			DMD	16	strength	No	
Leiden Úniversity Medical Center	Wokke et al	JMRI	2013	3		x			DMD	13	semi-quantitative and mspec mod	No	
Los Angeles	Wren et al	Skelet Radiology	2008	1,5					DMD	9	strength		