

## Biomarkers and surrogate endpoints in Duchenne

There is a lot of discussion ongoing in the DMD community about biomarkers and surrogate endpoints as was pointed out by Patient representatives during an ENMC meeting on biomarkers for DMD (Jan 24-26 2014, Naarden, the Netherlands). On their request this document will explain what biomarkers are and will address questions about biomarkers and surrogate endpoints.

### What is a biomarker?

The official definition of a biomarker is “a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes or pharmacological responses to a therapeutic intervention”.

### What types of biomarkers are there?

There are three types of biomarkers:

- Diagnostic biomarkers (telling you something is wrong)
- Prognostic biomarkers (predicting disease course and/or severity)
- Therapeutic monitoring biomarkers (can predict whether a therapy will work or is working)

It is possible that one single biomarker is diagnostic, prognostic and allows for therapeutic monitoring. However generally, a biomarker is either diagnostic, or prognostic or allows for therapeutic monitoring.

### Which biomarkers are used for DMD already?

The biomarker most commonly used for DMD is serum creatine kinase (CK), and it is used as a diagnostic biomarker. CK is a muscle enzyme that leaks into the bloodstream upon muscle damage. As such, elevated CK in the blood is a marker for muscle damage. CK is not specific for DMD or even muscular dystrophy, since it is elevated in blood even in healthy people after muscle damage due to injury or heavy exercise. In clinical practice when patients present with muscle weakness, this can have several causes (e.g. problems in the nervous system or in the muscle), and detecting elevated CK levels helps clinicians, because this points the diagnostic thinking to a problem in the muscle rather than in the nervous system.

There are currently no prognostic or therapeutic monitoring biomarkers available for DMD, but finding biomarkers like these is an important part of ongoing research.

### What research is ongoing for prognostic biomarkers for DMD?

Prognostic biomarker research for DMD focuses primarily on the identification of genetic modifiers that can influence the disease course. The major gene that influences disease course in DMD is the dystrophin gene itself since the type of mutation determines whether a patient has Duchenne or

Becker. There are other genetic variations called SNPs that can modify the outcome of disease progression. For instance, when a SNP is located in a gene involved in inflammation, this can lead to differences in how strongly the immune system responds to muscle damage (e.g. a patient carrying one type of SNP will have a stronger immune response than patients carrying the other type – the stronger immune response will lead to more muscle damage and more fibrosis and thus will lead to a more severe disease course, as e.g. reflected by earlier loss of ambulation). Since these SNPs may influence how patients function, knowing about these prognostic biomarkers could allow for patient stratification in clinical trials.

So far SNPs in the genes *SPP1* (osteopontin) and *LTBP4* (latent TGF-beta binding protein 4) have been reported to influence the age at loss of ambulation for DMD patients. However, analysis in larger, independent patient groups are needed to confirm whether these are true findings or whether they happened by chance and how their effect is influenced by the use of corticosteroids. This work is currently ongoing.

### **How can biomarkers be used in clinical trials?**

There are several ways biomarkers can be used in the context of new drug development in a clinical trial. Predictive biomarkers can be used to identify patients who will respond to the intended treatment before starting the trial. For example, this approach is used in breast cancer, to assess whether the tumour is responsive to a certain type of chemotherapy. Similarly, biomarkers can also be used to assess whether patients are responding to treatment during the trial. With this information, those patients most likely to respond can be recruited or continue to be in the clinical trial. Optimizing which patients enter and remain in a trial is expected to make it easier to detect clinical benefit.

Biomarkers can also be used to demonstrate that a treatment worked as hypothesised. This can be done with pharmacodynamic (PD) biomarkers, which are markers that change upon treatment. A specific subset of pharmacodynamic biomarkers can be used to confirm the drug mechanism. For example, increased dystrophin levels in the muscles of DMD patients are expected to be a pharmacodynamic biomarker for therapeutic approaches aiming to restore dystrophin expression, while reduced fibrosis could be a pharmacodynamic biomarker for antioxidants or other anti-fibrotics.

Some biomarkers can be used as surrogate endpoints, meaning they can be used instead of a functional outcome measure. In order **for a biomarker to be accepted as a surrogate endpoint, appropriate data have to show that the biomarker correlates with current or future clinical benefit**. Surrogate endpoints can predict a response to therapy and are tools that can be used to accelerate the process of making a drug available on the market. They show less variation than functional tests (like measuring muscle strength or walk distance, which can depend also on patient motivation) and they may also show quicker than functional tests whether the drug works or not.

At the moment there is no surrogate endpoint validated for use in DMD trials.

### **What is the current status of using dystrophin as a surrogate endpoint?**

A surrogate endpoint needs to be shown to correlate with clinical benefit, since **the regulators can only approve drugs that provide clinically important benefit to the patients, outweighing their**

**risks.** This correlation can be very difficult to demonstrate, and in fact, there are very few surrogate endpoints that have been approved for any indication.

DMD is caused by lack of dystrophin and one could argue that restoring dystrophin should lead to clinical benefit. However, it is not as simple as that. Due to lack of dystrophin, DMD patients continuously lose muscle tissue, which is replaced by fat (adipose) and scar (fibrotic) tissue. This means that by the time patients are included in a clinical trial and are exposed to a potentially effective test drug, they have only a limited amount of muscle tissue left. A therapeutic approach may bring back dystrophin in the muscle that is left, but restoring dystrophin is not expected to help with the fat and fibrosis that have already replaced the damaged muscle tissue. The ability to produce clinical benefit will depend not only on the efficacy of the test drug but also on whether enough muscle is present at the time the therapy starts. When too much muscle is lost, dystrophin restoration may not lead to observable clinical benefit during the duration of the clinical trial. Additionally, the amount of dystrophin restored in the remaining muscular fibers may be enough to be detected via laboratory testing, but not sufficient to produce any measurable improvement in muscle function. These are only a couple of explanations for why levels of dystrophin may not necessarily correlate with objective clinical benefit and this is why regulators at present cannot accept dystrophin as a surrogate endpoint.

As explained earlier, dystrophin restoration can be used as a pharmacodynamic biomarker (i.e. a marker for drug activity) for those drugs which are designed to restore its expression in the muscle. There have been advances in technologies to quantify dystrophin levels in muscle biopsies but many challenges remain. For example, the levels of fibrosis and fat will differ between muscles but also within a single muscle. So it is possible to take a biopsy from a very fibrotic area or from a relatively well preserved area of the muscle. A biopsy is only a small sample from a single muscle at a single time point, so it can only tell whether dystrophin was restored in that area of that muscle. Likewise the amount of muscle fibers, fat and fibrosis in the muscle biopsy does not necessarily reflect the state of the muscle in the whole body.

These issues will need to be resolved and work needs to be conducted to assess whether dystrophin can be suitable as a surrogate endpoint. If dystrophin levels can be correlated with clinical benefit in a clinical trial setting it could be a critically important surrogate endpoint for smaller DMD populations where it will be much harder to show significant clinical benefit.

### **What research is ongoing to identify surrogate biomarkers for DMD?**

A lot of work is ongoing to try and identify biomarkers that can be used as surrogate endpoints. However, this work is challenging, as the development needs to be done in the context of a therapy (and there currently is none for DMD). The focus is on markers that are minimally invasive (e.g. MRI, serum and urine analysis), rather than muscle biopsies, which are invasive. There are certain criteria that these biomarkers have to fulfill:

- They need to be measurable in a reproducible, reliable and standardized way
- They should be able to discriminate between DMD and healthy people (e.g. higher or lower in DMD compared to controls)

- They need to have prognostic value for the course of disease and preferably correlate with disease severity/progression. This is complicated in DMD because blood levels of many markers are higher in patients compared to healthy people at early stages of the disease, but when the disease progresses and patients lose muscle mass, the levels go down – this makes it challenging to use these markers as surrogate endpoints because when the levels go down it is not certain whether this is because patients improved, or because they got worse. For this reason the blood candidate biomarkers need to be first evaluated in longitudinal studies (with multiple samples obtained from the same patient at different time points) to confirm that their levels correlate with disease progression.
- They need to correlate to or be predictive of clinical benefit and have to respond to treatment with the test drug

Most work on candidate biomarker discovery focuses on serum. Discovery can be done either with a targeted approach (i.e. selecting potential proteins that could differ between patients and healthy people based on the disease pathology and comparing serum levels of those proteins) and a non-targeted approach (comparing all proteins between patients and healthy people and see what differs). Usually as a first step a few samples belonging to patients and healthy people are compared for both the targeted and non-targeted approach. Then the potential candidates are further tested in larger sample sets, in longitudinal sample sets and/or by other investigators. To confirm whether a biomarker responds to treatment, they are finally tested in clinical trial samples. This can be done retrospectively in serum samples of patients from a completed trial, or during the trial (where the candidate marker is taken along as an exploratory biomarker).

When a biomarker shows potential as a surrogate endpoint, it has to also be qualified and accepted as a surrogate endpoint by the regulatory agencies, before it can be used in the confirmatory stages of drug development programme aiming at marketing authorization. This involves validating the assay to measure the biomarker (showing good reproducibility, specificity etc), assessing how the biomarker is influenced by external factors, such as geographic location, diet, age etc. Finally, data has to be presented to the regulators to show that the biomarker has the discriminative power to predict clinical benefit of the drug.

For DMD the work is mainly in the discovery and early confirmation phase, with MMP-9 being the most advanced. MMP-9 (matrix metalloproteinase 9) is a protein that involved in tissue remodelling and is connected to the fibrosis that occurs in DMD. Serum MMP-9 levels are higher in Duchenne patients than in age-matched controls and have been shown to increase further with age. Currently work is ongoing to confirm these findings in larger sample sets and to assess MMP-9 levels in clinical trial samples.

*UPPMD gratefully acknowledges the effort of all the experts who commented on the draft and especially dr Annemieke Aartsma-Rus for coordinating the drafting of this document*