Updates on biomarker research in DMD
Disclosure

Chair of the Working Group on Biomarkers

Coordinator of FINGER (FP5)
BIO-NMD (FP7)
Partner of NMD-CHIP,
NEUROMICS (FP7)
SIGN (Slovenia-Italy transborder project)
Cost Action Exon Skipping

EMQN and ISS quality assessment Schemes

Principal Investigator of various grants on RDs

Associate Professor in Medical Genetics

Principal Investigator of clinical trials on Duchenne Muscular Dystrophy

Member of the ENMC scientific Committee

Medical Genetic Group
Biomarkers: definition and types

What is a Biomarker?

‘A characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes or pharmacological responses to a therapeutic intervention.’

Biomarkers definition working group, Clin. Pharm. Ther. 2001;69:80-95

Genomic Biomarkers

EMA-ICH (International Conference of Harmonization) 2007/2008

Proteomic Biomarkers

DNA
- Genome
- Epigenome

RNA
- Transcriptome

Proteome

DISCOVERY

CK
MMP9
SPP1
LFTB4

myomiR

VALIDATION
Biomarkers: characteristics and meaning

**Analytical validity**
accurately measuring a specific parameter,
clearly distinguishing between diseased and normal status.

**Analytical performance**
methodology used for biomarker detection, sample preparation, etc.

**Clinical validity**
– finely measuring changes in disease characteristics with a bidirectional correlation (biomarker change > disease phenotypic change and vice versa)

*Included concept:* **STABILITY** (not affected by diet, exercise, stress, or age, sex or genetic determinants)

**Clinical utility**
It is the likelihood that the biomarker will lead to improved outcome with a given intervention.
it is the value that the biomarker provides in predicting drug response or prognostic evaluation of a certain disease of a marker defined population over and above other standard clinical features.

**Feasibility**
A biomarker should be practical to identify and measure, and not variable, depending on type of sample collection, methods, or platform used for its identification.

*Included concept:* **LOW INVASIVITY**

**Time and cost effectiveness**
The cost/effect relationship is important especially if a biomarkers can be widely used in large populations and for monitoring frequent diseases (as diabetes or cancer, for example).
Biomarkers: types and applications

**Diagnostic**

- DISEASE DIAGNOSIS (GENE MUTATIONS)
- DISEASE PROGRESSION
- DISEASE STRATIFICATION
- DISEASE SCREENING
- DISEASE MECHANISMS

**Therapeutic**

- PHARMACODYNAMIC
  - (“what the drug does to the body”)
  - Confirm predicted mechanism of action

- PHARMACOKINETIC
  - (“what the body does to the drug”)
  - Drug biodistribution, half-life, elimination mode

- PROGNOSTIC
  - surrogate endpoints

- THERAPIES MONITORING
  - Toxicity & Safety
  - Efficacy (responders vs non-responders)
  - Efficiency (effective dose regimen evaluation)
IDEAL BIOMARKER (EMA)

- Non Invasiveness
- Analytical Validity
- Clinical Validity
- Clinical Utility
- Feasibility
- Time and Cost Effectiveness
Why biomarkers are crucial in NMDs (and in all Rare Diseases)

- Hereditary NMDs (and RDs) are chronic and often progressive disorders

- the gold standard clinical outcome measures might not be able to detect small changes in the short running time of clinical trials

- helping in patients enrollment (personalised T)
BIOMARKERS DISCOVERY

• TARGETED SEARCH
Proceeding by candidate (, hypothesis driven by functions, animal studies, ...)
Single biomarker approach
*In silico* selection of candidates
Less demanding but low effective

• OMICS
• NGS methods
• many biomarkers identified
• risk of information overflow, need of biostatistical tools
• expensive

OMICS
Neologism, fields of study in biology ending in the suffix -omics, such as genomics or proteomics
-omes are the objects of study of the field such as the genome or proteome etc.
Biomarkers discovery by high throughput OMICS approaches: News in the field

Proteomic biomarkers (multiplexes immunoassay)

DNA biomarkers (profilomics)
**BIOMARKERS**

- **discovery**
- **validation**
- **regulation**
- **development**

**FLOW CHART**

**RESEARCH**

- OMIC or combined science tools
- Population studies
- Technology assessment

**R &PPP**

- Predictive value
- Sensitivity
- Specificity
- Validation in cells and animal models
- Regulatory validation procedures

**INDUSTRIES**

- Orphan drug designation
- Planning personalized treatment
- Identifying sub-populations
- Get on the market

**TARGETED SEARCH OMICS COMBINED PROFILOMICS**

**EXPLORATORY BIOMARKERS**

**TECHNICAL VALIDATION**

- Repeatability

**FUNCTIONAL VALIDATION**

- Animal models, in vitro assay, etc.

**DATA MEANING**

- Statistical tools

**GO TO**

- Translation in clinical practice
BIOMARKERS REGULATORY ACTIVITIES IN EU: EMA
(briefly....)

EMA does support Biomarkers/Pharmacogenomics/Personalised Medicine and BMs qualification, through:

1) Dedicated Biomarker Qualification Procedure (specific EMA Document)
2) Development of regulatory guidance
3) Scientific advice process
(Through: PGxWP-Pharmacogenetics Working Party, SAWP-Scientific Advice Working Party..)
4) Innovation task force Briefing Meetings
5) Regulatory support to projects in the Innovative Medicines Initiative (IMI) and Critical Path
(eg Joint EMEA/FDA VXDS)
<table>
<thead>
<tr>
<th>Biomarker</th>
<th>Type</th>
<th>Source</th>
<th>Clinical mirroring</th>
</tr>
</thead>
<tbody>
<tr>
<td>Creatine kinase (CK)</td>
<td>protein</td>
<td>serum</td>
<td>muscle damage (?)</td>
</tr>
<tr>
<td>Serum matrix metalloproteinase (MMP9)</td>
<td>protein</td>
<td>serum</td>
<td>Disease progression</td>
</tr>
<tr>
<td>Dystrophin</td>
<td>protein</td>
<td>muscle</td>
<td>Drug response (pharmacodynamic biomarker)</td>
</tr>
<tr>
<td>Fibronectin</td>
<td>protein</td>
<td>serum</td>
<td>Disease severity (age dependent)</td>
</tr>
<tr>
<td>myomiRs</td>
<td>RNA</td>
<td>serum</td>
<td>Disease severity</td>
</tr>
<tr>
<td>Osteopontin-SSP1</td>
<td>SNP</td>
<td>DNA</td>
<td>Loss of ambulationSteroid response</td>
</tr>
<tr>
<td>LFTB4</td>
<td>SNP</td>
<td>DNA</td>
<td>Loss of ambulation</td>
</tr>
</tbody>
</table>
### NOVEL biomarkers in Dystrophinopathies (patients and/or animal models)

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>Type</th>
<th>Source</th>
<th>Clinical mirroring</th>
</tr>
</thead>
<tbody>
<tr>
<td>Annexin 1</td>
<td>SNP DNA</td>
<td>Sarcolemma repair</td>
<td></td>
</tr>
<tr>
<td>Titin</td>
<td>TTN protein urine</td>
<td>Disease severity</td>
<td></td>
</tr>
<tr>
<td>carbonic anhydrase III</td>
<td>CA3 protein Plasma/serum</td>
<td>Respiratory function</td>
<td></td>
</tr>
<tr>
<td>myosin light chain 3</td>
<td>MYL3 protein Plasma/serum</td>
<td>Disease progression</td>
<td></td>
</tr>
<tr>
<td>malate dehydrogenase 2</td>
<td>MDH2 protein Plasma/serum</td>
<td>Disease progression</td>
<td></td>
</tr>
</tbody>
</table>
**IMAGE biomarkers in Duchenne muscular dystrophy (DMD)**

<table>
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<th>Biomarker</th>
<th>Type</th>
<th>Source</th>
<th>Clinical mirroring</th>
</tr>
</thead>
<tbody>
<tr>
<td>STRAIN profile (LV)</td>
<td>cardiac magnetic resonance (CMR)</td>
<td>HEART</td>
<td>cardiomyopathy</td>
</tr>
<tr>
<td>IMAGING</td>
<td>MUSCLE MRI</td>
<td>MUSCLE</td>
<td>MUSCLE damage</td>
</tr>
<tr>
<td>IMAGING</td>
<td>MUSCLE MRI SPECTROSCOPY</td>
<td>muscle</td>
<td>Muscle damage</td>
</tr>
</tbody>
</table>
DMD BIOMARKERS

• VALIDATED
  (technically and functionally)
Circulating miRNAs as biomarkers
(Francesco Muntoni & Irina Zharaieva, UCL)

- Quantification of miR-1, miR-206, miR-31, miR-133a and miR-133b in serum from 44 DMD, 16 UCMD, 5 BMD patients and controls
Correlation of miRs with severity in DMD patients:

- Low levels of **miR-1** and miR-133b in patients with **low forced vital capacity** (FVC) scores;

![Graph showing correlation between miR-1 and FVC]

- Low levels of **miR-1**, miR-31 and miR-133a,b in patients with **severe scoliosis**;

![Box plots showing miRNA levels in patients with and without scoliosis surgery]

- Considerably lower levels of **miR-1**, miR-133a and miR-133b miRNAs in older patients possibly reflecting the **loss of muscle fibres**.

![Graphs showing miRNA levels across different ages]
miRNAs (miR-1)

• TAKE HOME MESSAGE
• promising biomarkers
• Reflect multiple clinical characteristics
Proteomics non-targeted approaches for biomarker discovery

PROTEOMIC STUDIES
Serum fractionation by functionalized beads followed by MALDI-TOF-MASS SPECTROMETRY

DMD PATIENTS SERUM
CONTROL SERUM

Fibronectin is a serum biomarker for Duchenne muscular dystrophy

1 Department of Human Genetics, Leiden University Medical Center (LUMC), RC, Leiden, The Netherlands
2 Center for Proteomics and Metabolomics, Leiden University Medical Center (LUMC), RC, Leiden, The Netherlands
3 Institute of Genetic Medicine, Newcastle University, International Centre for Life, Newcastle upon Tyne, UK
4 Department of Neurology, Leiden University Medical Center (LUMC), RC, Leiden, The Netherlands
Serum fibronectin is a biomarker for DMD severity, age dependent.

A. Age effect $p < 0.01$

B. Time effect $p < 0.01$
MULTIPLEXED ANTIBODY ARRAY ASSAYED PLASMA PROTEINS IN DMD

345 samples
DMD, BMD and female carriers

4 COHORTS
(geographically dispersed)

vs
phenotype category
control plasmas

Body fluids (plasma and serum)
Small volumes of serum or plasma
High-multiplexing capacity
**Method**

Antibody-based array platform (315 protein-384 abs)

- **SAMPLES**
  - SERUM
  - PLASMA

*prioritized by*

- Human Protein Atlas
- MedScan Pathway Studio

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**The Human Protein Atlas**

- 384 Antibodies
- Suspended Bead Arrays
- 345 Samples Serum/Plasma
- Protein Profiles

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**Novel Approach to Meta-Analysis of Microarray Datasets Reveals Muscle Remodeling-related Drug Targets and Biomarkers in Duchenne Muscular Dystrophy**

Ekaterina Kotelnikova, Maria A. Shkrob, Mikhail A. Pyatnitskiy, Alessandra Ferlini, Nikolai Daraselia
RESULTS: MYL3 and CA3 are disease severity exploratory biomarkers

Overexpressed in plasma of DMD
CA3 (carbonic anhydrase III)

Overexpressed in diseased status (both DMD/BMD)
MYL3 (myosin light chain 3)
MDH2 (malate dehydrogenase 2)
RESULTS: ETFA, MDH2, TNNT3 are DMD disease progression exploratory biomarkers
RESULTS: CONSISTENCY IN DIFFERENT COHORTS

- CA3
- MDH2
- MYL3
- ETFA
QUESTIONS

• HOW TO ANALYSE THIS HUGE FLOW OF INFORMATION?

• HOW TO VALIDATE BIOMARKERS TO BE TRANSLATED IN CLINICAL PRACTICE?
BIO-NMD: the importance of the INTERACTOME leading to GENE PRIORITIZATION
Sub-Network Enrichment Analysis (SNEA)

Lower p-value (more significant)

- SNEA builds networks from all genes/proteins measured in the experiment using all relations in the database.
- SNEA can include indirect regulation i.e. expression regulatory cascades consisting of 2-3 steps.
- Significant network centers may be found that are not measured in the primary dataset.
- No prior curation of gene sets is required.
- Can work with partial information about TF targets. Does not require knowledge about all targets for TF.
- P-value is sensitive to the size of the chip.

Higher p-value (less significant)

DMD Candidate markers and drug targets selection

24 Suggested drug targets

140 Suggested biomarkers

Novel Approach to Meta-Analysis of Microarray Datasets Reveals Muscle Remodeling-related Drug Targets and Biomarkers in Duchenne Muscular Dystrophy

Ekaterina Kotelnikova¹, Maria A. Shkrob¹, Mikhail A. Pyatnitskiy¹, Alessandra Ferlini², Nikolai Daraselia³
Objective: We formed a multi-institution collaboration in order to compare dystrophin quantification methods, reach a consensus on the most reliable method, and report its biological significance in the context of clinical trials.

Conclusions: Considering the biological function of dystrophin at the sarcolemma, our data indicate that the combined use of quantitative immunohistochemistry and Western blotting are reliable biochemical outcome measures for Duchenne muscular dystrophy clinical trials, and that standardized protocols can be comparable between competent laboratories. The methodology validated in our study will facilitate the development of experimental therapies focused on dystrophin production and their regulatory approval. Neurology® 2014;83:1–8
SEVERAL VALIDATED BIOMARKERS AVAILABLE FOR DMD MONITORING

DYSTROPHIN MEASURED BY QUANTITATIVE PROTEIN ANALYSIS IS A RELIABLE PHARMACODYNAMIC BIOMARKER FOR DMD THERAPIES

MEASURING DYSTATROPHIN ACCURATELY AND NON-INVASIVELY COULD BE A TURNING POINT FOR CLINICAL TRIALS
204th ENMC International Workshop:
Title: Biomarkers in DMD
Date: 24 – 26 January 2014
Naarden (NL)

Organizers
Alessandra Ferlini
Kevin Flanigan
Francesco Muntoni
Hanns Lochmueller
Peter Bram ‘t Hoen
Elizabeth McNally
Summary and future directions

Many exploratory biomarkers in DMD

Small cohorts analysis might generate controversial results

Validation needs a large number of Duchenne patients: cooperation is a must

Establishing a biomarkers databases it is recommendable to avoid duplication and to speed up research and translational output in clinical trials

Qualification is highly demanding (time/cost/effort) and requires cooperation and dialogue between industries

There are no biomarkers approved by the EMA or FDA for clinical use in DMD
- Biomarkers validation: the needs

- **Data sharing** (mandatory)
- **Biomaterials** (DNA/RNA, plasma/serum) collection for biomarker studies
- Sharing **large patients series** for biomarker **validation**
- Establishing validation procedures (number of samples, strategies, techniques)
- Dissecting patient clinical **phenotypes** by identifying subcategories (i.e. muscle force, cardiac failure, loss of ambulation, progression and ageing, etc.) in which validating biomarkers

- **Milestone**: improve biomarker validation in larger cohorts to speed up translation of data into clinical practice
Future plans

Many exploratory biomarkers in DMD

Small cohorts analysis might generate controversial results

Validation needs a large number of Duchenne patients: cooperation is a must

Polygenic model (more than one biomarker associated to disease severity or drug response is likely applicable)

Genetic modifiers important to stratify patients for clinical trial readiness

Establishing a biomarkers databases it is recommendable to avoid duplication and to speed up research and translational output in clinical trials

Qualification is highly demanding (time/cost/effort) and requires cooperation and dialogue with Industries

There are no biomarkers approved by the EMA or FDA for clinical use in DMD
THE IRDiRC PERSPECTIVE
THE BIOMARKER WORKING GROUP

• Alessandra Ferlini, Ferrara, Italy (Chair)
• Giles Campion, Leiden, Netherlands
  (member of the Therapy Steering Committee)
• Anne Bechet, Leiden, Netherlands
• Gillian Butler-Browne, Paris, France
• Marc Walton, Silver Spring, USA
• Marlene Haffner, FDA Rockville, USA
• Spiros Vamvakas, EMA London, UK
• David Wishart, Edmonton, Canada

• Sophie Höhn, Scientific Secretariat
• Sandra Peixoto, Scientific Secretariat
ACKNOWLEDGMENTS

MEDICAL GENETICS

HUMAN GENETICS

FP7 - NEUROMICS
Integrated European Project on Omics Research of Rare Neuromuscular and Neurodegenerative Diseases

BIO-NMD
Bridging basic research and clinical research with the aim of discovering and validating biomarkers for neuromuscular disease.

What is BIO-NMD?
BIO-NMD is an EU-funded project devoted to the discovery and validation of biomarkers in muscle dystrophies with the aim of improving disease and therapy monitoring. It is a translational project which will focus on Duchenne and collagen VI myopathies.