# Insights and ongoing efforts from Of Mice and Measures: A collaborative project to improve preclinical methodology in Duchenne muscular dystrophy

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Optimizing preclinical tools and methods to evaluate therapeutic candidates is critical to improve decision-making about advancing therapies to clinical testing. In 2016, in collaboration with leading experts in the neuromuscular community and TREAT-NMD network, Charley's Fund, a patient-founded research nonprofit, launched an effort to address a timely topic of this nature: how to utilize a promising newer mouse model, the D2.B10-*Dmd*$^{mdx}$/J (D2/*mdx*), speculated to recapitulate human pathology better than the commonly used C57BL/10ScSn-*Dmd*$^{mdx}$/J (Bl10/*mdx*). The group convened working groups and collected and analyzed D2/*mdx* and Bl10/*mdx* data from multiple academic and industry sources. Nearly 18,000 datapoints on mutant and WT strains were gathered spanning 10 labs worldwide, 650 individual mice, and 230 different functional, histological, imaging, biochemical and molecular parameters. In addition to information about best practices and data gaps in the two models, the cross-lab data comparison yielded findings with broader implications. Even in labs of leading experts, notable differences in practices and results were identified. Since this founding workshop and data collection effort, progress has continued. A workshop report has been published to distribute initial findings. A natural history study has been designed and launched to address data gaps and update best practices. And in pursuit of a tool with the potential to sensitively and quantitatively evaluate muscle condition and response to therapy across both preclinical and clinical testing, 3 universities, a medical device company, and Charley's Fund have developed a plan to incorporate and validate the potential of a technology called EIM in the natural history study.

## Background

- Charley's Fund was founded in 2004 to accelerate drug development for DMD
- The current DMD research landscape features an increasingly active pipeline of therapies; accordingly, particular importance exists around developing research tools and improved processes that benefit all development efforts
- Research-focused nonprofits like Charley's Fund —with sole focus on driving benefit to patients — are particularly poised to address opportunities of this nature
- A particular challenge in DMD is translatability of preclinical animal data to human clinical trials: best practices and better methods are urgently needed
- Charley's Fund engaged experts from academia and industry to develop a program to:
  - a) help establish a grounding perspective on the present state of the data
  - b) align on best practices for selecting the best model and measures
  - c) ensure data on natural history of the new D2/*mdx* model is complete
  - d) establish rigorous criteria to graduate a therapy to human clinical trials

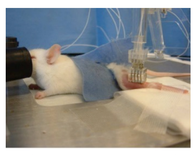
## Methods

Ongoing since fall 2016, 'Of Mice and Measures' is a collaborative initiative among academic, industry, and nonprofit partners in the DMD research community. Charley's Fund serves as a central coordinating party and works collaboratively with a Scientific Organizing Committee and contributing partners to identify opportunities, develop strategies, convene contributors, and undertake action steps. An initial October 2017 workshop in Paris provided a key grounding step. It is acknowledged that success in achieving the overall initiative's objectives will require significant, ongoing work from the parties involved.

10 labs across the world contributed data to the initial workshop. It is hoped that more will add their data as the effort continues.

**Key steps since the initial 2017 workshop**

- October 2017: Initial 1.5 day workshop convened
- Winter-Spring 2018: Results + workshop report generated
- Workshop report published in *Journal of Neuromuscular Diseases*
- EIM preclinical-to-clinical outcome measure evaluation initiated, training conducted, device optimization and testing plan designed
- Natural History Study launched



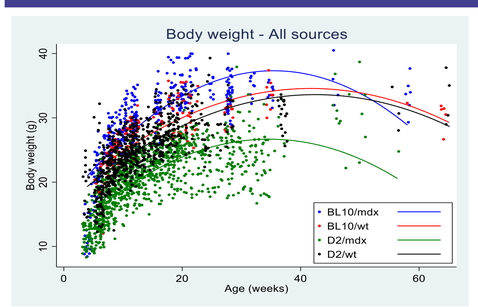
As in humans, EIM can be performed over time in mice

## Data Collection

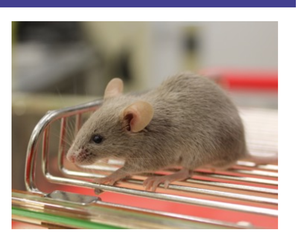
| Outcome Class | Outcome | Data Points | Age range (weeks) | Strain Measured: BL10/mdx | BL10/wt | DB2/mdx | DB2/wt | Contributors |
|---|---|---|---|---|---|---|---|---|
| Natural History | Lean and fat mass | 394 | 6 - 12 | | | * | * | Pfizer |
| | Body weight | 2800 | 4 - 65 | * | * | * | * | Aartsma-Rus, De Luca, Duan, Jax, Nagaraju, Pfizer, Sarepta, Spencer |
| | Tissue weights (excluding heart) | 2135 | 4 - 65 | * | * | * | * | Aartsma-Rus, De Luca, Duan, Nagaraju, Pfizer |
| | Tibia length | 18 | 23 - 30 | | | * | * | Duan |
| Functional | Wheel (voluntary and exhaustion) | 318 | 8 - 58 | * | * | * | * | De Luca, Nagaraju |
| | Open field (all assessments) [1] | 376 | 8 - 65 | | * | * | * | Jax, Nagaraju, Spencer |
| | Grip strength (including normalized & hang test) | 4142 | 4 - 65 | * | * | * | * | Aartsma-Rus, De Luca, Jax, Nagaraju, Sarepta, Spencer |
| | Rotarod | 612 | 6 - 65 | * | * | * | * | Jax, Nagaraju, Sarepta |
| | Hindlimb sonography [2] | 72 | 28 | * | * | * | * | De Luca |
| | Force (all muscles, all conditions) | 1816 | 8 - 65 | * | * | * | * | De Luca, Duan, Jax, Nagaraju, Pfizer |
| Cardiac | Functional assessments [3] | 761 | 16 - 58 | | | * | * | De Luca, Duan, Nagaraju |
| | Size/weight assessments | 288 | 4 - 65 | | | * | * | Aartsma-Rus, Duan, Nagaraju |
| Histology | All assessments [4] | 1240 | 8 - 78 | | | * | * | Aartsma-Rus, Duan, Jax, Nagaraju, Yokota |
| Respiratory | All assessments | 1174 | 4 - 34 | | | * | * | Aartsma-Rus, Spencer |
| Therapeutics | Gene expression in response to Exon skipping | 71 | 13 | * | | * | | Aartsma-Rus |
| Biomarkers | CK | 574 | 8 - 64 | * | * | * | * | Aartsma-Rus, De Luca, Jax, Nagaraju |
| | LDH | 76 | 9 - 64 | * | * | * | * | De Luca |
| | Serum biomarkers [5] | 37 | 13 - 36 | | | * | * | Duan, Nagaraju |
| | Dystrophin level | 12 | 13 | * | | * | | Aartsma-Rus |
| | Gene expression[6] | 939 | 10 - 34 | * | * | * | * | Aartsma-Rus, Jax |

[1]Includes open field distance run, Digiscan measurements, and # of rearings. [2]Includes volume and % vascularization. [3]Shortening fraction, ejection fraction, stroke volume, and cardiac output also measured in Bl10 mice. [4]Fiber area, fibrosis area, and % calcification also measured in Bl10 mice. [5]Includes WBCs, macrophages, and neutrophils. [6]Gene expression values from 12 genes in two different tissues (gastrocnemius and diaphragm).

## Natural History



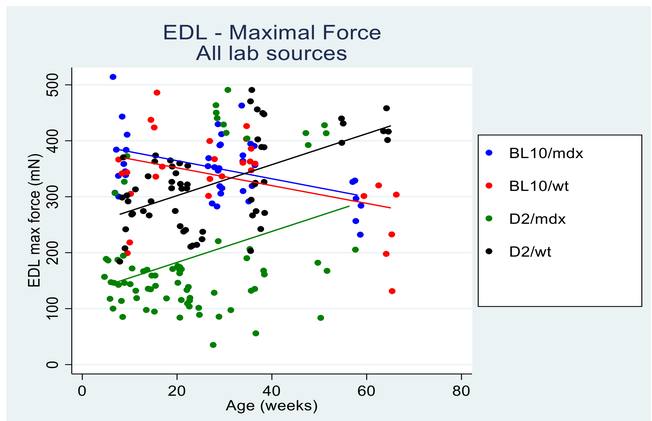

The commonly used classic C57BL/10ScSn-*Dmd*$^{mdx}$/J (Bl10/*mdx*) mouse model



The newer D2.B10-*Dmd*$^{mdx}$/J (D2/*mdx*) mouse model

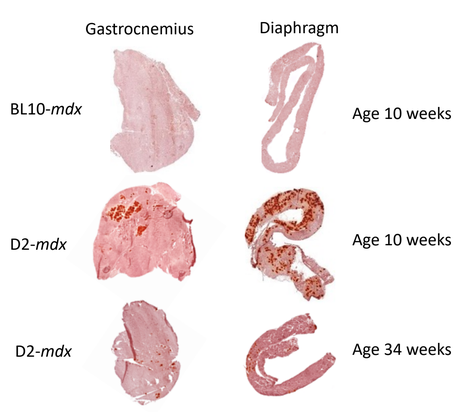
This figure clearly shows a difference in body weight between Bl10 and D2 mice. However it also shows a difference of up to 10g in D2/*mdx* mice from different sources. This variability could be attributed to several factors including the husbandry at each facility, genetic drift in the mice, or stress from transportation. If we observe up to a 10g difference between mice of the same strain, detecting a difference due to treatment effects becomes very challenging.

## Functional Measures




This figure shows overall differences between mouse strains, a decrease in maximal force with age in Bl10 mice, yet an increase with age in D2 mice. The variability, however, makes distinguishing between strains at a given age difficult. At 38 weeks of age, force values from all four strains completely overlap. Any treatment expected to show a strain difference at 38 weeks would be problematic and would require a very large sample size.

## Histological Measures

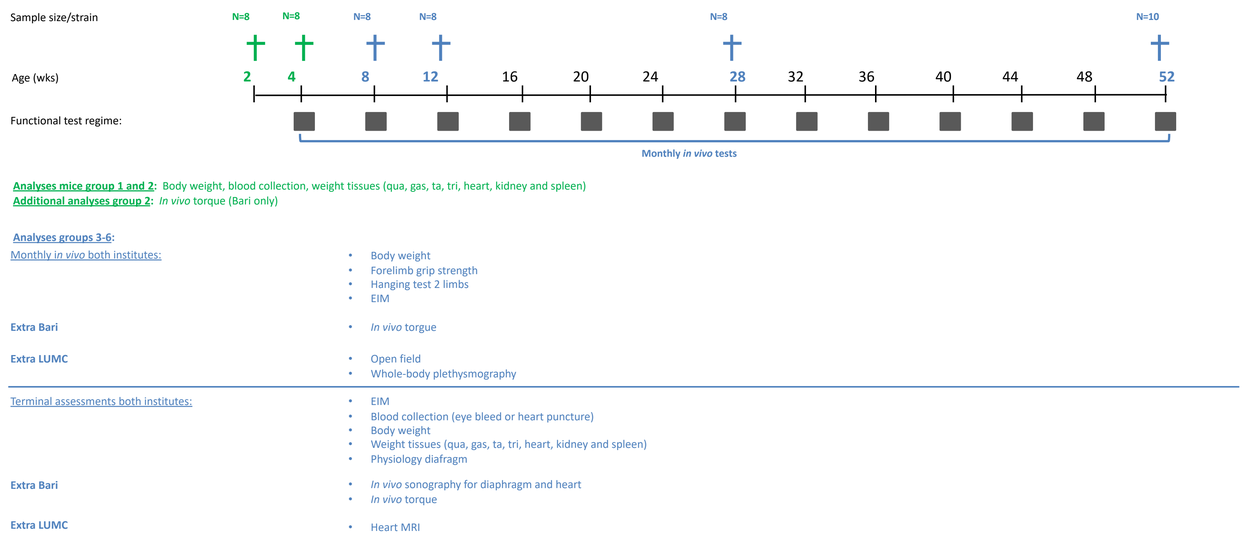



Histological assessments such as inflammation, fibrosis, degeneration, regeneration, and central nucleation are not consistently measured in all the labs. One of the striking histological features of the D2/*mdx* model is extensive calcifications in skeletal muscle. Here we show an assessment of calcifications in skeletal muscle and the diaphragm that are prominent early (10 weeks) but spontaneously resolve as the mice age (34 weeks). Calcification is not a major histological feature of Bl10/*mdx* mice.

## Recommendations

- Investigators should follow established SOPs for the collection of data to decrease variability due to collection method
- Investigators should choose outcomes based on ability to assess efficacy given the mouse model and mechanism of action of the treatment, rather than the ease or comfort with collection method
- Investigators should follow blinding procedures, especially for outcomes that have a component of subjectivity involved
- The statistical analysis of preclinical data should be performed thoughtfully. This includes:
  - Using nonparametric tests where warranted
  - Using an analysis method that is appropriate for the outcome's distribution
  - When assessing an outcome over time or age, use methods that account for repeated assessments and evaluate non-linear relationships
  - Clearly define the dependent and independent variable(s)
  - Use time-to-event analysis methods where appropriate

## 52-Week Natural History Study Set-up




**Analyses mice group 1 and 2**: Body weight, blood collection, weight tissues (qua, gas, ta, tri, heart, kidney and spleen)
**Additional analyses group 2**: *In vivo* torque (Bari only)

**Analyses groups 3-6**:

| | |
|---|---|
| Monthly *in vivo* both institutes: | • Body weight<br>• Forelimb grip strength<br>• Hanging test 2 limbs<br>• EIM |
| Extra Bari | • *In vivo* torgue |
| Extra LUMC | • Open field<br>• Whole-body plethysmography |
| Terminal assessments both institutes: | • EIM<br>• Blood collection (eye bleed or heart puncture)<br>• Body weight<br>• Weight tissues (qua, gas, ta, tri, heart, kidney and spleen)<br>• Physiology diafragm |
| Extra Bari | • *In vivo* sonography for diaphragm and heart<br>• *In vivo* torque |
| Extra LUMC | • Heart MRI |

This figure describes the timeline and assessments that will be performed over the course of the natural history study designed to compare *in vivo* functional performance and several other *ex vivo* dystrophy-related outcomes over time in the Bl10 and D2 mice. This study will be performed at 2 different sites in 2 identical cohorts of 200 mice (50 mice/strain).

## Results

The first step in Of Mice and Measures provided insights on:

(1) Findings and recommendations that can be implemented immediately to improve how the DMD research community utilizes the D2/*mdx* and Bl10/*mdx* models to evaluate therapies

(2) Broader findings about opportunities to gather missing data, to identify best practices to be standardized, and to ensure effective communications to disseminate important information

Now the team is focusing on:

- The natural history study to fill in identified gaps across D2/*mdx* and Bl10/*mdx* mice
- Incorporation and validation of EIM as a preclinical-to-clinical tool to measure drug response
- Improved summary guidance materials for DMD mice and preclinical protocols
- Consideration of future efforts such as a single searchable DMD mouse database and broader minimal preclinical data standards in DMD

**Please join our efforts. For citations and more information about OMAM, EIM, and how to get involved, contact laura@charleysfund.org or visit charleysfund.org/mda2020**

Some data herein has been previously presented at the World Muscle Society 2018 congress in Mendoza.