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Experimental design for translational studies

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Vital role of experimental animals in developing new treatments

BUT

- Lack of reproducibility
- Successful rodent treatments fail in the clinic

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What is lack of reproducibility due to?

Sample size not statistically robust

Inadequate randomisation

Inadequate blinding

Different effects in males and females

Microbiological status

Husbandry

For some experiments, the sex of the investigator!

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Practical steps to blinding

Blinding can be difficult for some academic research groups

Practical steps:

- Have independent person make up the water/feed/drug and code so assessor blinded.
- Code tissue samples
- Blind slides
- Second independent assessor

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Failure to translate to the clinic

As before – lack of reproducibility.

Failure to confirm results in independent labs

Poor understanding of the model

Perhaps the wrong model!

The “Nature” effect – dosed for effect rather than dosed for safety

- Mice are MUCH tougher than humans!
- Inappropriate routes of administration
- Unrealistic doses

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What is the relationship between mouse dose and human dose?

The dose given to mice can be converted to the human equivalent dose (HED) using the following formula

HED (mg/kg) = Mouse dose (mg/kg) x (Mouse k_m / human k_m).

Where k_m is body weight (kg) divided by body surface area, thus converting mg/kg to mg/m^2.

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Animal models

Genotypic vs phenotypic models?

What are you modelling?

Size matters for translation?

Inbred and induced models?

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What are rare diseases?

Rare diseases are those that affect less than 1 in 2000

80% have a genetic origin

Estimated 7,000 rare diseases

Approximately 7% of EU population may be affected by a rare disease

Often chronic with substantial health care and associated societal costs

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Duchenne muscular dystrophy as an example

An X-linked recessive muscle wasting disorder .

Due to the loss of dystrophin which renders the muscle vulnerable to exercise induced damage.

Progressive necrosis and fibrosis of muscle leads to loss of function and loss of ambulation by 12.

The CNS, cardiac and smooth muscle are also affected.

Affects approx. 1 in 5,000 male births worldwide.

Corticosteroids, cardiac medication, surgery and assisted ventilation all prolong survival and quality of life.

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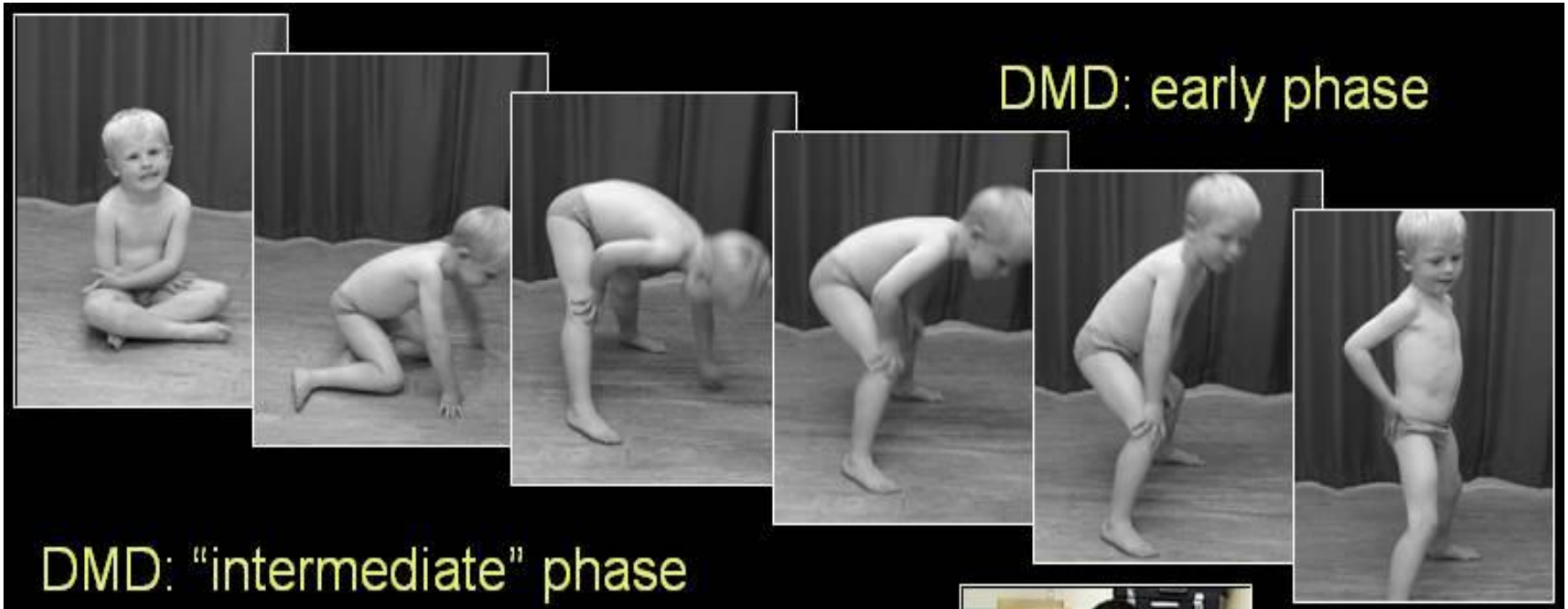
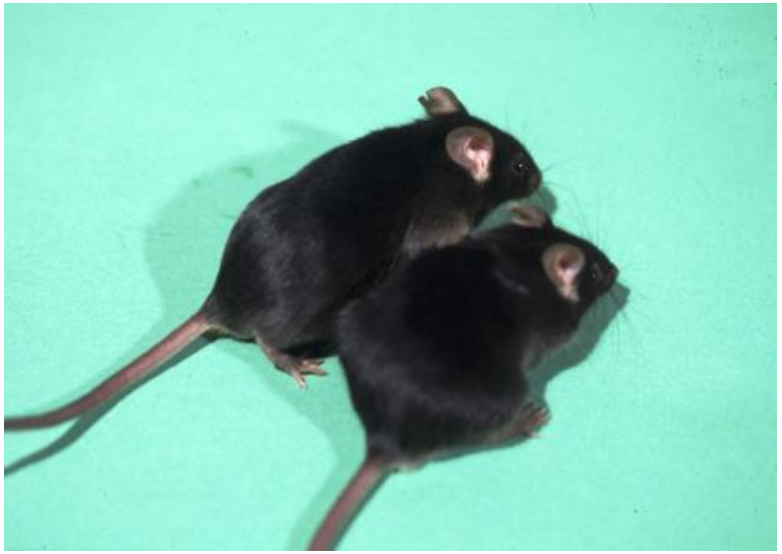


DMD: "intermediate" phase

DMD: 'late' phase

Images provided by Prof Francesco Muntoni

The *mdx* mouse model of DMD



Extensively used model > 2600 papers to date

Spontaneous mutant discovered in 1984

Dystrophin deficient due to stop mutation in exon 23

Near normal lifespan and mobility

Normal until onset of acute myopathy at 3 weeks

Muscle damage associated with movement

Limited fibrosis and fatty infiltration

Little if any clinical signs of disease

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Problems with assessing treatments

Timing of treatment relative to the pathological process and comparison to man.

Very mild clinical signs – challenging the model

Pathology less severe than man –effect on treatment efficacy

Unrealistic doses and route of administration

Selection and conduct of effect assessment.

Motivational issues with functional assessments.

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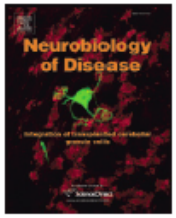
Outcome measures and SOPs for the *mdx*

Neurobiology of Disease 31 (2008) 1–19

Contents lists available at ScienceDirect

Neurobiology of Disease

journal homepage: www.elsevier.com/locate/ynbdi



Review

Towards developing standard operating procedures for pre-clinical testing in the mdx mouse model of Duchenne muscular dystrophy

Miranda D. Grounds,[a,*] Hannah G. Radley,[a] Gordon S. Lynch,[b] Kanneboyina Nagaraju,[c] and Annamaria De Luca[d]

http://www.treat-nmd.eu/research/preclinical/dmd-sops/

TREAT-NMD
Neuromuscular Network

Wellstone Muscular Dystrophy Center
Washington, DC

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Amyotrophic lateral sclerosis (ALS)

- Fatal neurodegenerative disorder, specifically affecting motor neurons in spinal cord and brainstem.
- Muscle weakness, atrophy and progressive paralysis.
- Survival time after diagnosis (usually at ~50 years of age) often only 2-4 years.
- 90% of cases are sporadic, the rest familial.
- ~20% of familial cases caused by mutations in Cu/Zn superoxide dismutase (SOD1) gene.
- Gain of function mechanism.

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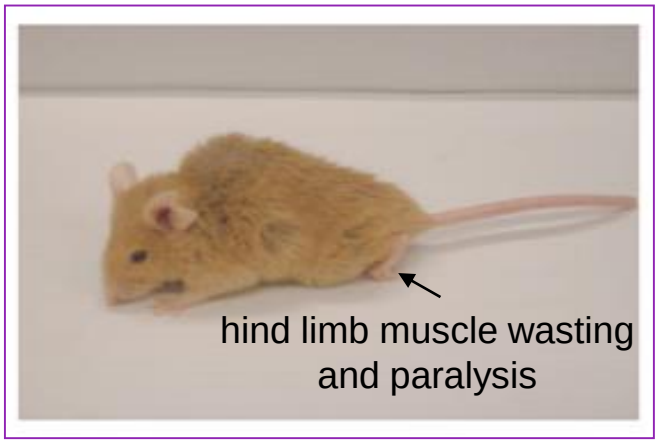
The SOD1 G93A mouse

- A range of transgenic mice expressing mutant forms of human SOD1 have been developed.
- A high copy number SOD1 G93A (glycine to alanine change) line is most commonly used.



SOD1G93A disease timeline: days

0	35	90	120
	• Mitochondrial abnormalities • mSOD aggregation	• Hindlimb tremor • Muscle wasting and conversion to progressively slower phenotype	• Hindlimb paralysis • Humane end point

Severely affected mouse

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Amyotrophic lateral sclerosis

The $SOD1^{G93A}$ transgenic mouse has been the model of choice for studies of ALS (>1000 papers).

Nearly 100 “successful” treatments in the mouse but only one approved in man.

Scott et al., 2008 clearly demonstrated that reasons for lack of translation was poor experimental design and analysis.

Very disappointing that the field has generally failed to build on this work

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Issues identified by Scott et al.

Inadequate numbers – mixed genetic background therefore minimum of 12 per group.

12 males and 12 females for each treatment as there are sex differences in rate of progression.

Need to account for possible litter to litter variation

Need to censor non-ALS related deaths

Need to check copy number of the transgene

Inappropriate statistics.

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Statistical issues

Key is to estimate the effect size and make appropriate power calculations BEFORE the experiment.

Get statistical advice – especially as relates to use of parametric or non-parametric tests.

May be litter to litter variation so either mix groups at weaning (if treatment is in food or water) or use a randomised block design.

Consider how age at treatment may affect results.

Avoid mixing the sexes within a group.

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Other translational issues in rare diseases

Man is not a big mouse. Consider use of larger animal models to confirm effective therapies.

Wishful thinking – if the effect in mouse is small it is unlikely to get better in man.

Bridging the gap between academia and industry. Particular problem in rare diseases.

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Further guidance

Contact a good (biological) statistician BEFORE the experiment.

NC3Rs website page for experimental design papers (e.g. Festing and Altman, 2002)

NC3Rs Experimental Design Assistant (in prep)

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Thank you

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