Royal Veterinary College
University of London

Combination therapy

Dominic Wells
Professor in Translational Medicine,
Royal Veterinary College, London, UK
7th November 2015

Disclosures

Member of the Scientific Advisory Board for Akashi Therapeutics.

Grant funding for DMD work from a range of charities, DoH and research councils.

Have conducted DMD mouse model studies for Proximagen and AstraZeneca and have consulted for a number of other companies.

Member of MDUK, Telethon, AFM grant committees.

RVC

Outline

The case for combination therapies.

An approach to standardised testing of combinations.

Examples

Possible combinations for the future

Cautions

Conclusions

RVC

Why combination therapies?

Even with dystrophin replacement there is still existing pathology.

Also currently unlikely that dystrophin replacement methods will be 100% efficient.

For treatment of downstream pathology there are multiple targets.

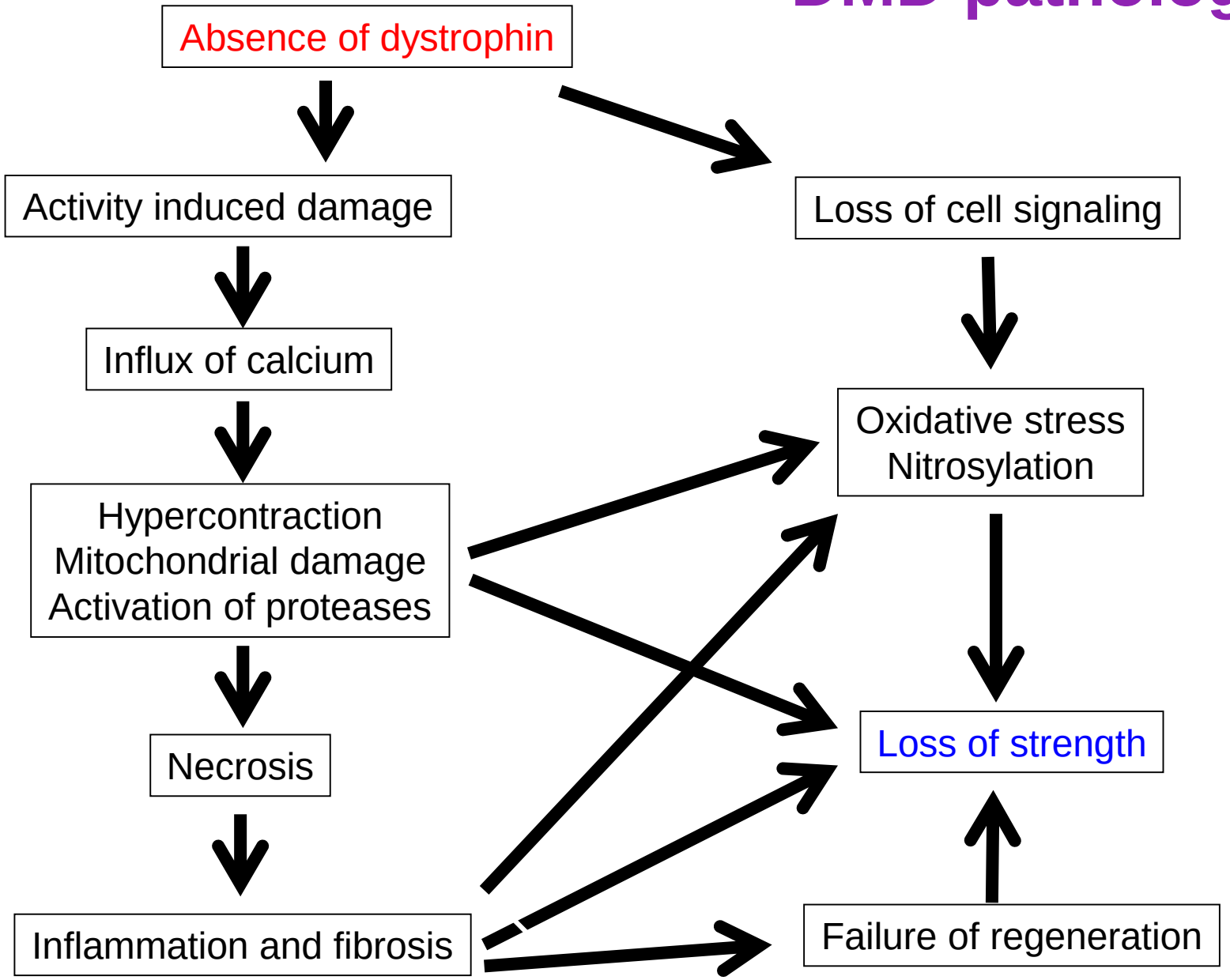
Should be applicable to all DMD patients as downstream pathology is not mutation specific.

Widespread use of corticosteroids means we already do combination therapies in most clinical trials but rarely test this rigorously in pre-clinical models.

Potential to repurpose existing drugs in medical use thus shortening time to the clinic.

RVC

DMD pathology



RVC

Targets

Restore dystrophin

Substitute utrophin

Increase stability of muscle membrane.

Increase muscle strength.

Reduce inflammation

Reduce fibrosis

Improve blood supply in exercise

Improve muscle regeneration

Improve calcium buffering

Etc….

RVC

A standardised method for evaluating potential therapies

Mdx mice with established disease. We use 12 week old males.

Dose for a long enough period to see full effects (and off-target effects). We do 12 weeks. Route of delivery should match route in man.

Primary outcome measure should be one that reflects the goal(s) of therapies and is not subject to mouse motivational state.

Secondary outcome measures can include a variety of readouts but should include histopathology and quantitative image analysis.

Choice of muscles is important. Diaphragm behaves differently to the limb muscles. Contralateral muscles are not a perfect match.

Useful to look at serum biomarkers.

RVC

Muscle physiology 1

Advantages of this as a primary outcome measure:

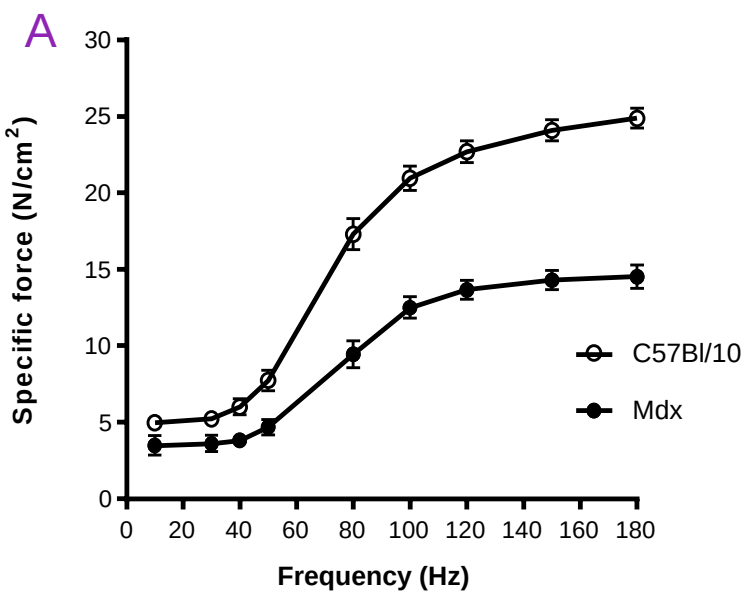
1. Ability to resist damage following eccentric exercise is a good measure of the reduction in activity induced damage and thus likely reflects prevention of further disease progression.
2. Once the disease is stabilised, we would like to increase the force that can be generated by the remaining muscle in DMD and this is best measured by changes in specific maximal force (force per unit quantity of muscle).
3. In-situ or ex-vivo muscle physiology is not affected by the motivational state of the mouse (unlike a treadmill).

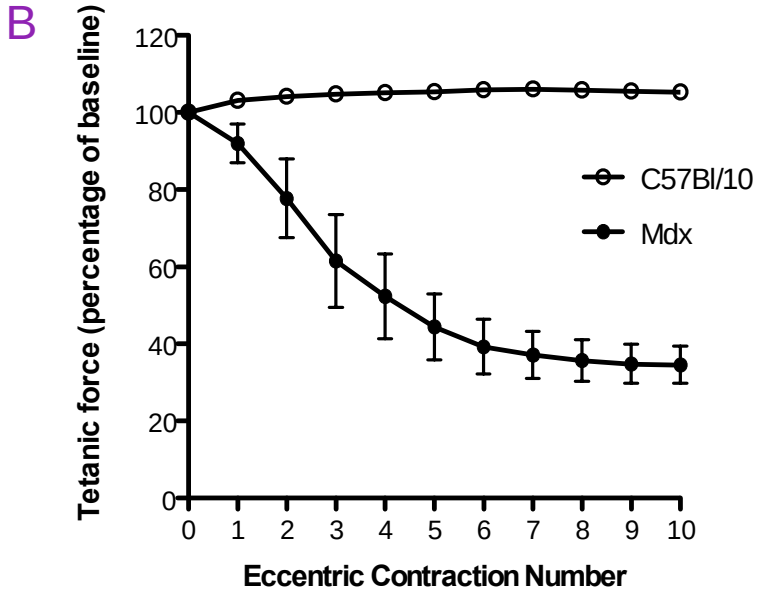
RVC

Muscle physiology 2

In situ protocol tibialis anterior – muscle with intact nerve and blood supply

A Force frequency analysis - muscle held at fixed optimal length (isometric)
B Eccentric exercise protocol - muscle stretched while contracting in each of ten cycles. Tetanic force reported as percentage of initial baseline value



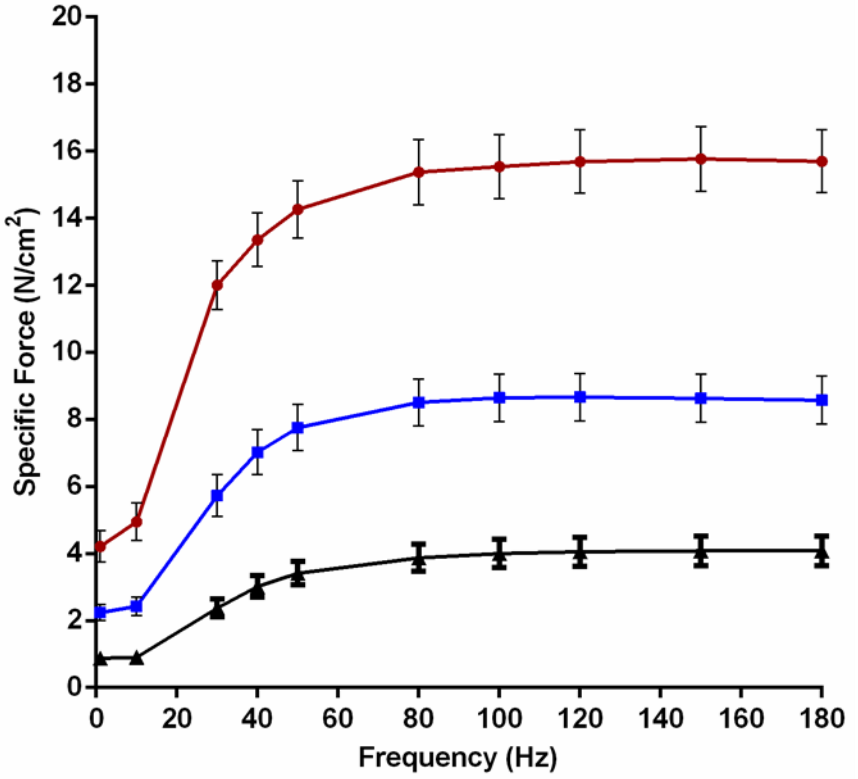


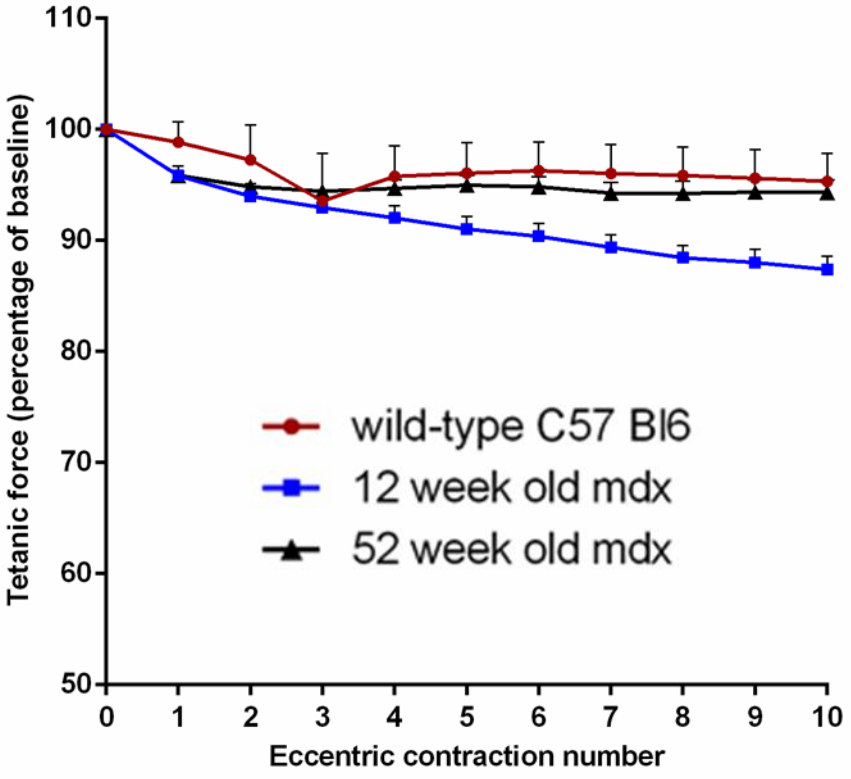
RVC

Muscle physiology 3

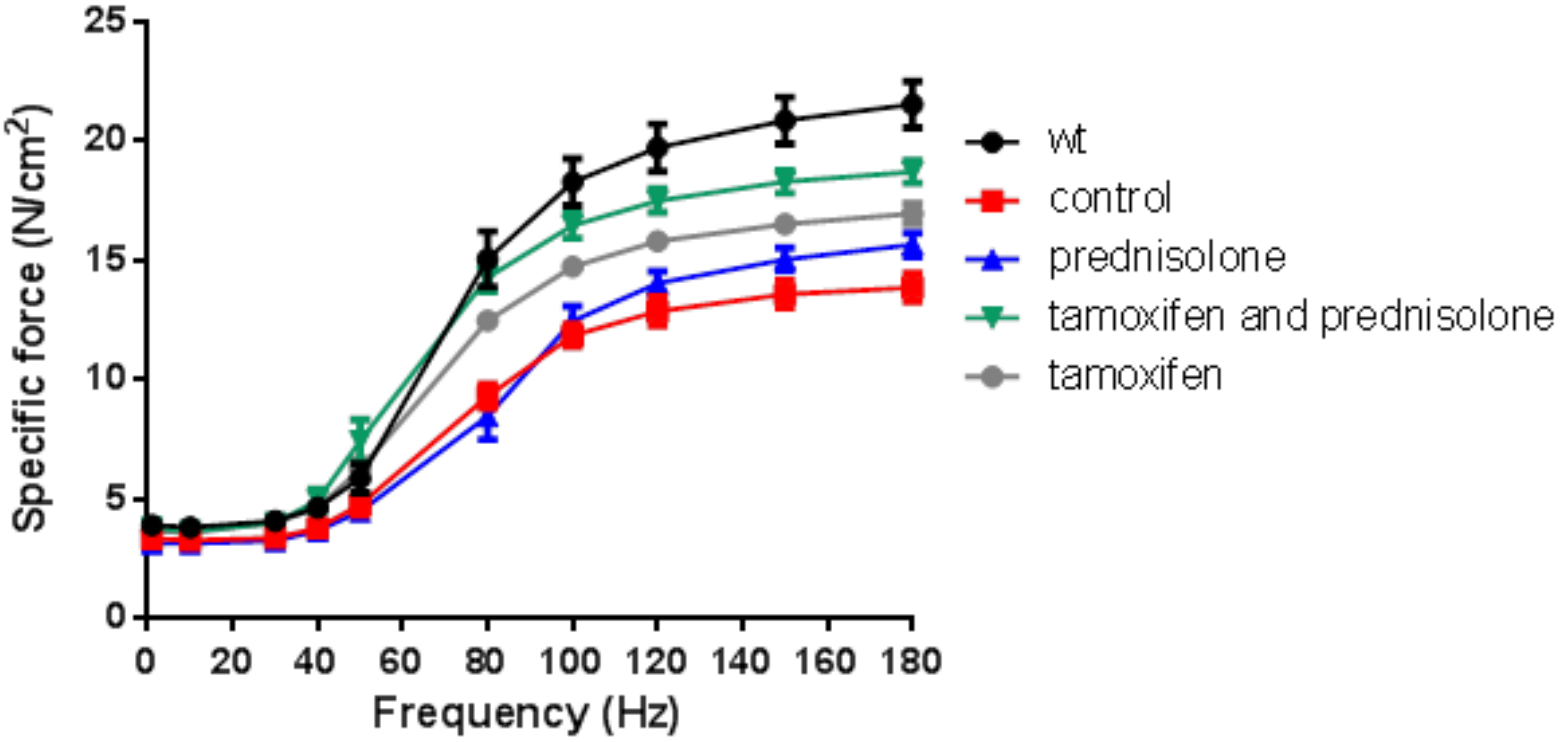
Ex vivo (in vitro) physiology using strips of diaphragm.

Most affected muscle in the mdx, dramatically more fibrosis than the limb muscles.



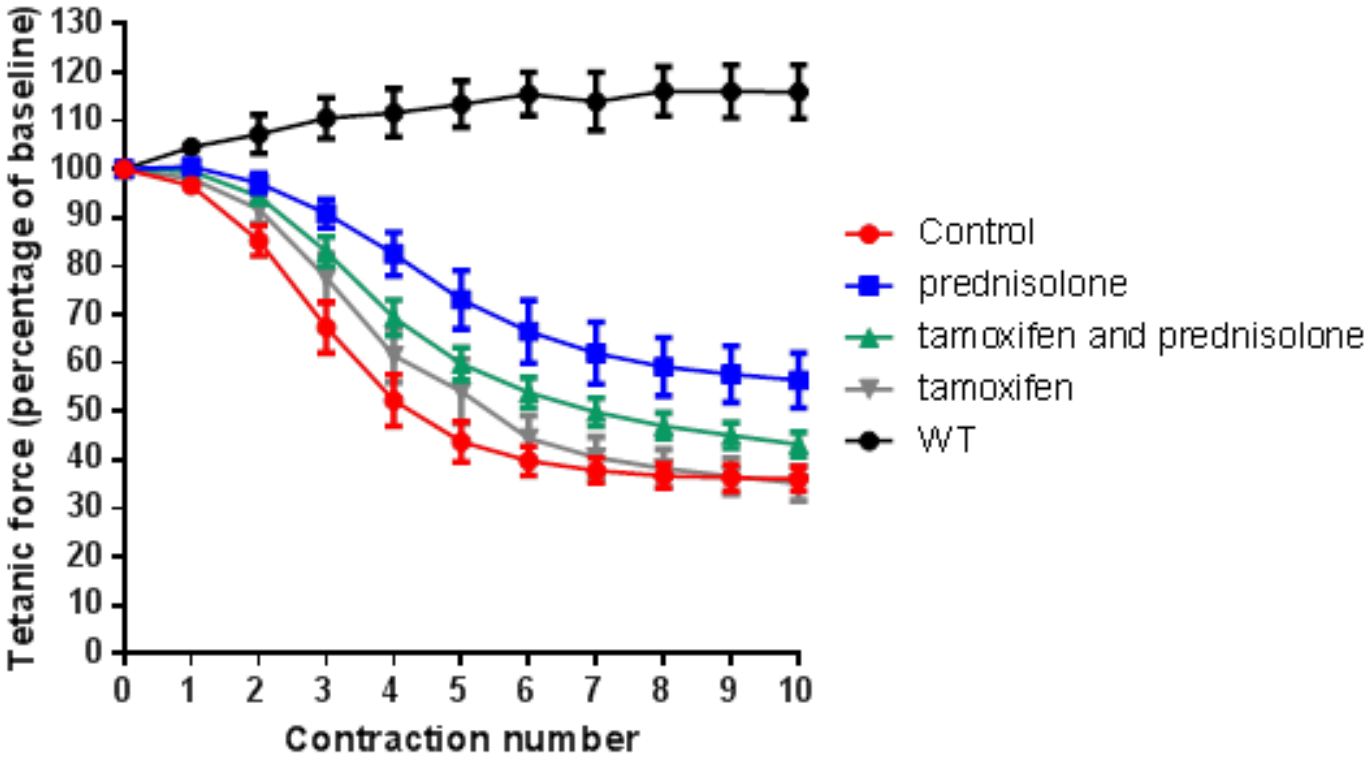


Muscle force (Tibialis anterior) following combination therapy



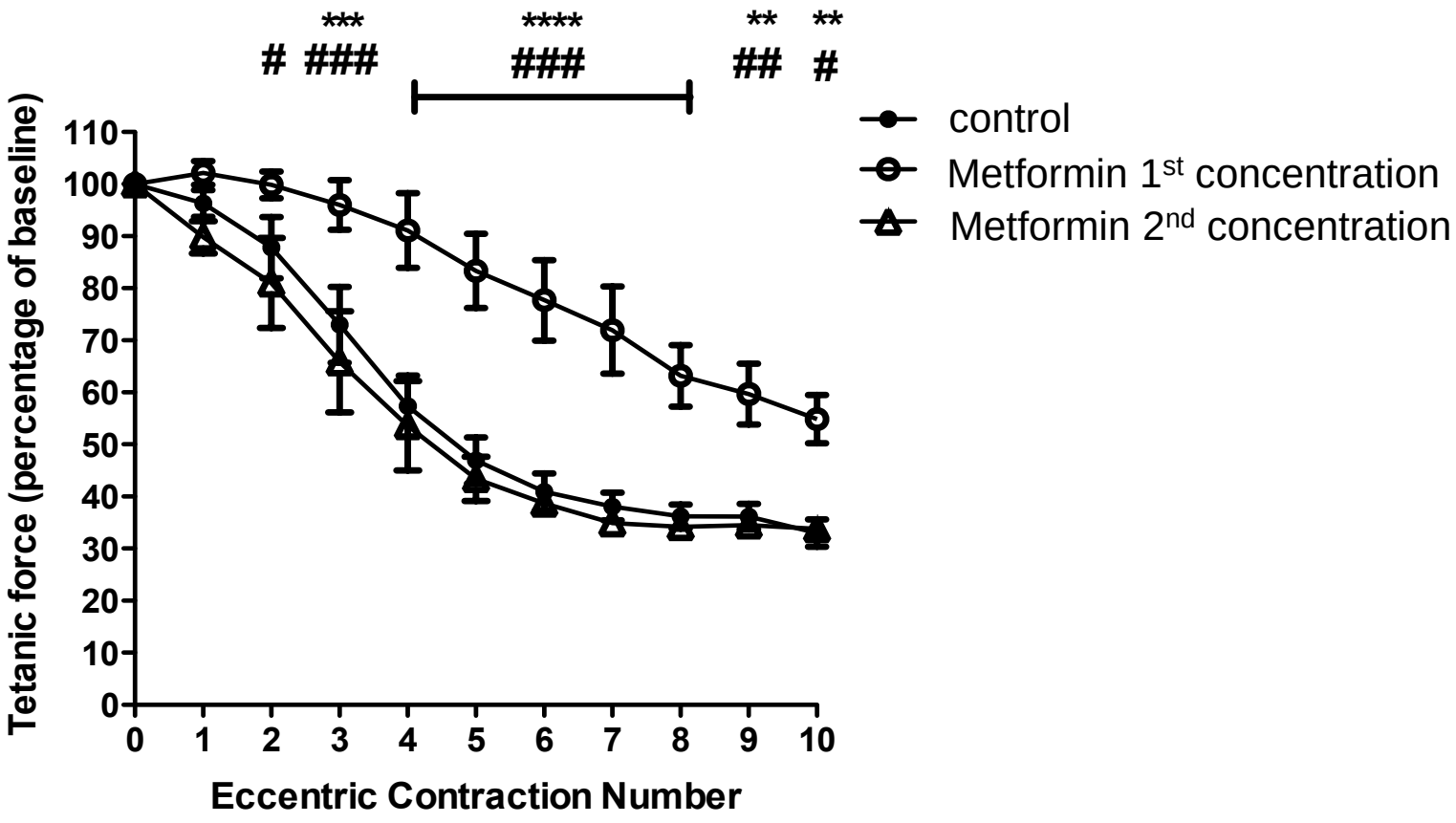
RVC

Response to eccentric exercise following combination therapy



RVC

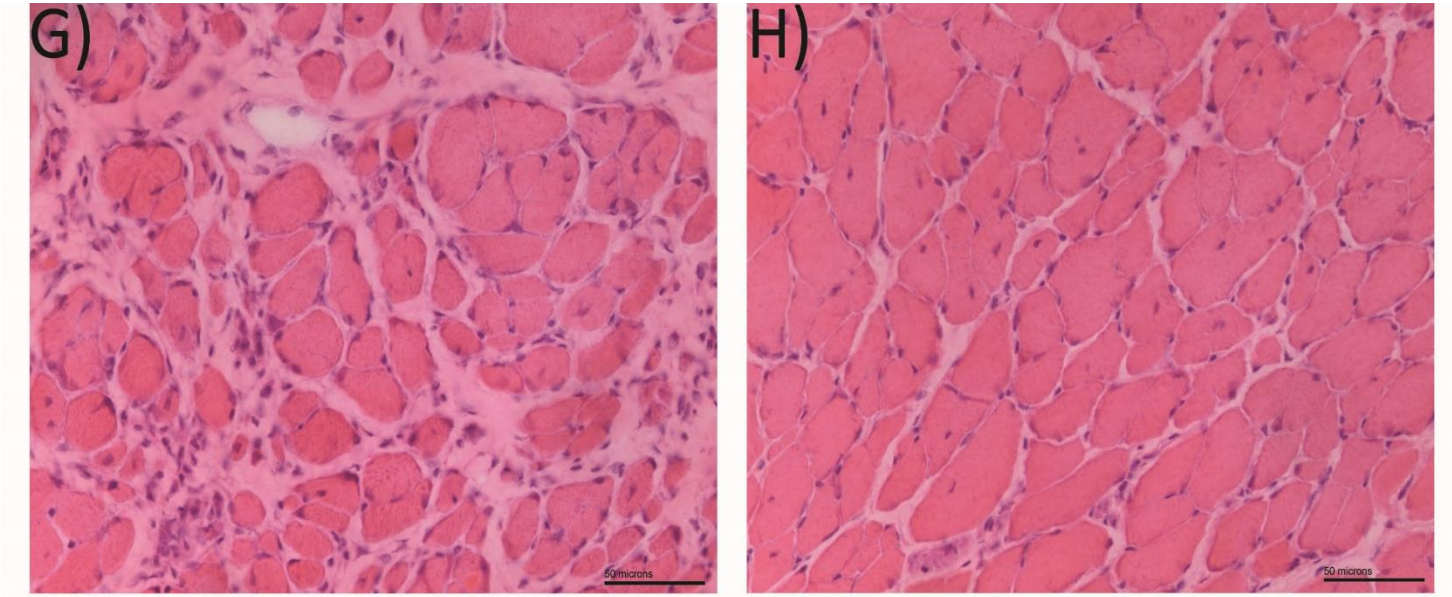
Another repurposed drug (single therapy)



This drug had no effect on specific force

RVC

Histopathology 1

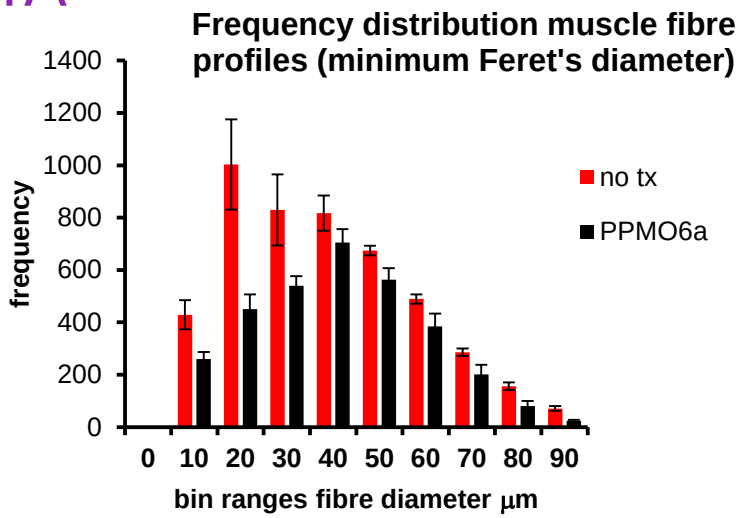


Look at fibrosis, variation in fibre size, degree of inflammation.
Overview with standard histological stains then specific immuno-stains for specific features.
As far as possible analyse whole muscle and multiple muscles.

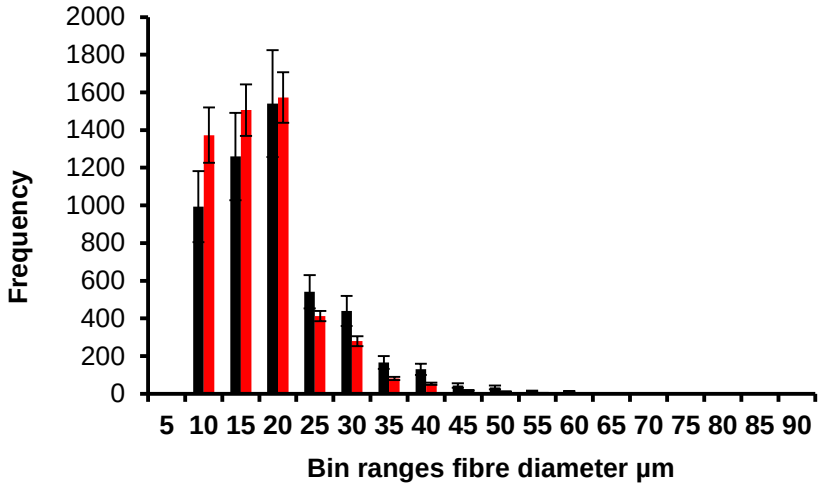
RVC

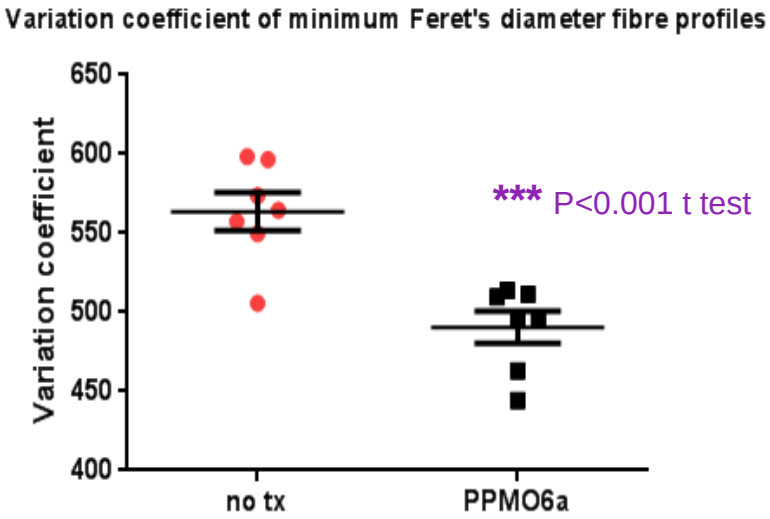
Example of different effects in different muscles

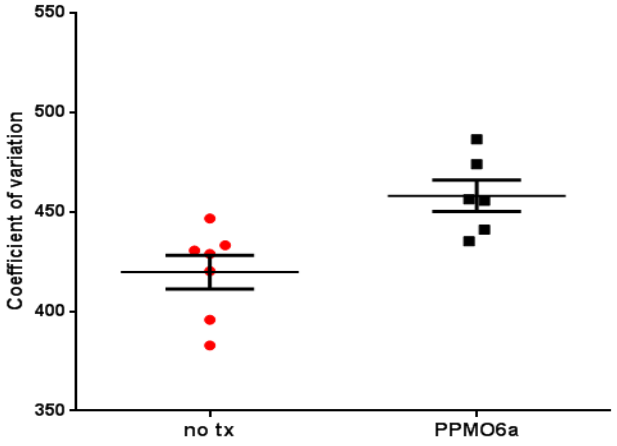
TA



Diaphragm







RVC

Possible next step

Consider a combination of tamoxifen plus metformin plus or minus prednisolone. Potential to address both muscle deficits.

All are drugs approved for use in man for other indications.

Need to consider potential interactions that may not been seen in mice and may require regulatory toxicology prior to clinical trial.

RVC

Reasons for a standardised assessment

By using a standardised assessment methodology we can compare the effects of single drugs and combinations between experiments.

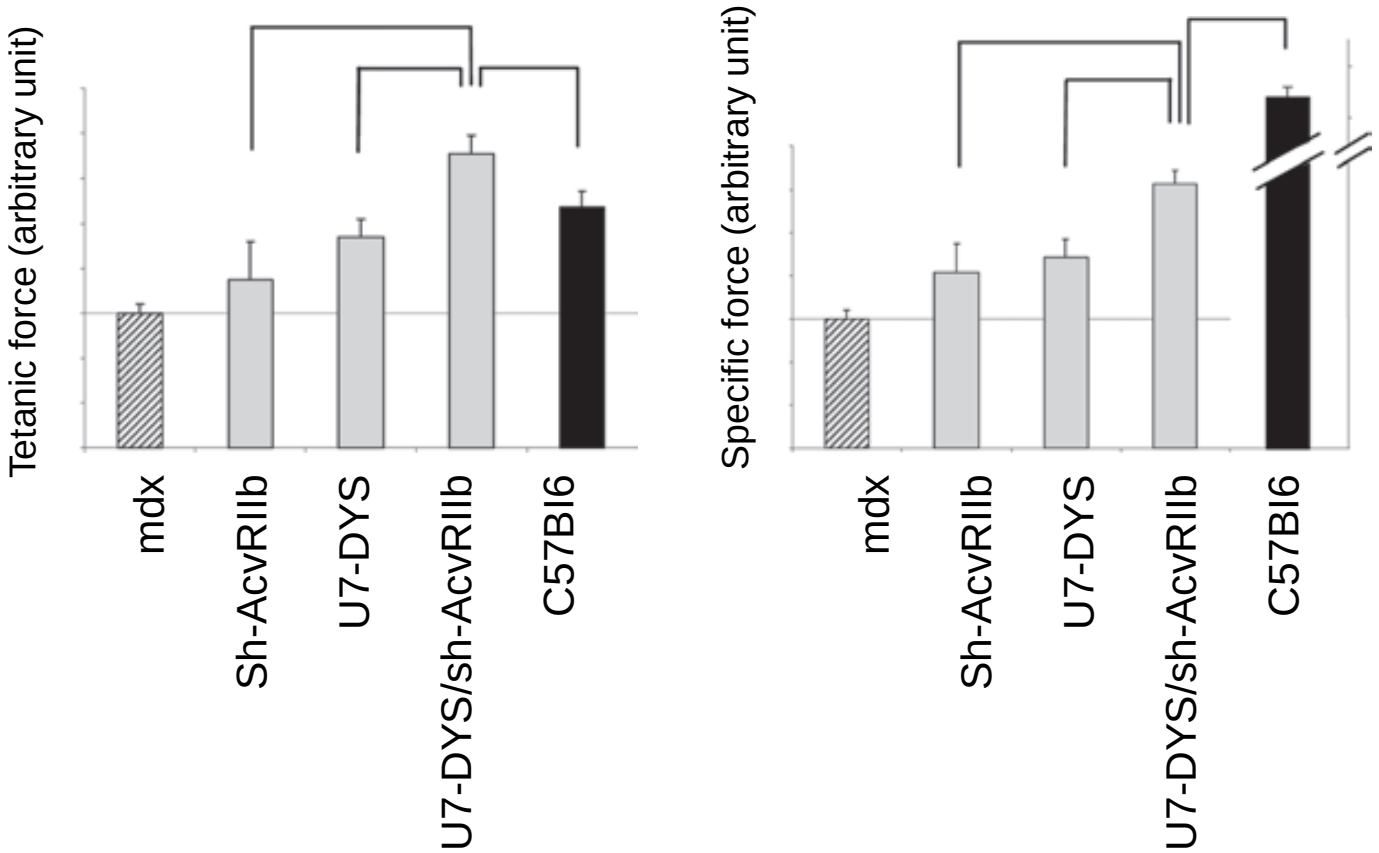
The same methodology can be reproduced in other labs.

The approach outlined in previous slides is focussed on translation to the clinic.

RVC

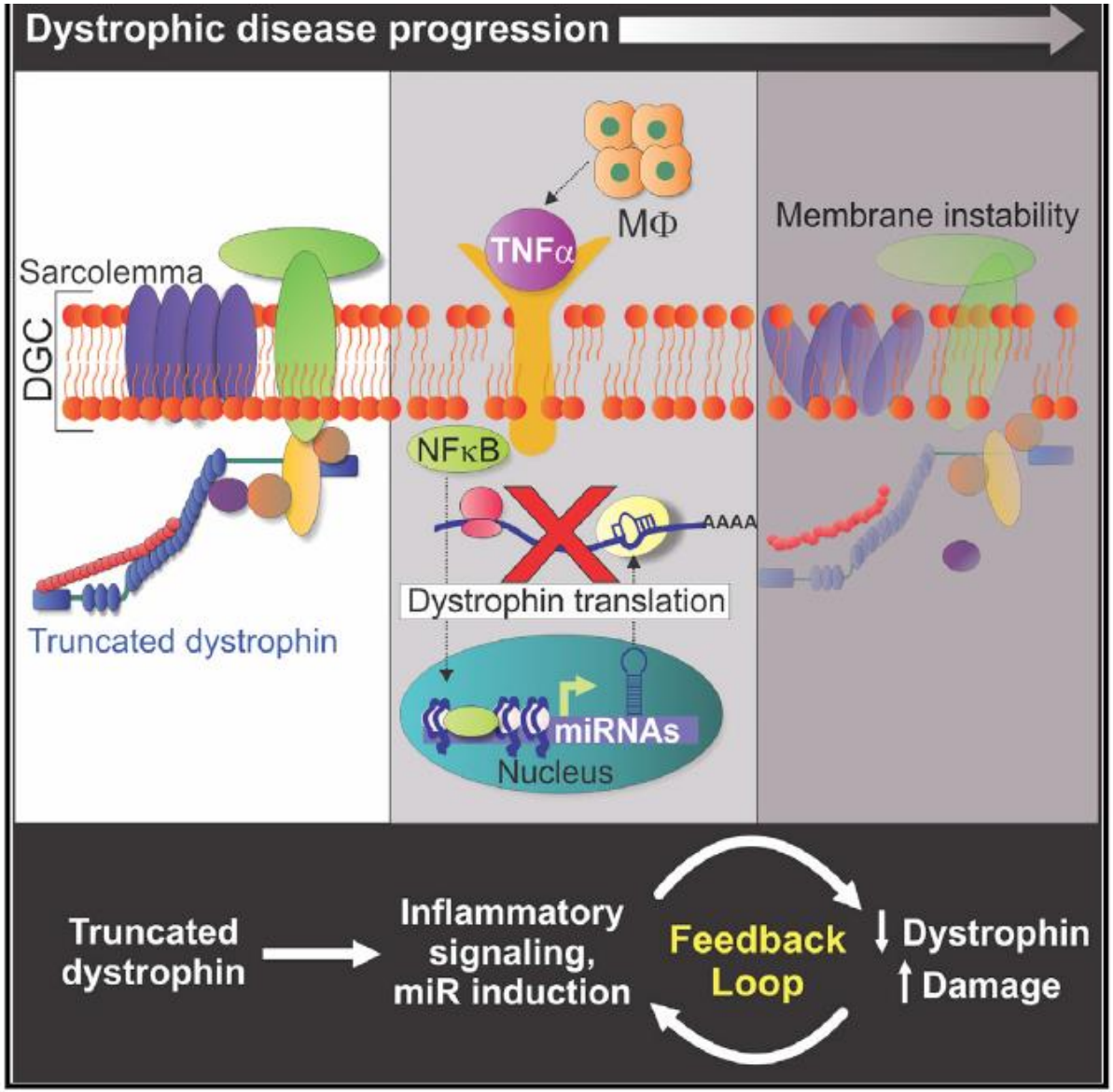
Dystrophin and myostatin

Restoration of dystrophin to stop muscle damage and blocking myostatin to increase muscle mass and function (Dumonceaux et al., 2010).



RVC

Inflammation represses dystrophin translation



Fiorillo et al., 2015, Cell Reports 12: 1678-1690.

Strongly suggests that anti-inflammatories are likely to improve the efficiency of translation of dystrophin following exon-skipping or read-through of premature stop mutations

RVC

Further combination therapy examples

Genetic therapy and myostatin inhibition

Genetic therapy and NFkB inhibitor

Upgreulation of utrophin and Tadalafil

Upregulation of utrophin and mitochondrial boost (e.g. idebenone)

NFκB inhibitor and Tadalafil

NFκB inhibitor and myostatin inhibition

Statin and Tadalafil

+/- steroids

RVC

Different drugs for different stages of the disease

Very early treatment might prevent accumulation of pathology and therefore combinations may be less important.

Older patients need treatments to address accumulated pathology – so combinations may be very important.

Increasing loss of muscle will reduce the effectiveness of treatments targeting the muscle so different combinations may be more appropriate.

Increasing importance of cardiac treatments in older patients.

RVC

Cautions

It is possible that the positive effects seen in the mouse may not be seen in DMD. Important to keep the doses within the normal therapeutic range in man.

Side effects of combinations may not have been seen in the mouse.

Repurposed drugs may not have been previously used in children.

Combinations of unapproved drugs may have an increased regulatory burden – toxicology and safety pharmacology of each drug and combination.

Collaboration with or between companies are likely to be difficult (IP issues and concerns with any AE).

RVC

Conclusion

Cure not really in sight.

Unlikely any one therapy will be fully effective and there are multiple targets for treatment.

Combination therapies are the likely future, perhaps a specific combination based on the patient's genotype and stage of the disease.

RVC

Possible next steps

Ideally drug companies will collaborate and regulators will apply a light touch to combination therapies.

Alternatively, single drugs will be approved and it will be up to the medics to decide which combinations to use.

As we understand better how reliable the animal models are, it may be possible to guide medics on combinations via high quality pre-clinical studies.

RVC

Questions?

RVC