Current Research Strategies and Therapeutic Approaches in Duchenne Muscular Dystrophy

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Ultimately, a multi-drug target, multi-drug approach is the key to developing an effective treatment regimen for DMD.
Current Duchenne muscular dystrophy therapeutic targets can be grouped into five categories. Only the first addresses the primary genetic defect (resulting in the loss of dystrophin protein). The rest address downstream aspects of the pathogenesis.

1) Replacement of dystrophin/utrophin
2) Increasing muscle mass and regeneration
3) Decreasing inflammation and fibrosis
4) Correcting blood flow regulation
5) Correcting perturbations in calcium handing
Duchenne Muscular Dystrophy: Molecular Basis and Possible Treatment Strategies

Functional Roles of Dystrophin:

• **Mechanical** - transmits force from the contractile apparatus to connective tissue/tendon

• **Organizer** - positions a number of proteins at the muscle membrane (NOS, ion channels, etc.)

• **Signaling** - likely plays a number of signaling roles, including a key role in calcium homeostasis

• Contraction causes rupture of the muscle membrane, which allows calcium inflow. There also may be increased flux through ion (TRP) channels and leakiness of the internal calcium storage compartment (SR) via the ryanadine receptor (SR-calcium release channel).

• Excessive calcium activates breakdown of muscle (via calpain and other proteases) and may trigger cell death program.

• Cell death triggers an inflammatory response. Activation of fibroblasts can lead to fibrosis, which prevents muscle regeneration (modulated by IGF-I and myostatin).
Possible Approaches to Therapy for DMD

• **Gene Replacement Therapy** -
  Stem cells
  Viral delivery of dystrophin/utrophin mini-gene
  Viral-directed exon skipping (U7-snRNA)

• **RNA manipulation** -
  Oligonucleotides (“oligos”) to alter RNA splicing
  Drugs that will cause read-through of a premature stop codon in the mRNA

• **Pharmacological agents** -
  *Drugs/factors to promote muscle repair (IGF-I; anti-MSTN)
  *Drugs/biologics that will increase the amount of utrophin or integrin
  Drugs/biologics to decrease membrane damage (e.g. poloxamers) or decrease damage from calcium overload (e.g. SERCA activator*, TRP channel inhibitor, etc.)

*Project Catalyst Targets*
Targeted Development of New Drugs for Muscular Dystrophy
In the clinic:

1) Nonsense Suppression (PTC124): Phase 2b in DMD completed
2) Exon skipping using oligonucleotides: Prosensa/GSK and AVI BioPharma
3) AAV delivery of micro-dystrophin (Phase 1 completed)
4) Myostatin/Activin inhibition (soluble activin receptor/myostatin antibody)
5) Mesangioblasts (cell therapy)
6) AAV delivery of follistatin (BMD)
7) PDE5 inhibition for skeletal and cardiac muscle
Nearing the clinic:

1) Utrophin up-regulation
2) Anti-fibrotic (halofuginone)
3) Anti-inflammatory drugs targeting NFκB
4) Drugs to normalize calcium “leaks”
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A New Paradigm for Treating Genetic Disorders: Mutation-based, rather than Disease-based, Therapy

Drug (PTC124)

Nonsense (Premature Termination) Codon

Normal Termination Codon

mRNA

Full-length Protein
Eccentric contractions reveal large deficit between healthy and diseased muscle.

Deficit from lack of dystrophin.

Petrof et al. PNAS, 1993
Low level dystrophin upregulation provides modest improvement in fragility

~5% dystrophin
Protection against contractile injury is dependent upon extent of dystrophin upregulation.

~10% dystrophin
~5% dystrophin
Protection against contractile injury is dependent upon extent of dystrophin upregulation and number of repeated contractions.
A Pivotal Phase 2b Randomized, Placebo-Controlled Study Was Designed to Evaluate Ataluren Safety and Efficacy in DBMD

Eligibility Criteria:
- DBMD
- Nonsense mutation
- Males ≥5 years
- Able to walk ≥75 m
- Stable corticosteroid (if receiving)

Randomization & Stratification:
- Age
- Corticosteroid use
- Baseline 6MWD

Primary Endpoint:
- 6-minute walk distance

High-Dose Ataluren*
N=~55

Low-Dose Ataluren**
N=~55

Placebo
N=~55

Open-Label Extension Study
High-Dose Ataluren*

* High-dose ataluren = 20 mg/kg (morning), 20 mg/kg (midday), 40 mg/kg (evening)
** Low-dose ataluren = 10 mg/kg (morning), 10 mg/kg (midday), 20 mg/kg (evening)
6MWD Results Showed Low-Dose Ataluren Slowed Loss of Walking Ability Compared to Placebo*

Low-Dose vs. Placebo
Mean Difference at Week 48: +29.7 m
ITT p = 0.0584
PP p = 0.0462

High-Dose vs. Placebo
Mean Difference at Week 48: +0.8 m
ITT p = 0.9788
PP p = 0.9412

* Post hoc analysis

ITT: Intent to treat
PP: Per protocol
Time to Persistent 10% 6MWD Worsening Indicated Slower Disease Progression in the Low-Dose Ataluren Group

Low-Dose vs placebo
Log-rank p=0.039
(HR =0.52, 0.28-0.97)

High-Dose vs Placebo
Log-rank p=0.606
(HR=1.15, 0.67-1.98)

74% not progressed
56% not progressed
52% not progressed
Human Myotube* Data Demonstrate a Bell-Shaped Ataluren Dose-Response Curve for Dystrophin Expression

Mean Increase in Full-Length Dystrophin Expression Relative to Control

* Pretreatment samples (n=35) from Phase 2a DMD trial patients
Nonsense read-through
PTC124 (Ataluren) has undergone a Phase 2b clinical trial in DMD patients. (Saturday: Research Session #4)
The drug provided clinical benefit at the low dose in the trial. At the low dose, patients were relatively stable (6 minute walk) over the course of a year. There likely were variable and low levels of dystrophin made in the patients as a result of the combined effects of nonsense-mediated decay, the type of premature stop codon and the overall RNA context of the mutation.

Low level dystrophin expression will likely slow but not stop disease progression. This approach applies to ~15% of DMD patients.
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RNA manipulation

RNA splicing modification
Oligonucleotides directed at splice junctions/enhancers
to produce exon slipping
-production of truncated dystrophin

Essentially, the objective is to turn DMD into a mild BMD phenotype.

Conceptually, this means that skipping has to be developed for a
number of exons, and that the resulting protein and clinical benefit
will depend upon the patient’s specific deletion.
Phase of DMD exons showing deletion of exon 50

DP427

Actin-binding

Rod domain

DP260

Rod domain

DP140

Rod domain

End of Rod domain

DP71

CYS-rich

Dystroglycan binding site

Zz

Dystrophin domains

mono-skippable exons
Phase of DMD exons showing deletion of exon 50 and skipping of exon 51

mono-skippable exons
Clinical Progress

RNA splicing modifications

Clinical trials have occurred and are ongoing using either O-methyl-oligonucleotides (Prosensa/GSK) or morpholinos (AVI BioPharma). (Friday: Research Session #2)

The heart does not take the oligos up to a great degree (likely because its membranes are not as leaky) and thus there may be no cardiac impact.

Furthermore, not all truncated dystrophin molecules will be equally efficacious (as in the case of BMD). That is to say, while some skipping may produce mild BMD-like phenotypes, while others will produce more severe phenotypes.

Lastly, many deletions in the rod will make the resulting dystrophin unable to localize NOS (nitric oxide synthase).

A major unresolved problem is whether or not some of the resulting dystrophin proteins will cause an immune response in some patients.
In the clinic:

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Gene Replacement Therapy

Viral gene therapy
   AAV / micro-dystrophin or micro-utrophin
   AAV / U7-directed exon skipping
Schematic of a Dystrophin Mini-gene for Viral (AAV)-Based Gene Therapy

- CMV or Muscle-Specific Promoter
- U7-exon mask or Human μ-dystrophin
- PolyA
Clinical Development:

The completed AAV-μ-dystrophin trial was not designed for efficacy. The data that has been presented to date has not shown expression, possibly due to immune response(s) against either vector or transgene or both. At least in some patients, an immune response was detected against the μ-dystrophin.

It will be necessary to develop immune suppression procedures to allow repeated virus administrations and better methods of preventing presentation of neoantigens in the transgene.
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Skeletal Muscle Growth and Repair uses Satellite Cells (muscle stem cells)

HGF leads to activation of satellite cells.

IGF-I stimulates satellite cell proliferation and differentiation.

Myostatin, Activin A and TGF-β can antagonize IGF-I actions.
Myostatin K.O. Mouse

Wild Type

Myostatin Null

Specific Force Decreased
Signaling via the Activin Receptors
Strategies to Achieve Post-natal Myosatin Inhibition


- Delivery of N-terminal myostatin propeptide

- Delivery of follistatin or FLRG (myostatin binding proteins)

- Delivery of soluble Activin IIB receptor (Acceleron/Shire clinical trial)
Inhibition via Soluble Activin IIB Receptor
Myostatin / Activin Inhibition-Based Therapy for Muscular Dystrophy

Clinical Development:
The Phase I activin IIB decoy receptor (Acceleron/Shire; ACE-031) was for safety in normal volunteers, but muscle hypertrophy was observed. The Phase 2 trial in DMD was stopped due to bleeding. A Phase I study with a myostatin neutralizing antibody has begun (Pfizer), with DMD as a possible target for later phase development.

The expectation is that this approach will result in bigger and stronger muscles in DMD patients and may lessen fibrosis. The muscle will still be unstable, so satellite cell repair will continue, but will be more successful. Ultimately, humans (unlike mice) will be exhausted of satellite cells and the muscle function will be lost. However, it is thought that this approach will extend the number of years that the muscle can successfully repair.

The heart will not benefit from this approach, so other drugs must be used to protect the heart. Furthermore, new drugs need to be developed to slow the destruction of the skeletal muscle.
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Major Hurdles Facing Viral-Based Gene Therapy for Muscular Dystrophy

Clinical Development:

The idea is to use a molecule that inhibits myostatin and activin signaling within skeletal muscle (follistatin), but to have it made only in skeletal muscle that has been injected with recombinant AAV that is designed to produce follistatin.

The main drawback of this approach is that while the muscle will become larger and stronger, the muscle will still be unstable, and as it is repaired by satellite cells, the viral transgenes will be lost (i.e. follistatin expression will decrease over time). This is why the trial is being done in BMD, where muscle turnover is slower and thus the effects will last longer. Since follistatin is not a neoantigen, an immune response is not expected.
In the clinic:

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Nearing the clinic:

1) Utrophin up-regulation
Major Hurdles Facing Stem Cell-Based Therapy for Muscular Dystrophy

Clinical Development:

While stem cells seem like the ultimate answer for treating DMD, the approach is a number of years away from being a treatment reality.

The questions that must be answered include:

1) How do we get the stem cells to the right place and have them adopt the correct fate (skeletal muscle)? In diseased tissue, the cells may become fat or fibrosis and make the situation worse instead of better.

2) Will there be an immune response to donor cells, or even to modified patient cells if dystrophin is seen as a neoantigen? Will life-long immune suppression be needed?

3) Can enough cells be obtained and expanded to treat the patient’s muscles without losing their stem cell properties.
In the clinic:

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Nitric Oxide in DMD Muscle

Dystrophin

NOS

NO → cGMP

Many cellular functions

PDE5

Sildenafil or Tadalafil inhibit PDE5 (Viagra® or Cialis®)

GMP (inactive)

NO = nitric oxide
cGMP = cyclic GMP
PDE = Phosphodiesterase
Development of PDE5 Inhibition Therapy for DMD

Clinical Development:

PPMD is sponsoring a trial to compare the effects of sildenafil and tadalafil on activating blood flow in DMD skeletal muscle. In parallel, we are comparing their effects on cardiomyopathy in the dystrophic (GRMD) dog. *(Friday: Research Session #1)*

Ongoing trial (sponsored by Charlie’s Fund) at Johns Hopkins to examine impact of sildenafil on the cardiomyopathy in DMD.

The goal is to ascertain whether either tadalafil or sildenafil have skeletal muscle and/or cardiac benefits in DMD.
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Dystrophin and Utrophin structural similarities

**Dystrophin**

**Utrophin**

**β-spectrin**

**α-spectrin**

**α-actinin**
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   (Saturday: Research Session #4)
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   (Saturday: Research Session #4)
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Phases of skeletal muscle injury and repair

1. Degeneration
   - Necrosis of myofibers

2. Inflammation
   - Neutrophils
   - Macrophages
     - M1: Pro-inflammatory cytokines production
     - M2: Satellite cell activation

3. Regeneration
   - Satellite cell activation
   - Stem cell recruitment
   - Regenerating fibers

4. Remodelling/Repair
   - Extracellular matrix remodeling
   - Angiogenesis
   - Functional recovery
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Duchenne Muscular Dystrophy:

Molecular Basis and Possible Treatment Strategies

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• Mechanical - transmits force from the contractile apparatus to connective tissue/tendon

• Organizer - positions a number of proteins at the muscle membrane (NOS, ion channels, etc.)

• Signaling - likely plays a number of signaling roles, including a key role in calcium homeostasis

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• Excessive calcium activates breakdown of muscle (via calpain and other proteases) and may trigger cell death program.

• Cell death triggers an inflammatory response. Activation of fibroblasts can lead to fibrosis, which prevents muscle regeneration (modulated by IGF-I and myostatin).
By understanding the pathophysiology of Duchenne Muscular Dystrophy, therapeutic targets are unveiled.

1) Replacement of dystrophin/utrophin
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4) Correcting blood flow regulation
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Parent Project
Muscular Dystrophy
LEADING THE FIGHT TO END DUCHENNE