Modulating fast skeletal muscle contraction as a novel therapeutic strategy for muscular dystrophy

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This presentation concerns product candidates that are under clinical investigation, and which have not yet been approved for marketing by the U.S. Food and Drug Administration (FDA). It is currently limited by federal law to investigational use, and no representation is made as to its safety or effectiveness for the purposes for which it is being investigated.
Dystrophin connects the sarcomere of muscle to the extracellular matrix.

- Dystrophin connects the z-disc of the sarcomere to the dystroglycan membrane/matrix complex, connecting the contraction of fibers together.

- Absence or mutation of dystrophin destabilizes the dystroglycan complex leading to stress and injury with contraction and causing Duchenne or Becker muscular dystrophy.
BMD and DMD Represent a Continuum of Severe Dystrophinopathies

Spectrum of Severity Across Dystrophinopathies

Functional Dystrophin

Normal Levels

Severe

BMD and DMD represent a continuum of the same disease, driven by level of functional dystrophin

References: Waddell LB et. al., Neurology Genetics, 2021; Brandsema JF and Darras BT, 2020
Contraction Leads to Extensive Degeneration in Dystrophic Muscle

- Exaggerated force loss with eccentric contraction
- Associated with calcium influx and activation of apoptotic, necrotic and proteolytic pathways

WT EDL muscle

*mdx* EDL muscle

*Sue Brooks, Dennis Claflin, Sunny Yu, University of Michigan*
Immobilization of Muscle Prevents Degeneration

• Complete immobilization of DMD (Sap) zebrafish with high concentrations of myosin inhibitor, BTS (50mM) prevents histological fiber breakdown

• Immobilization of hindlimbs in mdx mice by casting also prevents histological injury

Li and Arner, PLoS ONE, DOI:10.1371/journal.pone.0139483, 2015

Mokhtarian et al., J. Appl. Physiol. 86(3): 924–931, 1999
Skeletal Muscle is Comprised of Slow (type I) and Fast (type II) fibers

Many components of slow muscle are also shared with the heart

Humans are ~ 50/50% fast/slow
Fast Fibers Show Early Degeneration in DMD

Fast Muscle Fibers Are Preferentially Affected in Duchenne Muscular Dystrophy

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Enrichment of muscle regeneration biomarker eMHC in fast but not slow muscle fibers of young DMD boys

Black shading indicates eMHC-positive fibers

Embryonic myosin
Expressed mostly in fast but not slow fibers
Fast but not Slow Fiber Muscle Biomarkers are Elevated in BMD and DMD

132 DMD samples from Newcastle University Biobank, 52 BMD samples from the CINRG consortium and 52 healthy volunteers

‡ The majority of healthy volunteers (83%) had TNNI2 levels below the lower level of detection of the ELISA (<0.1 ng/ml), while only 4% of BMD and 6% of DMD patients had non-measurable levels of TNNI2

132 DMD samples from Newcastle University Biobank, 52 BMD samples from the CINRG consortium and 52 healthy volunteers

Can We Take Advantage of these Observations to Protect Dystrophic Muscle?

Contraction causes degeneration

Fast fibers are injured

Is selective modulation of fast fibers sufficient to protect muscle while maintaining skeletal and cardiac muscle function?

Absolute inhibition of contraction required?
The Target – Fast Skeletal Muscle Myosin

• Fast myosins are highly conserved (93-94%)
• Cardiac myosin approx. 80% homology with fast myosin isoforms at aa level
Development of a Potent, Selective Fast Skeletal Muscle Myosin Inhibitor, EDG-5506

Selective inhibition of fast skeletal muscle myofibril ATPase

Selective inhibition of fast myosin S1 subfragment ATPase

Concentration-dependent inhibition of force in skinned fast rabbit psoas fibers

Concentration-dependent inhibition of force in mouse EDL muscle ex vivo
Modest Inhibition of Contraction with EDG-5506 Is Sufficient for Protection Against Contraction Injury Ex Vivo

Linear relationship between EDG-5506 concentration and force pre-injury

Non-linear relationship between EDG-5506 concentration and injury force drop

Maximal protection at 1 μM = <20% inhibition of force
Modest Inhibition of Contraction with EDG-5506 is also Sufficient for Protection Against Contraction Injury \textit{In Situ}

- Oral dose EDG-5506
- Track isometric force change \textit{in situ}
- in situ TA muscle
  - 2x 20%
  - Lengthening at \(2L_0/\text{sec}\)
- Measure force change post-injury

\textbf{Maximal protection at 0.3 \(\mu\)M = <10\% inhibition of force}

Protection associated with decreased CK
Protection Prevents Multiple Aspects of Fiber Breakdown

DMD muscle (*mdx* mouse) no treatment

DMD muscle (*mdx* mouse) 0.3 mM EDG-5506

*mdx* mouse lumbrical muscle – 20, 1 second maximal isometric contractions (video sped up)

Claflin, Su and Brooks. U Michigan
Protection prevents calcium entry

*mdx* vehicle

*mdx* 0.3 mM EDG-5506

Decreased resting calcium entry with EDG-5506

*mdx* mouse lumbrical muscle
EDG-5506 Reduces *mdx* Mouse CK After Exercise Testing without Altering Performance

- Effect of EDG-5506 on coordination and endurance in a rotarod exercise challenge test
- Exercise performed 3 hrs post single PO dose
- Blood sample taken 1 hr post exercise for CK activity measure
- Similar results obtained for grip strength challenge
EDG-5506 Protects Muscles from Membrane Disruption in the Absence of Exercise

- Effect of 3 weeks PO EDG-5506 on whole-body Evans blue dye incorporation in juvenile *mdx* mice
- *Unexercised* mice
Fibrosis of Muscles Improved in DBA/2J *mdx* Mice in Medium-Term Dosing Studies

- 11 weeks treatment of juvenile DBA/2J *mdx* mice (starting at 8 weeks old)
- EDG-5506 administered with incorporated chow (50 ppm, approx. equivalent to 1 mg/kg PO dose)
- Fibrosis quantified with picrosirius red
One-year EDG-5506 also improves muscle size and strength in DBA/2J mdx mice

- Trends towards increased muscle size
- Increased grip strength after 12 months treatment
EDG-5506 Decreases Creatine Kinase in DMD Dogs

Pete Nghiem, Alexis Rutledge. Texas A&M

**Circulating CK**
Plasma CK across ALL GRMD dogs (N=4)

- **Vehicle Baseline**
- **EDG-5506 Dosing**
- **Washout**
- **Vehicle Repeat**

* CK Activity (U/L)

- 0
- 5000
- 10000
- 15000
- 20000
- 25000

- **ns**
- **✱**
- **✱✱✱✱**
- **✱✱✱**
- **✱✱✱✱**
- **ns**

• EDG-5506 administered to 7 month-old Golden Retrievers with Muscular Dystrophy (GRMD) for two weeks as an oral suspension
• Dosing with 3 mg/kg EDG-5506 for 3 days then 1 mg/kg
• Blood samples taken every 2-3 days and CK activity measured
• Compared with a similar duration 6-week control period 6 month later
EDG-5506 Increases Habitual Activity in DMD Dogs

• EDG-5506 administered to same 18-month-old GRMD for two weeks as a capsule
• Dosing with 2 mg/kg EDG-5506 for 3 days then 2 mg/kg every other day
• Average daily activity measured with a collar-bound Fitbark 2 monitor
• Baseline activity represents a 1-month period while dogs acclimated to activity collars

Pete Nghiem, Alexis Rutledge. Texas A&M
SOMAscan Aptamer Technology Enables Relative Measurement of 7000 Analytes for a Single Plasma Sample

- ‘Slow Off-rate Modified Apatamer Scan’
  - Aptamers are modified with a biotinylated photocleavable linker developed by Somalogic in Boulder, CO
  - Aptamers are mixed with plasma samples then added to biotinylated beads
  - Bead-bound proteins are biotinylated and beads are then washed and linker cleaved
  - Protein/Aptamer complexes are bound to streptavidin beads and aptamers are then dissociated and quantified on a chip
  - Note – SOMAscan optimized against human proteins, aptamers may not cross-react with proteins from other species

- SOMAscan analysis was performed on longitudinal samples from GRMD and compared to published datasets from DMD
EDG-5506 treatment positively alters proteome signatures associated with the dystrophic state in DMD Patients

- SOMAscan® analysis of patient plasma has previously been used to generate a common serum protein signature for DMD patients\(^1\)
- EDG-5506’s response fingerprint in GRMD was compared to the DMD patient signature

\(^1\) Hathout Y, et. al., Sci Rep, 2019

* Proteins selected by overlap between GRMD and published DMD signature biomarkers\(^1\): 40 increased and 9 decreased
Clinical Teaser – Robust Decreases in Elevated BMD Biomarkers with EDG-5506

Changes in BMD biomarkers vs. placebo* (Day 14)

- Effect of 14 days treatment with EDG-5506 on elevated SOMAscan biomarkers in participants with Becker muscular dystrophy


- Additional SOMAscan data - Ben Barthel, PhD. “Use of an Exercise Challenge System to Define a Universal Proteomic Signature of Muscle Injury in Adult Individuals”. Wed, 10:40 AM, Tennessee Ballroom

* EDG-5506 20 mg
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