Selective Inhibition of Fast Skeletal Muscle Myosin as a Novel Therapeutic Strategy for Muscular Dystrophy

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New Directions in Biology and Disease of Skeletal Muscle Conference
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Forward-Looking Statements

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We have based these forward-looking statements largely on our current expectations and projections about future events and trends that we believe may affect our financial condition, results of operations, business strategy, and financial needs. These forward-looking statements are subject to a number of risks, uncertainties, and assumptions, including, among other things: negative impacts of the COVID-19 pandemic on Edgewise’s operations, including clinical trials; risks associated with the process of discovering, developing, and commercializing drugs that are safe and effective for use as human therapeutics and operating as an early clinical stage company; Edgewise’s ability to develop, initiate, or complete preclinical studies and clinical trials for, obtain approvals for, and commercialize any of its product candidates; changes in Edgewise’s plans to develop and commercialize EDG-5506 or any other product candidates; the potential for clinical trials of EDG-5506 or any other product candidates to differ from preclinical, preliminary, or expected results; Edgewise’s ability to enroll patients in its ongoing and future clinical trials; operating results and business generally; Edgewise’s ability to raise funding it will need to continue to pursue its business and product development plans; regulatory developments in the United States and foreign countries; Edgewise’s reliance on third parties, contract manufacturers, and contract research organizations; Edgewise’s ability to obtain and maintain intellectual property protection for its product candidates; risks associated with access to capital and credit markets; the loss of key scientific or management personnel; competition in the industry in which Edgewise operates; Edgewise’s ability to develop a proprietary drug discovery platform to build a pipeline of product candidates; general economic and market conditions; and other risks. These risks are not exhaustive. New risk factors emerge from time to time, and it is not possible for our management to predict all risk factors, nor can we assess the impact of all factors on our business or the extent to which any factor, or combination of factors, may cause actual results to differ materially from those contained in, or implied by, any forward-looking statements. You should not rely upon forward-looking statements as predictions of future events. Although we believe that the expectations reflected in the forward-looking statements are reasonable, we cannot guarantee future results, levels of activity, performance, or achievements. Except as required by law, we undertake no obligation to update publicly any forward-looking statements for any reason after the date of this presentation.

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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.

NB. EDG-5506 is an investigational drug and is not approved in any territory.
Mutations in Dystrophin or Other Proteins of the Sarcoglycan Complex Lead to a Family of Severe Myopathies

**DMD: non-functional dystrophin**
- 12K-15K patients in US
- Muscle damage from birth; functional deficit by 4-6 yrs.
- Nearly all patients will be wheelchair-bound by early teen yrs.
- Death by respiratory/cardiac failure at 20-30 yrs. old

**BMD: partially functional protein**
- 4K-5K patients in US
- Later onset versus DMD, typically 8-15 yrs.
- Variable progression for mobility (late 30s) and cardiomyopathy
Contraction Leads to Injury in Dystrophic Muscle

**Ex vivo contraction of mdx diaphragm**

Relationship between peak force and membrane injury, measured by procion orange uptake in mdx vs WT diaphragm ex vivo

Petrof et al PNAS 90:3710-3714, 1993

**mdx mouse lumbrical muscle, 20 isometric contractions**

Sue Brooks, Dennis Claflin, Sunny Yu, University of Michigan
Contraction of Dystrophic Muscle Leads to Membrane Stress, Calcium Entry, Hypercontraction, and Necrosis

Dystrophin connects the membrane to the contractile machinery

With contraction, dystrophin anchors the membrane

Sue Brooks, Dennis Claflin, Sunny Yu, University of Michigan
Skeletal Muscle is Comprised of Slow (Type I) and Fast (Type II) Fibers

Type I: 50%
- Slow/Cardiac

Type II a: 35%
- Fast Fatigue-Resistant

Type II x/d: 15%
- Fast Fatigable

A Twitch
- Top row (A) shows tension developed during single twitches.

B Unfused tetanic force
- Middle row (B) shows the tension developed during an unfused tetanus.

C Fatigability
- Bottom row (C) shows the degree to which each fiber type can sustain force during continuous stimulation.

Key: ——— Electrical stimulus

Top row (A) shows tension developed during single twitches. The middle row (B) shows the tension developed during an unfused tetanus. The bottom row (C) shows the degree to which each fiber type can sustain force during continuous stimulation.
Type II Fibers Show Early Degeneration in Patients with DMD

Fast Muscle Fibers Are Preferentially Affected in Duchenne Muscular Dystrophy

We show that Duchenne muscular dystrophy (DMD) selectively affects a subset of skeletal muscle fibers specialized for fast contraction. Muscle fiber types were characterized immunohistochemically with monoclonal antibodies that distinguish isoforms of fetal and adult-fast or adult-slow myosin heavy chain present in the same fiber. Fetal myosin expression increased with patient age and was not due to arrested development but rather to de novo synthesis, which served as a sensitive indicator of muscle regeneration. A subset of fast fibers were the first to degenerate (type IIb). Extensive fast fiber regeneration occurred before slow fibers were affected. These results

Humans are ~ 50% (fast) Type II fiber

Distribution of Fetal MHC by Fiber Type

Normal muscle
Embryonic myosin disappears after birth

Dystrophic muscle
Embryonic myosin enriched in type II fibers after birth
Susceptible Fast Fiber Muscle Biomarkers are Elevated in BMD and DMD

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

Barthel et al., Muscle and Nerve.
Therapeutic Hypothesis: 
*Selectively limit contraction in susceptible fast muscle fibers*

Reduce muscle contraction in susceptible type II (fast) muscle fibers to a level sufficient to prevent muscle breakdown and improve dystrophic muscle health

- Demonstrate that other types of striated muscle contraction (e.g. cardiac) are not impaired
- Demonstrate that protective levels of muscle inhibition do not impair strength or coordination
- Demonstrate that long-term protection improves muscle health
The Target: Fast Skeletal Muscle Myosin

Myosin Hydrolyzes ATP to bind actin and generate force
EDG-5506: A Potent, Selective Fast Skeletal Muscle Myosin Inhibitor

Muscle myofibril ATPase

Skinned fast rabbit psoas fibers

Isolated myosin S1 subfragment ATPase

Mouse EDL muscle ex vivo
Low Levels of Inhibition are Required to Protect \textit{mdx} Muscle \textit{ex vivo}

\textbf{DMD muscle (mdx mouse) no treatment}

\textbf{DMD muscle (mdx mouse) 0.3 \textmu M EDG-5506}

\textit{Suffers extensive contraction-induced injuries}

\textit{One hour incubation reduces maximal force by 15\%}

\textit{Contraction-induced injuries completely prevented}

\textit{Claflin, Su and Brooks. U Michigan}
Treatment is Associated with Reduced Calcium Entry
Protection with EDG-5506 Also Present after Eccentric Contraction *ex vivo*

Lengthening contraction of mouse EDL muscle *ex vivo*
In vivo Exercise Injury Proof of Concept

Modeling muscle injury in a more natural environment

• In fasted *mdx* or WT mouse, single oral dose of compound
• 2-4 hours post dose, mouse run on rotarod or performs a grip test
• One hour post “exercise,” blood sample taken for analysis of CK
EDG-5506 Reduces \textit{mdx} Mouse Creatine Kinase Response After Strength-Testing without Altering Performance

**Physical Performance**

*Grip strength (best of 5) 4 hours post-PO dose*

**Circulating Biomarker**

*Plasma CK activity 1 hour post-grip strength test*

Graphs show mean +/- 1 SEM. Significance calculated by one-way ANOVA with Dunnet’s multiple comparison: ns: p > 0.05, **: p <= 0.01, ***: p <= 0.001, ****: p <= 0.0001.
EDG-5506 Protects Muscles from Membrane Disruption in *mdx* Mice

**Ex vivo** Dye Uptake by Contracting Muscle

- **Vehicle**
- **1 μM EDG-5506**
- **5 μM EDG-5506**

*Red* = fluorescent procion orange. Taken up by leaky muscle fibers

*Green* = wheat germ agglutinin (outlines fibers)

**In vivo** Dye Uptake by Leaky Muscle Fibers

*Evans blue uptake in non-exercised *mdx* mice after 3 weeks of treatment*

Graph shows mean +/- 1 SEM. Significance calculated by one-way ANOVA with Dunnet’s multiple comparison (** **0.0001)
Eight Weeks of Dosing with EDG-5506 Reduces Diaphragm Fibrosis

- 8 weeks dosing of mdx mice starting at 5 weeks
- EDG-5506 given at 1 or 3 mg/kg PO by gavage
EDG-5506’s Impact on Cardiac Fibrosis is a Significant Finding Since Cardiac Myopathy is a Common Driver of Mortality in DMD and BMD

15 Months Dosing of DBA/2J mdx Mice Demonstrated Lower Cardiac Fibrosis Compared to Vehicle Controls*

<table>
<thead>
<tr>
<th>12 months</th>
<th>15 months</th>
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<tbody>
<tr>
<td>Control</td>
<td>EDG-5506</td>
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EDG-5506 Led to a Reduction in the Incidence of Cardiac Hypertrophy*

* Graph shows mean +/- SEM. Significance calculated by one-way ANOVA with Dunnet’s multiple comparison (*<0.05; **<0.01; ***<.001; ****<0.0001)
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 of treatment
Natural History of Disease Progression in Golden Retrievers with Muscular Dystrophy (GRMD)

**Duchenne Muscular Dystrophy**

<table>
<thead>
<tr>
<th>CM</th>
<th>SM</th>
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<tbody>
<tr>
<td><strong>0-5 Yrs</strong></td>
<td><strong>0-3 Mos</strong></td>
</tr>
<tr>
<td>Limited data; subclinical ECG conduction changes [121]</td>
<td>Neuronal respiratory [129]; weight gain; flexor weakness [130]</td>
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<tr>
<th>5-10 Yrs</th>
<th>3-6 Mos</th>
</tr>
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<tbody>
<tr>
<td>Abnormal ECG conduction by 10 years: ↑ QT:PQ ratio (cardiomyopathic index) [122]</td>
<td>Marked progression; postural changes; extensor weakness [130]</td>
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<table>
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<tr>
<th>10-15 Yrs</th>
<th>6-9 Mos</th>
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<tbody>
<tr>
<td>Increasing incidence of clinical cardiac disease (28% before 18 but 57% afterwards) [122]; diastolic predates systolic changes [129]</td>
<td>Skeletal muscle phenotype tends to stabilise; many GRMD dogs live well into adulthood</td>
</tr>
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<table>
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<tr>
<th>15-20 Yrs</th>
<th>9-12 Mos</th>
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<tbody>
<tr>
<td>Delayed milestones [124]</td>
<td>Cardiac phenotype is stable, with no evidence of congestive heart failure and normal LVEF [127, 128]</td>
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**Golden Retriever Muscular Dystrophy**

Kornegay. Skeletal Muscle (2017) 7:9
EDG-5506 Decreases Creatine Kinase and Increases Habitual Activity in a GRMD

Circulating Biomarker Throughout Study
Plasma CK across ALL GRMD dogs at 7 months

Daily Average Activity
GRMD dogs at 18 months

Pete Nghiem, Alexis Rutledge. Texas A&M
EDG-5506 Treatment Reverses Proteome Signatures Associated with the Dystrophic State

1.3K SOMAscan® plasma analysis

* Proteins selected by overlap between GRMD and published DMD signature biomarkers\(^1\): 40 increased and 9 decreased.

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

Significance calculated by one-way ANOVA with Tukey’s multiple comparison correction. * p<0.05, **p<0.01, ***p<0.001, ****p<0.0001
Clinical Progression with EDG-5506

Study Objectives
- To assess safety of single and multiple oral doses of EDG-5506 in healthy participants and in participants with BMD
- To assess pharmacokinetics of single and multiple oral doses of EDG-5506 in healthy participants and in participants with BMD
- To assess blood biomarkers in participants with BMD

Single ascending doses in healthy participants: Completed

Multiple ascending doses (14 days) in healthy participants: Ongoing

Multiple doses (14 days) in participants with BMD at levels well tolerated in healthy participants

Up to 130 participants

Up to 12 participants

*Review*
## Next Steps

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<tr>
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<th>2021</th>
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<tr>
<td><strong>BMD Cohort Phase 1</strong></td>
<td><strong>Second half of 2021</strong></td>
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<tr>
<td><strong>BMD Phase 2</strong></td>
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<td><strong>First half of 2021</strong></td>
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<tr>
<td><strong>DMD Phase 2</strong></td>
<td></td>
<td><strong>Second half of 2021</strong></td>
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Thank You!

For questions or comments please email us:

info@edgewisetx.com