Fibrosis in DMD

Rationale for anti-fibrotic therapy in DMD

Action Duchenne Conference

November 7th, 2015
WHAT IS FIBROSIS?
Hardening and scar formation of tissues that results from uncontrolled wound-healing processes in response to chronic tissue injury and inflammation.
WHAT CAUSES FIBROSIS IN DMD?
Fibrosis in DMD is caused, in part, by chronic inflammation triggering abnormal repair.

General fibrotic response to chronic inflammation

Fibrosis in DMD muscle

Mann et al. Skeletal Muscle 2011 1:21
Fibrosis is also caused by pro-fibrinogenic response due to the absence of dystrophin. The Dys-Syn-NOS pathway of miRNA regulation.
Why should we care?

WHAT ARE THE CONSEQUENCES OF FIBROSIS IN DMD MUSCLE?
Fibrosis causes derangement of muscle architecture.

A healthy muscle environment is needed for force transduction, exercise, and muscle regeneration.

Fibrosis is a physical barrier for normal blood flow.

- **Oxygen**
- **Nutrients** (Glucose, Fatty Acids)
Fibrosis physically separates muscle fibers decreasing muscle force production.

Altered structure prevents a coordinated and aligned contraction, decreasing ability to transfer force to the tendons and bones.

Fibrosis decreases blood supply to muscle cells

Separation of capillary blood vessels and muscle cells decreases supply of oxygen and nutrients to muscle.

Fibrosis decreases formation of new vessels (angiogenesis)

Decrease blood flow interferes with exercise and muscle repair processes

In DMD, blood flow is abnormal during exercise due to mislocalization of nNOS

Fibrosis causes decrease blood supply even at rest
Fibrosis perpetuates chronic inflammation

Perpetuation of chronic inflammation maintains an abnormal regeneration process toward fibrous tissue, instead of normal muscle cells.
Fibrosis decreases muscle regeneration

Abnormal structure of extracellular matrix creates a poor platform for satellite cells (main cells activated when muscle needs regeneration and/or repair) to become active
FIBROSIS DECREASES EXERCISE CAPACITY

- Decreased blood flow, thus oxygen, to muscle
- Decreased supply of needed nutrients to muscle during increase energy demands (glucose and fatty acids)
Fibrosis hinders the results of other experimental therapeutic approaches

- Gene therapy
- Cell therapy
- Exon skipping

Muscle cells with dystrophin, but not organized and not connected to blood vessels, to each other, to tendons.
Fibrosis is the only pathological feature that correlates with DMD prognosis, muscle strength and motor function

Desguerre et al. J Neuropathol Exp Neurol 68(7), July 2009

- Endomysial fibrosis is the only myopathologic parameter that significantly correlated with poor motor outcome (quadriceps muscle strength, manual muscle testing of upper and lower limbs at 10 years, and age at ambulation loss (all p < 0.002)).

- Endomysial fibrosis does NOT correlate with age at first muscle biopsy

A. 2% fibrosis at age 4yr, MRC score 3.5 at age 10
B. 19% fibrosis at age 6, MRC score 1.5 at age 10.
Targeting fibrosis in DMD

HT-100
How does HT-100 work?

HT-100 binds to a tRNA synthetase (PROtRNA) and by doing this it orchestrates natural physiological processes, restoring homeostasis.

- Decreases only pathological (abnormal) pathways
  - Inflammation
  - Fibrosis
- Augment Restoration pathways
  - Muscle regeneration
- Augments Homeostatic pathways
HT-100 tips the balance

DMD muscle
- Normalization
- Degeneration

HT-100 + DMD muscle
- Normalization
- Degeneration
- Inflammation
- Necrosis
- Fibrosis
- Regeneration

Regeneration
HT-100 Interim Clinical Data
HALO-DMD 01-02-03
Clinical Trials Progress Status Update

Cohort 1 (n=6): 10 µg/kg/d

Cohort 2 (n=6 safety; n=4 efficacy): 20 µg/kg/d

Cohort 3 (n=6): 40 µg/kg/d

Month 3 of Open extension

Month 1 of Open Extension

7+ months continuous dosing

Cohort 4a (n=6) 60 µg/kg/d. MAD phase

- Cohort 4b (n=6), 60 µg/kg/d: Starting enrollment in February 2016
- Cohort 5 (n=24), 1500 µg/d: Starting enrollment in November 2015

Patients included in the trial:
- Ambulant or non ambulant
- Any mutation, confirmed DMD dx
- Age 6-20 years
- Steroid-treated (stable dose) or naive
<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Safety</th>
<th>Efficacy</th>
<th>External Control³</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>25</td>
<td>16</td>
<td>32</td>
</tr>
<tr>
<td>Dose (HT-100 µg/kg/day)</td>
<td>10, 20, 40, 60</td>
<td>10, 20, 40</td>
<td>Steroid only</td>
</tr>
<tr>
<td>Tx Duration (mos.)</td>
<td>12, 12, 7, single dose</td>
<td>10, 10, 5</td>
<td>12</td>
</tr>
<tr>
<td>Baseline Age mean (SD)</td>
<td>10.7 (2.7)</td>
<td>10.4 (2.5)</td>
<td>10.2 (2.8)</td>
</tr>
<tr>
<td>Steroid treated?</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Ambulant at baseline</td>
<td>60%</td>
<td>60%</td>
<td>100%</td>
</tr>
<tr>
<td>Baseline QMT score Mean (SD)</td>
<td>37.28</td>
<td>35.2(10.36)</td>
<td></td>
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Preliminary Safety Results

- Based on a cumulative >11.5 years of dosing, with 6 patients dosed 15 months total, 10 patients dosed at least 12 months continuously, and 6 patients dosed one time.
- 191 adverse events (AEs) have been reported by 18 subjects
  - ~58% deemed not related to study drug
  - ~28% unlikely related
  - ~14% possibly related (26 AEs)
- Majority of treatment-related AEs were mild and resolved without sequelae.
- No serious AEs have been reported and no deaths have occurred.
- Most frequent possibly-related AEs are vomiting, diarrhea, and headaches.
HT-100 Improves Muscle Strength in Ambulant and Non-Ambulant Boys with DMD

- Muscle Strength % change from baseline after 12 months (Cohorts 1 and 2) and 7 months (Cohort 3) of continuous dosing.
Preliminary Efficacy Findings Show *Improvement* in Muscle Strength

*Strength is most sensitive measure of HT-100 effect*

<table>
<thead>
<tr>
<th>Cohort (n)</th>
<th>Months of Continuous Dosing (ongoing)</th>
<th>QMT score %CFRB* (%CFRB) (SD)</th>
<th>% CFRB* over External Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (6)</td>
<td>12</td>
<td>+14.4 (25.3)</td>
<td>+25% (at 12mo)</td>
</tr>
<tr>
<td>2 (4)</td>
<td>12</td>
<td>+13.8 (27.5)</td>
<td>+24.4% (at 12mo)</td>
</tr>
<tr>
<td>3 (6)</td>
<td>7</td>
<td>-5.7 (7.9)</td>
<td>+4.9 % (at 7 mo)</td>
</tr>
<tr>
<td>Matched external steroid-treated cohort¹ (32)</td>
<td>12 (placebo+ stable steroid dose)</td>
<td>-10.6 % (9.8) (change at 12 months)</td>
<td></td>
</tr>
</tbody>
</table>

*%CFRB: Percent change from Restart baseline. This is the baseline value before continuous dosing begins.*

- Strength increase correlates with changes in clinical observations
- Clinically meaningful improvement in muscle strength defined by corticosteroid trials: 24-30% increase at 12 months in steroid-naïve DMD

¹ From a previously-published study, not a randomized control from this program. Neurology. 2012 Mar 20;78(12):904-13.
Muscle strength highly correlates with overall Motor Function Measure (MFM)

Combined Cohorts 1-3 (n=19)

$rho = 0.82$
$p < 0.002$
$N=19$
HT-100 Reduces Cardiac Fibrosis:
Change over 18 months, only 7 months total and 4 months of continuous dosing

Baseline before Treatment

Area of focus

Following HT-100 Treatment

- White areas represent fibrosis
- Red/pink spots mark areas of intense fibrosis
- Subject: 10 years old at entry. Last scan: 11 ½ years old
Many contributing pathways to fibrosis in DMD

Other potential anti-fibrotics therapies
Other pathways identified in DMD muscle that can lead to fibrosis are targets for anti-fibrotic therapy

- **Myostatin inhibitors**
  - Myostatin stimulates proliferation of fibroblasts and can directly stimulate muscle fibroblasts to proliferate and expresses ECM proteins
  - Myostatin inhibitors might increase muscle size and decease fibrosis
    - ACE-031 (Acceleron/Shire): Program stopped due to side effects
    - MYO29 (Wyeth): Phase II study. Program stopped due to side effects
    - Pfizer Phase I Clinical Trial in Normal volunteers of specific anti-myostatin antibody
    - Follistatin Gene therapy

- **Sunphenon Epigallocatechin-Gallate (EGCg)(antioxidant, anti-fibrotic)**
  - A clinical trial of EGCG in Duchenne patients is under way at Charité Hospital in Berlin

- **Pentoxifylline (TNFα, TGFβ antagonist)**
  - Phase III RTC study was negative

- **ACE-inhibitors**
  - Losartan decreases fibrosis in mdx
Acknowledgements