Calcium and Sodium exchange in Duchenne
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Disclosures/Disclaimers

- Florence Porte Thomé is an employee of EspeRare, a non-for-profit organization committed to rare pediatric diseases.
- Rimeporide is a Sodium/Proton exchanger inhibitor, being developed by EspeRare for the treatment of Duchenne muscular dystrophy, and not approved for treatment.
- This presentation does not provide medical advice, diagnosis or treatment.
- This presentation is not intended to be a substitute for professional medical advice, diagnosis, or treatment.
- All other informations contained in this presentation is based on literature research and may not be exhaustive nor up to date.
The dystrophin associated protein complex in skeletal and cardiac muscles

- Structural role in linking the actin cytoskeleton to the ECM
- Stabilizing the sarcolemma during contraction and relaxation
- Transmitting force generated in the muscle to the ECM
- Plays an essential role in maintaining muscle integrity
The role of Calcium & Sodium in the Contraction and relaxation processes in normal muscle fibers

**Contraction**
- An action potential arrives at the neuromuscular junction.
- ACh is released, binds to receptors, and opens sodium ion channels, leading to an action potential in sarcolemma.
- Action potential travels along the T-tubules.
- Calcium is released from the sarcoplasmic reticulum.
- Troponin interacts with actin, allowing for thick and thin filament interaction and muscle contraction.
- Thick and thin filament interaction leads to muscle contraction.
- Muscle shortens and produces tension.

**Relaxation**
- Calcium is resorbed, beginning relaxation cycle; ATP is required.
- Thick and thin filament interaction relaxes.
- Muscle lengthens and relaxes.
In dystrophic muscle fibers, Na & Ca pathways are disrupted

Reduced or absent dystrophin

Mechanically weakened plasma membrane prone to focal tears during contraction/relaxation

Massive entry of extracellular Ca/ Na via sarcolemmal lesions & dysregulated channel

Activation of proteolytic enzyme (calpain) leading to unregulated protein degradation

Increased production of reactive oxygen species (ROS) and inflammatory processes

Induction of necrosis through opening of the mitochondrial permeability transition pore with mitochondrial rupture
Dysregulation of Calcium homeostasis in DMD

- Elevations in Ca2+ is associated with increased SOCE (store-operated calcium entry), stretch-activated calcium entry, ROCE (receptor-operated calcium entry), and Na/Ca exchanger. The L-type calcium channel might also be responsible.

- Sarcoplasmic reticulum (SR), Ca2+ reuptake is also reduced with decreased function of the SERCA pump.

- In DMD, this gradient promotes Ca2+ overload, resulting in mitochondrial uncoupling, calpain activation & production of ROS.

- The biochemical downstream effects are the accumulation of acidic metabolites & production of cytokines.

- Finally, the cellular integrity is unsustainable. Myofibre necrosis and inflammation lead to fibrotic tissue remodelling.

Na+: the other deleterious ion in DMD

$[\text{Na}^+]_i$ serves as a co-regulator of $\text{Ca}^{2+}$ influx through the NCX and NHE-1, \textit{(Burr et al, 2015)}

In physiological conditions: NCX is in forward mode

In DMD conditions: NCX is in Reverse mode
Na+: the other deleterious ion in DMD

- The Na gradient is a key determinant of net Ca2+ influx or efflux by NCX1
- NHE-1 is overexpressed in mdx myotubes and blocks the Na+/Ca+ exchanger (NCX1) in “reverse mode”
- Persistent overload of Na+ has been reported in mdx mice & DMD patients (Weber et al, 2012) & may lead to muscle weakness and degeneration
- DMD muscles are hypertrophic due to edema which leads to inflammation
- Edema-like changes are reported in cardiac and skeletal muscles in DMD

(Weber et al., 2012)
The regulation of Ca$^{2+}$/ Na$^{+}$ homeostasis offers multiple sources of therapeutic opportunities

### Modulating Calcium Homeostasis in DMD (1/2)

<table>
<thead>
<tr>
<th>outcomes</th>
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| **L-type calcium channels blockers:** | **Diltiazem, verapamil, nifedipine, flunarizine**  
1 week of treatment of mdx mice with nifedipine, intracellular Ca2+ was decreased and grip strength and swimming times were increased. mdx mice treated with diltiazem, or verapamil showed decreased levels of circulating CK and decreased necrosis in the diaphragm. Metaanalysis of clinical studies failed to show a significant beneficial effect on muscle function in DMD (low n). 2nd generation drugs with better side effect profile should be studied. |
| **Store operated Calcium Entry Channels** | **Streptomycin:** Long-term treatment had a positive effect on limb muscle pathology, reduced fibrosis, increased sarcolemmal stability, and promoted muscle regeneration in older mice. However, streptomycin treatment did not show positive effects in diaphragm or heart muscle, and heart pathology was worsened. |
| **Sarcoplasmic/endoplasmic reticulum Ca(2+)-ATPase (SERCA)** | **BGP-15** (Hsp 72 inducer) increased SERCA activity in dystrophic skeletal muscles, improved muscle architecture, strength and contractile function in severely affected diaphragm muscles in mdx dystrophic mice. In dko mice, BGP-15 decreased kyphosis, improved the dystrophic pathophysiology in limb and diaphragm muscles and enhanced systolic function in the hearts of dko mice. Excitingly, later stage treatment also reduced fibrotic deposition. This study was the first to demonstrate that BGP-15 improves the cardiac pathology of dystrophic mice and further supports BGP-15 as a promising prospective therapy for DMD patients |
## Modulating Calcium Homeostasis in DMD (2/2)

<table>
<thead>
<tr>
<th>Inhibitor of mitochondrial pore</th>
<th><strong>Outcomes</strong></th>
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<tr>
<td><strong>Cyclosporine A (CsA):</strong> Is a potent immuno-suppressant. Effective in reducing mitochondrial abnormalities in patients lacking collagen VI. During 8 weeks of treatment in mdx mice, CsA significantly increased tetanic force and maximum voluntary contraction.</td>
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<td><strong>Debio-025 (Db25):</strong> CsA analog (10X more potent). Already proved to be safe in humans for treatment of Hepatitis C virus. Positive data in mdx mice.</td>
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<tr>
<th>Inhibition of Na+/Ca2+ exchangers, Na+/H+ exchanger</th>
<th><strong>Outcomes</strong></th>
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<tr>
<td><strong>Cariporide/ Rimeporide:</strong> Potent anti fibrotic and anti inflammatory effects seen in skeletal, cardiac muscles and diaphragm in mdx mice. Improved survival in Cardiomyopathic Syrian Hamsters (decreased hypertrophy and necrosis).</td>
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<td><strong>Rimeporide:</strong> Ongoing Phase Ib in DMD - Safety &amp; PK</td>
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<th>RyR 1 complex</th>
<th><strong>Outcomes</strong></th>
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<td><strong>Dantrolene</strong> treatment alone has no significant beneficial effects at the tested doses in young mdx mice.</td>
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<th>Inhibition of Stretch-Activated Ca2+ Channels</th>
<th><strong>Outcomes</strong></th>
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<tr>
<td><strong>AT-300/GsMTx-4:</strong> is the only known specific inhibitor of stretch activated class of calcium ion channels. Extract from Tarentula venom. In vivo efficacy study in mdx had modest results, new studies ongoing in mdx mice.</td>
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## Modulating Sodium Homeostasis in DMD

<table>
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<th>Method</th>
<th>Outcomes</th>
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<td>Selective Aldosterone Receptor Antagonist</td>
<td><strong>Eplerenone</strong>: preserved cardiac and skeletal muscle function in mdx mice. In patients, a 12 month treatment was associated with significant change in myocardial strain and attenuation in the decline of ejection fraction.</td>
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<td>Inhibition of Stretch activated channel</td>
<td><strong>Gadolinium</strong>: was efficient to reduce the Na influx in muscle fibers but cannot be used chronically</td>
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<td>Inhibition of reverse mode NCX1, NHE-1 exchanger inhibitor</td>
<td><strong>Cariporide/Rimeporide</strong>: refer to previous slide</td>
</tr>
<tr>
<td>Inhibition of reverse mode NCX1 and Nav1,4</td>
<td><strong>Ranolazine</strong>: in Sgcd -/- (mutation in sarcoglycan) mice that exhibit early mortality with elevated creatine kinase serum levels and muscle degeneration, histological examination of quadriceps muscles showed noticeably less pathology with significantly less fibrosis and central nucleation with ranolazine treatment, as well as fewer infiltrates and less fiber size irregularity</td>
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The repurposing of Rimeporide, a Sodium/Proton (NHE-1) inhibitor, from congestive heart failure to Duchenne Muscular Dystrophy
The regulation of Ca$^{2+}$/ Na$^+$ homeostasis offers multiple sources of therapeutic opportunities

Initial preclinical evidence for NHE-1 inhibition efficacy in DMD

Cariporide: NHE-1 inhibitor (discontinued development)

1. Cariporide induces mdx muscle fiber survival (22 days oral treat@3mg/kg/day)
2. Cariporide treatment induces a reduction of circulating creatine Kinase in mdx
3. Cariporide increased mdx muscle strength as seen by increased time in grip test. (22 days oral treat@3mg/kg/day)
4. Cariporide decreases significantly Na+ influx in BIO14.6 muscle fibers
5. Cariporide significantly decreases excessive Ca2+ influx in BIO14.6 fibers (hamster model of DMD) – link to NCX
Rimeporide has a good safety and toxicology profile

- A comprehensive nonclinical safety package was completed
  - GLP Toxicology studies:
    • No major sign of acute or chronic toxicity in rodent and non-rodent after acute and long-term studies (chronic and acute, genotoxicity, mutagenicity, fertility)
  - Safety pharmacology:
    • No safety findings in cardiovascular, CNS and respiratory organs
  - Pharmacokinetics and TPK
    • ADME in animals is well characterized and shows that the compound is well absorbed through the GI tract, poorly bound to plasma protein, rapidly cleared by the kidney (half life 4 hours) and not metabolized

- Rimeporide has been evaluated in two animal models relevant to different clinical manifestations of DMD:
  - Significant improved survival was established in cardiomyopathic hamsters (CM) with dilated cardiomyopathy similar to that experienced in DMD
  - Decreased fibrosis and inflammation was established in mdx mice in a large panel of muscles

- Rimeporide is currently being tested in GRMD dogs to support the design of the phase II/III study
Rimeporide has excellent human safety and PK profiles

- **Summary of Safety Data**
  - Adverse events: mostly mild in intensity, no clear dose-dependency, most frequent:
    - headache (≈15%)
    - dizziness (≈ 5%)
    - chest discomfort (≈10%)
    - paresthesias, vaso-vagal attacks (3 in total moderate intensity)
    - skin reactions (only in single study mainly local reactions)
  - Vital signs, ECGs, Laboratory: no clinically relevant changes
  - Gastrin, Gastroscopy, Gastric biopsies: no signs of parietal cell toxicity

- **Summary of PK data**:
  - Absorption was rapid, complete and dose proportional
  - There was a food interaction on $C_{\text{max}}$ but not on the extent of absorption after intake of a standard high fat breakfast
  - The half life of rimeporide in plasma is 4H
  - Elimination takes place by glomerular filtration
  - There is no metabolism by the CYP450
  - Decreased creatinine clearance (<30mL/min) may require dose adaptation (prolonged half life)
Rimeporide prevented Cardiac Necrosis in CM hamsters and regulated [Ca\textsuperscript{2+}]i and [Na\textsuperscript{+}]i.

Effects of 198-days treatment of cardiomyopathic hamsters (CMHs) with Rimeporide on calcium (A) and sodium (B). Wild = wild type hamster, CM = untreated CMHs, EMD = Rimeporide treated CMH, n=3, ***p<0.001.

Acknowledgements: Chahine et al., 2005, Journal of Molecular and Cellular Cardiology

Rimeporide is a cardioprotective agent and is expected to be of benefit in the treatment of cardiomyopathy in patients with DMD.

Representative myocardial longitudinal sections of hearts. Arrows show necrosis and (*) shows mural thrombus. 198-day treatment of 30-day-old CMHs with Rimeporide significantly (p<0.001) prevented development of cardiac necrosis.
Rimeporide 9-month treatment leads to clinically relevant anti-fibrotic effect in mdx mice

Acknowlegdement: Children’s Hospital Washington DC, Pr Nagaraju
Rimeporide promotes potent anti-inflammatory effect in mdx mice (5 w and 9 M)
A Phase Ib trial is ongoing to evaluate the safety, tolerability, PK and explore PD endpoints in patients with DMD after a 4-week oral treatment.

**Study objectives**

1. To determine the safety and tolerability profile of multiple oral administrations of rimeporide patients with DMD.
2. To evaluate the pharmacokinetic profile of rimeporide in pediatric patients with DMD.
3. Exploratory: NMRI indices (T2, fat fraction ...), Plasma/Serum cytokine levels, PD biomarkers to monitor muscle damage.

**Study design and treatment**

- 20 ambulant DMD patients (6 to 14)
- Multiple dose escalating study
- 4 weeks oral treatment
- Hardgel capsules of 25 or 50 mg

More information on www.clinicaltrials.gov
Phase Ib clinical trial in DMD: recruitment plan

- Cohort 1 (n=5)  
  50-75mg TID  
  4 weeks
- Cohort 2 (n=5)  
  100-150mg TID  
  4 weeks
- Cohort 3 (n=5)  
  150-200mg TID  
  4 weeks
- Cohort 4 (n=5)  
  200-300mg TID  
  4 weeks

Safety Review and Pharmacokinetics

- France
- 5 ambulant patients aged 6 to 10 enrolled
- No treatment-related adverse events
- No tolerability issues
- Good compliance
- PK measures in line with adults

- Spain and Italy and UK
- 5 ambulant patients aged 6 to 10 enrolled
- No treatment-related adverse events
- No tolerability issues
- Good compliance
- PK measures dose proportional and in line with adults
- Decrease in CK levels
Conclusions

- The sodium and calcium hypothesis have matured greatly over the past decade and offer today several new opportunities for drug development in DMD. Many of these options have shown benefit either in the mdx mice and now more work is needed to translate these options in the clinic.

- Molecules, such as Rimeporide, that are able to regulate intracellular gradients of Calcium and Sodium are interesting targets for maintaining muscle integrity. They can prevent the long term accumulation of fibrosis and inflammation both in skeletal and cardiac muscles regardless of the mutation.

- However, given the complex natural history of DMD, cocktails will be the order of the day to achieve a robust therapeutic effect, with some molecules correcting genetic defects while others ameliorate downstream ion homeostasis, inflammation and fibrosis.
Questions?

Thank you for your attention!