**Utrophin modulators improve muscular dystrophy in the mdx mouse diaphragm**

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**UtroDMD Alliance:** This work is part of the UtroDMD Alliance, a multi-year strategic collaboration that combines the extensive biology, chemistry and drug-discovery expertise of the University of Oxford, Summit plc, supported by the MRC and Muscular Dystrophy Campaign, with the sole aim to accelerate the development of first-in and next generation utrophin modulator therapies for all DMD patients.

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**Introduction**

Dystrophic muscular dystrophy (DMD) is a severe muscle degenerative disease caused by genetic mutations in the dystrophin gene resulting in loss of dystrophin function. Affected individuals often succumb to heart or respiratory failure by 30 years of age. Currently, there is no effective treatment for DMD and only a limited number of disease modifying drugs are progressing in clinical trials.

We have previously demonstrated that the dystrophin-related protein utrophin, a structural and functional autosomal analogue of dystrophin, can act as an effective surrogate to compensate for the loss of dystrophin in mdx mice. By oral administration of small molecules specifically designed to target the utrophin A promoter, we aim to develop a treatment for all the DMD patients irrespective of their mutation. In partnership with Summit Therapeutics, we have previously developed SMT C1100, an oral utrophin modulator that restores dystrophic symptoms in the mdx mouse and successfully completed a Phase 1b trial with an excellent safety profile in DMD patients.

We are now exploring in the mdx mouse, future generations of utrophin modulators from the SMT C1100 series with improved physicochemical properties and a more robust metabolism profile. Unlike skeletal and cardiac muscles, the mdx diaphragm exhibits a highly dystrophic pathology and closely recreates the degeneration observed in DMD patients suggesting that the early dystrophic changes are most prominent in this muscle. Therefore the mdx diaphragm is the most reliable pre-clinical indicator of damage or recovery post-treatment. In this poster, we present the benefits of the 2nd generation utrophin modulators from the SMT C1100 series, namely SMT022353 and SMT022357, in the mdx diaphragm and discuss their relevance to potential clinical development.

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**1. 2nd generation utrophin modulators show promising in vitro activity**

A. New compounds structurally similar to SMT C1100 demonstrate transcriptional modulation in murine myoblasts

- SMT022353 increased luminescence 3-fold at 1 µM
- SMT022357 increased luminescence 2.4-fold at 0.1 µM
- SMT022353 increased luminescence 2.5-fold at 10 µM
- SMT022357 increased luminescence 2.6-fold at 10 µM

Murine myoblast luciferase reporter cell lines 1 and 2 are controlled by partial utrophin promoter or full utrophin promoter respectively.

B. SMT022353 and SMT022357 increase utrophin protein expression in murine myoblasts

- SMT022353 treatment increased utrophin expression 2.6-fold at 1 µM
- SMT022357 treatment increased utrophin expression 2.5-fold at 1 µM

Increase in luminescence from reporter assay correlated well with increase in utrophin protein.

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**2. SMT022353 & SMT022357 improves systemic exposure in mdx mouse**

**Time course of serum compound exposure levels after treatment**

![Graph showing time course of serum compound exposure levels after treatment](image)

The 24-h time course of serum exposure levels demonstrated better bioavailability of SMT022353 and SMT022357 post treatment.

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**3. SMT022353 and SMT022357 treatment modulates utrophin protein levels in the mdx mouse diaphragm**

**Immunofluorescence of utrophin in compound treated mdx mice**

- After 5 weeks of daily treatment (30 mg/kg/day) in 2-week old mdx mice, an increase of utrophin in the diaphragm was observed in both SMT022353 and SMT022357 treated groups.

**C. Quantification of utrophin protein by Western Blotting**

- SMT022353 treatment increased utrophin expression in the mdx diaphragm by 1.5-fold (p < 0.05).
- SMT022357 treatment increased utrophin expression in the mdx diaphragm by 1.2-fold.

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**4. Histological improvements in mdx diaphragm muscle pathology**

**A. Hematoxylin & Eosin (H&E) staining of mdx diaphragm after treatment**

- SMT022353 showed a 41.5% (p < 0.05) reduction in necrotic area compared to the vehicle group.
- SMT022357 showed a 36.4% (p < 0.05) reduction in necrotic area compared to the vehicle group.

**B. Percentage of necrotic area**

- SMT022353 treatment showed a 27.3% (p < 0.001) reduction in centronucleated fibres compared to the vehicle group.
- SMT022357 treatment showed a 36.5% (p < 0.001) reduction in centronucleated fibres compared to the vehicle group.

**C. Percentage of centronucleated myofibres**

- There were a drastic reduction of calcium deposits in compound treated mdx mice.
- Specifically, mdx mice in the SMT022357 treated group showed complete exclusion of calcium deposits in the diaphragm.

**5. Protection against diaphragm myofibre damage and fibrosis**

In the mdx mice, muscle membrane damage causing an influx of retrolateral calcium. This disrupts normal contractile signalling and activates the muscle repair process, leading to eventual aberrant degeneration of myofibres.

- **A. Alcian Red staining**
  - There was a drastic reduction of calcium deposits in compound treated mdx mice.
  - Specifically, mdx mice in the SMT022357 treated group showed complete exclusion of calcium deposits in the diaphragm.

- **B. Collagen I (4X)**
  - Sirius Red staining showed a decrease in collagen infiltration into the diaphragm of compound treated mdx mice.
  - This suggests that SMT022353 and SMT022357 treatment effectively protects the diaphragm muscle from injury and subsequent fibrosis.

- **C. Sirius Red (2X)**
  - Sirius Red staining reveals collagen fibres as red and elastin/collagen type II fibres in brown.

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**Conclusions**

- We have identified 2 compounds in the same chemical series as SMT C1100, with improved physicochemical profiles.
- Daily oral administration of these utrophin modulators show efficient distribution to all skeletal muscles, heart and diaphragm of the mdx mouse and reduced muscular dystrophy (Refer to poster G.P.245 for details).
- In particular, SMT022353 and SMT022357 provide an effective rescue of calcium dysregulation and are able to protect against muscle damage, necrosis and fibrosis in the highly dystrophic mdx diaphragm, one of the most severely affected muscle in DMD.