Second-generation compound for the modulation of utrophin in the therapy of DMD


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Introduction: DMD is a progressive X-linked muscle-wasting disorder caused by lack of the cytoskeletal protein dystrophin. Utrophin is the closest sequence homologue related to dystrophin and studies with transgenic utrophin expression in the mdx mouse have shown that constitutive utrophin expression compared to the natural regeneration levels seen in the mdx muscle can compensate for the lack of dystrophin and successfully prevent the dystrophic pathology. Deliberately over-expressing utrophin protein showed no detrimental effect in a broad range of muscle tissues. Thus, utrophin can act as an effective surrogate for dystrophin in mdx muscles.

By pharmacologically modulating utrophin, we aim to develop a systemic therapy applicable to all DMD patients, regardless of their dystrophin mutation, by targeting the primary defect and restoring sarcolemmal stability. In partnership with Summit Therapeutics, we previously developed ezutromid (formerly SMT C1103), a small molecule utrophin modulator that reduced dystrophic symptoms in the mdx mouse. As a potential first-in-class molecule, ezutromid is being tested in a Phase 2 proof of concept study in DMD patients. We are now developing next generation utrophin modulator molecules which exhibit favourable solubility, stability, oral absorption and which are well tolerated in the mouse. Pre-clinical in vivo studies in mdx mice with one such modulator (SMT022357), structurally related to ezutromid, demonstrated that oral administration of SMT022357 leads to increased utrophin expression in skeletal, respiratory and cardiac muscles. This results in improved sarcolemmal stability and reduces dystrophic pathology through a significant reduction of regeneration, necrosis and fibrosis. All these improvements combine to protect the mdx muscle from contraction induced damage and enhance physiological function. The results from some of these studies are presented in this poster.

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 and Summit Therapeutics, with additional support from the MRC, MDA and Muscular Dystrophy UK to accelerate the development of first and next generation utrophin modulator therapies for all DMD patients.

1. In vitro activity of second generation compound

A. SMT022357 demonstrated positive results in utrophin screening assay

- Second generation compounds, related to ezutromid present better chemistry properties (solubility) and a more robust metabolism profile.
- SMT022357 shows a maximal increase of three fold in lucerasease compared to vehicle (Fig. 1A). No stabilisation or inhibition of the lucerase activity was noted (data not shown). Furthermore, no change in proliferation was observed after a treatment with SMT022357.

B. SMT022357 demonstrates increased utrophin mRNA and protein expression

- In vitro dosing of murine myoblasts with 3μM of SMT022357 led to a 50% increase in utrophin mRNA when compared to vehicle.
- Treatment of murine DMD cells with SMT022357 showed a 2.5-fold increase in utrophin protein level at an optimal concentration of 10μM.

2. In vivo potential of second generation compound

A. Improved systemic exposure with second generation compound

B. Increased utrophin expression in skeletal muscle fibers

- Increased utrophin expression in skeletal muscle fibers: In fibre type I (19%), type IIa (9%) and IIb (9%) muscles, Utrophin was localised along the entire length of the membrane with drug treatment, Independent of regeneration and change in fibre-type composition.
- Reconstruction of the dystrophin protein complex and restoration of membrane stability in dystrophin-negative mdx fibers following the increased level of utrophin at the sarcolemma.

3. Benefits of daily dosing of sedentary mdx mice with SMT022357

A. SMT022357 significantly reduces the muscular dystrophy in skeletal muscle

- A decrease in muscle pathology: regeneration (10% reduction, p=0.01) and necrosis (35% reduction, p=0.02).
- Expression of different regeneration/myogenic differentiation markers are reduced after drug treatment, Similar results were obtained in Soleus muscle (fibre-type I).

B. SMT022357 modulates utrophin in the mdx diaphragm and heart

- In diaphragm, SMT022357 increases the utrophin by 20%, decreases fibre regeneration (36%, p=0.01) as determined by reductions in centrally nucleated myofibers and reduced necrosis (15%) and the fibrosis by 15%.
- SMT022357 treatment completely prevented the accumulation of calcium-rich deposits demonstrating a significant decrease in membrane damage (P<0.04).
- SMT022357 treatment results in an 50% increase of the utrophin protein level in the heart.

C. SMT022357 treatment protects muscle

- SMT022357 results in a 50% decrease in force drop (p=0.003), Increased utrophin levels after SMT022357 treatment results in a significant improvement in membrane stability and resistance to damage.

Conclusions

Oral administration of SMT022357, a molecule structurally related to ezutromid with more favourable exposure properties, modulates utrophin expression in skeletal, respiratory and cardiac muscles which leads to a significant reduction in dystrophic pathology in the mdx mouse. These data further validate the approach of utrophin modulation for therapy of DMD.

Future plans

As demonstrated here, therapeutic benefits with SMT022357 validate the ezutromid drug series. Continued formulation development of ezutromid has identified an alternative formulation which achieves over a six-fold increase in average maximum plasma levels in patients but with a reduced oral dose compared to the current microfluidised formulation. It is planned to evaluate both of these formulations in the ongoing Phase 2a DMD Phase 2b clinical trial being conducted in DMD patients with the aim of establishing proof of concept for ezutromid and utrophin modulation. We are now focussed on the development of future generation utrophin modulator molecules that are structurally distinct from the ezutromid generation molecules and which may act through a different mechanism of action.