Abstract

Duchenne muscular dystrophy (DMD) is an inherited muscular disease characterized by progressive muscle weakness and cardiac dysfunction due to the lack of dystrophin. This disease is notably characterized by a systemic myotonic defect central to disease etiology. Sarcones (API BIO101) is a first-in-class Mas activator currently in clinical development (Phase IIb) for sarcopenia. Twelve-week-old C57BL10-mxd mice were orally treated with vehicle or BIO101 at 50mg/kg/day for 8 weeks. Immortalized human skeletal muscle cells KMS71 derived from DMD patient (exon 52 mutated) were also employed and treated with various doses of BIO101 ranging from 1 to 10 µM. Myoblast differentiation was investigated by fluorescent microscopy. Signaling pathway activation status was assessed by western blot. Oxygen consumption rate was measured using a Seahorse XF Analyzer.

Results

- **In vivo functional study: mdx mouse model**

- **In vivo study: Exercise tolerance test**

- **In situ study: Maximal isometric TA strength**

- **In situ study: Myoblast differentiation**

Introduction

About DMD: DMD is a X-linked inherited muscular disease, characterized by progressive muscle weakness and cardiomyopathy, leading to premature death. DMD is caused by an abnormality of dystrophin. Muscle fibres are repeatedly damaged, necroses/repair and are replaced by connective and adipose tissues. Glycocorticoids and supportive therapy are the current standard of care leaving patients with an untreated medical need.

About Sarcones: Sarcones is a first-in-class drug candidate based on the activation of the MAS receptor (major player of the renin-angiotensin system) that had demonstrated meaningful activity in animal models of muscular dystrophies. Sarcones has already entered clinical Phase IIB (SARA-IHT) in patients with sarcopenia, an age-related degeneration of skeletal muscles, leading to loss of mobility in elderly people. BIO101 is the active principal ingredient of Sarcones.

BIO101 received an Orphan Drug Designation (ODD) for Duchenne muscular dystrophy in EU on the 27th of June 2018 and in US on the 10th of May 2018. FDA IND application will be submitted by the end of 2018. Biochemical clinical programme for DMD (MYOFA) will start in the first quarter of 2019.

Methods

**Animal model:** 12-week-old C57BL10 and C57BL10 mdx male mice were treated orally for 8 weeks with either vehicle or BIO101 (at 50 mg/kg/day) under normal diet. Exercise tolerance test: the animals from all groups were submitted to running exercise and their muscle running distance was recorded at completion of the experiment (after 8 weeks of treatment). The running test consists in 2 minutes of warm-up session in which the speed of the treadmill is increased from 0 to 20 m/min. Then, the speed of the treadmill is increased by 5 cm/s every 10 minutes. Muscle force measurement: tibialis anterior (TA) distal tendon was attached to a force recorder. The sciatic nerve was stimulated by a bipolar electrode (10 V, 0.1 ms, 75-150 Hz, duration of 500 ms). Oxygen consumption rate: Oxygen consumption (mitochondrial respiration) was recorded using a Seahorse XF Analyzer following sequential addition of oligomycin, FCCP and rotenone/antimycin A.

Sarcones differentiation: Immortalized healthy (AB11727FL2) and DMD (KMS751DMD10FL) human skeletal muscle cells (fascia lata) derived from a 10-year-old DMD patient (exon 52 mutated) were employed and treated with 10µM of BIO101. Myoblast differentiation was investigated by fluorescent microscopy.

Signaling pathways: Cells were lysed, equal amounts of proteins were electroblotted on 4-12% SDS-PAGE and transferred following sequential addition of oligomycin, FCCP and rotenone/antimycin A.

**Myoblast differentiation:** Immunostained healthy (AB11727FL2) and DMD (KMS751DMD10FL) human skeletal muscle cells (fascia lata) derived from a 10-year-old DMD patient (exon 52 mutated) were employed and treated with 10µM of BIO101. Myoblast differentiation was investigated by fluorescent microscopy. Signaling pathways: Cells were lysed, equal amounts of proteins were electroblotted on 4-12% SDS-PAGE and transferred following sequential addition of oligomycin, FCCP and rotenone/antimycin A.

**Conclusions**

These results demonstrate the efficacy of BIO101 in the improvement of mdx muscle functionality supported by cellular and molecular changes. BIO101 significantly increased running distance of mdx mice when compared with untreated mice, as well as improving the absolute strength of dystrophin-deficient mdx mice. Most interestingly, (1) energy metabolism (mitochondrial respiration), (2) myoblast differentiation as well as (3) the activation of signaling pathways involved in anabolism (Akt/mTOR) and regeneration (MAPK) known for being impaired in DMD muscle, are all significantly improved by BIO101.

Taken together, these results warrant further preclinical and clinical developments of BIO101 in DMD.

Sarcones (API: BIO101), already in clinical development, could offer a new option, alone or in combination with gene therapies, for the treatment of DMD.