Effects of SARCONEOS (API BIO101) on *in vitro* and *in vivo* models of Duchenne muscular dystrophy

P. Dilda[1], M. Serova[1], S. On[1], B. Didry-Barca[1], M. Latil[1], S. Veillet[1], R. Lafont[2]

[1] Biophytis, Sorbonne Université – BC9, 4 place Jussieu, 75005 Paris, France.
[2] Sorbonne Université, UPMC Univ Paris 06, Paris-Seine Biology Institut (BIOSIPE), CNRS, 75005 Paris, France

Abstract

Duchenne muscular dystrophy (DMD) is an inherited muscular disease characterized by progressive muscle weakness and cardiomyopathy leading to premature death. It is believed that DMD is notably characterized by a systemic mitochondrial defect central to disease aetiology. Sarconeos (API BIO101) is a first-in-class Mas activator currently in clinical development (Phase IIb) for sarcopenia.
Twelve-week-old C57BL10-mdx mice were orally treated with vehicle or BIO101 at 50mg/kg*day for 8 weeks. Immortalized human skeletal muscle cells KM571 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.
BIO101 treatment significantly improved physical performances of C57BL10-mdx mice when compared to vehicle-treated mdx animals: running distance (2.4-fold increase); TA muscle maximal isometric force (+ 15%, p<0.01). KM571 human myotubes exposed to BIO101 for two days displayed an improved basal and maximal mitochondrial respiration (+20% in OCR, p<0.01). Additionally, BIO101 stimulated KM571 differentiation according to increased myofibres diameter, number of nuclei per myotube and fusion index (+21%, p<0.001, +34%, p<0.01 and +7%, p<0.01, respectively), consistent with a rapid and significant activation of AKT/mTOR and MAPK signaling pathways both involved in muscle anabolism.
Our study demonstrates that Sarconeos (API BIO101), increases muscle performance consistently with improved mitochondrial respiration and anabolism. These results warrant further investigation notably on mitochondrial biogenesis and energy metabolism. Sarconeos, for which Orphan Drug Designation applications have been lodged could offer a new option, alone or in combination with gene therapies, for the treatment of DMD.

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 absence of *dystrophin.* Muscles undergo repeated cycles of necrosis/regeneration and are replaced by connective and adipose tissues. Glucocorticoids and supportive therapy are the current standard of care leaving patients with an unmet medical need.
About Sarconeos: Sarconeos 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. Sarconeos has already entered clinical Phase IIb (SARA-INT) 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 Sarconeos.
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. Biophytis clinical programme for DMD (MYODA) 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 maximal running distance was recorded at the 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 cm/s. Then, the speed is increased by 5cm/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 XF24 Analyzer following sequential addition of oligmycin, FCCP and rotenone/antimycin A.

Myoblast differentiation: Immortalized healthy (AB116720FL) and DMD (KM571DMD10FL) 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 electrophoresed on 4-12% SDS-PAGE and transferred to nitrocellulose membranes. Protein expression was revealed by ECL then quantified by densitometry and normalized against GAPDH.

Results

In vivo functional study: mdx mouse model

- *In toto* activity: Exercise tolerance test

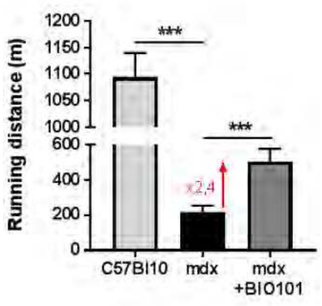

Figure 1: **Effect of BIO101 chronic treatment on exercise tolerance test.** Mdx mice (n=15) ran significantly less than control C57 mice (n=5) (-80.4%, p<0.001).
After 8 weeks of treatment with drugs, *mdx* mice which received BIO101 (n=9) ran significantly more than *mdx* control mice (+136%, p<0.001).

- *In situ* activity: Maximal isometric TA strength

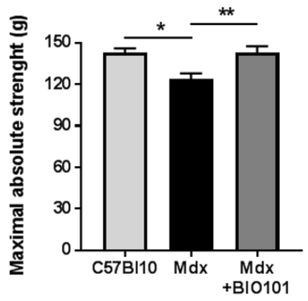

Figure 2: **Effect of BIO101 chronic treatment on tibialis muscle maximal absolute isometric strength (P0).** P0 is decreased in mdx mice (n=15) compared to control C57 mice (n=5) (p<0.05). A significant increase in P0 of the TA was observed in mdx animals daily treated with BIO101 (n=9) (+ 15.3%, p=0.004) compared to non-treated *mdx* mice.

→ BIO101 compensates for the functional loss due to dystrophin deficiency. The overall physical performance of mdx dystrophic mice (in toto activity) is markedly improved by BIO101 (2.4 fold). Consistently, the maximal absolute strength of TA muscle (in situ activity) is also significantly improved.

In vitro study: Mitochondrial respiration

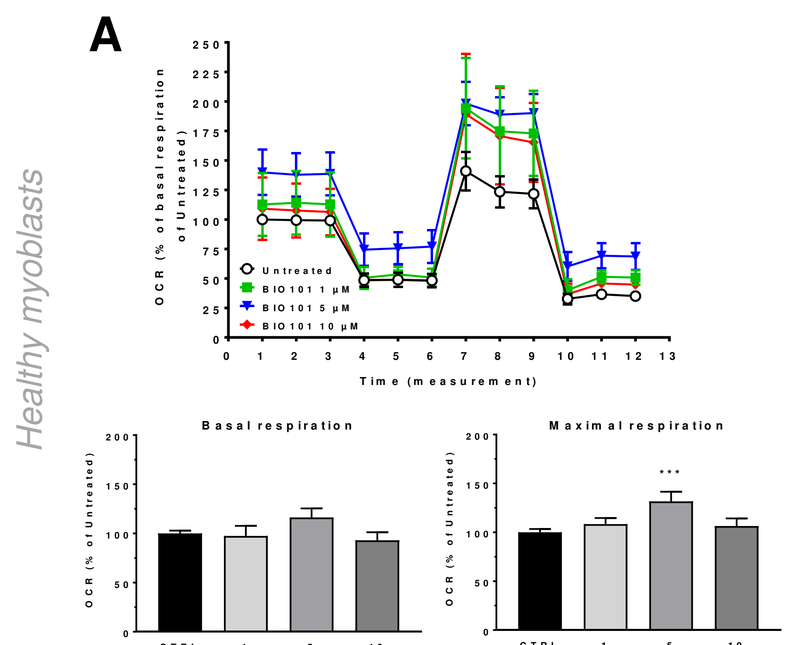

Figure 3: Effects of BIO101 on oxygen consumption in healthy and DMD myotubes. AB1167C20FL (normal human myoblasts, A) and KM571DMD10FL (DMD human myoblasts, B) cells were differentiated for 4 days than BIO101 at 1, 5 and 10µM was added for additional 2 days. Mitochondrial respiration was measured using Seahorse XF24 analyzer at the end of the treatment. Data are normalized over protein content. Bars represent Mean ± SEM of 5 independent experiments. Differences are significant when p<0.05 (*), <0.01 (**), <0.001 (***) using Mann-Whitney test.

→BIO101 demonstrates beneficial effects on mitochondrial respiration in human myocytes regardless their dystrophin gene status.

In vitro study: myoblast differentiation

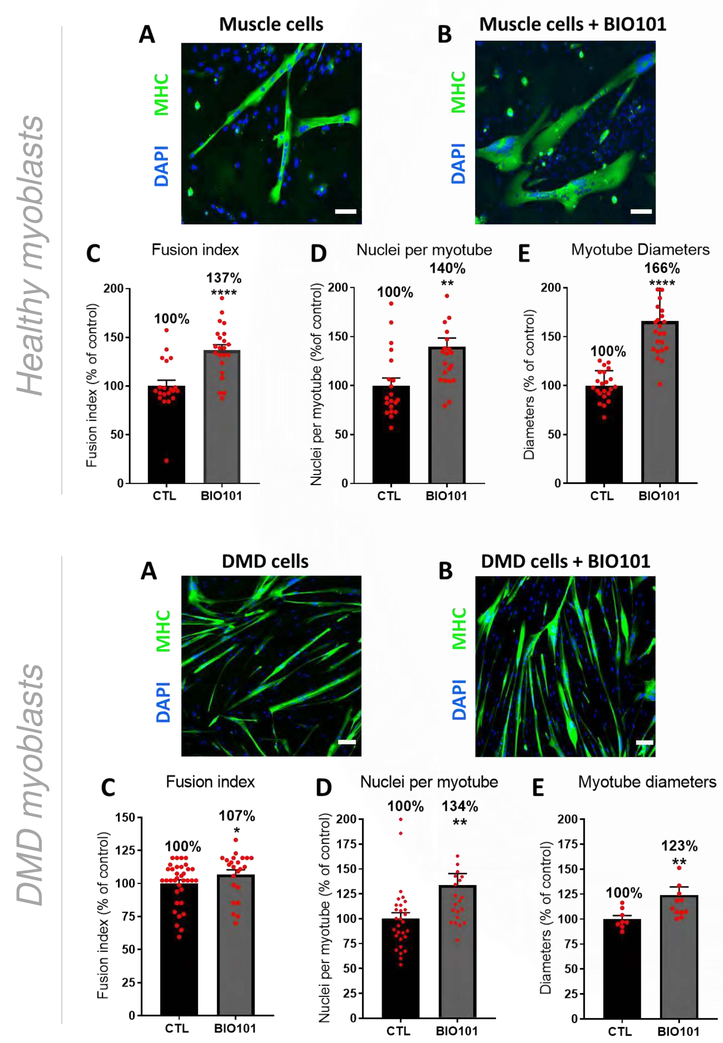

Figure 4: **Effects of BIO101 on normal and DMD myoblast differentiation**. Human normal and DMD skeletal muscle cells were differentiated for 3 days and then incubated with or without BIO101 at 10 µM for 3 days. (**A**) Myosin Heavy Chain (MHC) immunofluorescence of untreated DMD cells (MHC: green; DAPI: blue). (**B**) MHC immunofluorescence of DMD cells treated with BIO101 10 µM for 3 days. Scale bar represents 150 µm. (**C**) Fusion index represents the percentage of nuclei incorporated into MHC positive myotubes over the total number of nuclei. (**D**) Number of nuclei per myotubes (600 myotubes counted). (**E**) Myotube diameter (300 myotubes were measured per condition). Data are shown as mean ± SEM and represents 3 independent experiments. Statistical analysis shows significance between treated cells (BIO101 10 µM) and untreated cells (CTL) using a Mann-Whitney test, *p*<0.05 (*); *p*<0,01 (**) ; *p*<0.001 (***).

→ BIO101 demonstrates beneficial effects on myoblast differentiation regardless their dystrophin gene status.

In vitro study: signaling pathways

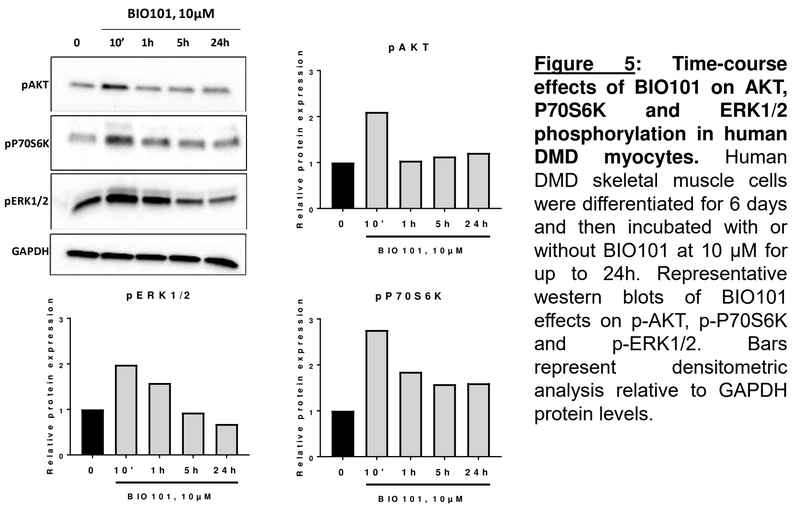

Figure 5: **Time-course effects of BIO101 on AKT, P70S6K and ERK1/2 phosphorylation in human DMD myocytes.** Human DMD skeletal muscle cells were differentiated for 6 days and then incubated with or without BIO101 at 10 µM for up to 24h. Representative western blots of BIO101 effects on p-AKT, p-P70S6K and p-ERK1/2. Bars represent densitometric analysis relative to GAPDH protein levels.

→ BIO101 induces a significant and early activation of AKT and MAPK signaling pathways in human DMD myoblasts.

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 mdx 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.

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