SARCONEOS, a drug candidate in clinical development for sarcopenia, demonstrates sharp functional improvement and anti-fibrotic properties in an animal model of Duchenne muscular dystrophy

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Abstract

Duchenne muscular dystrophy (DMD), is an inherited muscular disease, characterized by progressive muscle weakness and cardiomyopathy, leading to premature death. Muscles undergo repeated cycles of necrosis/regeneration and are replaced by connective tissues. Unfortunately, glucocorticoids remain the only standard of care. Sarconeos, a first-in-class Mas activator in clinical development (Phase 2b) for Sarcopenia, demonstrated improved muscle anabolism and a good safety profile in young and elderly adults after oral administration.

Sarconeos, as well as its derivative BIO103, were daily administered *per os* to 12-week-old control C57BL10 and mdx mice for 8 weeks. At completion of treatment, we observed an improvement of mdx muscle functionality, supported by histological and molecular changes. Indeed, in the exercise tolerance test, Sarconeos and BIO103 significantly increased running distance of mdx mice by 2.4- and 1.7-fold, respectively, when compared with mdx untreated mice. Muscle maximal force was also significantly improved by 15.3% and 22.5% with Sarconeos and BIO103 treatments, respectively. In the heart, gene expression of fibrosis (CTGF) and hypertrophy (myh7, BMP4) markers were reduced in response to the two drug candidates. Strikingly, histopathological analysis revealed a clear reduction in muscle lesion profile and fibrosis development in treated mdx animals.

These results demonstrate the efficacy of Sarconeos and BIO103 in the improvement of muscle functionality and in the prevention of fibrosis development in a DMD animal model. Sarconeos, already in clinical development, 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 many 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), restoring muscular anabolism, inhibiting myostatin, that had demonstrated meaningful activity in animal models of muscular dystrophies. Sarconeos is developed for the treatment of sarcopenia, an age-related degeneration of skeletal muscles, leading to loss of mobility in elderly people. In a Phase I clinical trial (SARA-PK), Sarconeos showed favourable pharmacokinetics and pharmacodynamics profiles. It is currently entering a clinical Phase 2b trial named SARA-INT. BIO101 is the active principal ingredient of Sarconeos.

Methods

12 week-old C57BL10 and C57BL10 mdx male mice were treated orally for 8 weeks with either vehicle, BIO101 or BIO103 (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 of 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).

Gene expression: gene expression of selected markers was evaluated by semi-quantitative PCR. Heart muscle RNAs were extracted, purified and converted into cDNA to allow gene expression analysis.

Histological studies: TA muscles of all animals were frozen then serial sections (7 µm) were realised and stained using either hematoxyline eosin (HE) or sirius red (SR). A detailed anatomopathological study of the sections stained by HE was performed. The lesional profile of each section was determined. A histopathological study of the sections stained by SR allowed us to quantify the percentage area of fibrosis.

Protein expression: protein expression of Col1a1 was evaluated by Western Blot analysis. Proteins were extracted from gastrocnemius muscle. Protein expression was revealed by chemiluminescence then quantified by densitometry after normalization against GAPDH.

Results

▪ Functional study

- *In toto* activity: Exercise tolerance test

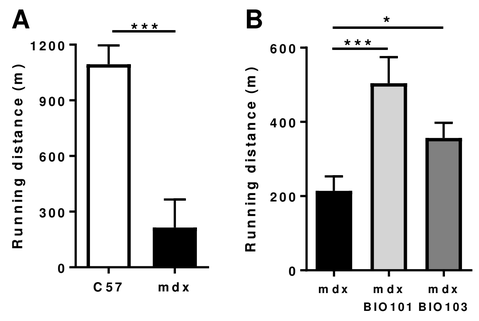

Figure 1: mdx mice (n=15) ran significantly less than control C57 mice (n=5) (**A**; -80,4%, p<0,001). After 8 weeks of treatment with drugs, *mdx* mice which received BIO101 (n=9) or BIO103 (n=9) ran significantly more than *mdx* control mice (**B**; +136%, p=0,0007 and +67%, p=0,0229 respectively).

- *In situ* activity: Maximal isometric TA strength

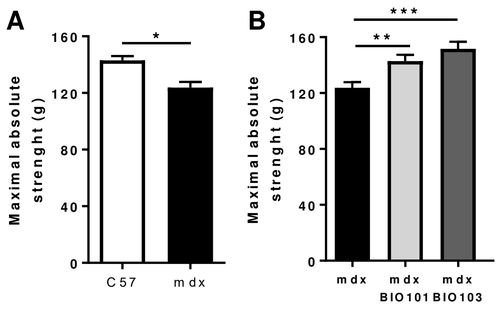

Figure 2: Maximal absolute isometric strength (P0) is decreased in mdx mice (n=15) compared to control C57 mice (n=5) (**A**; p<0,05).
A significant increase in P0 of the TA was observed in mdx animals daily treated with BIO101 (n=9) (+ 15.3%, p=0.004) and BIO103 (n=9) (+ 22.5%, p <0.0001) compared to non-treated *mdx* mice (**B**).

→ BIO101 and BIO103 compensate for the functional loss due to dystrophin deficiency. The overall physical performances of mdx dystrophic mice (in toto activity) is markedly improved by BIO101 (2.4 fold) and BIO103 (1.7 fold), as well as maximal absolute strength of TA muscle.

▪ Histological analysis of TA muscle

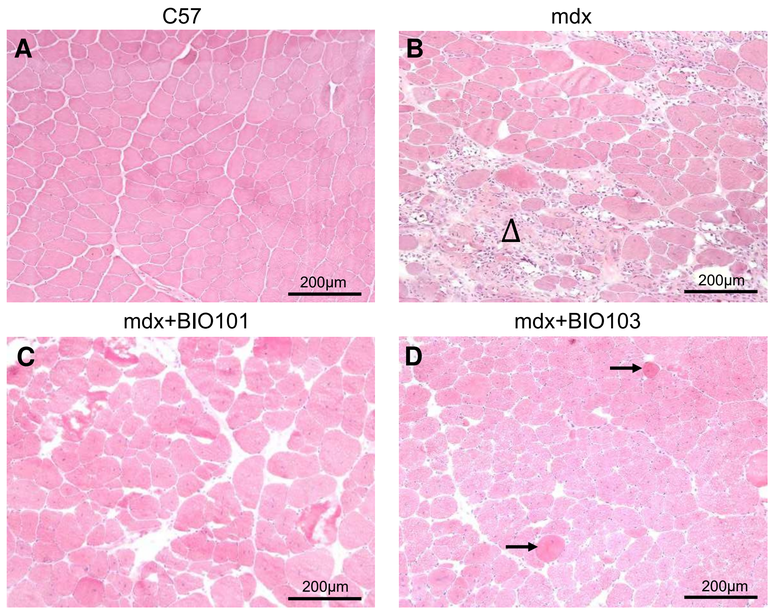

Figure 3: Hematoxilin Eosin coloration of muscle sections reveal that while C57Bl10 TA muscles show no lesion (**A**), untreated mdx mice show marked and diverse muscular lesion profiles. Some muscles harbor a moderate anysocotosis with numerous necrotic fibers. Others show a severe multifocal anysocytosis, with a large myocyte atrophy, as well as an important chronic inflammatory area associated with fibrosis (black triangle) and mononuclear cells (especially macrophages) (**B**). Interestingly, mouse TA muscle treated with BIO101 (**C**) harbor only two types of lesional profiles: a so called "light profile" in which muscle harbor very few anisocytosis, few inflammatory cells and very little necrotic area (37.5%, 3 out of 8 of TA) and a more severe profile with anisocytosis, spread necrotic fibers and variable inflammation (62.5%, 5 out of 8 of TA). Strikingly, mouse TA muscle treated with BIO103 (**D**) present unambiguously fewer muscular lesions with a so called "light profile" (67%, 6 out of 9 of TA). Histologically, a large majority of myocytes are centronucleated (showing different cycles of necrosis/regeneration processes), a light to moderate anisocytosis and rare and sparse necrotic hypercontracted myofibers (initial phase of necrosis; black arrows). Very few inflammatory cells are located in the endomysium.

→BIO101 and BIO103 decrease the severity of muscle lesional profile in dystrophin-deficient mdx mice

▪ Fibrosis analysis in TA muscle (1)

- *Histological study*

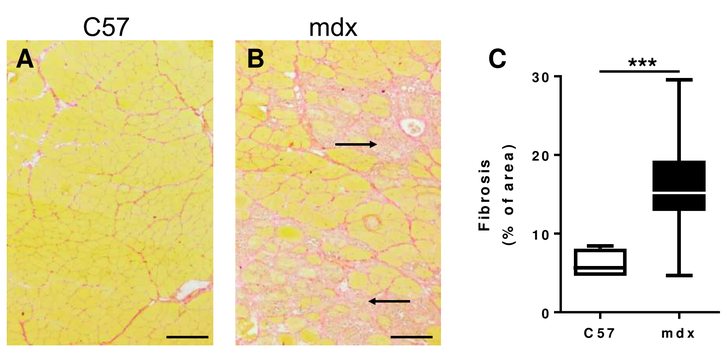

Figure 4: Histological analysis of Sirius red coloration of untreated mdx TA muscles show large fibrotic areas (red coloration, black arrows) compared to C57 normal muscle (**A** and **B**). Percentage of fibrosis area is significantly increased in mdx (p<0.001) compared to C57 control mice (**C**).

▪ Fibrosis analysis in TA muscle (2)

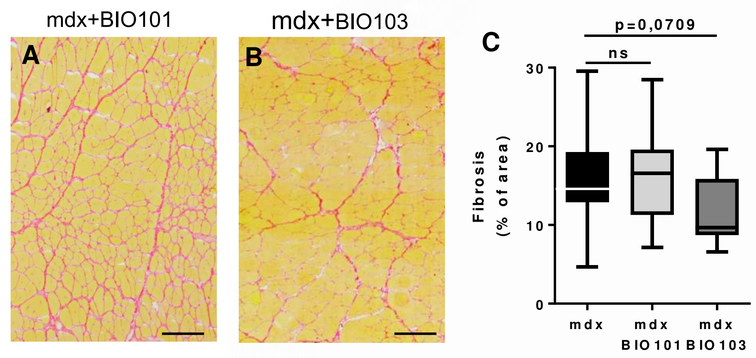

Figure 5: Histological analysis of Sirius red coloration of TA from BIO101 and BIO103 treated mdx TA muscles show a decrease in fibrosis (**A** and **B**) compared to untreated mdx mice, confirmed by the quantification of the fibrosis percentage area (**C**) in TA muscle.

→BIO103 tends to decrease TA muscle fibrosis in mdx mice.

- *Molecular analysis*

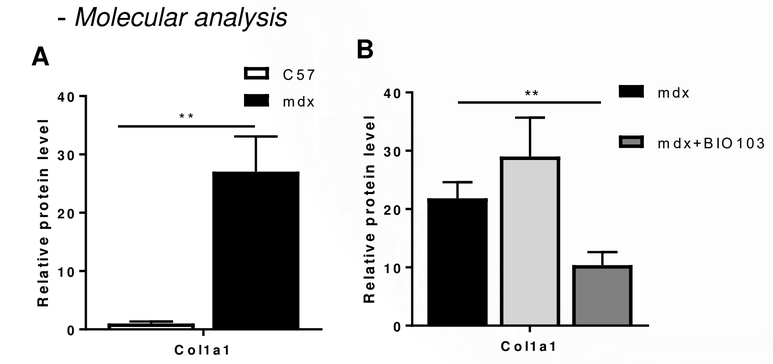

Figure 6: Relative protein expression of collagen 1a1 in mdx muscle was significantly increased (27 fold) compared to control C57 mice (p<0.01) (**A**).
BIO103 treatment of mdx mice showed a significant decrease of relative protein level of Collagen 1a1 compared to mdx untreated mice (p<0.01) (**B**).

→ BIO103 tends to prevent muscular fibrosis in dystrophin deficient mdx mouse model as shown by histological analysis, fibrosis quantification and molecular markers.

▪ Myocardial fibrosis and heart hypertrophy

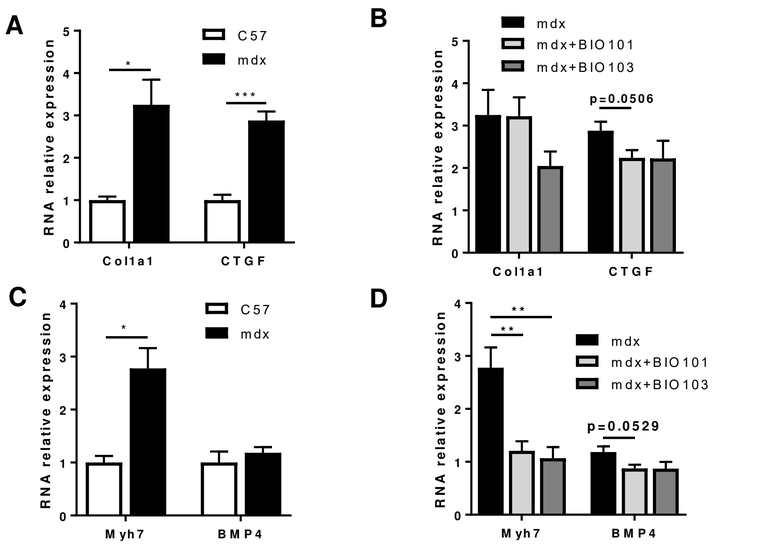

Figure 7: Molecular analysis of fibrosis markers (Col1a1 and CTGF) showed that these genes are strongly upregulated in mdx mice compared to C57 control mice (p<0.05 and p<0.001 respectively) (**A**). Gene expression of these two markers tended to decrease in mdx treated with BIO103. CTGF gene expression tended to decrease (p=0.0506) when mice were treated with BIO101 (**B**).
Cardiac hypertrophy marker evaluation (Myh7 and BMP4) revealed that Myh7 was significantly increased in mdx mice (p<0.01) (**C**). Interestingly, after BIO101 or BIO103 treatments, Myh7 gene expression was significantly reduced (p<0.01). Moreover, BIO101 tended to reduce (p=0.0529) the expression of BMP4 compared to mdx vehicule treated mice.

→ BIO101 and BIO103 decrease cardiac fibrosis of dystrophine deficient mdx mice and reduced heart hypertrophy.

Conclusions

These results demonstrate the efficacy of BIO101, as well as its derivative BIO103, in the improvement of mdx muscle functionality, supported by histological and molecular changes.
BIO101 and BIO103 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.
In the heart, gene expression of fibrosis and hypertrophy markers were reduced in response to the two drug candidates. Strikingly, histopathological analysis revealed a clear reduction in muscle lesion profile and fibrosis development in treated mdx animals.
BIOI101 and BIO103 improve functionality and allow the prevention of fibrosis development in a DMD animal model.

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