Skeletal muscle function

In vitro

4: Effect of BIO 101 treatment on mdx mice

BIO101 reduces the loss of running distance in mdx mice (A)

Respiratory function

Protocol design (II)

Figure staining reveals vehicle-treated muscle as an important chronic inflammatory area associated with fibrosis and mononuclear cells (especially macrophages). In contrast, the BIO 101 two-month-treated mdx TA muscle and variable inflammation (62.5%, 5/8 TA).

Results (I) – Skeletal muscle function

• Protocol design (I)

Oral gavage of mice with BIO101 (50 mg/kg/day) or Vehicle (cycloDEXtrin)

Exercise Habituation

Chronic exercise (2x/week)

12 week-old mdx mice
n=15 mdx Vehicle
n=9 mdx BIO101
n=5 C57Bl/10 vehicle

• Protocol design (II)

Oral gavage of mice with BIO101 (50 mg/kg/day) or Vehicle (cycloDEXtrin)

Exercise Habituation

Chronic exercise (2x/week)

12 week-old mdx mouse
n=10 mdx Vehicle
n=10 mdx BIO101
n=5 C57Bl/10 vehicle

Results (II) – Histological analysis

A

Control

B

mdx

C

mdx+BIO101

Figure 4: Effect of BIO101 treatment on Tibialis anterior (TA) lesion profile. Hematoxylin–Eosin staining reveals vehicle-treated mdx mice show marked and diverse muscular lesion profiles compared to control (C57Bl/10) TA muscles (A). Some muscles harbor a moderate atrophy with numerous necrotic myofibers. Others show a severe multilobular atrophy, with large myocyte atrophy, as well as an important chronic inflammatory area associated with fibrosis and mononuclear cells (especially macrophages). In contrast, the BIO101 two-month-treated mdx TA muscle (C) exhibit two types of lesion profiles; a "light profile" with few foci of atrophy, few inflammatory cells and very little necrotic area (37.5, 5/3 TA) and a more severe profile with atrophy, spread necrotic myofibers and variable inflammation (82.5, 5/3 TA). Scale bars represent 200μm.

Results (III) – In vitro differentiation

A

Untreated

B

+BIO101

C

+BIO101

Figure 5: Effects of BIO101 on DMD myoblast differentiation. Myogenin Heavy Chain (MHC) immunofluorescence of Human DMD (mdx) skeletal muscle cells (KM17/DMD1HL) differentiated during 6 days without (A) or with (B) BIO101 (50 μM). Scale bars represent 110μm. (C) Fusion index represents the percentage of nuclei incorporated into MHC-positive myotubes over the total number of nuclei. (D) Number of nuclei per myotube (%). (E) Myotube diameters (μm). (F) Flow cytometry analysis (G) Flow cytometry analysis of myoblasts from control and BIO101-treated mdx mice. (G) Flow cytometry analysis of myoblasts from control and BIO101-treated mdx mice. (H) Flow cytometry analysis of myoblasts from control and BIO101-treated mdx mice.

Results (IV) – Respiratory function

Oral administration of mdx mice with BIO101 (50 mg/kg/day) or no treatment in drinking water

Whole body plethysmography

Figure 6: BIO101 treatment improves respiratory functions of mdx treated mice. Mdx mice, but not C57Bl/10 mice, increase enhanced Pause (P0) with time (A and B) after 60 days. After 2 months of oral treatment, BIO101 decreased significantly elevated Pause (A0) at all age of mice (C and D).***p<0.001, **p<0.01, *p<0.05, n.s

Conclusions

These results demonstrate the efficacy of BIO101 in the improvement of mdx muscle functionality. BIO101 significantly increased running distance of mdx mice when compared with mdx untreated mice, as well as improving the absolute distance of dystrophin-deficient mdx limit or loss of muscle functionality over time. Interestingly, (1) muscle histology (lesional profile), (2) myoblast differentiation and (3) respiratory function, known for being impaired in DMD patients, 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 for the treatment of Sarcopenia, could offer a new option, alone or in combination with gene therapies, for the treatment of DMD.

Preclinical characterization of Sarconeos (API BIO101) in Duchenne muscular dystrophy

Mathilde Latil1, Baise Didy-BaHar1, Kamel Mamchaoui2, Maria Serova1, Sissi On1, Stanislav Veillet1, René Lafont1,3, Pierre Dilda1

1/ Biophytis, Sorbonne University – BC9, 4 place Jussieu, 75005 Paris, France; 2/ Center for Research in Myology, GH Pitié-Salpêtrière, Paris 75013, France; 3/ Sorbonne University; Paris-Seine Biology Institute (BIOSIPE), CNRS, 75005 Paris, France

Abstract Number: P11-116-351

Introduction

About Duchenne Muscular Dystrophy (DMD): DMD is a X-linked inherited muscular disease, caused by an absence of dystrophin. DMD is characterized by progressive muscle weakness and cardiomyopathy, respiratory failure and cardiomyopathy, leading to premature death. Muscles undergo repeated cycles of necrosis/regeneration and are replaced by connective and adipose tissues. Glucocorticoids and supportive therapies are the current standard of care leaving many patients, primarily those suffering from respiratory function defect, 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) which demonstrated meaningful activity in animal models of muscular dystrophies. Sarconeos is being tested in an ongoing Phase 2b (SARA-INT) clinical trial in elderly patients with sarcopenia, an age-related degeneration of skeletal muscles, leading to loss of mobility. BIO101 is the active principal ingredient of Sarconeos.

In 2018, BIO101 received Orphan Drug Designation (ODD) in the United States and the European Union. Biophytis is preparing for the clinical development of Sarconeos in DMD through its MYODA program. See posters P11-128-4459 and P11-129-4460 for more information on the design of the MYODA program.

Results (I) – Skeletal muscle function

• Protocol design (I)

Table 1: Effect of BIO 101 treatment on mdx mice (A)

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<th>Group</th>
<th>Running distance (m)</th>
<th>Vehicle (C57Bl/10)</th>
<th>MDX (Control)</th>
<th>BIO101 treated (MDX)</th>
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• Protocol design (II)

Table 2: Effect of BIO 101 treatment on mdx mice (A)

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Results (IV) – Respiratory function

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