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

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Introduction

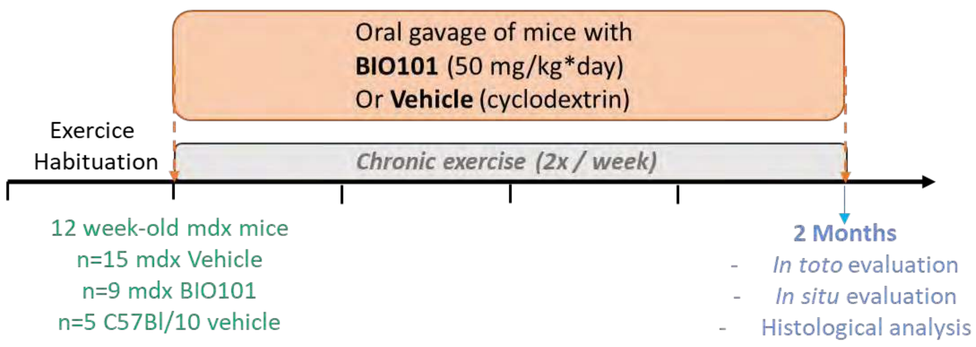
About Duchenne Muscular Dystrophy (DMD): DMD is a X-linked inherited muscular disease, caused by an absence of dystrophin. DMD is characterized by progressive muscle weakness and cardiomyopathy, respiratory failure and cardiomyopathy, leading to premature death. Muscles undergo repeated cycles of necrosis/regeneration 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-#459 and P11-129-#460 for more information on the design of MYODA program.

Results (I) – Skeletal muscle function

▪ Protocol design (I)



- *In toto* activity: Exercise tolerance test

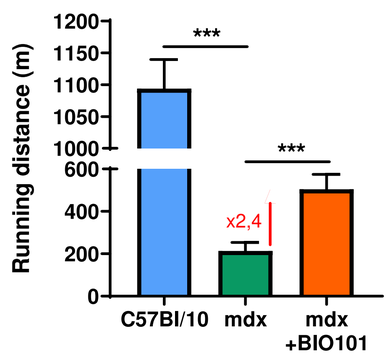


Figure 1: Effect of BIO101 chronic treatment on exercise tolerance test. Mdx mice ran significantly less than control C57Bl/10 mice) (-80.4%, p<0.001). After 8 weeks of treatment, mdx mice which received BIO101 ran significantly more than mdx control mice (+136%, p<0.001).

- *In situ* activity: Maximal isometric TA strength

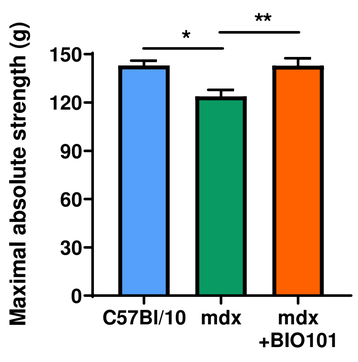
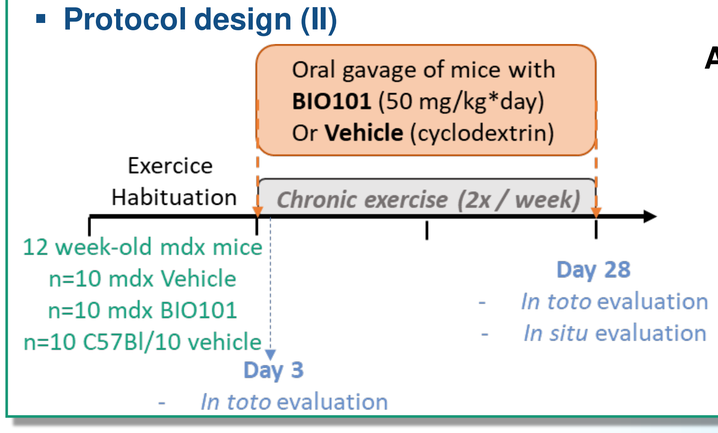


Figure 2: Effect of BIO101 chronic treatment on *Tibialis anterior* (TA) muscle maximal absolute isometric strength (P0). P0 is decreased in mdx mice compared to control C57Bl/10 mice (p<0.05). A significant increase in P0 of the TA was observed in BIO101 daily treated mdx mice (+ 15.3%, p=0.004) compared to non-treated *mdx* mice.

▪ Protocol design (II)



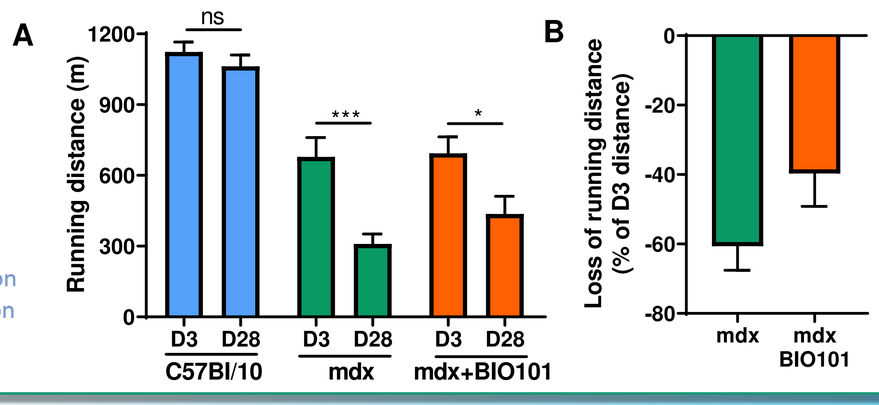


Figure 3: BIO101 reduces the loss of running distance in mdx mice (A) In control (C57BL/10) mice there was no significant loss of running performance (-5.5%, p=ns). In vehicle treated mdx mice, there was a significant decrease of running distance between D3 and D28 (-54.3%, p<0.001) that was less pronounced in BIO101-treated mdx mice (-37.1%, p<0.01), **(B)** Subgroup analysis of only those mice with decreasing performance between D3 and D30 revealed that vehicle-treated mdx mice lost as much as 60.6% of their running performance compared to only 39.7% in BIO101-treated mdx mice *p<0.05, ***p<0.001, student t test.

→ The overall physical performance of mdx dystrophic mice is markedly improved by BIO101 (2.4 fold) after 8 weeks of treatment. Consistently, the maximal absolute strength of TA muscle (in situ activity) is also significantly improved.

Results (II) – Histological analysis

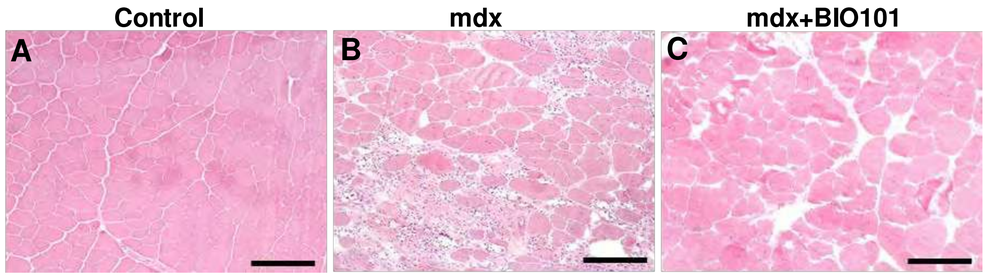


Figure 4: Effect of BIO101 treatment on *Tibialis anterior (TA)* lesion profile. Hematoxylin Eosin staining reveals vehicle-treated *mdx* mice show marked and diverse muscular lesion profiles compared to control (C57BL/10) TA muscles (**A,B**). Some muscles harbor a moderate anisocytosis with numerous necrotic myofibers. Others show a severe multifocal anisocytosis, 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 anisocytosis, few inflammatory cells and very little necrotic area (37.5%, 3/8 TA) and a more severe profile with anisocytosis, spread necrotic myofibers and variable inflammation (62.5%, 5/8 TA). Scale bars represent 200µm.

→ BIO101 improved muscle histology by limiting muscle lesional profile

Results (III) – *In vitro* differentiation

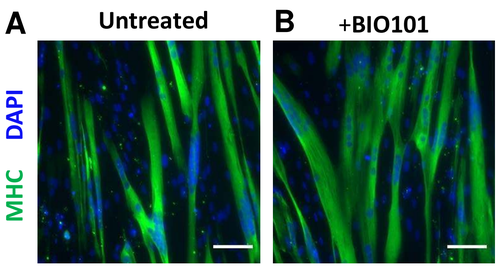
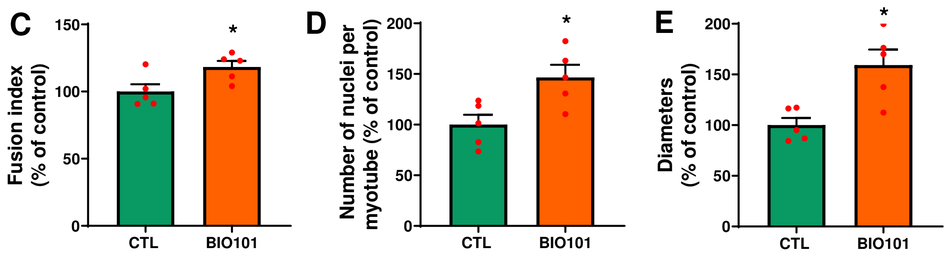


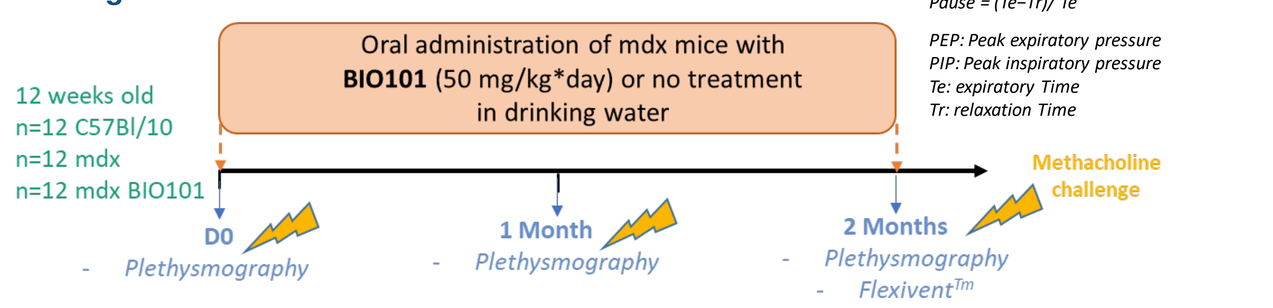
Figure 5: Effects of BIO101 on DMD myoblasts differentiation. Myosin Heavy Chain (MHC) immunofluorescence of human DMD skeletal muscle cells (KM571DMD10FL) differentiated during 6 days without **(A)** or with (**B**) BIO101 at 5µM. Scale bars represent 110µm. (**C**) Fusion index represents the percentage of nuclei incorporated into MHC positive myotubes over the total number of nuclei. (**D**) Number of nuclei per myotubes (600 myotubes counted). (**E**) Myotube diameters (300 myotubes were measured per condition). n=5 experiments, *p<0.05, Mann-Whitney test.



→ BIO101 demonstrates beneficial effects on DMD myoblast differentiation

Results (IV) – Respiratory function

▪ Protocol design



▪ Whole body plethysmography

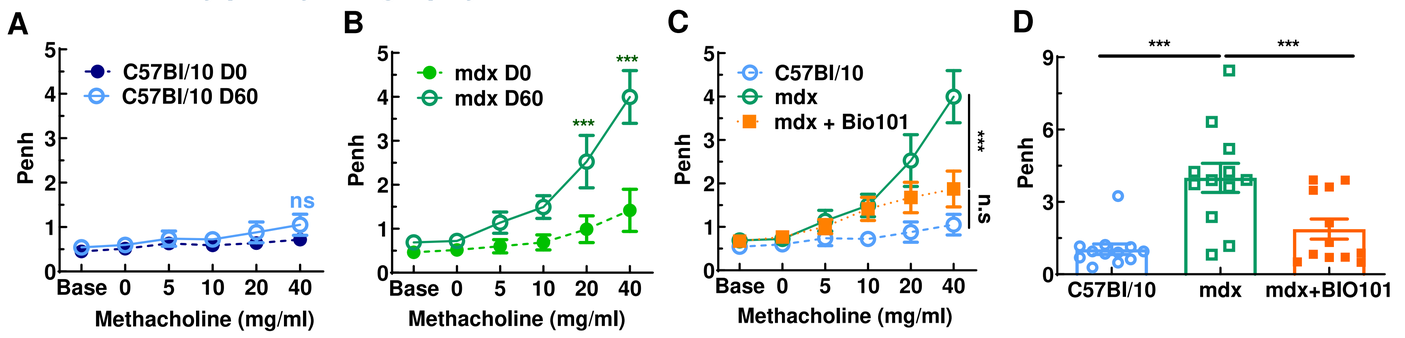


Figure 6: BIO101 treatment improves respiratory functions of mdx treated mice. *Mdx mice, but not C57Bl/10 mice, increase enhanced Pause (Penh) with time **(A and B)** after 60 days. After 2 months of oral treatment, BIO101 decreased significantly elevated Penh of mdx at 40mg/mL of methacholine (**C and D**). n= 10-12 mice per group, *p<0.05, **p<0.001, ***p<0.0001, bonferonni test.*

▪ **Flexivent analysis** (after 2 months of BIO101 treatment)

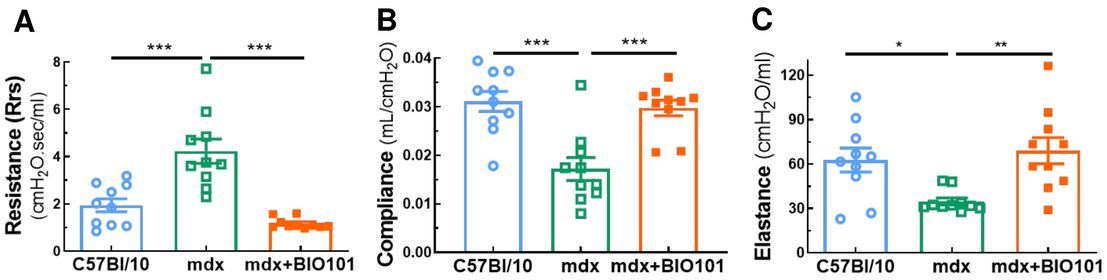


Figure 7: BIO101 treatment ameliorates lung mechanical properties. The lung resistance **(A)**, compliance **(B)** and elastance **(C)** of mdx, BIO101-treated mdx and C57Bl/10 was measured at 20 mg/mL of methacholine by Flexivent. n=10 mice per group, *p<0.05, **p<0.001, ***p<0.0001, bonferonni test.

→ BIO101 treatment significantly improves airway responsiveness as well as deep airway structure as shown by modifications of mechanical properties (resistance, elastance and compliance) in mdx mice and protects from lung function degradation in a time-dependent manner.

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 strength of dystrophin-deficient mdx mice or limit the 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.