BIO101 drug candidate development in rare neuromuscular diseases: Cardiorespiratory and motor unit preclinical evaluation in DMD and in SMA

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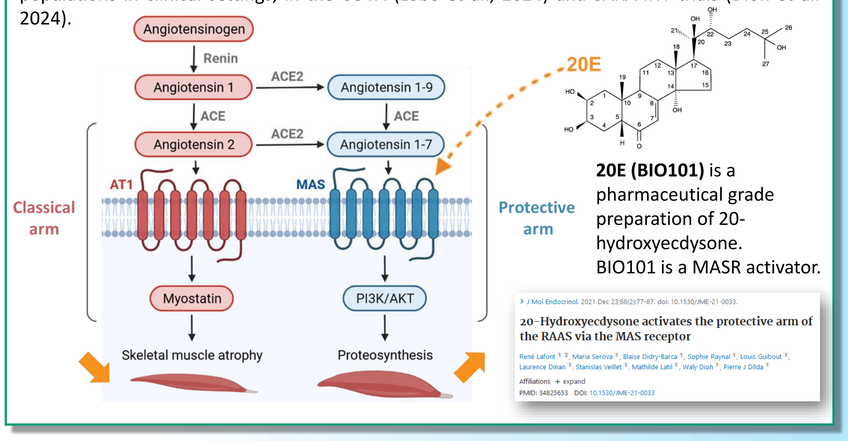
8th International congress myology april 22-25 2024 Palais des Congrès de Paris

Abstract P083

20-Hydroxyecdysone (20E-BIO101)

20E (BIO101) is a new oral drug candidate activating the MAS receptor (a major receptor in the renin-angiotensin system) leading to muscle anabolism, which already demonstrated meaningful activity in animal models of muscular dystrophies (Lafont et al., 2021) and respiratory function. Furthermore, 20E (BIO101) has also demonstrated beneficial effects in two vulnerable populations in clinical settings, in the COVA (Lobo et al., 2024) and SARA-INT trials (Dioh et al. 2024).



20E (BIO101) is a pharmaceutical grade preparation of 20-hydroxyecdysone. BIO101 is a MASR activator.

> J Mol Endocrinol. 2021 Dec 23;68(2):77-87. doi: 10.1530/JME-21-0033.
>
> **20-Hydroxyecdysone activates the protective arm of the RAAS via the MAS receptor**
>
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> PMID: 34825653 DOI: 10.1530/JME-21-0033

Duchenne Muscular Dystrophy preclinical data on respiratory function in mdx mice

- Whole body Plethysmography

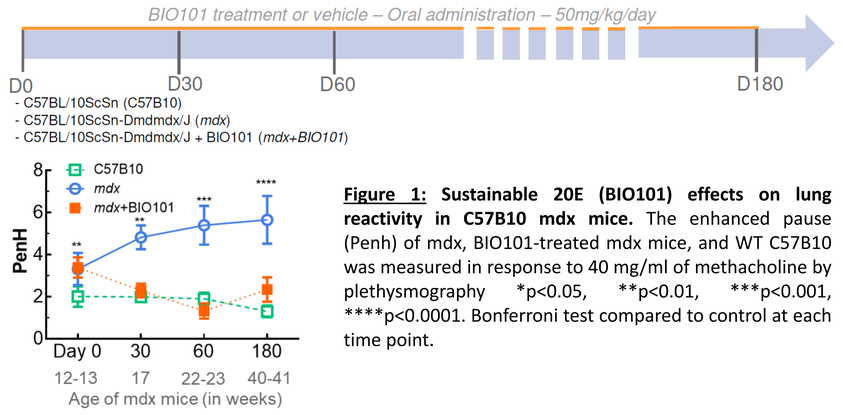


Figure 1: Sustainable 20E (BIO101) effects on lung reactivity in C57B10 mdx mice. The enhanced pause (Penh) of mdx, BIO101-treated mdx mice, and WT C57B10 was measured in response to 40 mg/ml of methacholine by plethysmography *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001. Bonferroni test compared to control at each time point.

➢ *BIO101 treatment significantly improves airway responsiveness as measured by PenH (normalization of Penh values vs control mice).*

➢ *BIO101 effects are sustained for 6 months.*

- Flexivent

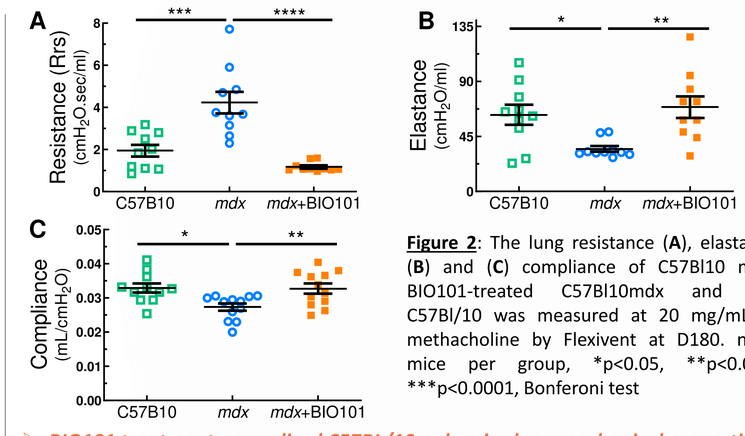
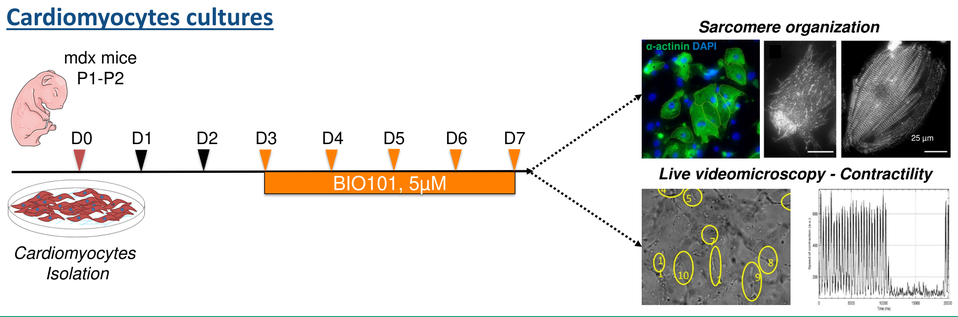


Figure 2: The lung resistance (**A**), elastance (**B**) and (**C**) compliance of C57Bl10 mdx, BIO101-treated C57Bl10mdx and WT C57Bl/10 was measured at 20 mg/mL of methacholine by Flexivent at D180. n=10 mice per group, *p<0.05, **p<0.001, ***p<0.0001, Bonferoni test

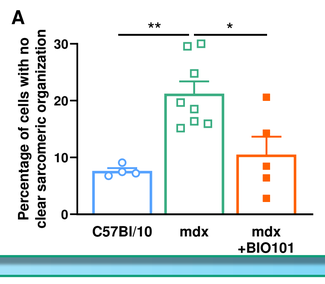
➢ *BIO101 treatment normalized C57BL/10 mdx mice lung mechanical properties such as resistance, elastance and compliance.*

Duchenne Muscular Dystrophy preclinical data on neonatal mdx-derived cardiomyocytes phenotype and function

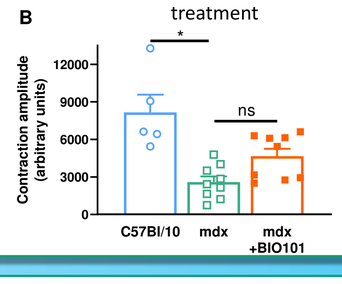
Cardiomyocytes cultures



✓ Sarcomeres organization is improved with BIO101 treatment



✓ Contraction amplitude is not significantly improved with BIO101 treatment



✓ Arrytmias are abolished with BIO101 treatment

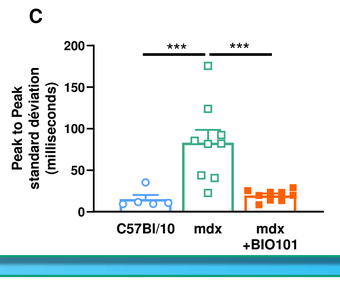


Figure 3: A: Percentage of mdx neonatal cardiomyocytes after 7 days of culture (alpha-actinin immunolabeling) with no clear sarcomere organization **B-C**: Contractility analysis of control and mdx neonatal cardiomyocytes at 7 days of culture, after treatment with vehicle or BIO101 (5 µM) for 4 days. Results are expressed as mean ± SEM. Statistical analysis was performed using t-test, *p<0.05 and **p<0.01.

➢ *BIO101 treatment significantly improves mdx cardiomyocytes functionality*

Spinal Muscular Atrophy preclinical data

BIO101 monotherapy in severe SMA-like mice

Hsieh-Li *et al*. (2000)
Smn $^{-/-}$, *SMN2* $^{2tg/-}$
Median survival of 10 days

From birth
Growth – Survival – Motor function
BIO101 or cyclodextrin (vehicle, VH) 50mg/kg/day
10,5 *post*-natal days (P10,5)
Cellular and molecular analysis

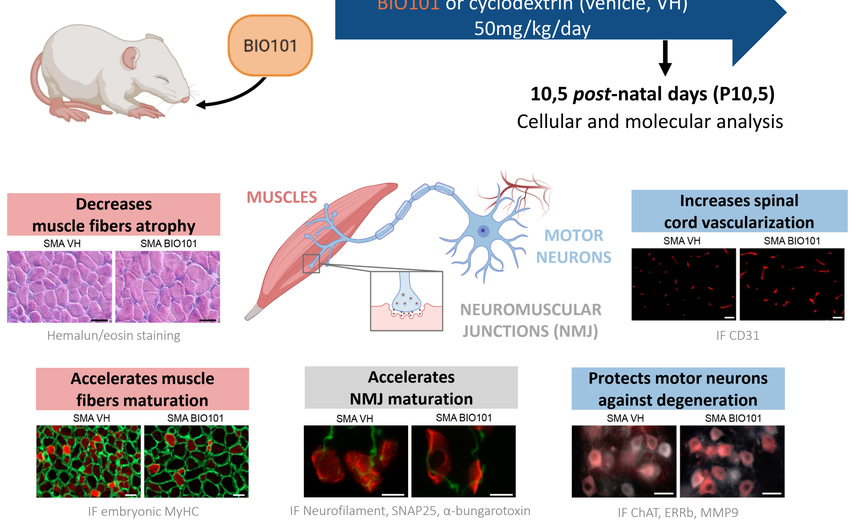


Figure 4: Illustration of the different beneficial effects of BIO101 monotherapy in severe SMA-like mice on the entire motor unit (muscles, motor neuros and neuromuscular junctions)

- Effects on growth, survival and motor function

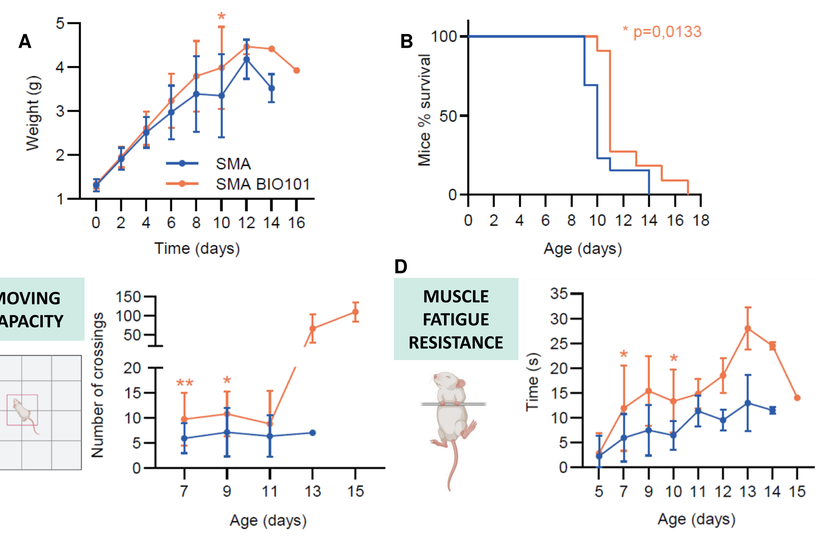


Figure 5: **Effect of BIO101 chronic treatment on growth, survival and motor-function in severe SMA-like mice.** (**A**) Weight curve and (**B**) Survival analysis of VH- and BIO101-treated severe SMA-like mice, (**C**) Open-field quantitative analysis to evaluate moving capacity and (**D**) Grip-test quantitative analysis to evaluate muscle fatigue resistance of VH- or BIO101-treated severe SMA-like mice (Data are presented as mean +/- SD with *p<0,05, **p<0,01).

- Effects on SMN protein expression in skeletal muscles and spinal cord

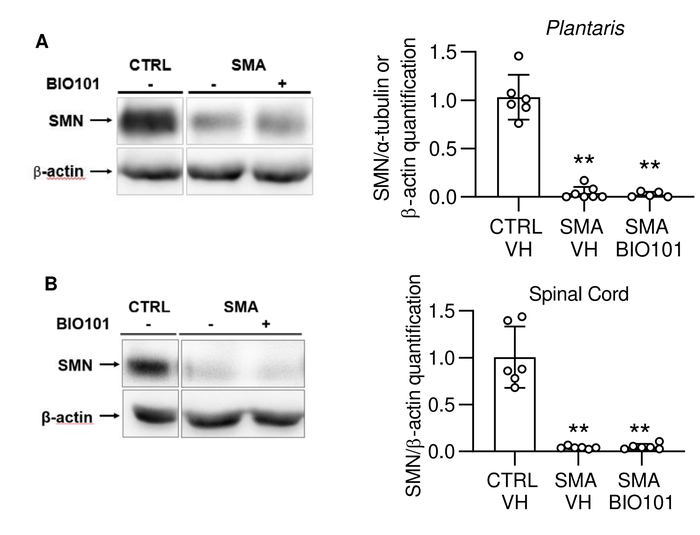
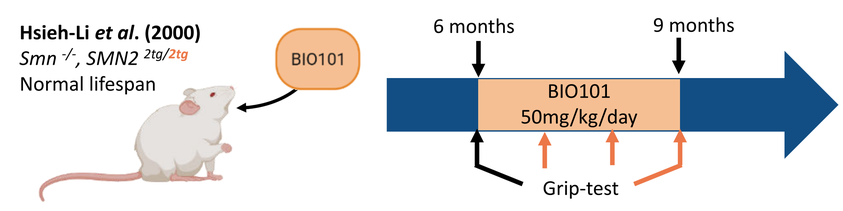


Figure 6: **Effect of BIO101 on SMN expression in the *plantaris* and the lumbar spinal cord of SMA-like mice.** Western Blot analysis and quantification of SMN protein expression in (**A**) the *plantaris* and (**B**) the spinal cord of VH-treated control mice and VH- or BIO101-treated SMA-like mice at P10,5 (Data are presented as mean +/- SD with **p<0,01).

➢ *BIO101 induces beneficial effects on the entire motor unit and delays the weight loss, increases survival, improves moving capacity and muscle fatigue resistance in severe SMA-like mice. BIO101 does not increase SMN protein expression in the plantaris and in the lumbar spinal cord of SMA-like mice. Similar results are found in the tibialis and the soleus (not shown).*

BIO101 monotherapy in mild SMA-like mice

Hsieh-Li *et al*. (2000)
Smn $^{-/-}$, *SMN2* $^{2tg/2tg}$
Normal lifespan



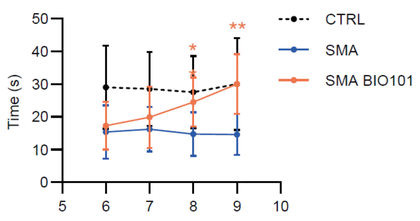
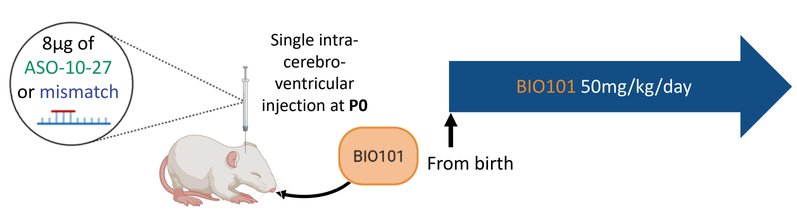
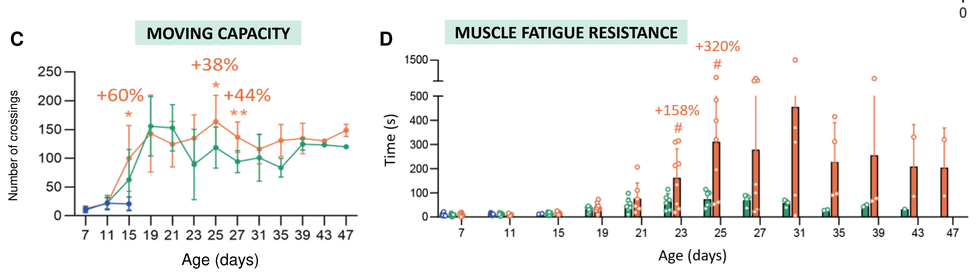


Figure 6: **Effect of BIO101 chronic treatment on muscle fatigue resistance in mild SMA-like mice.** Grip-test quantitative analysis to evaluate muscle fatigue resistance of VH- or BIO101-treated mild SMA-like mice (Data are presented as mean +/- SD with *p<0,05, **p<0,01 compared to the vehicle-treated SMA-like mice).

➢ *BIO101 restores muscle fatigue resistance of symptomatic mild SMA-like mice to control level after 3 months of treatment.*

Combinatorial therapy with BIO101 and ASO-10-27 in severe SMA-like mice





- Effects on growth, survival and motor function

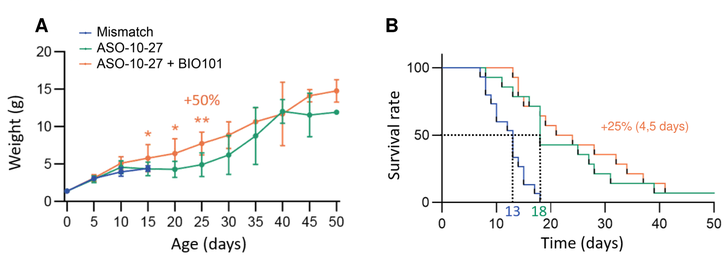


Figure 7: **Effect of BIO101 in combination with ASO-10-27 on growth, survival and motor-function in severe SMA-like mice.** (**A**) Weight curve, (**B**) Survival analysis, (**C**) Open-field quantitative analysis to evaluate moving capacity and (**D**) Grip-test quantitative analysis to evaluate muscle fatigue resistance of severe SMA-like mice treated with ASO mismatch, ASO-10-27 or ASO-10-27 + BIO101. (Data are presented as mean +/- SD with * or # p<0,05, **p<0,01 compared to the ASO-10-27-treated SMA-like mice).

➢ *BIO101 increases lifespan, improves growth, enhances moving capacity and resistance to muscle fatigue in SMA-like mice treated with ASO-10-27.*

Conclusion

➢ **20E (BIO101) is a promising oral treatment for DMD patients with respiratory deterioration and SMA patients.**
➢ Favorable safety profile (SARA-PK phase 1, good safety data on 149 SARA-INT participants with at least 6 Months of dosing)
➢ Beneficial effects on motor function in sarcopenic patients (SARA-INT phase 2b) and beneficial effects on COVID-19 patients with respiratory failure (COVA phase 2/3)
➢ ODD granted in Europe and US, Biophytis intends to start the Phase 1/2 MYODA clinical trial in the upcoming months (**see Poster P360**).
➢ For SMA patients, alone or in combination with approved therapies, BIO101 may improve survival, growth and motor function, especially resistance to muscle fatigue which plays a key role in the quality of life of SMA patients.