In vivo effects of Sarconeos (API BIO101) on a mouse model of severe spinal muscular atrophy

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Introduction

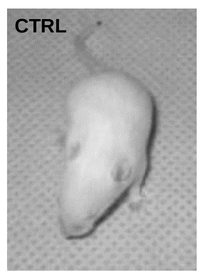
Spinal Muscular Atrophy (SMA) is an autosomal recessive neurodegenerative disease characterized by the loss of spinal cord motor neurons and progressive muscular atrophy, due to insufficient level of Survival of Motor Neuron (SMN) protein (Crawford and Pardo, 1996; Lefebvre *et al*, 2005). SMA is now defined as a non-cell autonomous disease, involving numerous tissues and cell-types, including skeletal muscles (Tony Frugier *et al*, 2001).

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.

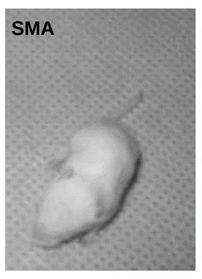
In SMA-like mouse models, it has been shown that NMDA receptor activation and partial invalidation of IGF1-R result in lifespan and motor functions improvement, motor-neurons protection and SMN overexpression in the spinal cord by activating the AKT/CREB pathway and inhibiting the ERK/Elk-1 pathway (Branchu *et al*, 2013; Biondi *et al*, 2015). In this context, using BIO101 administration could improve the neuromuscular functions in SMA by stimulating muscular anabolism and increasing SMN expression through the activation of the Pi3K/AKT pathway via the MAS receptor.

Severe SMA-like mouse model

- $Smn^{\Delta7/\Delta7}$; $huSMN2^{+/-}$ (Hsieh-Li *et al*. 2000)
- Mean survival of 12 days post-natal (P12)
- 50% loss of spinal motor neurons
- Severe muscle aplasia and atrophy

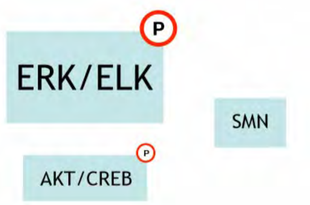


$Smn^{\Delta7/+}huSMN2^{+/-}$



$Smn^{\Delta7/\Delta7}$,$huSMN2^{+/-}$

In pathological conditions:



From birth (P0) to 11 days of age (P11): per os treatment with BIO101 (50mg/kg*day) or vehicle (VH; cyclodextrin)

Results

1. Systemic benefits

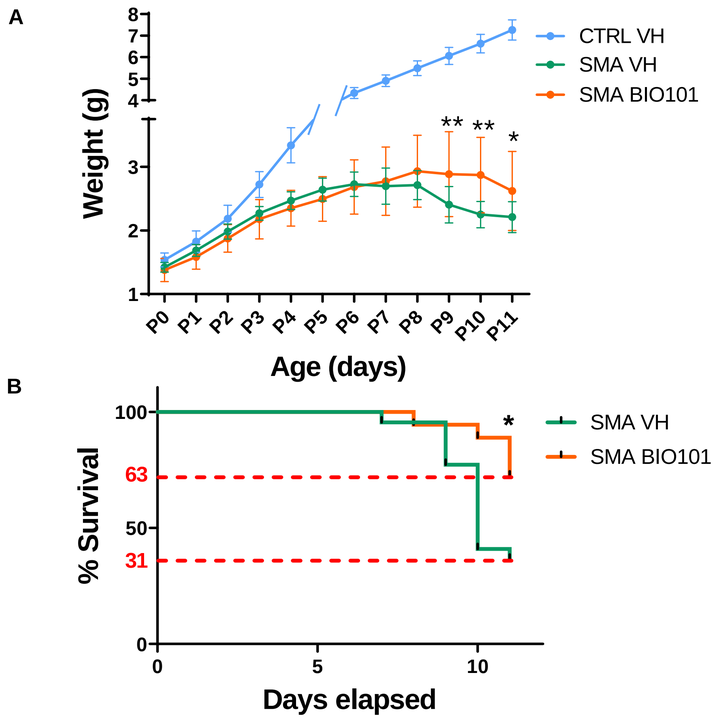


Figure 1: **Effect of BIO101 chronic treatment on body weight and survival in SMA-like mice.** (A) Weight curve of vehicle treated control mice and VH- or BIO101-treated SMA-like mice (n=16 in each group, *p<0.05, **p<0.01) and (B) survival analysis of SMA mice treated with BIO101 or vehicle (n=16 in each group, *p<0.05, **p<0.01).

→ *BIO101 induces a statistically significant limitation of weight loss from P9 and an increase in survival at P11 in SMA-like mice.*

Results

2. Tissue effects

- In muscles: Plantaris

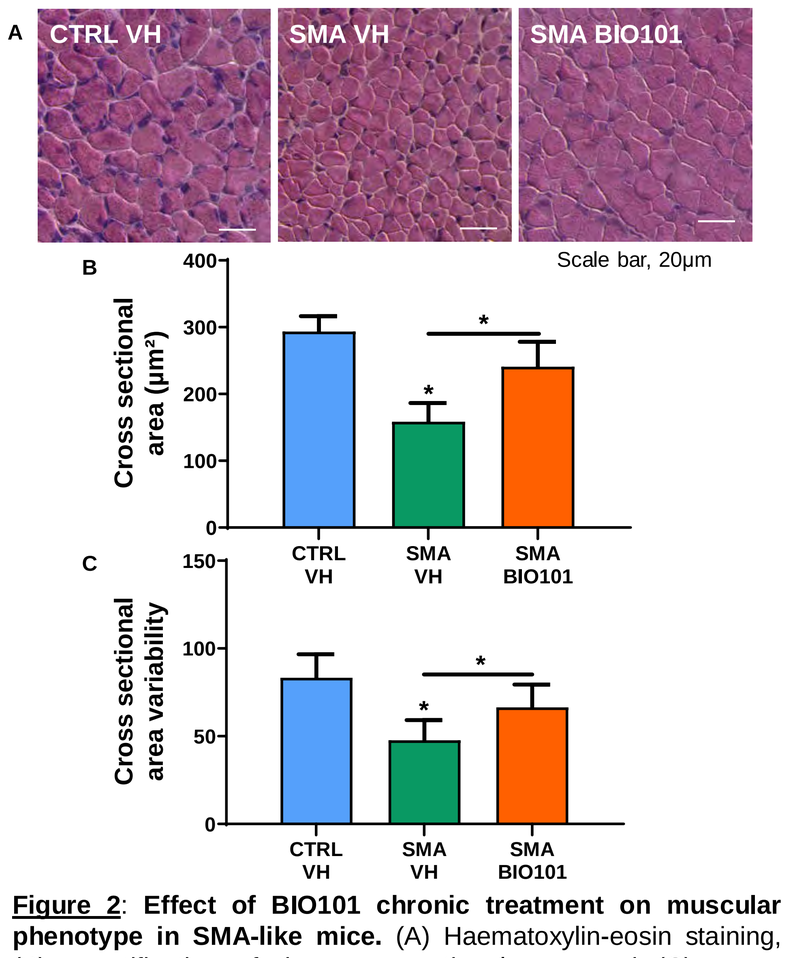


Figure 2: **Effect of BIO101 chronic treatment on muscular phenotype in SMA-like mice.** (A) Haematoxylin-eosin staining, (B) quantification of the cross-sectional area and (C) cross-sectional area variability analysis of myofibers in the *Plantaris* of vehicle treated control mice and VH- or BIO101-treated SMA-like mice at P11 (n=4 in each group, *p<0.05).

→ *BIO101 limits the muscular atrophy, and restore the myofiber size variability in the fast-twitch extensor plantaris of SMA-like mice. Similar results are found also in the fast-twitch flexor Tibialis and the slow-twitch extensor Soleus.*

- In lumbar motor neurons

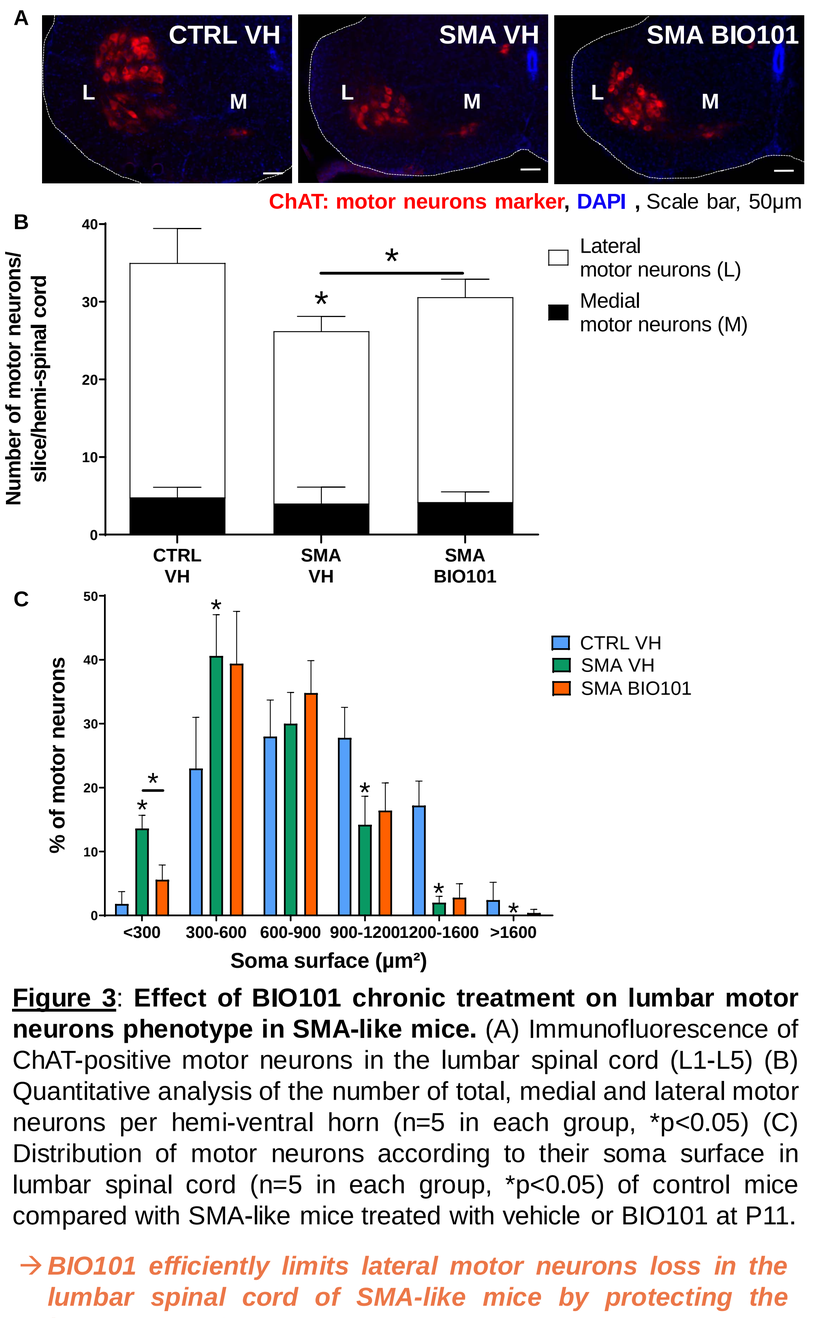


Figure 3: **Effect of BIO101 chronic treatment on lumbar motor neurons phenotype in SMA-like mice.** (A) Immunofluorescence of ChAT-positive motor neurons in the lumbar spinal cord (L1-L5) (B) Quantitative analysis of the number of total, medial and lateral motor neurons per hemi-ventral horn (n=5 in each group, *p<0.05) (C) Distribution of motor neurons according to their soma surface in lumbar spinal cord (n=5 in each group, *p<0.05) of control mice compared with SMA-like mice treated with vehicle or BIO101 at P11.

→ *BIO101 efficiently limits lateral motor neurons loss in the lumbar spinal cord of SMA-like mice by protecting the large-area motor neurons.*

3. Molecular effects

- In muscles: Plantaris

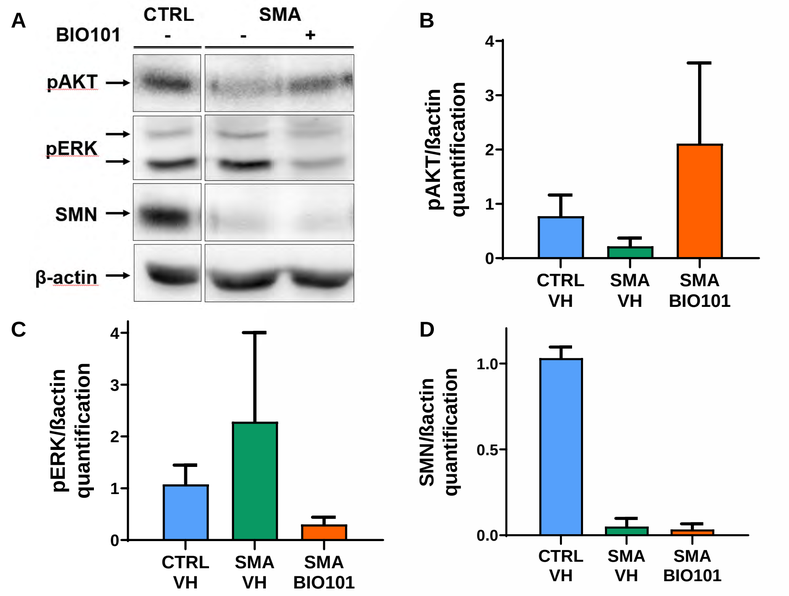


Figure 4: **Effect of BIO101 on AKT, ERK pathways and on SMN expression in SMA-like mice.**
(A) Western Blot analysis and quantification of (B) AKT phosphorylation, (C) ERK phosphorylation and (D) SMN expression in the *Plantaris* of vehicle-treated control mice and VH- or BIO101-treated SMA-like mice at P11 (n⩾2 in each group with ⩾6 mice in each group)

→ *BIO101 activates Pi3K-AKT signaling pathway and inhibits ERK signaling pathway without having any effect on SMN expression in the Plantaris of SMA-like mice. Similar results are also found in the Tibialis and the Soleus.*

- In lumbar motor neurons

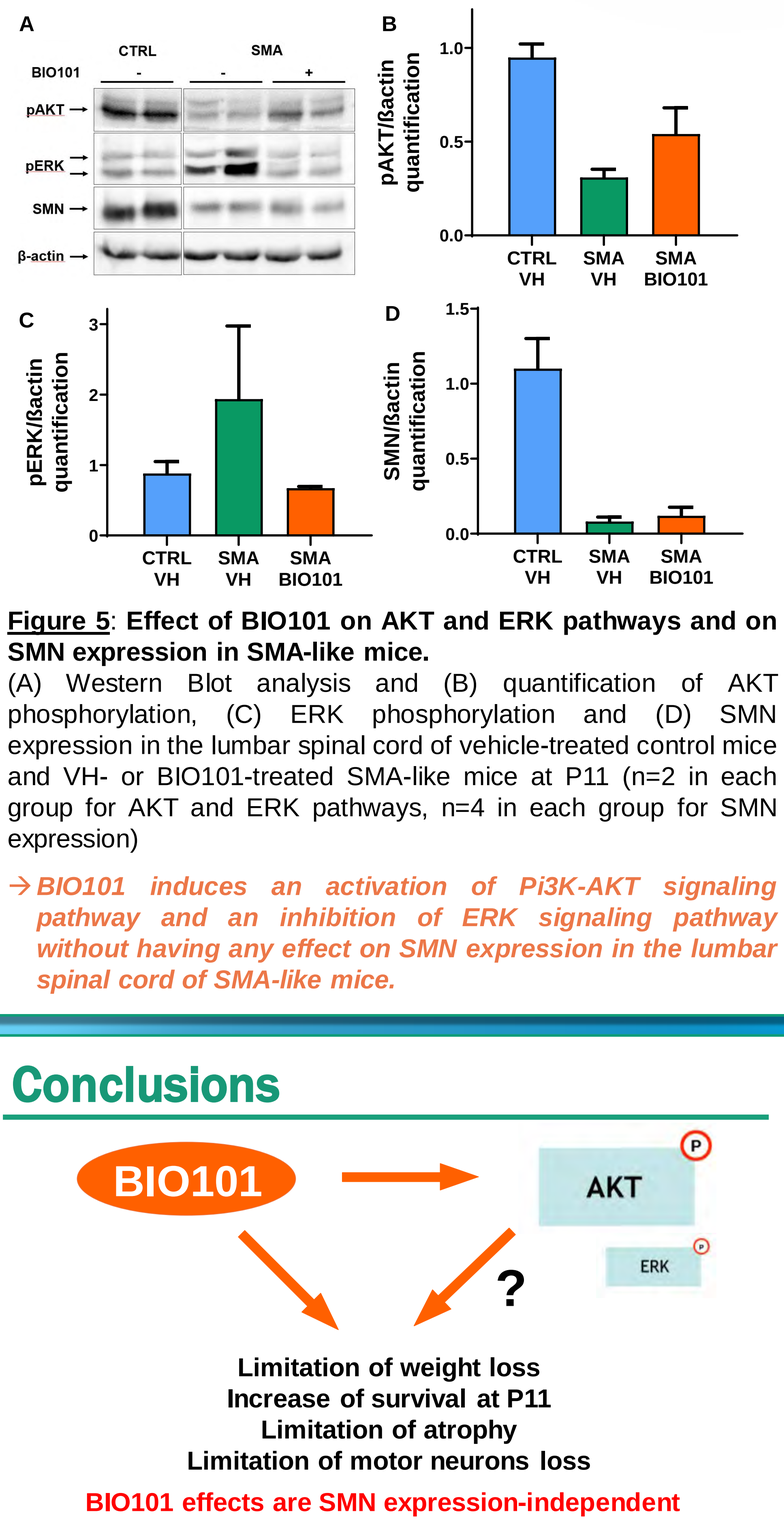


Figure 5: **Effect of BIO101 on AKT and ERK pathways and on SMN expression in SMA-like mice.**
(A) Western Blot analysis and (B) quantification of AKT phosphorylation, (C) ERK phosphorylation and (D) SMN expression in the lumbar spinal cord of vehicle-treated control mice and VH- or BIO101-treated SMA-like mice at P11 (n=2 in each group for AKT and ERK pathways, n=4 in each group for SMN expression)

→ *BIO101 induces an activation of Pi3K-AKT signaling pathway and an inhibition of ERK signaling pathway without having any effect on SMN expression in the lumbar spinal cord of SMA-like mice.*

Conclusions

Limitation of weight loss
Increase of survival at P11
Limitation of atrophy
Limitation of motor neurons loss

BIO101 effects are SMN expression-independent

BIO101, for which Orphan Disease Designation has been granted in Duchenne Muscular Dystrophy, could be considered as a key molecule for a new therapeutic strategy in combination with gene therapies for SMA patients.

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