Intramuscular transplantation in non-human primates of human muscle-derived stem cells (MuStem cells), a promising candidate for cell therapy of muscular dystrophies

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Introduction

Muscular dystrophies (MDs) are a group of severe disorders characterized by fiber degeneration, progressive muscle loss and premature death. **No curative treatment exists** despite the development of pharmacological, molecular-based, and cell therapy strategies. Intramuscular (IM) delivery of myoblasts could be an elective treatment for small to medium size muscles accessible for injections. Nevertheless, it seemed inappropriate to treat numerous large ones, considering the invasiveness of the injection protocol, which prompted the search for alternative cell types able to be distributed by the circulation. **Some stem cells have been described as exhibiting myogenic fate after delivery into damaged or diseased muscle, opening up new therapeutic opportunities.**

In this context, we isolated a muscle-derived stem cell population (termed **MuStem cells**) from healthy dogs and showed that their systemic delivery into immunosuppressed dystrophic dogs leads to muscle regeneration and long-term clinical stabilization. Recently, **we isolated the human counterpart (hMuStem cells) and showed a contribution to myofiber formation** after IM injection into damaged muscle of immunodeficient mice, placing these cells as an attractive candidate for cell therapy of MDs. Considering the specificities of the mouse that regularly lead to poor clinical predictability of studies done in this species, **the hMuStem cell behavior now needs to be investigated into a clinically more relevant context.**

Thus, the aim of the present study is to explore the regenerative potential of hMuStem cells after IM administration in non-human primates (NPHs), using a protocol of cell administration and immunosuppression similar to that already used in the clinical trials that gave the best results in terms of molecular correction in dystrophic patients.

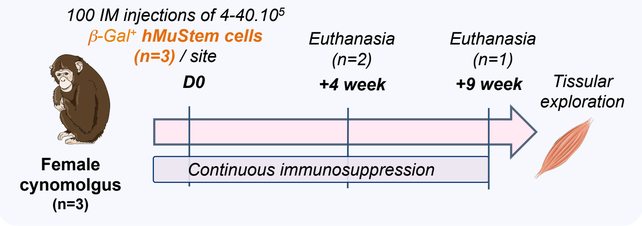
Materials & Methods

Animals. The study included 3 female cynomolgus (*Macaca fascicularis*, age range 3 to 4 years). Immunosuppression was started 5-7 days before transplantation) with a combination of: (a) Advagraf (Astellas Pharma Inc.), an extended release oral formulation of tacrolimus for clinical use: once a day, starting with 5 mg/kg/day and adjusting the dose to reach different tacrolimus blood levels (quantified by liquid chromatography tandem mass spectrometry in blood samples taken just before the Advagraf administration of the sampling day). (b) Dexamethasone: oral, 1 mg/day. For transplantation, monkeys were kept under general anaesthesia. All the procedures was authorized by the Laval University Animal Care Committee.

hMuStem cell isolation and characterization. Human muscle-derived cells were isolated from skeletal muscles of 9 to 15-year-old patients free of known muscle disease. Cells were isolated based on a modified version of preplating protocol initially described by Rouger et al., 2011. hMuStem cells were then cultivated on coated-plastic flasks in proliferation medium containing 10% human serum, PSF and human recombinant growth factors. For phenotypic characterization hMuStem cells were analyzed by FACS using a panel of myogenic, mesenchymal and pericytes markers. Myogenic differentiation was assessed based on cell morphology and the sarcomeric myosin heavy chain (sMyHC) expression. For *in vivo* myogenic potential demonstration, 3.10^5 hMuStem cells (n=4, batches) were independently injected in cryodamaged *Tibialis anterior* muscle of Rag2^{-}IL2rβ^{-} mice (n=3 per cell batch). Three weeks later, tissue localization of hMuStem cells was determined by using co-immunolabelling dedicated to specific human and murine proteins. Nuclei were stained with DAPI.

hMuStem cell transplantation. In vitro expanded hMuStem cells were labeled with a nuclear *lacZ* expression using recombinant nuclear-localizing site (*nls-lacZ)* retroviral particles. Three batches of *nls-lacZ*-transduced cells were independently injected into the left *Quadriceps femoris* of three monkeys (n=9 injected site). Transplantation was performed percutaneously using matrices of 100 parallel equidistant injections of 5 µL in about 1 cm^3 of muscle with 250-µl Hamilton syringe attached to a PB600-1 repetitive device (Hamilton, Reno, NV, USA). A range of 4.10^5 to 4.10^6 cells were delivered per site. To identify the injected muscle sites for later necropsis, stitches of suture were placed on both sides of each transplantation site.

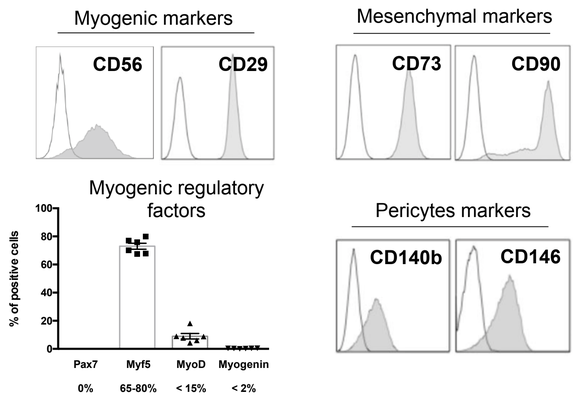
Experimental design.



Tissular exploration. Necropsis of hMuStem cell-injected muscle were taken at 4 weeks (n=2) and 9 weeks (n=1) post-transplantation and mounted in embedding medium, snap-frozen in liquid nitrogen, and stored at -80ºC until performing serial sections of 10 µm in cryostat. Tissue localisation of hMuStem cells was determined using enzymatique revelation of β-galactosidase activity. Histological analysis were performed using hematoxylin eosin safran (HES) and Kernechtrot staining.

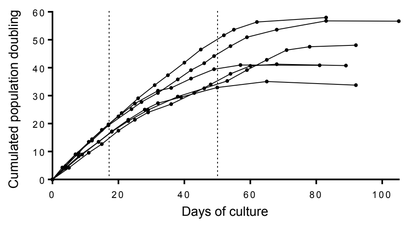
In vitro qualification of human MuStem cells

Expression of lineage surface markers



✓ Early myogenic - committed progenitors with mesenchymal / pericyte signature.

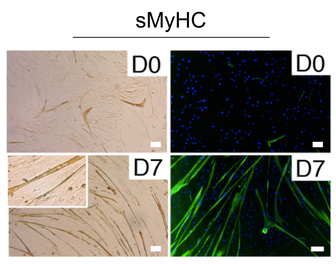
Proliferation capacities



✓ High proliferation rates (20.3±4.2 population doubling level in 38 days, n=6).

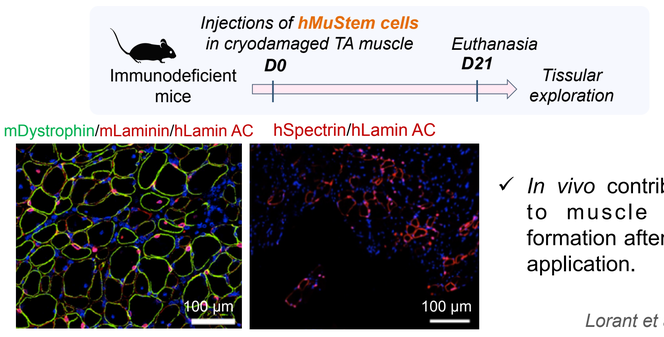
Myogenic potential

***In vitro* demonstration**



✓ *In vitro* contribution to myotube formation

***In vivo* demonstration**



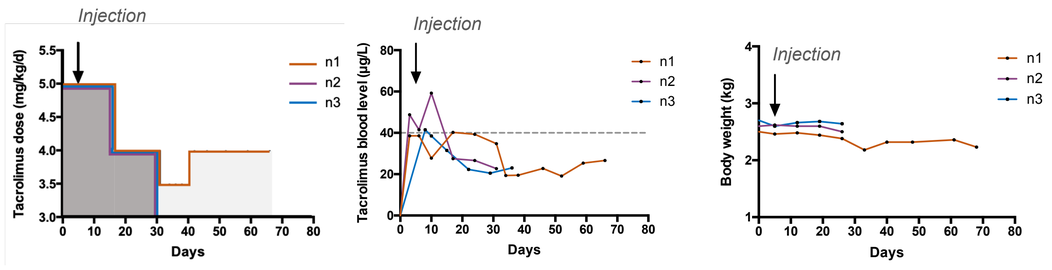
✓ *In vivo* contribution to muscle fiber formation after local application.

Lorant et al., 2018

hMuStem cells: a promising source of adult muscle-derived stem cells for muscle disease therapy

Validation of the experimental context

Clinical settings

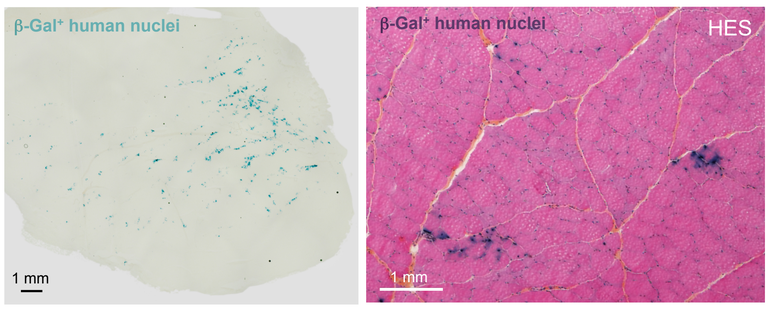


✓ Maintenance of stable Tacrolimus blood level under 40 µg/L.

✓ Maintenance of stable body weight.

Validation of an immunosuppressive protocol to obtain tacrolemia compatible to those obtained in clinic

Histological validation of graft take



Representative pictures of n=9 hMuStem cells-injected sites at 4 weeks (A, x10) and 9 weeks (B, x20) post-transplantation. Histoenzymatic revelation of β-galactosidase activity.

✓ Detection of graft-derived myonuclei (blue nuclei) on every injected muscle sites (n=9).

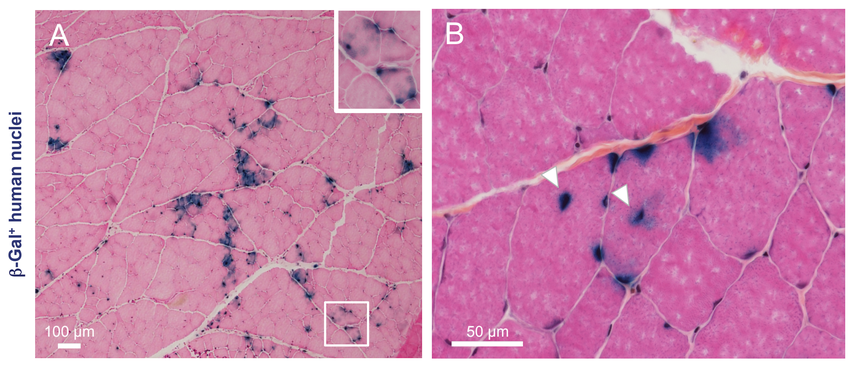
✓ No immune infiltration colocalized with transplanted cells after 4 and 9 weeks.

No sign of immune graft rejection

Successful graft acceptance of hMuStem cells at 4 and 9 weeks after administration

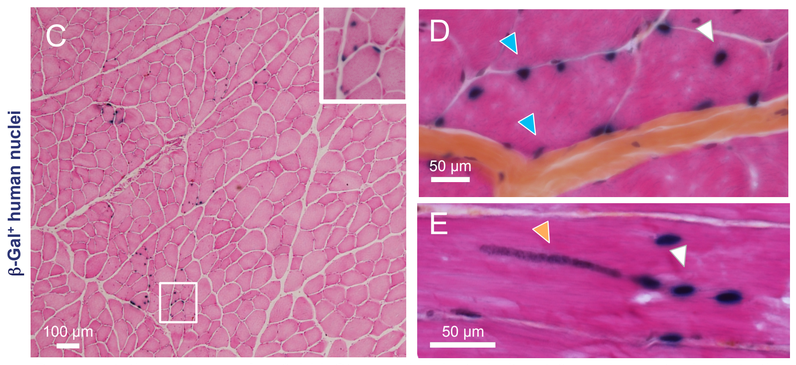
Engraftment capacities of hMuStem cells in NHP muscle tissue

+ 4 weeks post-transplantation



Representative pictures of n=6 hMuStem cells-injected site at 4 weeks post-administration. Histoenzymatic revelation of β-galactosidase activity with Kernechtrot (A, x40) and HES (B, x400) staining.

+ 9 weeks post-transplantation



Representative pictures of n=3 hMuStem cells-injected site at 9 weeks post-administration. Histoenzymatic revelation of β-galactosidase activity with Kernechtrot (C, x40) and HES staining on transverse (D, x400) and longitudinal (E, x400) muscle section.

✓ Numerous hMuStem cell implantation foci in NHP muscle tissu at 4 and 9 weeks (A and C). Each foci are composed of several positive muscle fibers (inset, x400).
→ Implantation demonstration

✓ hMuStem cell nuclei almost exclusively within NHP fibers in peripheric (blue arrows) and central (white arrows) position at 4 and 9 weeks (B, D and E).
→ Fusion capacity and regeneration participation

✓ Chimeric myotubes (E) composed of human (white) and NHP (orange) nuclei in central position.
→ Capacity to interact with host fibers for regenerative process

Demonstration of hMuStem cell contribution to muscle fiber regeneration

Conclusion

- Characterization of hMuStem cells as **early myogenic progenitors** with **myogenic potential**
- **Proof of feasibility** of the hMuStem cell **implantation** into immunosuppressed NHP muscle
- Demonstration of ability to **fuse** with host muscle fibers and **participate to regeneration**

MuStem cells exhibit regenerative potential positioning them as a promising tool for cell-based therapy of muscular dystrophies

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