INTRACEREBROVENTRICULAR TRANSPLANTATION OF NEURAL STEM CELLS IN AN EXPERIMENTAL MODEL OF AMYOTROPHIC LATERAL SCLEROSIS

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BACKGROUND

Amyotrophic Lateral Sclerosis (ALS) is a fatal and progressive neurodegenerative disorder. It is characterized by the loss of both primary and secondary motor neurons (MNs), typically leading to paralysis and eventually death from respiratory failure in less than 5 years from diagnosis.

To date, the pathophysiology of the disease remains largely unknown and for this devastating disease we still have no cure. However, several studies have demonstrated the ability of NSCs to integrate in the host CNS after transplantation, differentiate and elicit pleiotropic healing actions[1], thus fostering their application in the development of experimental treatment for neurological disorders, including ALS.

In accordance, data from our group have demonstrated that the intraspinal transplantation of cGMP clinical grade hNSCs (produced in our Cell Factory certified by AIFA)[2,3] in SOD1^{G93A} rats can prolong animals' survival and reduce the main histopathological markers of the disease[4]. Moreover, we successfully concluded a Phase I Clinical Trial on ALS patients (NCT01640067) by intraspinal transplantation of hNSCs. Promising safety and pioneering efficacy data were obtained[5].

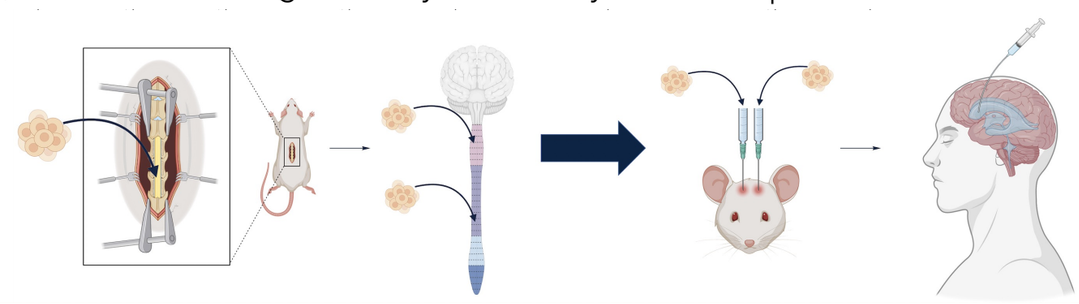
In order to proceed with Phase II Clinical Trial, it is important to deepen our knowledge on the molecular and cellular mechanisms sustaining hNSCs therapeutic activities and to refine the delivery strategy in order to increase cell dosage.

AIMS

Our previous data, both on animal models and ALS patients, suggest that intraspinal transplantation of hNSCs can transiently slow down the disease progression. The duration of the putative beneficial outcomes could be improved and extended by increasing cell dosage. However, the backbone destabilization consequent to the surgery limits the number of spinal cord injections that can be performed. Moreover, this strategy allows to target only the secondary MNs, thus leaving the primary MNs in the motor cortex, recently described as the first to be damaged in a pre-symptomatic stage of the disease[6], degenerating.

Intracerebroventricular injections could represent a novel strategy to treat ALS by increasing cell dosage and favoring a broader spread of the transplanted cells through cerebrospinal fluid, thus targeting both primary and secondary MNs.

Here, we aim at evaluating the safety and efficacy of this transplantation method.



RESULTS

1. hNSCs biodistribution after intracerebroventricular transplantation in immunodeficient mice

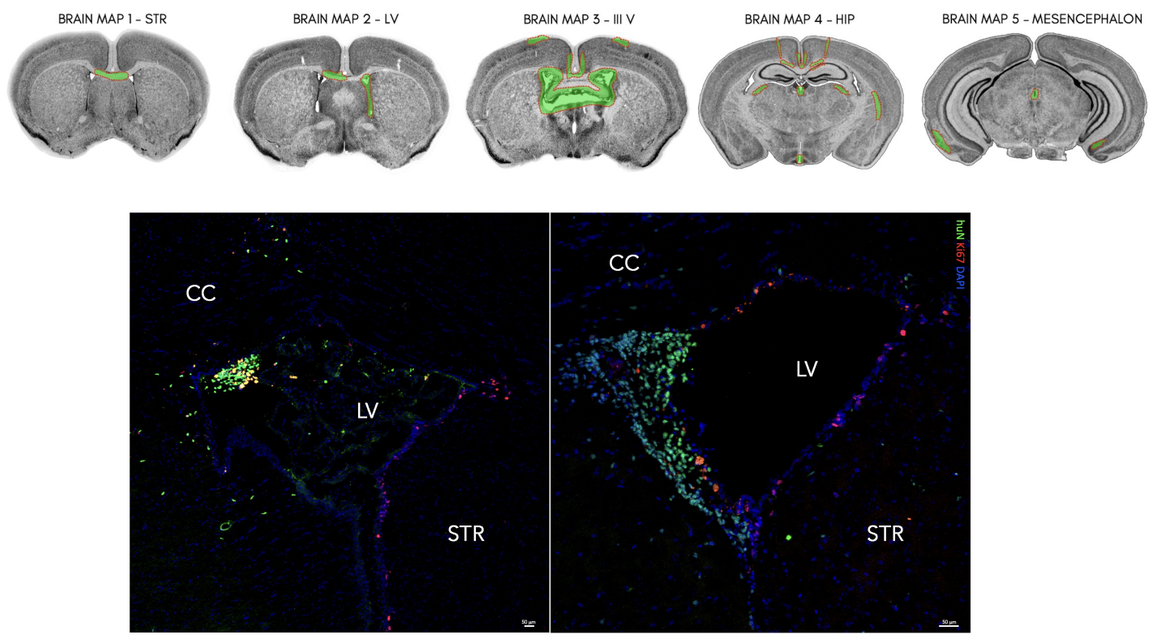


Fig. 1) hNSCs biodistribution map after unilateral transplantation in end-stage mice and representative confocal images showing huN$^+$ (green)/Ki67$^+$ (red) cells transplanted into the lateral ventricle of an immunodeficient mouse. Nuclei are shown by DAPI staining (blue). I hNSCs are well tolerated by the host nervous system and not tumorigenic after 6 months (300,000 cells/mouse). They have an extensive migratory capacity with the possibility to adhere to the ventricular wall, occasionally migrating into the parenchyma. CC: Corpus Callosum; LV: Lateral Ventricle; STR: Striatum.

2. Immunosuppression regimen evaluation

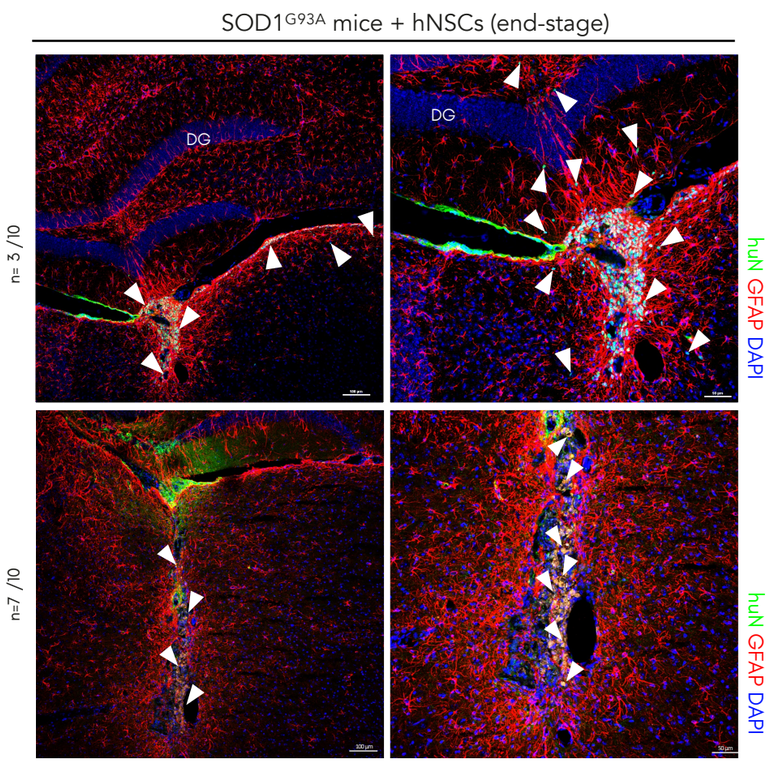


Fig. 2) Representative confocal images of huN$^+$ (green)/GFAP$^+$ (red) cells transplanted into the lateral ventricle (unilateral ICV injection) of ALS mouse models (SOD1^{G93A} mice) sacrificed at end-stage. Nuclei are shown by DAPI staining (blue).

These mice were treated with a 30mg/kg/daily transient immunosuppression regimen for 15 days. Living cells were found in 30% of the analyzed animals.

DG: Dentate Gyrus.

3. Behavioral analysis: end-stage mice

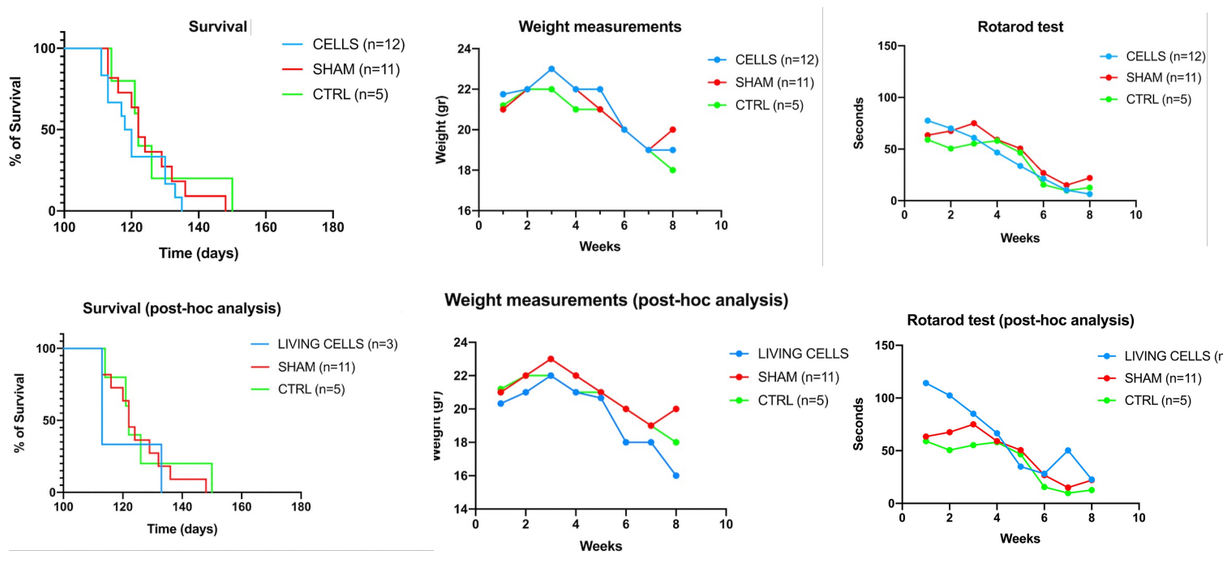


Fig. 3) Interestingly, despite no better outcomes regarding survival and weight analysis, only the 3 end-stage animals with living cells displayed much higher rotarod scores compared to sham and control (SOD1G93A) ones. I CELLS: SOD1G93A mice + hNSCs; LIVING CELLS: SOD1G93A mice + hNSCs found alive after analysis; SHAM: SOD1G93A mice + HBSS; CTRL: SOD1G93A mice.

4. Evaluation of increasing cell dosage

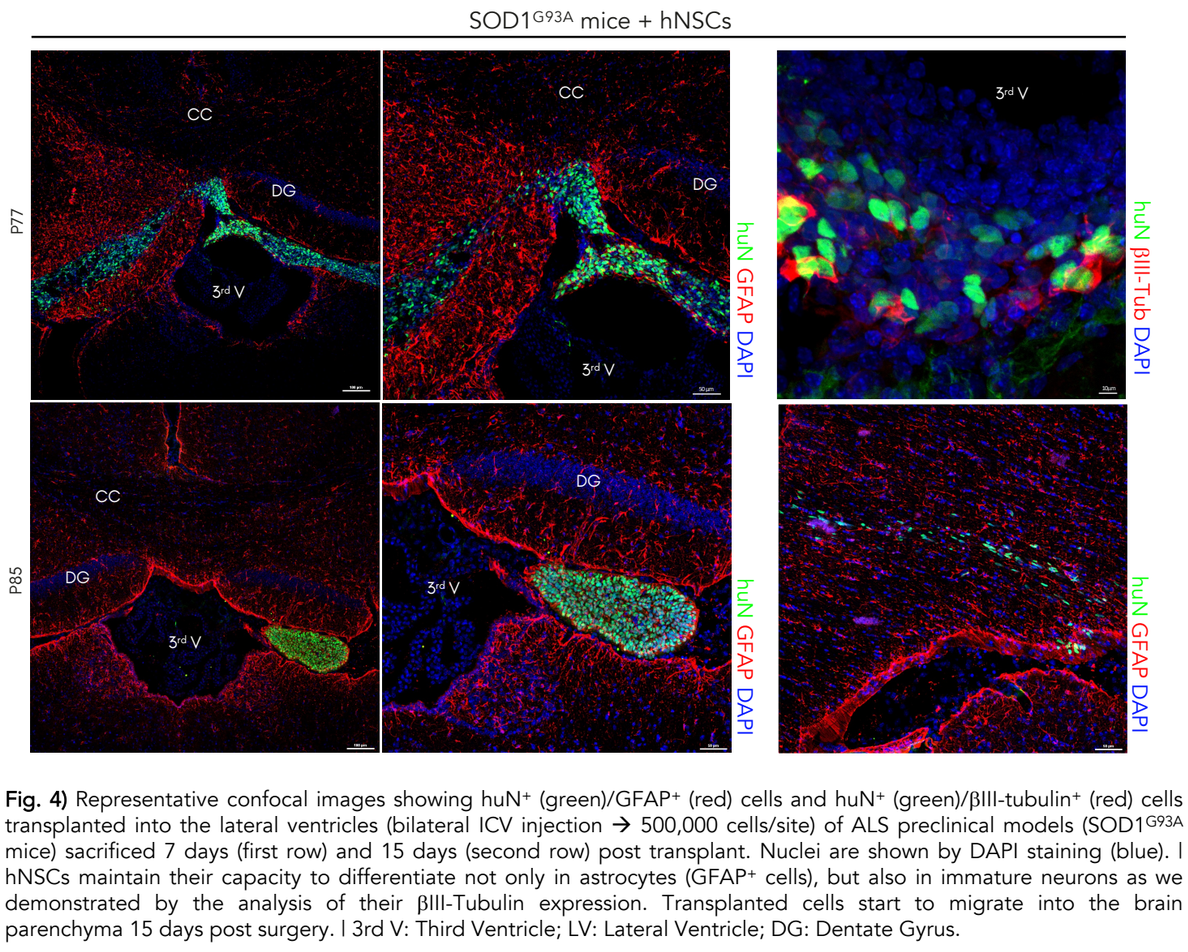


Fig. 4) Representative confocal images showing huN$^+$ (green)/GFAP$^+$ (red) cells and huN$^+$ (green)/βIII-tubulin$^+$ (red) cells transplanted into the lateral ventricles (bilateral ICV injection → 500,000 cells/site) of ALS preclinical models (SOD1^{G93A} mice) sacrificed 7 days (first row) and 15 days (second row) post transplant. Nuclei are shown by DAPI staining (blue). I hNSCs maintain their capacity to differentiate not only in astrocytes (GFAP$^+$ cells), but also in immature neurons as we demonstrated by the analysis of their βIII-Tubulin expression. Transplanted cells start to migrate into the brain parenchyma 15 days post surgery. I 3rd V: Third Ventricle; LV: Lateral Ventricle; DG: Dentate Gyrus.

METHODS

Transplantation

hNSCs were grafted into the lateral ventricle of immunodeficient and SOD1^{G93A} mice. Hsd:Athymic Nude-Foxn1nu mice are provided by Envigo. B6SJL-Tg(SOD1*G93A)1Gur/J mice are provided by The Jackson Laboratory.

Cells were harvested, counted and resuspended with HBSS at ~300,000 cells/3µL. The transplantation was made by using a stereotaxic apparatus (coordinates: - 0,1 mm posterior; ± 0,8 mm lateral, 2 mm ventral from Bregma) with a slow injection procedure (~300,000 cells/3µL/mouse).

Immunohistochemistry

Athymic Nude mice were sacrificed 6 months after hNSCs transplant. SOD1 mice+hNSCs used for the differentiation analysis were sacrificed 7 days after transplantation. SOD1 mice+hNSCs (n=12), SOD1 mice+HBSS (sham; n=11) and SOD1 mice (controls, n=5) used for behavioral analysis were sacrificed at end-stage. All animals were sacrificed via intracardiac perfusion with PFA 4%. Brains were frozen in OCT with nitrogen vapours and sliced by using a cryostat at 20mm and at 30mm with the free-floating technique. Immunohistochemistry stainings were performed by using the following primary antibodies: huN (Millipore, Ms, 1:200); GFAP (Dako, Rb, 1:500); Ki67 (Novus Biologicals, Rb, 1:500); βIII-tubulin (BioLegend, Rb, 1:400).

CONCLUSIONS

A) In the animal model, hNSCs intracerebroventricular transplantation is feasible and safe.

B) The transplanted cells can spread throughout the brain, differentiate and occasionally migrate into the cerebral parenchyma.

C) A 30mg/kg/daily transient immunosuppression regimen for 15 days after surgery might be sufficient to guarantee cell survival.

D) The post-hoc analysis of the end-stage SOD1^{G93A} mice with living cells underlined a better rotarod performance, compared to sham and control animals, that might suggest a positive healing effect exerted by our cells.

E) hNSCs transplanted bilaterally into the lateral ventricles of ALS animal models are perfectly viable after 7 and 15 days post-surgery and they maintain their capacity to differentiate.

FUTURE PERSPECTIVES

- To verify cell viability in later timepoints after transplant.
- To analyze MNs survival in mice motor cortex.
- To study cell biodistribution after bilateral ICV injection: spinal cord-analysis.
- To analyze MNs survival in mice spinal cord.
- To analyze behavioral data from bilateral ICV injection in order to possibly corroborate the data obtained after a unilateral ICV injection.

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