Preliminary data of ICV delivery of human-Neural Stem Cells in a mouse model of Amyotrophic Lateral Sclerosis

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Background & Aim

Amyotrophic lateral sclerosis (ALS) is a relentlessly fatal neurodegenerative disease affecting both upper and lower motor neurons in motor cortex, brainstem and spinal cord.
To date, the pathogenesis is largely unknow and effective therapies still lacking[1].
Neural Stem Cell (NSCs) transplant is under evaluation as a promising strategy to tackle this devastating disease. In accordance, intra-spinal cord transplantation of hNSCs showed beneficial effects during pre-clinical studies on SOD1^{G93A} rats[2]. Their therapeutic potential is likely relying on their neuroprotective paracrine effect. These encouraging results led to phase I Clinical Trials (NCT01640067, NCT01348451) which demonstrated that this approach is feasible and safe and that could transiently decrease the progression of the disease. Despite these positive outcomes, subsequent cell-dose- escalation studies highlighted several limits of this procedure[3,4].
We are investigating the use of the intracerebroventricular (ICV) transplant of hNSCs as a less-invasive method for cell delivery in the CNS and as an alternative strategy for the intraparenchymal injection. ICV transplant is a valuable standardized surgical procedure that can be leveraged to increase cell dosage (thanks to the large ventricular lumen) and to possibly maximize the spread of the hNSCs putative healing factors throughout the entire neuroaxis by exploiting the cerebrospinal fluid (CSF) circulation. Here, we are evaluating the safety and the putative efficacy of this procedure in a non-clinical setting by performing behavioral and histopathological analysis on immunodeficient and SOD1^{G93A} mice thus supporting the translational potential of ICV injection for ALS.

1. CELL ISOLATION and CHARACTERIZATION

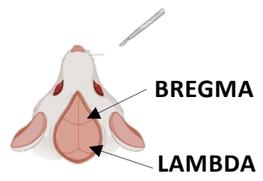
14/16 weeks human foetal brain [5]

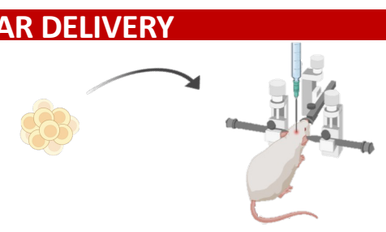
Human Neural Stem Cells [6] (hNSCs)

- Growth curve
- Clonal efficiency
- Multipotency test

2. BILATERAL INTRACEREBROVENTRICULAR DELIVERY

SOD1^{G93A} mice





Automatic stereotaxic apparatus

Safety and Biodistribution of hNSCs in immunodeficient mice

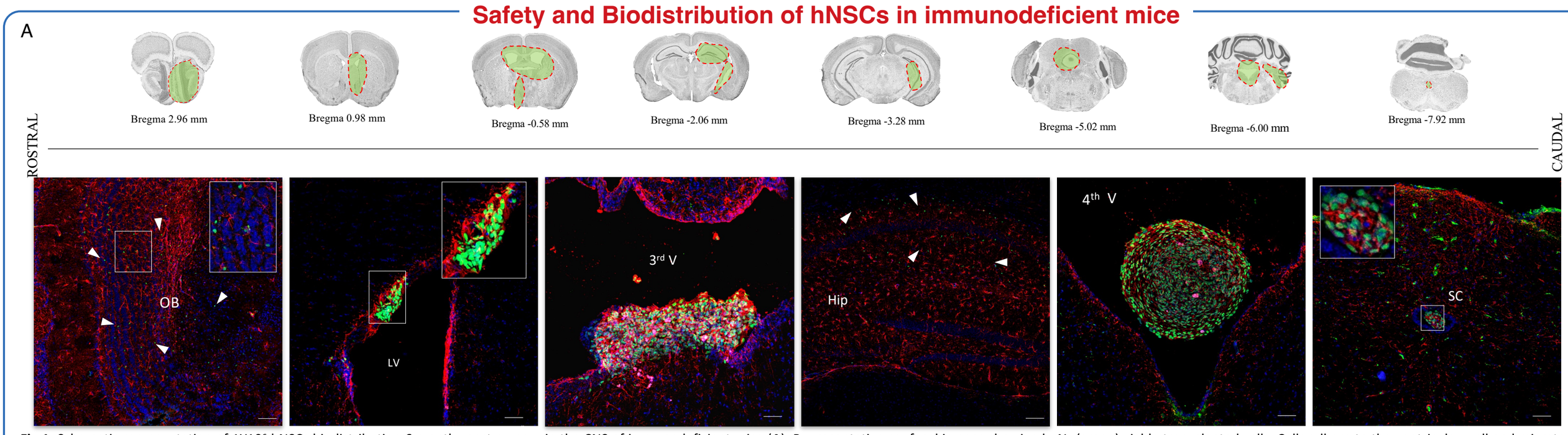
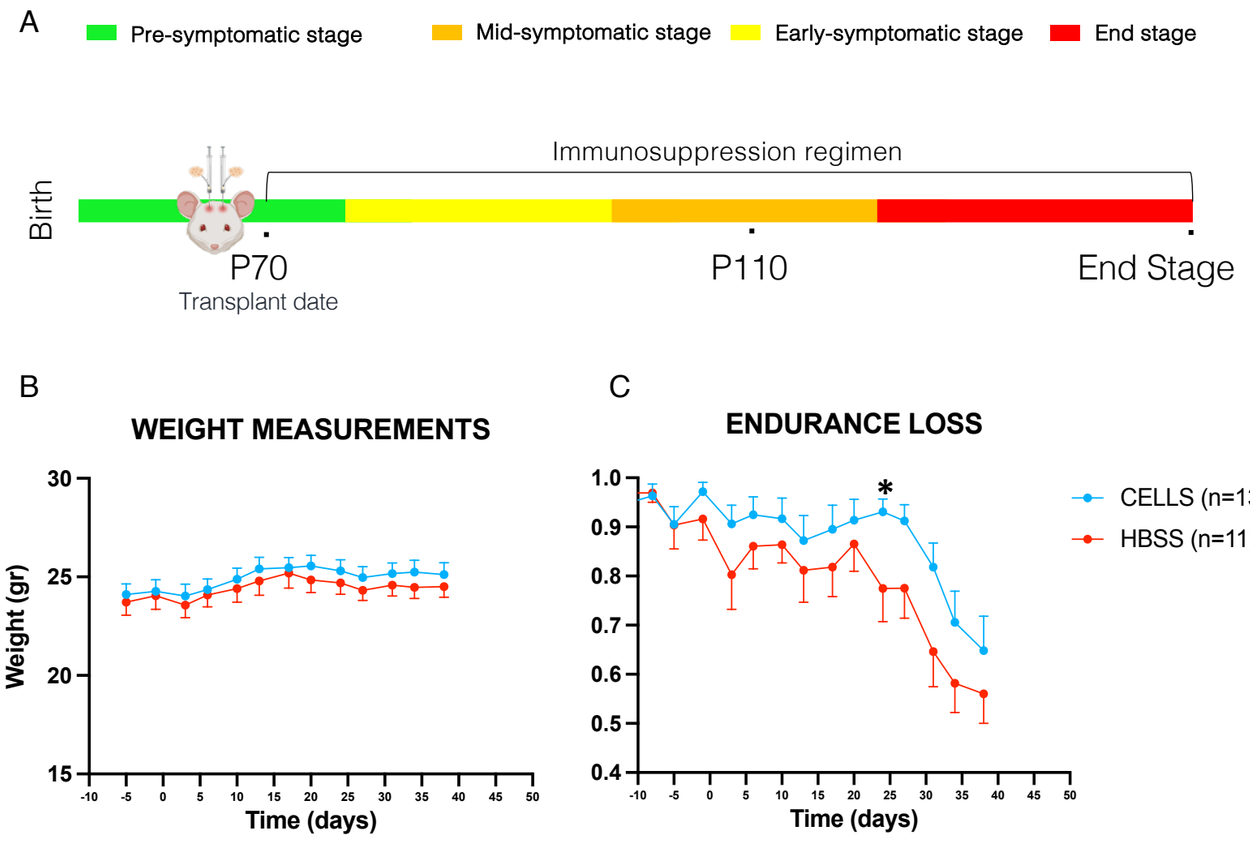


Fig.1. Schematic representation of 1X10^6 hNSCs biodistribution 6 months post-surgery in the CNS of immunodeficient mice (**A**). Representative confocal images showing huN$^+$ (green) viable transplanted cells. Cells adhere to the ventricular wall and migrate in the cerebral parenchyma reaching the corpus callosum (CC), striatum (Str), lateral septal nucleus (LSN), septofimbrial nucleus (SFi), hippocampus (Hip), Sylvius Aqueduct (Aq), lateral recess of the 4th ventricle (LR4V) and 4th ventricle until the central canal of the spinal cord (SC). Of note, transplanted cells can differentiate in astrocytes (GFAP$^+$, red). Scale bars: 100 µm. Nuclei are shown by DAPI staining, blue.

Efficacy evaluation in SOD1^{G93A} mice: Behavioral Analysis



Neuroinflammation Analysis

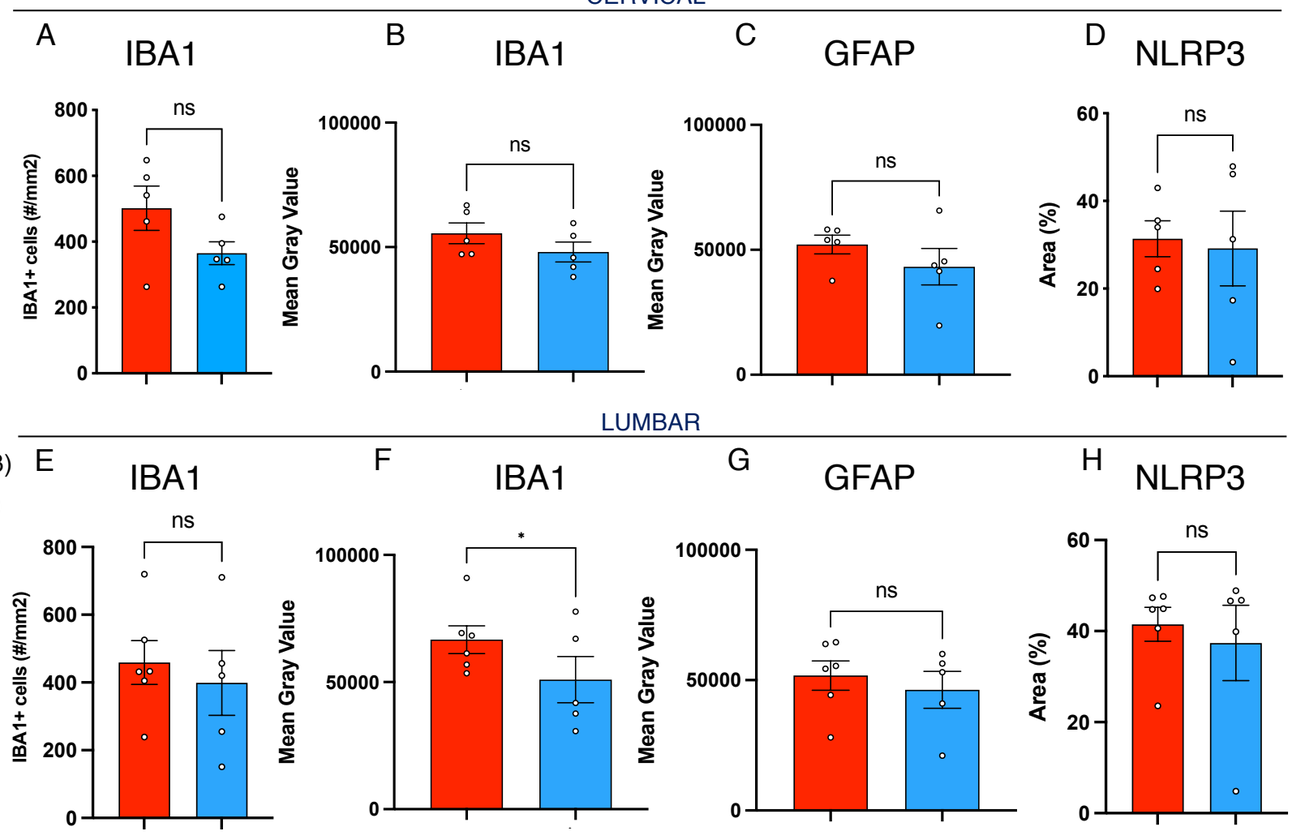


Fig. 2. Disease time course of SOD1^{G93A} mice under a permanent immunosuppression regimen. SOD1^{G93A} mice underwent a bilateral ICV injection of 1x10^6 hNSCs at post-natal day 70 (P70). Behavioral and histopathological analysis were performed until P110 (**A**). Trend of weight measurements (**B**); (**C**) Endurance loss measured by rotarod test. The curves of all the behavioral analysis were obtained by plotting the mean time spent on the rotarod by each group at each time point. (CELLS, n=13, sky blue, HBSS, n=11, red).

Fig. 3. Neuroinflammation analysis on cervical and lumbar segment of the spinal cord. Microgliosis has been evaluated by plotting IBA1$^+$cells and IBA1$^+$ mean gray value (**A, B, E, F**). Astrogliosis has been evaluated by measuring GFAP$^+$ mean gray value (**C, G**). Statistical analysis was performed by t-test (*p < 0.05, **p < 0.01). Data are reported as mean ± SEM.

Spinal cord Stereological Analysis

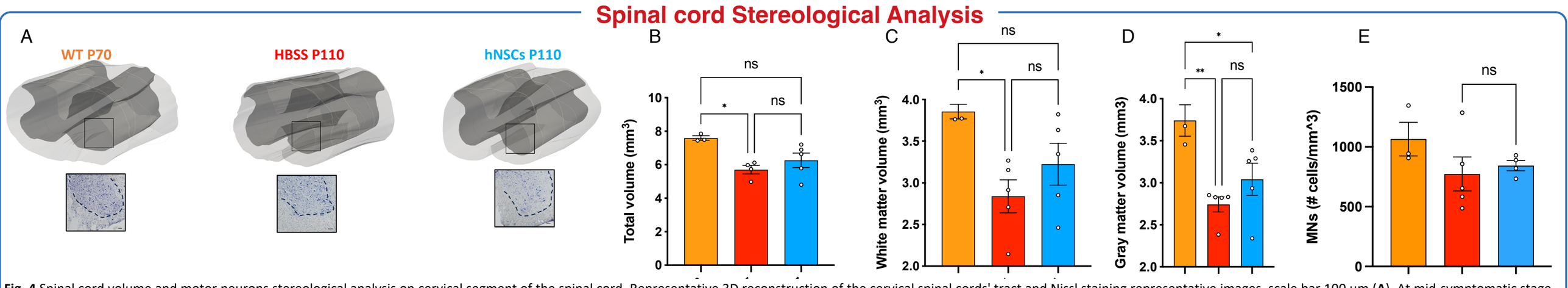


Fig. 4 Spinal cord volume and motor neurons stereological analysis on cervical segment of the spinal cord. Representative 3D reconstruction of the cervical spinal cords' tract and Nissl staining representative images, scale bar 100 µm (**A**). At mid-symptomatic stage (P110) of the disease the total area, white and gray matter area of the cervical part of the spinal cord are notably decreased in SOD1 ^{G93A} mice+ HBSS compared with WT P70 control. (**B-D**). The motor neurons (MNs) count in the ventral horn of the cervical part of the spinal cord (**E**). Statistical analysis was performed by one-way ANOVA (*p < 0.05, **p < 0.01). Data were reported as means ± SEM.

CONCLUSIONS

Our preliminary data suggest that ICV transplantation of hNSCs is a safe and feasible procedure. Moreover, hNSCs are well tolerated and not tumorigenic up to 6 months post transplantation. Transplanted animals show a positive trend of motor performance in the behavioral test and neuroinflammation analysis suggest a slight reduction of microgliosis in the cervical and lumbar part of the spinal cord. Spinal Cord stereological analysis highlighted an increasing trend in the cervical portion of SOD1^{G93A} mice + hNSCs. Although, further studies are necessary to confirm these hypothesis.

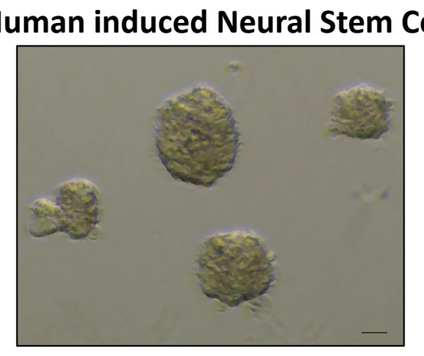
FUTURE PERSPECTIVES

Dissecting metabolic profile of ALS patients-derived Cells

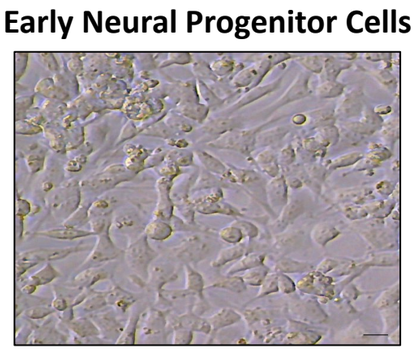
Seahorse XFE 96 Analyzer

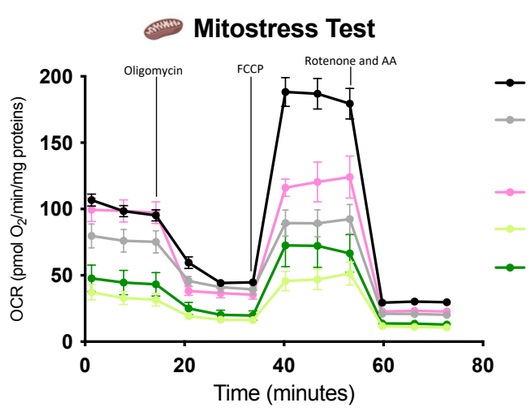
Operetta CLS

Human induced Neural Stem Cells



Early Neural Progenitor Cells





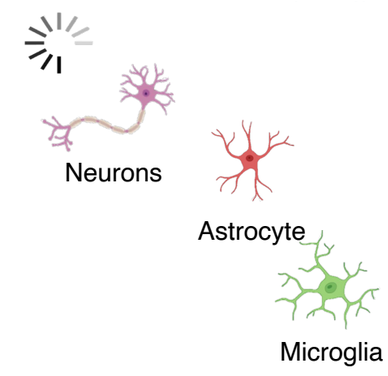


Fig. 5 Characterization of ALS patients-derived human induced Neural Stem cells (hiNSCs), scale bar 100 µm. Unrelated and familial controls, asymptomatic, early and late stage ALS patient-derived hiNSCs were cultured and maintained to obtain an *in vitro* model of early Neural Progenitor Cells (NPCs), scale bar 50 µm **(B)**. After differentiation, early Neural Progenitors were analysed using Seahorse XFE 96 Analyzer aims at evaluating the metabolic signature of ALS cells compared with controls. Mitostress Test was performed to assess mitochondrial functionality and metabolism's key parameters such as non-mitochondrial oxygen consumption rate, basal respiration, maximum respiration and spare respiratory capacity over the course of the disease (**C**).

References: [1] Brown RH, Al-Chalabi A., Amyotrophic Lateral Sclerosis. (2017) doi: 10.1056/NEJMra1603471. [2] Zalfa, C., et al. Transplantation of clinical-grade human neural stem cells reduces neuroinflammation, prolongs survival and delays disease progression in the SOD1 rats. Cell Death (2019). doi: 10.1038/s41419-019-1582-5; [3]Glass JD et al., Transplantation of spinal cord-derived neural stem cells for ALS: Analysis of phase 1 and 2 trials. Neurology. 2016 doi: 10.1212/WNL.0000000000002889. [4] Mazzini L. et al, Results from Phase I Clinical Trial with Intraspinal Injection of Neural Stem Cells in Amyotrophic Lateral Sclerosis: A Long-Term Outcome. (2019) doi: 10.1002/sctm.18-0154. [5] Leone et al., Phase I clinical trial of intracerebroventricular transplantation of allogeneic neural stem cells in people with progressive multiple sclerosis, Cell Stem Cell (2023), doi: 10.1016/j.stem.2023.11.001; [6] Gelati et al., Culturing and expansion of "clinical grade" precursors cells from the fetal human central nervous system. (2013).

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