



Preclinical Validation of Multilevel Intraspinal Stem Cell Therapy for Amyotrophic Lateral Sclerosis (ALS)

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Background

- ALS is a fatal and relentlessly progressive neurodegenerative disease with a median survival after symptom onset that ranges from 2 to 5 years. The only FDA approved treatment, riluzole, prolongs this survival by a matter of months.

- Cell therapies for ALS attempt to restore motor function through replacement of neuronal and non-neuronal cells. Graft localization, survival, and migration are crucial for the outcome and efficacy of spinal cord cell therapies. Multiple clinical trials using this approach are now underway in many countries around the world, however many gaps remain in our knowledge. These include: 1) The tolerance of the spinal cord to increasing volume and number of cell injections, 2) Understanding the immune response to cell transplantation in the spinal cord 3) Optimizing immunosuppression treatment to minimize transplant rejection. **The current study tested the spinal cord's tolerance to increasing volumes and total number of injections in Göttingen minipigs.**

Methods

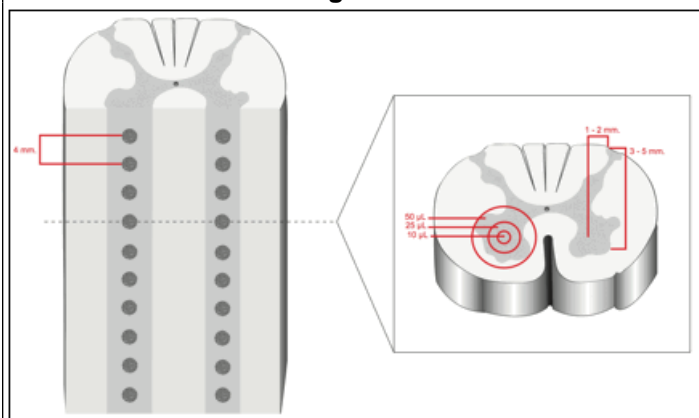
Cell Culture

- Human fetal cortex-derived neural progenitor cells (provided by the Clive Svendsen's Lab at Cedars Sinai, Los Angeles, CA) cultured as free-floating neurospheres and resuspended for transplantation between passage 25 and 35 at a concentration of 10,000 cells/ μ L.

Rationale

- Different volumes and total number of injections were selected based on the degree of displacement they would inflict in the normal anatomy of the anterior grey matter of the spinal cord (Figure 1). Specifically, the ventral horn of the grey matter in the spinal cord contains the motor neurons that undergo degeneration in ALS patients.

Figure 1



Transplantation strategy

Post-operative period

- Sensory and motor function, as well as general morbidity was assessed for 21 days. Motor function was evaluated using a four point Tarlov scale: (0) no voluntary limb function; (1) only perceptible joint movement; (2) active movement but unable to stand; (3) to be able to stand but unable to walk; (4) complete normal hind-limb motor function. At endpoints, full necropsy was performed; spinal cords were analyzed for graft survival (using stereological methods) and microscopic tissue damage.

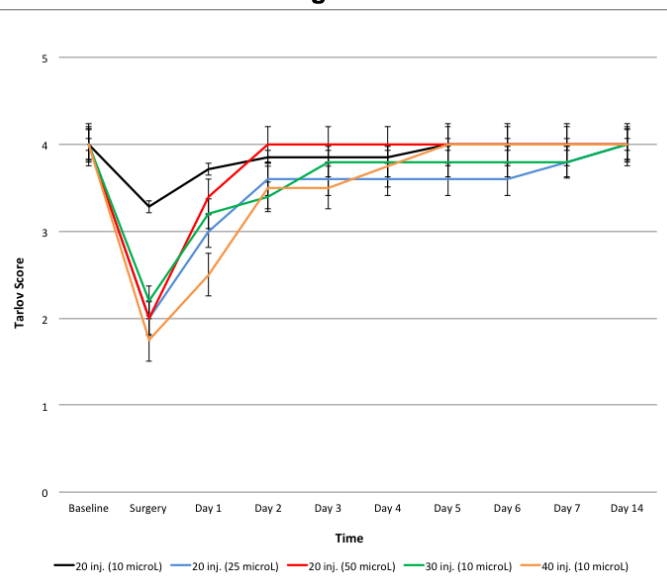
Study design

- Twenty-seven female minipigs received human neural progenitor cell injections using a stereotactic platform device developed by the Emory group. Cell transplantation in groups 1 to 5 (n = 5-7 pigs each) was undertaken with the intent of assessing the safety of an injection volume escalation (10, 25, and 50 microL) and an injection number escalation (20, 30 and 40 injections). One animal from group 5 (twenty 50 microL injections) was eliminated for the tissue analysis because of extensive damage caused to the spinal cord during sectioning leaving this group with an N of 4.

Results

- In all pigs assessed in groups 1 to 5, baseline motor function was regained by postoperative day 14 and maintained until postoperative day 21 when they were sacrificed (Figure 2).

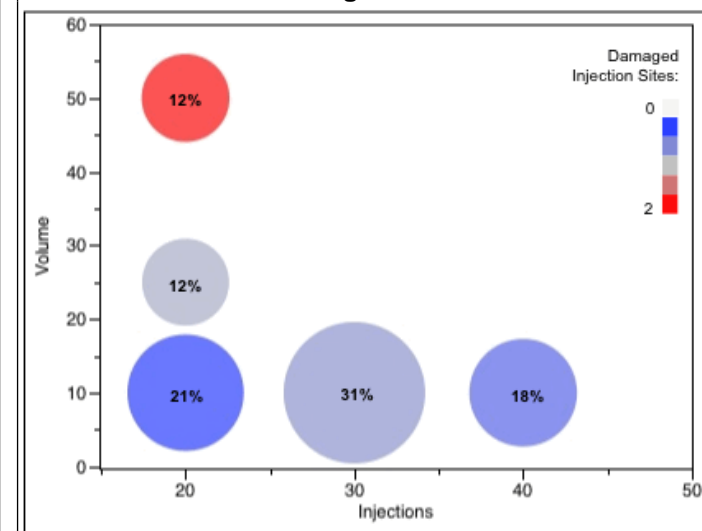
Figure 2



Motor function assessment in the post-operative period.

- When comparing total number of injections against injection volume (Figure 3), as volume increases the number of damaged injection sites increases and the engraftment percentage remains similar between groups (12-21%). Additionally, as number of total injections increases microscopic tissue damage stays relatively the same, but at 30 total injections the best engraftment percentage is achieved (31%).

Figure 3



Comparison of injection number and volume sized by engraftment percentage, and colored by microscopic tissue damage.

Conclusion

- This series supports the functional safety of various injection volumes and numbers in the spinal cord, and gives critical insight to important safety thresholds. The results from this study are relevant to all translational programs delivering therapeutics to the spinal cord.

References

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- Lamanna JJ, Miller JH, Riley JP, et al. Cellular therapeutics delivery to the spinal cord: technical considerations for clinical application. *Therapeutic delivery* 2013;4(11):1397-410.

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