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In vivo MPI/BLI tracking of genome-edited-patient derived neural precursor cells in a transgenic ALS mouse model

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Abstract

Amyotrophic lateral sclerosis (ALS) caused by superoxide dismutase 1 (SOD1) mutations may benefit from a gene-edited induced pluripotent stem cell (iPSC)-based therapy, provided cell fate after administration can be monitored precisely, non-invasively and longitudinally for effective optimization. This study evaluated tracking of neural precursor cells (NPCs) derived from gene-edited iPSCs using magnetic particle imaging (MPI) and bioluminescence imaging (BLI). Luciferase-expressing Nanoluc-NPCs were magnetically labeled and stereotaxically transplanted into the motor cortex of SOD1^{G93A} transgenic mouse models. BLI confirmed luciferase activity and cell viability, while MPI enabled quantitative tracking of labeled cells *in vivo*. Regression analysis estimated an iron content of ~10 pg per cell, with MPI-derived cell numbers aligning closely with injected values. BLI signals persisted for two weeks, and behavioral testing revealed delayed disease progression for treated vs. non-treated controls. These results demonstrate successful non-invasive visualization and quantification of transplanted NPCs, supporting the use of MPI/BLI for imaging-guided cell therapies in ALS.

I. Introduction

Approximately 20% of ALS cases are caused by a mutation in the gene SOD1, also known as familial ALS. iPSC-based therapies, aimed at restoring defect motor neurons in ALS patients, hold promise as a therapeutic approach, contingent upon genetic correction if derived from patients [1]. To pursue mutant SOD1-edited iPSC therapy effectively, it is crucial to have non-invasive imaging techniques available that can provide longitudinal data on the fate of transplanted cells [2, 3]. MPI enables the visualization and quantification of superparamagnetic iron oxide (SPIO)-labeled stem cells in a specific manner [2]. BLI is another method for cell tracking that enables monitoring the viability of transplanted cells over time [4]. The objective of this study was to track and quantify neural precursor cells (NPCs) derived from mutant SOD1-edited iPSCs using MPI/BLI *in vivo* in a SOD-1 transgenic mouse model of ALS.

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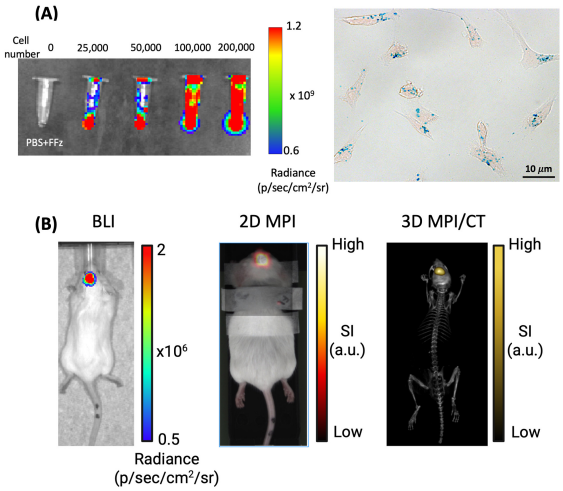
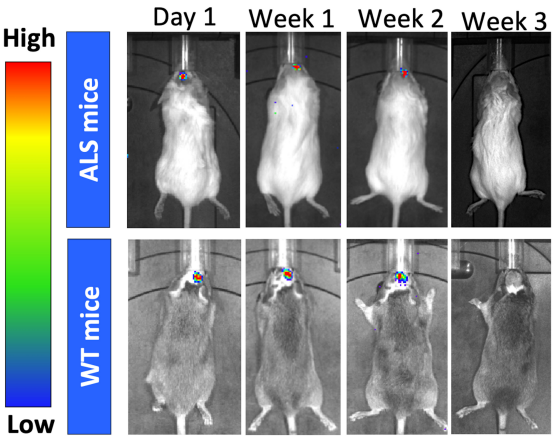


Figure 1: (**A**) BLI of Nanoluc-expressing NPCs at varying cell densities (left) and representative bright-field image of SPIO-labeled cells stained with Prussian blue, confirming efficient SPIO uptake (right). (**B**) Representative *in vivo* BLI and 2D MPI signal overlaid on a photographic image, and 3D MPI/CT scan of an ALS mouse 30 min after receiving SPIO-labeled Nanoluc-NPCs. MPI and CT were co-registered using fiducial markers and 3D Slicer.



	Day 1	Week 1	Week 2	Week 3
ALS-labeled	3.7E+6	2.7E+6	1.7E+6	0
WT-labeled	3.8E+6	6.3E+6	5.7E+6	0

Figure 2: Representative BLI showing stronger and more persistent signal in WT mice compared to ALS mice, indicating enhanced cell viability or retention in normal brain. Quantification of BLI signal intensity (photons/sec/cm²/sr) is provided in the table (bottom). BLI signal corresponds to representative individual mice and were not averaged across cohorts.

II. Methods and materials

The human 39B2.5 iPSC cell line [1], originally derived from an ALS patient carrying a mutant SOD1 allele and subsequently gene-edited to correct the mutation to the wild-type SOD1 sequence, was differentiated into NPCs. Differentiation of gene-corrected iPSCs into NPCs was performed using a previously established directed neural induction protocol [5]. NPCs were engineered to express luciferase through the introduction of the Nanoluc gene. Luciferase activity in NPCs was studied *in vitro* using the fluorofumarizine (FFz) substrate. Nanoluc-NPCs were labeled with SPIO (ferucarbotran, 28 µg Fe/ml, 2 h) and then transplanted into 12-week-old transgenic SOD1^{G93A} mice. 200,000 labeled Nanoluc-NPCs were stereotaxically transplanted into the motor cortex (MC). Serial *in vivo* imaging was performed using Magnetic Insight Momentum and Caliper Sciences IVIS Spectrum/CT scanners. For MPI data co-registration, two fiducials with 60,000 and 30,000 cells were positioned next to the mice in the same field of view. Cell numbers for both transplantation and fiducial preparation were determined using an acridine orange/propidium iodide viability assay with automated counting using a LUNA cell counter. To determine the iron content of the fiducials and calculate the amount of iron per cell, a linear regression analysis was conducted using reference samples of ferucarbotran. Latency to fall was measured weekly on a rotarod (starting speed of 4 rpm reaching the final speed of 40 rpm in 3 min) to assess motor coordination and endurance in ALS and wild type (WT) control mice treated with vehicle (phosphate-buffered saline, PBS, n=3), unlabeled NPCs (n=3), or SPIO-labeled NPCs (n=8), for a total of 14 ALS and 14 WT mice.

III. Results and discussion

We successfully generated gene-edited iPSC-derived NPCs and confirmed luciferase expression within these cells through *in vitro* validation with the FFz substrate and SPIO-labeling of Nanoluc-NPCs was accomplished successfully (Figure 1A). Upon transplantation of these labeled NPCs into the brains of ALS mice, we were able to track their localization and viability *in vivo* using MPI/CT and BLI (Figure 1B).

The anatomical accuracy of transplantation in the MC could be verified. Through regression analysis, we determined that the iron content of transplanted cells was approximately 10 pg Fe per cell. By correlating the MPI signal intensity of the brain with that of the fiducials, we estimated cell numbers of 237,000 in a representative mouse, close to that what was injected. Discrepancies between the theoretical and experimental cell numbers may arise from errors during pipetting, cell counting, loading cell suspensions into syringes, transplantation, and the presence of blooming artifacts. BLI signal confirmed cell viability for two weeks post-transplantation (Figure 2). Our intervention also led to a significant de-

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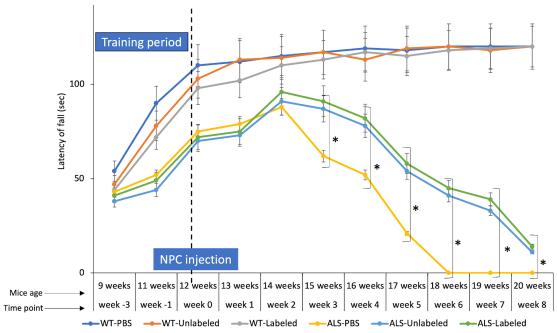

Figure 3: Rotarod performance in ALS and WT mice following NPC injection. ALS mice receiving NPCs exhibited significantly improved performance compared to ALS-PBS cohort at multiple time points post treatment. Asterisks (*) indicate time points at which statistically significant differences were observed between groups (*p<0.05). Data are presented as mean±SD.

lay in disease progression, as evidenced by a noticeable increase in latency to fall in rotarod testing compared to sham controls (Figure 3).

IV. Conclusion

In addition to promising therapeutic results, our data demonstrate successful tracking and quantification of SPIO-labeled Nanoluc-NPCs in an ALS mouse model. These findings may contribute to advance non-invasive cell tracking methodologies for imaging-guided treatment of neurodegenerative diseases.

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Author's statement

Conflict of interest: J.W.M.B is a shareholder of SuperBranche. This arrangement has been reviewed and approved by the Johns Hopkins University.

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