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Long-term stability and computational analysis of migration patterns of L-MYC immortalized neural stem cells in the brain

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Preclinical studies indicate that neural stem cells (NSCs) can limit or reverse central nervous system (CNS) damage through delivery of therapeutic agents of cell regeneration. Clinical translation of cell-based therapies raises concerns about long-term stability, differentiation and fate, absence of tumorigenicity, and manufacturing time required to produce therapeutic cells in quantities sufficient for clinical use. Allogeneic NSC lines are in growing demand due to challenges inherent in employing autologous stem cells, including production costs limiting availability to patients. Here, we demonstrate the long-term stability of L-MYC immortalized human NSCs (LM-NSC008) cells *in vivo*, including engraftment, migration, and absence of tumorigenicity in mouse brains for over nine months. Our allogeneic LM-NSC008 lines were also expanded in the Quantum Cell Expansion (QCE) bioreactor from Terumo BCT and characterized for viability, genetic stability, identity, and growth kinetics in comparison with the cells grown in conventional cell culture. We also examined the distributions of engrafted LM-NSC008 cells, and present computational techniques to analyze NSC migration characteristics in relation to intrinsic brain structures. This computational analysis of NSC distributions following implantation provides proof-of-concept for the development of computational models that can be used clinically to predict NSC migration paths in patients. Previously, models of preferential malignant tumor cell migration along white matter tracts have been used to predict their final distributions. We suggest that quantitative measures of tissue orientation and white matter tracts determined from MR images can be used in a diffusion tensor imaging tractography-like approach to describe the most likely migration routes and final distributions of NSCs administered in a clinical setting. Such a model could be very useful in choosing the optimal locations for NSC administration to patients to achieve maximum therapeutic effects.

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