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PERSPECTIVE

Advancing mesenchymal stem/stromal cells-based therapies for neurologic disease

The past decade has seen a dramatic expansion in the development and implementation of experimental cellular therapies in human patients. Mesenchymal stem/stromal cells (MSCs) are at the forefront of this effort, with over 650 registered clinical trials that employ MSCs as the principle therapeutic agent (www.clinicaltrials.gov). MSCs represent a specialized type of stromal cells first identified in bone marrow based on their capacity to differentiate into skeletal cell types (adipocytes, chondrocytes, and osteoblasts) and support hematopoiesis. MSCs or MSC-like cells are now believed to reside in most tissues as perivascular cells or pericytes, with isolates from bone marrow, adipose tissue and umbilical cord being the most widely studied. In addition to their stem/progenitor properties, MSCs have also been shown to possess a broad range of effector functions including angiogenic, anti-inflammatory and immuno-modulatory activities that are associated, in large part, with secretion of paracrine acting proteins and exosomes/micro-vesicles. Therefore, while originally developed for treating skeletal and hematologic diseases, MSC-based therapies now target a diverse array of inflammatory, ischemic, and auto-immune diseases, with the most promising results coming from clinical trials in patients with steroid resistant graft versus host disease (Squillaro et al., 2016).

Shifting paradigms: Studies conducted in the late 1990's including work from my own laboratory, which showed MSCs injected directly into the CNS of newborn mice acquired characteristics of neural cells (Kopen et al., 1999), suggested that adult stem cells possess broader than expected plasticity. This spurred many labs to identify culture conditions that promoted neural commitment of MSCs for use in cell replacement strategies to treat neurologic diseases. Cell replacement strategies remain a viable option as evidenced by a recent study showing that embryonic stem cell-derived basal forebrain cholinergic neurons transplanted into two different mouse models of Alzheimer's disease functionally integrated into the endogenous basal forebrain cholinergic projection system resulting in improved learning and memory performance (Yue et al., 2015). However, the lack of definitive evidence showing that MSCs can be reprogrammed to generate electrically excitable neurons suggests their use in this regard may be limited. Nevertheless, the realization that chronic inflammation and pathogenic immune responses are prominent features of many neurological disorders has provided a new path forward to exploit the anti-inflammatory and immuno-suppressive properties of MSC as a therapeutic option for these diseases. To date MSC-based clinical trials have been evaluated in patients afflicted with cerebral ataxia, amyotrophic lateral sclerosis, multiple sclerosis, multiple systems atrophy, Parkinson's disease, and Alzheimer's disease , and the number of these clinical trials is exceeded only by those directed at skeletal-related diseases (Squillaro et al., 2016).

Non-human primates as a model system for translations studies: Translational studies conducted in large animal models can be extremely valuable for advancing human clinical trials due to their similarities to human anatomical structure, host immune responses, and disease pathophysiology. Over the past decade our laboratory conducted a series of pre-clinical studies in non-human primates to evaluate the engraftment kinetics, anatomical distribution and transplant immunology of MSCs following direct intracranial injection, with the goal of exploiting the cells to treat neurologic sequelae associated with lysosomal storage diseases. For example, we demonstrated that stereotactic-guided injection of unmatched MSCs from a male donor into the caudate putamen of female, infant macaques was safe and well-tolerated based on physical examinations, body weight measures, and serological testing (Isakova et al., 2007). Using a battery of age- and species-appropriate tests we further showed that MSC administration yielded no adverse effects on animal cognition, fine and course motor function, behavior, or neural development up to 6 months post-transplant. These findings were significant since animals were monitored throughout their first year of life during which social behavior, motor skills and cognitive abilities are rapidly developing. Analysis of brain tissue further revealed that overall MSC engraftment levels were 17.8-fold higher ($P < 0.05$) in infant *vs*. young adult transplant recipients with a maximal observed difference of 180-fold. This result was also highly favorable in that patients with storage diseases that involve neurodegeneration develop neurologic complications at an early age and therefore early intervention is critical toward retarding disease progression. In subsequent studies we showed that intracranial administration of allogeneic but not autologous MSCs induced a weak allograft response that involved expansion of NK, B and T cell subsets in peripheral blood, and that its magnitude was dependent upon the degree of MHC mismatch between the MSC donor and transplant recipient. This finding was further substantiated by the fact that secondary challenge with allogeneic donor MSCs induced allo-antibody production and elevated levels of $CD3^-$ $CD16^+HLADR^+$ myeloid dendritic cells, which play a major role in peripheral tolerance (Isakova et al., 2010, 2014).

Using knowledge gained from these studies, we then set out to test the efficacy of MSCs in a non-human primate model of early onset Krabbe disease (Baskin et al., 1998). Krabbe disease is one of over 50 types of lysosomal storage diseases in humans and is caused by a deficiency in galactosylceramidase (GALC) activity. The disease is characterized by loss of myelin-forming oligodendrocytes and progressive demyelination resulting in severely impaired motor function. Disease symptoms including marked irritability, spasticity, and seizures appear within 3–6 months of age in human infants and the disease is often fatal by the second year of life with few effective treatment options. Neuro-inflammation that manifests as robust astrogliosis, microglial activation, and macrophage recruitmentis also now recognized as a critical aspect of the pathophysiology of this disease (Potter and Petryniak, 2016). Consequently, MSC administration may disrupt the feed forward loop of microglia activation, oligodendrocyte death, demyelination, and inflammation characteristic of this disease.

To assess this we treated an infant rhesus macaque that exhibited symptoms consistent with severe early onset Krabbe disease in humans including a noticeable tremor, weak posture and impaired ambulation (Isakova et al., 2016). This diagnosis was confirmed by lack of GALC activity in the animal's peripheral blood cells. At seven weeks of age the animal was administered *via* direct intracranial injectionpartially matched MSCs from a healthy donor, which expressed a similar repertoire of Mamu A1 and Mamu E alleles in order to minimize the risk of allograft reaction. While we still observed a transient increase in peripheral blood lymphocyte counts several weeks post MSC administration, we were unable to detect evidence of allo-antibody production in the transplant recipient after secondary antigen challenge, suggesting that the donor cells were weakly immunogenic.

The afflicted infant was subject to neurodevelopment and behavior tests pre- and post-surgery and results were compared to age-matched normal infants. As expected, the afflicted infant scored significantly lower on tests assessing large motor skills as compared to age-matched controls. These scores improved between 0 and 2 months post-surgery, declined steadily thereafter, and showed improvement after the animal received a second intramuscular MSC injection. A significant difference between animals was also noted for predominant state (irritability, agitation, difficult to soothe) and scores for the afflicted infant also improved between 0.5 and 3 months post-surgery but then steadily declined. Neural development assessments further revealed that cognitive subset scores for the afflicted infant were well below that measured for normal age-matched control infants but then improved dramatically at 5 months

of age and remained elevated up to 7 months of age. This change was commensurate with noticeable improvements in ambulation, which allowed the infant to better navigate its play cage environment thereby enhancing learning. Improvements in these activities may have resulted from preservation of myelin content in critical brain regions, as MRI scans taken at 4.5 months of age showed clear evidence that the injected *vs.* contralateral hemisphere of the infant's brain contained observably greater amounts of myelin.

The infant also exhibited extremely low conduction velocities (CVs) for the tibial, ulnar and median nerves consistent with a diagnosis of severe Krabbe disease. At one month post MSC administration CVs for the tibial nerve, which controls hind limb movements and posture, increased by 3.7-fold, and this change was consistent with a measurable improvement in coordination, leg and arm resistance, and ambulation based on physical examinations. CV values for the ulnar nerve also increased by 1.5-fold over baseline and remained at or above pre-treatment levels for the duration of the study. These changes were accompanied by a marked decrease in distal latencies measured for the tibial and ulnar nerves at 30 days post MSC administration. Therefore, despite the severity of its symptoms, the afflicted infant responded rather rapidly to MSC administration based as evidenced by improved nerve conduction velocities, motor control, ambulation and learning. While this cases study did not assess effects on neuro-inflammation, outcomes are consistent with previous reports indicating that MSCs exert trophic effects that improve survival of myelin-producing oligodendrocytes when transplanted in a rodent model of toxicity-induced demyelination (Jaramillo-Merchan et al., 2013) and that MSC conditioned media reduces functional deficits in a mouse model of experimental autoimmune encephalitis by promoting maturation of oligodendroglial progenitors toward mature myelin producing cells (Bai et al., 2012). These positive outcomes provide a basis for further testing using this large animal model, which provides a means to rigorously pursue mechanistic-based studies that can inform clinical trials to improve efficacy in human patients.

Refinements for moving forward: A growing number of early stage clinical trials have demonstrated the safety of MSC-based therapies in humans, and completed trials to date suggest that MSCs may be efficacious in treating a range of neurologic disorders (Squillaro et al., 2016). However, while MSC-based therapies have shown a clear benefit in some patient populations, other trails have yielded suboptimal results or failed to meet their primary efficacy endpoints. Developing efficacious MSC-based therapies is critically dependent on a number of key factors, such as selection of the appropriate patient population, adequate rigor in trial design, development of appropriate dosing strategies, and selection of rigorous and well-defined metrics to assess patient outcomes. However, choice of the appropriate human MSC donor population and the manufacturing scheme employed to generate clinical cell doses is also important but often overlooked despite the fact that human MSC populations exhibit significant donor-to-donor heterogeneity. Therefore, there remains a critical need to develop metrics to assess the relative potency of clinical grade MSC preparations so they can be carefully matched to the appropriate patient populations. As a step toward this goal our laboratory recently described a Clinical Indications Prediction (CLIP) scale that predicts the therapeutic efficacy of different human MSC isolates for a given disease indication based on *TWIST1* expression levels. This scale arose from our studies showing that stem/progenitor and effector functions of MSCs are coordinately regulated by *TWIST1*, and that one could predictably alter these properties in MSCs by manipulating expressed levels of this protein (Boregowda et al., 2016). While continued validation of the CLIP scale is needed, the scale itself is advantageous over other potency assays as it predicts differences in growth, survival, stem/progentior, and effector functions of MSCs rather than just a single function, and can easily be correlated to quantifiable functional assays. For example, we reported that *TWIST1* levels are positively correlated with CFU-F activity. Therefore, use of a standard CFU-F assay provides a simple means to orient a given MSC preparation on the CLIP scale. Importantly, the scale can be expanded to incorporate additional metrics as the role of *TWIST1* in MSCs is further explored.

Conclusion: The number and scope of MSC-based clinical trials continues to show robust expansion both with respect to treatment of neurologic diseases as well as many other maladies. However, as more advanced phase trials that employ MSCs are completed their outcomes will dictate the long-term viability of the field. To ensure their success, it is essential to pursue development of potency assays that serve as reliable predictors of *in vivo* efficacy and that are also acceptable to the appropriate regulatory authorities who oversee trial approval. The difficulty in developing such assays to date clearly indicates the need for continued translational research using the most appropriate animal models available. Indeed, robust collaborative efforts that engage basic scientists, disease model experts and clinicians is needed in order to develop MSC-based therapies to their full potential.

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