

# ASNTR 2018 Abstracts

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1-41  
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## Presidential Symposium: Technologies Enabling Brain Repair

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Brain damage leads to irreversible behavioral deficits. Understanding the biology of neural dysfunction and loss defines the environment within which we aim to promote repair. The brain has a limited capacity to repair itself and understanding this process allows us to conceptualize novel therapeutic interventions that can abate or reverse these impairments. The delivery of these approaches is commonly highly dependent on technological developments. For the past 25 years, the American Society for Neural Therapy and Repair (ASNTR) has been at the forefront of this effort to define the biological environments and to devise strategies to modify these to promote recovery. Our meeting this year will showcase the latest technological developments to reshape disturbed neurovascular environments in experimental model systems to translational efforts in clinical trials. With many clinical trials progressing these exciting treatments, new hope for recovery can be provided to patients and their families.

## Regenerative Properties of Mesencephalic Astrocyte-derived Neurotrophic Factor in Developing Neurons

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Mesencephalic astrocyte-derived neurotrophic factor (MANF) is an endoplasmic reticulum (ER) resident protein with neuroprotective actions. We have recently studied its role in mammalian neurogenesis and found that MANF is highly expressed in neural stem cells (NSCs) in both the developing and adult brain. We discovered that endogenous or exogenous MANF does not affect NSC proliferation.

However, MANF-deficient cells have deficits in neurite extension when they are differentiated into neurons in vitro, and mechanistic studies indicate that impaired neurite extension is preceded by reduced de novo protein synthesis and constitutively activated unfolded protein response (UPR) pathways. We then studied the role of MANF in neuronal migration and found that both endogenous and exogenous MANF regulates neuroprogenitor cell (NPC) migration in vitro, and MANF overexpression in subventricular explant cultures increased signal transducer and activator of transcription 3 (STAT3) phosphorylation. Using a rat model of cortical stroke, intracerebroventricular injections of MANF did not affect cell proliferation in the subventricular zone, but promoted migration of NPCs towards the corpus callosum and infarct boundary on day 14 post-stroke. Long-term infusion of MANF into the peri-infarct zone increased the recruitment of NPCs in the infarct area. In conclusion, our data demonstrate beneficial effects of MANF and a neuroregenerative activity that facilitates differentiation and migration of NPCs, thereby increasing recruitment of NPCs into the stroke-affected cortex.

## Activation of the Cytomegalovirus (CMV) Promoter: Considerations for Viral Vector-Mediated Transgene Expression

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The transcriptional promoter of the cytomegalovirus (CMV) immediate early genes has been extensively exploited in mammalian vectors used for in vitro and in vivo transgene delivery. Transcriptional activation by the CMV promoter is dependent on the presence of certain cellular transcription factors, several of which are known to be altered in response to cellular stimuli. However, the stability of transgene



expression in model systems that depend on coincident stimuli has not previously been addressed. Here we monitored the activity of the CMV promoter in an adeno-associated virus (AAV) vector used to deliver constitutively secreted *Gaussia* luciferase (GLuc) to primary cortical neurons in vitro and the rat striatum in vivo. Using a technique for repeated sampling of cerebral spinal fluid (CSF) in rats, we observed a methamphetamine-dependent increase in GLuc activity in the CSF. We also found a methamphetamine-dependent increase in *GLuc* mRNA levels in the striatum where the AAV-GLuc was injected. In cultured primary cortical neurons, glutamate and kainic acid treatment caused an increase in the CMV-dependent expression of GLuc following viral transduction. Our results suggest that variations in transgene expression can serve as a confounding factor in studies where stimulatory substances are applied on biological systems that use the CMV promoter to drive transgene expression. However, the observed effect on CMV-driven transcription could also be exploited for therapeutic purposes. As an example, we provide evidence that methamphetamine administration induces upregulation of a previously characterized antibody designed to mediate virus-based passive immunization against methamphetamine toxicity, leading to higher concentrations of the antibody in the presence of its antigen (methamphetamine). Collectively, our data emphasize that the use of a CMV promoter for constitutive, stable viral vector-mediated transgene expression should be empirically evaluated in the model system.

### Diffusion Tensor Imaging to Assess Brain Injury and Repair Post Neurointerventional Stem Cell Therapies in a Canine Model of Stroke

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White matter damage is an important factor for functional outcome in ischemic stroke. To assess the microstructural changes in the white matter tracts (WMTs), more sensitive methods than conventional magnetic resonance imaging (MRI) is needed. Diffusion tensor imaging (DTI) produces a three-dimensional representation depicting the integrity of WMTs by capitalizing on the restricted diffusion of water in intact WMTs. These diffusion patterns change when axons are damaged or shortened, as in ischemia. From the diffusion tensor image, the fractional anisotropy (FA) can be computed. We hypothesize that canines receiving intra-arterial (IA) delivery of mesenchymal stem cells (MSCs) 48 h post-reversible middle cerebral artery occlusion (rMCAo) will show improved FA values than controls at 30 days post-stroke. Mongrel hounds ( $n=7$ ), aged 12–36 months, were included in this pilot study. rMCAo was achieved by a

detachable helical ultra-coil over 35–80 min. IA-MSCs or saline were infused 48 h post-stroke into the ipsilesional carotid artery. DTI-MR images were obtained pre-IA delivery and at 15 and 30 days. FA values of the right and left hemisphere corticospinal tracts (CSTs) were determined. Diffusion tensor tractography (DTT) allowed for visualization of the CSTs. Blinded neurological evaluations of canines were also performed. We observed a higher average change in the FA values from IA delivery to 30 days post-stroke in MSC-treated canines compared with controls. We observed thicker and more organized CSTs in the DTT of MSC-treated canines. FA improvement correlated with improved neurological outcomes of MSC-treated canines. Histological analyses may confirm microstructural changes in the WMTs. DTI/DTT imaging can be used to assess white matter ischemic brain injury and recovery, which may better predict functional outcomes. DTI/DTT imaging may also shed light on the mechanisms by which ischemia impacts axons and how stem cell therapies facilitate repair.

### Large Animal Modeling and Brain Imaging in Translational Stroke Research

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Large animal models are particular suitable for use with state-of-the-art imaging technologies. This allows for late-stage translational research on diagnostic and therapeutic paradigms in scenarios closely mimicking clinical reality. Hybrid positron emission tomography (PET)/magnetic resonance imaging (MRI) is a new-generation imaging technology with great potential to improve both clinical and experimental brain diagnostics, and we have utilized this technology in combination with an ovine stroke model. Here, we share results and insights from selected research programs. A sheep model of middle cerebral artery occlusion was used. A wide spectrum of PET tracers such as <sup>15</sup>O-H<sub>2</sub>O to quantify cerebral blood flow or <sup>18</sup>F-fluorodeoxyglucose (FDG) to quantify cerebral glucose consumption can be utilized with this model. A fully integrated 3 Tesla PET/MRI Siemens mMR system was available, allowing for anatomical investigation of acute and chronic stroke lesions. This combination was used to (i) develop a novel technique for non-invasive measurement of arterial input function, (ii) challenge the classical perfusion-diffusion mismatch in MRI using a parallel series of trials in sheep and patients, and (iii) investigate therapeutic effects of inhalative nitric oxide (iNO). Using post-hoc PET/MRI coregistration, a technology to obtain an arterial input function non-invasively by

analyzing time-of-flight MR angiography data has been developed. The method was successfully applied in sheep and humans. Performing acute stroke imaging in the hybrid PET/MRI system, we were able to provide initial evidence for a lack of accuracy of the perfusion-diffusion mismatch concept to describe the ischemic penumbra in MRI in both sheep and human patients. We were further able to show that inhaled iNO can preserve parts of the ischemic penumbra in acute stroke. iNO application does not lead to severe side effects (e.g. blood pressure decrease) and is well controllable, suggesting its future use in the pre-hospital environment. Although logistically challenging and just emerging, combining large animal research with cutting-edge imaging technologies has fostered our translational stroke research program. The setup allows investigation of pathophysiological and biochemical processes. It is reasonable to speculate that this translational tool box can also help to investigate transplantation strategies to treat stroke such as application of biomaterials together with regenerative cell populations. Our talk also provides examples on how such research could be organized and conducted, including most important challenges and opportunities.

## **Cerebral Dopamine Neurotrophic Factor: Advances in Clinical Development**

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Cerebral Dopamine Neurotrophic Factor (CDNF) is a member of a novel family of unconventional neurotrophic factors. CDNF protects midbrain dopaminergic neurons and restores motor function in several rodent and primate models of Parkinson's disease (PD). A novel therapy for PD is currently being developed based on intracerebral infusion of recombinant human CDNF protein (rhCDNF). Previous failures in neurotrophic factor clinical trials have provided important information on how to improve brain infusion of therapeutic proteins. Characteristics and location of infusion catheters, infusion protocol and the distribution properties of the therapeutic protein are among the key determinants of the efficacy of an intracerebral neurotrophic factor therapy. As a neurosurgical procedure is required for implantation of a drug delivery system (DDS) capable of intermittent intracerebral protein drug delivery, the first-in-human study with rhCDNF will be carried out in PD patients, not in healthy volunteers. This phase I–II clinical study [ClinicalTrials.gov identifier: NCT03295786] will enroll 18 patients with idiopathic PD of moderate severity. A crossover clinical study design, including a placebo group, will be used for assessment of safety and tolerability of the treatment, as well as for preliminary assessment of rhCDNF's effects on motor function (Unified Parkinson Disease Rating Scale; UPDRS) and dopaminergic function in the nigrostriatal pathway (positron emission tomography (PET) with dopamine transporter (DAT) tracer). In addition, data will be collected on the

safety and functionality of the DDS. First PD patients were implanted with the DDS in November 2017 and dosed with rhCDNF in January 2018. Challenges related to late preclinical and pharmaceutical development of rhCDNF-based therapy as well as regarding the clinical study design will be discussed.

## **Proteasome-Targeted Nanobodies Alleviate Pathology in a Synuclein-based Parkinson's Disease Model**

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Parkinson's disease (PD) is a synucleinopathy with a significant loss of dopaminergic neurons in the substantia nigra (SN) and abrogation of dopaminergic tone along the nigrostriatal pathway. Therapeutics designed to target  $\alpha$ -synuclein ( $\alpha$ -syn) aggregation may be critical in halting the progression of pathology in PD patients. Nanobodies are single-domain antibody fragments that can be expressed intracellularly and specifically bind to target regions critical for protein accumulation. Nanobody fusion with a proteasome-targeting proline-glutamic acid-serine-threonine rich (PEST) motif can modulate monomeric concentrations of aggregate proteins while maintaining aptamer stability. Here we aimed to validate and compare the in vivo therapeutic potential of gene therapy delivery of two proteasome-directed nanobodies selectively targeting  $\alpha$ -syn in a synuclein overexpression-based PD model: VH14 $\times$ PEST (non-amyloid component region) and NbSyn87 $\times$ PEST (C-terminal region). Stereotaxic injections of adeno-associated virus 5 (AAV5)- $\alpha$ -syn into the SN were performed on Sprague-Dawley rats that were sorted into three cohorts based on their pre-operative behavioral testing (cylinder test and stepping test). Rats were treated with unilateral SN injections of vectors for VH14 $\times$ PEST, NbSyn87 $\times$ PEST, or injected with saline 3 weeks post-lesion. Post-mortem assessments of the SN showed both nanobodies markedly reduced the level of phosphorylated-Serine129  $\alpha$ -syn labeling relative to saline-treated animals. VH14 $\times$ PEST showed considerable maintenance of striatal dopaminergic tone in comparison with saline- and NbSyn87 $\times$ PEST-treated animals as measured by tyrosine hydroxylase immunoreactivity (optical density), dopamine transporter immunoreactivity (optical density), and dopamine concentration (high performance liquid chromatography; HPLC). Microglial accumulation and inflammatory response, assessed by stereological density of ionized calcium-binding adapter molecule 1 (Iba-1)-labeled cells,

was modestly increased in NbSyn87×PEST-injected rats but not in VH14×PEST- or saline-treated animals. Both nanobody constructs significantly improved stepping test performance, with the NbSyn87×PEST-treated group also showing improvement in cylinder test compared with the saline-treated group, although there was pronounced variability among individual animals. These data show novel *in vivo* therapeutic efficacy of vector-delivered intracellular nanobodies targeting  $\alpha$ -syn misfolding and aggregation in synucleinopathies such as PD.

## Brain Repair via In Situ Astrocyte-to-Neuron Conversion

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Glial scar is widely associated with brain and spinal cord injury, stroke, glioma, and neurodegenerative disorders such as Alzheimer's disease. Reactive glia initially exert a neuroprotective role but later form a glial scar to inhibit neuroregeneration. Currently, there is no effective way to reverse the glial scar back to neural tissue. We have recently developed an innovative *in vivo* cell conversion technology to directly convert reactive glial cells into functional neurons inside the mouse brain. This is achieved through *in vivo* expression of a single neural transcription factor Neuronal Differentiation 1 (NeuroD1) in the reactive astrocytes in injured mouse brain or Alzheimer's disease mouse model. Our *in vivo* cell conversion technology makes use of internal glial cells to regenerate new neurons with 90% conversion efficiency, making it possible for the first time in history to reverse glial scar back to neural tissue. Such an internal cell conversion method will avoid cell transplantation and immune rejection. More importantly, our recent study in a stroke model demonstrated that *in vivo* neuroregeneration efficiency can be as high as 100 times that of the internal regeneration capability in the cerebral cortex after injury, an unprecedented efficiency in regenerative medicine. We have further discovered a cocktail of small molecules that can directly convert cultured human astrocytes into functional neurons, paving the way for a potential drug therapy for human brain repair. We have also successfully converted reactive glial cells into neurons using NeuroD1 in nonhuman primate brains, making an important step towards future clinical trials.

## Dopamine Release in the Nucleus Accumbens is Altered Following Traumatic Brain Injury

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Mild-to-severe traumatic brain injury (TBI) is frequently associated with prolonged dysfunction of reward circuitry, including motivation and salience, which suggests alterations of dopamine (DA) processing within the core and shell of the nucleus accumbens (NAC). Using fast-scan cyclic voltammetry in a rodent model of traumatic brain injury, we found that stimulus-evoked DA release is distinct in the core and shell of the NAC, with the shell being less responsive to tonic stimulation and more sensitive to the number of pulses when phasic stimulation is applied. Exposure to TBI was associated with major changes in both release and reuptake of DA in both the core and shell of NAC, with greater changes seen in the core. These alterations evolved over time, becoming most severe 1–2 weeks after injury with subsequent recovery, and the extent and progression of these abnormalities was correlated with severity of injury. Taken together, these data support behavior and anatomical studies suggesting the NAC core and striatum may serve parallel functions, whereas the shell is distinct. These data offer a unique window on how different neurological systems respond to TBI and may help explain affective and cognitive changes that are seen.

## Nogo Receptor-1 Regulates Post-Traumatic Cognitive, Motional, and Emotional Behaviors in Mice

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Traumatic brain injury (TBI) is a major public health issue involving the lesioned central nervous system. Among all the TBIs, ~75–90% are mild TBIs (mTBIs) and can cause various physical, cognitive, emotional, and psychological-related symptoms. However, there is currently no efficacious treatment for mTBI. Although some clinical signs and symptoms of mTBI resolve within the first few months after

injury, recovery is slow and frequently incomplete, and many patients continue to manifest prolonged or even permanent neurocognitive dysfunction, with lasting changes in cognition, motor function, and personality that may contribute to significant disability. Little is known about the mechanisms of recovery after a mTBI. If synaptic plasticity plays a significant role in the recovery, the plasticity regulating Nogo signaling system is likely to be involved. To test this, based on the finding that Nogo receptor 1 (NgR1) mRNA levels are rapidly downregulated in activated neurons, we used a mouse with inducible overexpression of a NgR1 transgene (Tg) in forebrain neurons, leading to impaired memory and cognition, and reduced dendritic spine density and dendrite complexity. We hypothesized that the inability to downregulate NgR1, inhibiting structural synaptic plasticity, would impair recovery from mTBI. To determine the role of NgR1 on cognitive, motion and emotional regulation after mTBI, we divided the wild-type mice and NgR1-Tg mice with doxycycline or not into six groups with or without weight-drop induced mTBI. Results show NgR1-Tg mice with doxycycline were more functional than NgR1-Tg mice without doxycycline after mTBI on balance, learning memory, cognition and anxiety. In addition, the NgR1-Tg mice after mTBI were worse than those with suppression of NgR1 for cognitive, motion and emotional aspects. These results support our hypothesis, and suggest that pharmacological measures to dampen Nogo-NgR1 signaling, might have beneficial effects.

### **Cerebral Organoids as a Novel Source of Dopaminergic Neuron Progenitors for Cell-Based Treatment of Parkinson's Disease**

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Tissue organoids grown from human induced pluripotent stem cells (hiPSCs) offer a novel source of dopaminergic neural progenitors which could be used in cell-based therapies to treat Parkinson's disease (PD). We have developed cerebral organoids from hiPSC cultured in Cell-Mate 3D hydrogel matrix in E8 medium. Over 8 weeks of growth in vitro, immunostaining of the cerebral organoids revealed cell markers characteristic of the developing midbrain, forkhead box A1 (FOXA1), LIM homeobox transcription factor 1 alpha (LMX1A), activated leukocyte cell adhesion molecule, nuclear receptor related 1 protein (NURR1), and orthodenticle homeobox 2 (OTX2), as well as markers for dopaminergic cells including paired-like homeodomain

transcription factor 3 (PITX3), dopamine transporter (DAT), and tyrosine hydroxylase (TH). Organoids were dissociated into a single-cell suspension for transplantation into the striatum of immunosuppressed 6-hydroxydopamine (6-OHDA) lesioned rats and unlesioned controls. Functional recovery was assessed every 2 weeks using the amphetamine-induced rotation task. Transplantation of cerebral organoids into 6-OHDA lesioned rats demonstrated varying degrees of functional recovery. After 8 weeks following transplantation, neither group displayed adverse effects and histological analysis revealed no tumorigenicity from the transplanted organoids. Immunostaining of the transplanted brains detected the presence of human cells using the STEM121 antibody, indicating engraftment and survival of the transplanted cells. Immunostaining also revealed the presence of myelin basic protein co-labeled with STEM121, indicating the presence of a surviving oligodendrocyte lineage among the transplanted cells. Future immunostaining to characterize the engrafted cells is underway. These results demonstrate that cerebral organoid-derived cell preparations can survive transplantation and produce functional improvements in 6-OHDA-lesioned rat models of PD. Future studies will interrogate the heterogeneity of cerebral organoids for markers associated with successful engraftment, including engrailed homeobox 1 (EN1), E26 transformation-specific variant 5 (ETV5), canopy fibroblast growth factor signaling regulator 1 (CNPY1), paired box gene 8 (PAX8), and Sprouty receptor tyrosine kinase signaling antagonist 1 (SPRY1).

### **An iPSC-Based Platform for Investigating Idiopathic Parkinson's Disease**

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Human induced pluripotent stem cells (iPSCs) are proving to be a valuable source of patient cells for generating neural phenotypes relevant to Parkinson's disease (PD). Here we characterize iPSCs, as well as iPSC-derived midbrain dopamine (DA) neurons derived from the skin fibroblasts of late-onset idiopathic PD subjects. Specifically, we comparatively analyzed the survival, differentiation, morphology, and alpha-synuclein protein expression, in cells obtained from idiopathic PD and age-matched control (AMC) subjects. Our data indicate that the iPSCs from PD subjects had lower viability rates, and a reduced capacity to generate neurons when induced to differentiate via a floorplate dual-mothers against decapentaplegic homolog (SMAD) inhibition

method. At day 42 post-differentiation, although the efficiency of tyrosine hydroxylase positive (TH<sup>+</sup>) DA neuron generation did not differ between the PD and AMC DA cultures, the efficiency of production of G protein-activated inward rectifier potassium channel 2-positive (GIRK2<sup>+</sup>) TH neurons (A9 DA neurons) was lower in the PD cultures. Furthermore, the morphology of DA neurons in the PD cultures appeared altered in that the cells displayed a smaller soma size, reduced number of neurites, lower neurite arborization, and shorter neurite lengths, compared with DA neurons in AMC cultures. In addition, it was found that the expression of the PD-relevant protein, alpha-synuclein, was higher in the DA neurons obtained from PD subjects than AMC cells. Our current studies are further extending these findings by examining the redox, mitochondrial, and electrophysiological profiles of the iPSC-derived PD and AMC DA neurons. In conclusion, our study develops an iPSC-based model that captures a phenotype relevant to the study of idiopathic PD, as well as biomarker and therapeutic testing.

### Mechanisms of Age-Related Cognitive Decline

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Currently, few molecular targets have been identified to treat age-related memory disorders such as mild cognitive impairment during normal aging, or Alzheimer's disease. We identified an isoform of the *Homer1* gene, *Homer1c* that plays an important role in age-related learning. Our goal is to understand the molecular mechanisms of age-related learning and memory formation by studying the role of *Homer1c* in age-related cognitive decline. Environmental enrichment (EE) preserves cognition in the senescent brain. Although humans with high cognitive activity have a lower risk for Alzheimer's disease, little is known concerning mechanisms giving rise to the functional benefits of EE. Rodent models of aging have been used to study the effects of environmental enrichment on cognition in normal aging and neurodegenerative disease. EE enhances performance in multiple well-established behavioral tasks, including the Morris water maze which measures spatial memory, and memory for objects and odors. Both spatial and object/odor memory declines with age in humans. In order to understand the molecular pathways involved in enhanced cognition and synaptic plasticity in EE in aged rats, we exposed aged rats to three different housing conditions: EE, socially enriched

(SE), or standard housing (SC). We found that aged rats exposed to 1 month of EE exhibit enhanced learning and memory in the Morris water maze and novel object recognition behavioral tasks. Moreover, we showed that EE rats perform significantly better than SE or SC rats in the radial-arm water maze, and display enhanced metabotropic glutamate receptor-dependent long-term potentiation (mGluR-LTP). Enhanced hippocampal function results from activity-dependent upregulation of mGluR5, *Homer1c*, and phospho-p70S6 kinase. These findings suggest a potential mechanism by which EE benefits overall cognition in the aging brain.

### Zika Virus as an Oncolytic Agent in Human and Murine Malignant Gliomas

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Patients with malignant brain tumors have limited options; the survival rate for grade IV glioblastoma multiforme is 5% at 5 years after the initial diagnosis. The emergence of Zika virus (ZKV) to the status of public health emergency led to increased funding towards mechanisms of ZKV-related microcephaly. Our data suggest that ZKV infection of fetal brain induced increased expression of autophagy and apoptosis-related genes. Given the similarities between neural stem cells and glioma stem cells, we sought to determine if ZKV is a viable therapeutic option for malignant gliomas. Characterization of in vitro cultured human and murine glioma cell lines demonstrate expression of putative receptors for ZKV entry. Analysis of the kinetics of infection and active viral replication in glioma cell lines through the plaque-forming assay reveal variable kinetics in ZKV infected cells; murine gliomas are relatively resistant to ZKV replication while highly passaged human gliomas are highly amenable to ZKV propagation and virus-induced lysis. To interrogate ZKV as an oncolytic agent in vivo, we induced brain tumors in immunocompetent C57BL/6 J mice through transplantation of the murine GL261 glioma cell line within the striatum followed immediately by injection of ZKV at the same coordinates. At all three concentrations tested, ZKV did not significantly prolong the overall survival of tumor-bearing mice, relative to untreated tumor-bearing

mice. We next sought to determine if ZKV can be used in conjunction with immunotherapies. Our lab previously developed a vaccine immunotherapy in which ex vivo cultured tumor cells are irradiated then infused into tumor-bearing mice which modestly extended overall survival. In the current study, we induced brain tumors in C57BL/6 J and immunodeficient non-obese diabetic-severe combined immunodeficient (NOD-SCID) mice. Mice in the treatment condition were immediately injected with ZKV at the same coordinates. At 3, 7, and 14 days following brain tumor induction, mice were subcutaneously infused with irradiated GL261 tumor cells previously infected with ZKV. We observed an increase in overall survival of immunocompetent treated mice with roughly half of all treated mice surviving long-term. These long-term survivors were re-challenged with tumor cells and the immune response was analyzed using flow cytometry. Relative to age-matched tumor-bearing controls, we observed increases in activated microglia and infiltrating T-lymphocytes in the brain of treated tumor-bearing mice. These results suggest that ZKV acts as an adjuvant, signaling an immune response to the infected tumor cells in the brain.

### **Delivery of a Zinc Finger Artificial Transcription Factor for *Ube3a* Reactivation via Mesenchymal Stem Cells in Angelman Syndrome**

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Angelman Syndrome (AS) is a genetically inherited neurodevelopmental disorder characterized by impaired cognitive development, lack of speech, seizures, and motor ataxia. The genetic cause for AS is usually due to a de novo deletion of the maternal ubiquitin protein ligase E3A (*Ube3a* also known as *E6AP*) gene in the chromosome 15q11-q13 region. Additionally, brain-specific postnatal imprinting of the intact paternal *Ube3a* gene results in complete loss of *Ube3a* in mature neurons due to the presence of a long antisense transcript driven by the neighboring small nuclear ribonucleoprotein polypeptide N (SNRPN) upstream reading frame (SNURF)/SNRPN promoter. Our group has previously shown reactivation of the paternally silent *Ube3a* gene in the brains of the E6-AP adult AS mouse following intraperitoneal injection of a Krüppel-associated box (KRAB)-fused Zinc Finger (referred to as S1 K) protein targeted

towards the *Snurf/Snrpn* promoter – effectively silencing expression of the antisense transcript. As an alternative delivery method, we have engineered artificial transcription factor (ATF)-secreting bone marrow-derived mesenchymal stem cells (BM-MSCs) following lentiviral reprogramming. Presently, we have engineered mouse BM-MSCs to secrete S1 K as confirmed by uptake into Neuro2a cells with MSC-S1 K conditioned media via fluorescent microscopy. Incubation of MSC-S1 K conditioned media with E15.5 E6-AP: yellow fluorescent protein (YFP) AS mouse primary neurons demonstrated significant reactivation of YFP-fused *Ube3a* as compared with a scramble Zinc Finger MSC (MSC-SR6) and non-transduced MSC (MSC-NT) 48 h post-treatment. We then bilaterally transplanted 250,000 MSCs from each treatment type into their respective treatment arms (MSC-S1 K, MSC-SR6, MSC-NT) into 8-week-old E6-AP: YFP mice. We observed significant reactivation of silent *Ube3a* via immunohistochemistry (IHC) and Western blotting for YFP expression in the hippocampus, cerebellum, and cortex as compared with the MSC-SR6 and MSC-NT treatment groups, 3 weeks post-transplantation. Future experiments will evaluate improvement of phenotypic outcome measures in the E6-AP mouse. Currently, we report the first-in-its-kind use of MSCs as a delivery platform for genetic modifiers in disease.

### **Mutant Allele Knockdown in the YAC128 Transgenic Mouse Model of Huntington's Disease Using an Artificial Transcription Factor**

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Huntington's disease (HD) is an autosomal dominant neurodegenerative disorder characterized by the presence of a misfolded mutant *Huntingtin* (muHTT) protein. Reduction of this protein via antisense oligonucleotide (ASO) therapies are now in clinical trials, however the current clinical approach targets both the normal and mutant *Huntingtin* allele. We have previously shown allele-specific silencing of the muHTT transcript in patient-derived fibroblasts via transcriptional activator-like effectors (TALEs) by targeting

a single nucleotide polymorphism that is highly associated with the mutant allele. Furthermore, we have demonstrated significant reduction of the muHTT (approximately 50%) and an observable reduction of the muHTT protein following unilateral striatal injection of the TALE into transgenic HD mice. In this study we examine the use of an adeno-associated virus (AAV) as a putative delivery vehicle for our therapeutic TALE transgene in the YAC128 transgenic HD mouse model. We have executed a longitudinal study evaluating AAV9-TALE for distribution of the TALE, duration of transgene expression over the course of 3 months, duration of mutant allele silencing, and attenuation of HD-related neuropathology and behavioral phenotypes. Identification of a potent, widespread delivery vehicle and assessment of the long-term duration of expression and effect of our therapeutic transgene will be vital in the evaluation of our TALE as a viable therapeutic for HD.

### **Characterization of the Effects of Conditioned Medium Derived from Endothelial Progenitor Cells on Cultured Striatal Neuronal Stem Cells**

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There is growing evidence that stem and progenitor cells exert regenerative actions by means of paracrine factors. Moreover, impairment of vascular tissues in the brain is known to be involved in the pathogenesis of neurodegenerative disorders. In line with these notions we recently demonstrated that endothelial progenitor cell (EPC)-derived conditioned medium (CM) substantially increased the viability of brain microvascular cells. In the present study we aimed to investigate whether EPC-CM supports striatal progenitor cell function and/or survival. For the preparation of EPC-CM, mononuclear cells were isolated from the peripheral blood of healthy human donors and consequently cultured under hypoxic conditions (1.5% O<sub>2</sub>) to stimulate the secretion of growth factors. Chloroform extraction and protein K treatment experiments were performed to narrow down key effectors of EPC-CM-mediated effects. The specific protein kinase B (AKT) inhibitor LY294002 and the extracellular signal-regulated kinase (ERK) inhibitor PD98059 were used to analyze for the involvement of these signaling pathways in the effects of EPC-CM. The effects of EPC-CM were monitored by immunocytochemical analysis for  $\gamma$ -amino butyric acid (GABA). We observed that primary cultures from fetal rat embryonic (E14) ganglionic eminence treated with EPC-CM demonstrated a significant increase in GABA-immunoreactivity (ir) cell densities. Inhibition of the AKT and ERK pathways suppressed the EPC-CM dependent increase in GABA-ir cell numbers. Similarly, proteolytic

digestion (protein K) and lipid extraction (chloroform) significantly reduced the effects of EPC-CM. Importantly, EPC-CM displayed neuroprotection against 3-nitropropionic acid (3-NP) induced toxicity. These findings demonstrate that EPC-derived paracrine factors substantially promote survival and/or differentiation of striatal progenitor cells by activating AKT and ERK signaling cascades. Furthermore, our results suggest the activity of EPC-CM to support cultured striatal progenitor cells involves proteinaceous factors and lipidic factors. A deeper characterization of EPC-CM constituents might represent a cell-free approach to explore novel strategies for the treatment of neurodegenerative diseases.

### **Kainic Acid and Glutamate Induce Endoplasmic Reticulum Calcium Depletion in Primary Cortical Neurons**

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The endoplasmic reticulum (ER) is a cellular compartment responsible for the folding and maturation of newly synthesized proteins; which functions as an intracellular store for calcium. The calcium concentration in the ER and the extracellular space are approximately 1000-fold higher than the cytosol and each compartment serves as a source of calcium for the cytosol. Calcium ions play an essential role in many functions, including cell-to-cell communication, synaptic plasticity, and cell survival. Our lab developed a bioluminescent reporter of ER calcium homeostasis, known as a secreted ER calcium monitoring protein (SERCAMP). This reporter enables longitudinal monitoring of ER calcium from a single biological sample. ER calcium homeostasis is essential for cellular function and health, and is compromised in several neurological disorders, including epilepsy. Epilepsy is characterized by unpredictable seizures caused by excessive neuronal activity. The contributions of extracellularly-sourced calcium to the neuronal activity associated with epilepsy are well established, however, the role of ER-sourced calcium in epilepsy pathology remains to be delineated. We induced an experimental epilepsy-like hyperexcitability in primary cortical neurons by administration of kainic acid (KA) or glutamate. We measured SERCaMP in the extracellular and intracellular compartments to examine the impact of epileptogenic agents on ER calcium homeostasis. We found that KA and glutamate significantly increased extracellular SERCaMP 24 h after treatment, which is consistent with impairment in ER calcium homeostasis. There was an increase in intracellular levels of SERCaMP, suggesting impairment in the secretory pathway of the cells or an increase in the transcriptional activity of our promoter following this treatment. Overall, our results indicate that these epileptogenic agents deplete ER calcium and support a role for ER calcium dysregulation in the pathology associated with

epilepsy-like neuronal activity. Further studies will examine the ability of ER calcium stabilizing compounds to mitigate the excitotoxic effects of epileptogenic agents.

### **Lewy Body-Like Alpha-Synuclein Inclusions Trigger Reactive Microgliosis Prior to Nigral Degeneration**

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It remains unclear whether neuroinflammation contributes to, or is merely a secondary consequence of nigrostriatal degeneration in Parkinson's disease. Our lab has characterized the accumulation of phosphorylated alpha-synuclein ( $\alpha$ -syn; pSyn) intraneuronal inclusions and nigrostriatal degeneration following intrastriatal injection of sonicated  $\alpha$ -syn preformed fibrils (PFFs) into rats. The time course of inclusion formation in this model offers the distinct advantage that nigral inclusions precede dopamine neuron loss by several months, allowing for evaluation of neuroinflammation prior to and following degeneration. To examine the neuroinflammatory signature, rats received unilateral intrastriatal injections of mouse  $\alpha$ -syn PFFs or vehicle (phosphate-buffered saline; PBS) and cohorts of rats (total  $n=114$ ) were euthanized at monthly intervals up to 6 months. pSyn inclusions in the substantia nigra pars compacta (SNpc) were most abundant at 2 months post-injection (p.i.). We observed a significant decrease in ipsilateral tyrosine hydroxylase immunoreactive (TH-ir) neurons at 5 and 6 months p.i. compared with controls ( $p \leq 0.027$ ). At 2 months, increases in microglial soma size ( $\sim 20\%$ ) and in thickness, and number of processes was observed in the SN of PFF-injected rats compared with controls ( $p < 0.02$ ). Major histocompatibility complex-II immunoreactive (MHC-IIir) microglia were observed in the ipsilateral SN in both  $\alpha$ -syn PFF and PBS control rats at all time points. Significantly higher numbers of MHC-IIir microglia were observed in the SN of  $\alpha$ -syn PFF-injected rats compared with controls at months 2, 4 and 5 ( $p < 0.006$ ) with the highest number of MHC-IIir microglia observed in the SN 2 months

p.i. ( $p < 0.02$ ). Significantly fewer MHC-IIir microglia were observed at months 5 and 6, the interval of SNpc neuron loss. To examine whether neuroinflammation in this model is associated with changes in inflammatory cytokine levels in the periphery, a separate cohort of male Fischer344 rats ( $n=120$ ) received unilateral injection of mouse  $\alpha$ -syn PFFs or vehicle (PBS), and the cerebral spinal fluid (CSF) and plasma were collected. Preliminary analysis suggests dysregulation of several inflammatory cytokines prior to, and following nigral degeneration. Collectively, our results show that Lewy body-like  $\alpha$ -syn inclusions trigger disturbances in local microglia months prior to loss of nigral dopamine neurons.

### **Preclinical Evaluation of iPSC-Derived Midbrain Dopaminergic Neurons for Parkinson's Disease**

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The possibility of replacing lost dopaminergic nigral neurons in Parkinson's disease has been a tantalizing goal for the field of brain repair. Investigators have relied on nonhuman primate studies to provide insight on the feasibility, safety, and efficacy of the approach, methods of cell collection, preparation, and viability, as well as ideal brain targets. Aiming towards clinical translation, our interdisciplinary team has developed protocols for generating human and nonhuman primate induced pluripotent stem cells (iPSCs) and subsequent differentiation into midbrain dopaminergic (mDA) neurons. We have demonstrated that autologous iPSC-derived mDA neurons can successfully integrate in the Parkinsonian monkey brain. Lastly, we have optimized methods of intracerebral cell delivery, applying real-time intraoperative magnetic resonance imaging (RT-IMRI) for improved accuracy and replicability. We have applied

these methods to evaluate the effects of autologous iPSC-derived mDA neurons in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced hemiparkinsonian rhesus monkeys. After 24 months from intrastriatal cell delivery, we found positive graft-induced functional effects by motor behavioral outcome measures and *in vivo* positron emission tomography (PET) with  $^{11}\text{C}$ -dihydrotetrabenazine. As a next step towards improved cell replacement, we used the site-specific genome editing method clustered regularly interspaced short palindromic repeats (CRISPR) to engineer iPSC lines to carry the excitatory (modified human M3 muscarinic (hM3Dq) receptor) or inhibitory (modified human M4 muscarinic (hM4Di) receptor) form of designer receptors exclusively activated by designer drugs (DREADDs). At 12 months post RT-IMRI grafting of iPSC-derived mDA-DREADDs, PET with the radioligands  $^{11}\text{C}$ -clozapine and  $^{11}\text{C}$ -raclopride showed changes in radioligand uptake associated with the administration of the designer drug clozapine-N-oxide or clozapine. Overall, our ongoing studies in nonhuman primates suggest that the combination of improved methods of producing and accurately delivering iPSC-derived mDA neurons hold promise towards the clinical application for brain repair in Parkinson's disease.

### Intracellular Calcium Leads to $\alpha$ -Synuclein Aggregation and Release in Dopaminergic Cells

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Sporadic and genetic forms of synucleinopathies like Parkinson's disease share invariant pathological hallmarks including loss of neurons, intracytoplasmic eosinophilic proteinaceous inclusions enriched in  $\alpha$ -synuclein ( $\alpha$ Syn) called Lewy bodies and neurites, and increased microglial activation.  $\alpha$ Syn is a 140 amino acid protein that misfolds and aggregates into oligomeric species which are considered pathogenic. Recently, research suggests that  $\alpha$ Syn pathology might spread through a prion-like mechanism and contribute to the development of synucleinopathies. However, what remains to be determined is the biochemical process behind release and transfer. Dopaminergic pacemaker neurons in the substantia nigra pars compacta show elevated levels of intracellular calcium, ( $[\text{Ca}^{2+}]_i$ ) due to a high density of L-type voltage-gated calcium channels (VGCCs).  $\alpha$ Syn can interact with calcium ions and form oligomers via its acidic-residue rich, calcium-binding domain at the C-terminus. Previous research showed that,  $\alpha$ Syn aggregation takes place in the presence of high  $[\text{Ca}^{2+}]_i$  within a neuroblastoma cell line, which is attenuated after treatment with VGCC blockers. Therefore, we hypothesize that increased neuronal calcium levels will lead to the release of  $\alpha$ Syn.

Employing a dopaminergic doxycycline-inducible (DOX)  $\alpha$ Syn-overexpressing cell line (MN9Dsyn) we demonstrated that 96 hours of DOX treatment led to maximum intracellular  $\alpha$ Syn expression and release. Monomeric and oligomeric conformers were detected in conditioned media. We also showed that MN9Dsyn cells express CaV1.2, the channel pore-forming subunit of L-type VGCCs. Furthermore, fluorescent calcium-imaging experiments, revealed a significant increase ( $p < 0.0001$ ) in peak amplitude fluorescence ( $\Delta F/F_0$ ) in  $\alpha$ Syn-overexpressing cells compared with vehicle-treated cells. Taken together, our studies show that  $\alpha$ Syn overexpression leads to an increase in  $[\text{Ca}^{2+}]_i$ , which in turn augments  $\alpha$ Syn aggregation and release. Next steps will be to investigate the effect of VGCC blockers on  $\alpha$ Syn release, as they represent potential therapeutics for synucleinopathies.

### Introducing a Novel Method of Intravascular Adeno-Associated Virus-Mediated Gene Delivery

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Adeno-associated virus (AAV) has shown therapeutic potential as a viral vector in various studies of gene therapy. However, research on its use in targeting intravascular cells in a localized manner is lacking. We introduce a novel method to deliver various AAV serotypes intravascularly and examine their efficiency in transducing cells of the murine carotid artery. The study aimed to examine the transduction efficiency of AAV-mediated gene delivery in cells of the murine carotid artery both with and without a fully formed aneurysm. Results of infection were visualized with green fluorescence protein (GFP) reporter gene. Naïve murine carotid artery or experimentally-induced murine carotid aneurysm was ligated distally and proximally. A small incision was made and 5  $\mu\text{l}$  AAV2, AAV5, AAV8, or AAV9 was microsurgically injected and allowed to incubate for 30 minutes. The incision was closed and tissue was excised 3 weeks following AAV injection. The carotid artery or aneurysm tissue was excised and fixed in 4% paraformaldehyde solution. On both naïve carotid artery tissue and aneurysm tissue, GFP was visualized by immunofluorescence using antibody against GFP. Overall, three out of four serotypes of AAV successfully transduced cells within both the murine aneurysm tissue and the naïve carotid artery tissue. AAV5- and AAV9-transduced aneurysm tissue showed the greatest presence of GFP, with AAV8 showing less overall fluorescence. AAV2 showed no fluorescence. AAV-mediated gene delivery is an effective way to transduce cells intravascularly with a transgene of interest.

Our method can be generalized across a wide variety of studies to further research or treat other vascular disease.

### Differential Cleavage of the Chemokine Fractalkine Alters its Effect on Primary Microglia

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Fractalkine (FKN) also known as chemokine (C-X3-C motif) ligand or CX3CL1) is an endogenous chemokine expressed throughout the body. It is a transmembrane protein containing the chemokine domain, a long mucin-like stalk, and small transmembrane and intracellular domains. FKN can be cleaved by several proteases (a disintegrin and metalloproteinase domain-containing protein 10 (ADAM10) and ADAM17) to signal as a soluble peptide, consisting of the chemokine domain and the mucin-like stalk domain (sFKN). In the central nervous system, FKN is expressed solely by neurons and binds its unique receptor, chemokine (C-X3-C motif) receptor 1 (CX3CR1), which is expressed only on microglia. FKN signaling reduces expression of proinflammatory cytokines such as tumor necrosis factor (TNF)- $\alpha$ . We have recently published studies in two models of Parkinson's disease examining the anti-inflammatory effects of the membrane-bound or soluble species of fractalkine. We demonstrated that only the soluble fragment was effective in reducing neurodegeneration. We have also shown that adeno-associated virus (AAV)-mediated overexpression of sFKN in tau transgenic (Tg4510) mice inhibited microglial activation, reduced tau pathology, and ameliorated neuron loss. However, a recent study in amyloid precursor protein/presenilin 1 transgenic (APP/PS1) mice expressing only the chemokine domain of FKN failed to rescue phospho-tau pathology present in APP/PS1;CX3CL1<sup>-/-</sup> mice. Given this discrepancy, we decided to investigate the differential microglial response to sFKN and the chemokine domain of FKN (ckFKN). We show that low nanomolar concentrations of sFKN and ckFKN significantly reduce TNF- $\alpha$  secretion by lipopolysaccharide (LPS)-stimulated primary rat microglia; however higher nanomolar concentrations of both FKN species significantly increase TNF- $\alpha$  secretion. Calcium-imaging data shows a significantly reduced calcium response to higher concentration sFKN than lower concentrations. Furthermore, we found ckFKN has an approximately 1000-fold higher EC50 than sFKN. These data suggest a differential response of microglia to high and low concentrations of FKN and that the chemokine domain alone has significantly reduced efficacy. These data may explain the discrepancies observed in the current published literature, suggesting that in future work, the form of FKN and concentration of FKN must be carefully considered.

### Advancing Stem Cell Therapy for Repair of Blood–Spinal Cord Barrier in Symptomatic Amyotrophic Lateral Sclerosis Mice

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Impairment of the blood–central nervous system barrier (B-CNS-B) via endothelial cell (EC) degeneration in amyotrophic lateral sclerosis (ALS), a fatal neurodegenerative disease, is supported by significant evidence. Such barrier damage has been recently recognized as a hallmark of this disease pathogenesis. Replacement of damaged endothelial cells with transplanted cells to restore B-CNS-B integrity may be a new therapeutic approach for ALS. Our recent study examined effects of intravenously transplanted unmodified human bone marrow CD34<sup>+</sup> (hBM34<sup>+</sup>) cells, a primary source of endothelial cells, into symptomatic G93A superoxide dismutase 1 (SOD1) mice towards blood–spinal cord barrier (BSCB) repair. Symptomatic G93A mice at 13 weeks of age received  $5 \times 10^4$ ,  $5 \times 10^5$ , or  $1 \times 10^6$  hBM34<sup>+</sup> cells or media. Results demonstrated that the highest dose of  $1 \times 10^6$  cells provided the most significant benefits at 4 weeks post-transplantation by: (1) improved behavioral disease outcomes; (2) differentiation into ECs and engraftment into spinal cord capillaries; (3) enhanced ultrastructural capillary morphology; (4) decreased Evans Blue extravasation into spinal cord parenchyma; (5) reduced astrogliosis and microgliosis; (6) improved perivascular end-feet astrocyte integrity; (7) greater spinal cord motor neuron survival; (8) decreased microhemorrhages in spinal cord gray and white matter. Also, the presence of hBM34<sup>+</sup> cells was determined after perfusion in blood smears of cell-treated animals by human nuclei marker. Additionally, elevated expression of tight junction proteins zonula occludens 1 (ZO-1), occludin, and claudin-5 were noted in cell-treated mice, yet no significant differences were found between media and hBM34<sup>+</sup> cell-treated mice. These novel data demonstrated benefits of unmodified hematopoietic stem cells derived from human bone marrow when treatment was initiated at the symptomatic disease stage, with results suggestive of potential BSCB restoration in ALS mice. Transplanted cells determined in blood circulation of treated late symptomatic ALS mice might indicate ongoing BSCB repair processes. Overall, hBM34<sup>+</sup> cell transplantation at an optimal dose may have therapeutic potential for B-CNS-B repair not only in ALS, but also in other neurodegenerative disorders with similar barrier damage.

## Biochemical and Genetic Methods for Enhancing Polysialic Acid on Transplanted Cells After Spinal Cord Injury

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Schwann cells (SCs) have shown promise as a therapeutic strategy for spinal cord injury (SCI) repair experimentally and are currently being evaluated for safety in phase I clinical trials for acute and chronic human SCI. We have shown that modification of the surface of SCs with polysialic acid (PSA) can significantly enhance their migration and support for supraspinal axon growth when transplanted into the injured spinal cord. The current investigation sought to compare the degree of transplanted and host cell polysialylation as well as axonal ingrowth when two different approaches were used to enhance PSA; (i) lentiviral vector (LV) transduction of SCs with polysialic transferase (PST) or (ii) incubation/co-injection of SCs with bacterial PST enzyme (PSTnm). For these studies a moderate (25.0 mm) spinal cord contusion injury was performed using the Multicenter Animal Spinal Cord Injury Study impactor in female Fischer rats and red fluorescent protein (RFP)-labeled SCs were transplanted into the lesion epicenter at 1 week post-SCI. Using immunohistochemistry for PSA, polysialylation of the SC implant-lesion site was found to be most pronounced when PST was expressed in the cells using LV, however, mixing SCs with PSTnm in suspension for co-injection produced pronounced polysialylation of adjacent host tissue in addition to the SC graft. Migration of SCs was greatest in the LV-PST group, though both LV-PST and PSTnm SCs migrated for significantly greater distances into adjacent host spinal tissue than RFP-SC only controls. Axon ingrowth into the transplant-lesion site, as measured by neurofilament-positive axon density, was similar among both biochemical and genetic methods of PSA enhancement and more than three-fold in amount compared with RFP-SC-only controls. These studies show that transient application of PSTnm may offer an alternative approach to genetic methods for enhancing PSA on transplanted SCs following SCI so as to enhance their migration and reparative efficacy.

## Sub-Additive Effects of Cell and Physical Therapy in a Rodent Model of Stroke

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Stroke caused by the occlusion of a cerebral artery can lead to death or severe, long-lasting functional impairments. Treatment options are extremely limited, extensive, and cost-intensive without any guarantee to restore lost functions. Physical therapy (PT) is currently the only treatment used to improve chronic behavioral impairments. Emerging approaches, such as stem cell therapies, are promising and can support tissue restoration and functional recovery. The aim of this study was to evaluate if the combination of human neural stem cell (NSC) transplantation with physical therapy will improve efficacy compared with either treatment alone. Adult male Sprague-Dawley rats underwent transient middle cerebral artery occlusion (MCAo). Success of MCAo was determined by T<sub>2</sub>-weighted magnetic resonance imaging (MRI) and animals were then randomly assigned to the following conditions: MCAo only, MCAo+NSCs, MCAo+PT, MCAo+NSCs+PT. Groups subjected to NSCs or NSCs+PT received a perilesional NSC graft (450,000 cells) at 2 weeks post-stroke. Experimental groups with PT underwent daily treadmill running at a speed corresponding to 80% of their maximum capacity. Functional deficits and improvements were followed over a time course of 10 weeks. The maximum capacity test measures the maximum speed that the rats can maintain on a treadmill and showed that animals in the exercise group improved their performance by over 50%, while the untreated and control groups saw a decline (−10%). Bilateral asymmetry testing revealed that NSCs, exercise and combination therapy groups reduced the sensorimotor neglect by 20%, 33% and 42% compared with MCAo only. The foot-fault test measured an animal's ability to integrate motor responses and demonstrated the most improvement in the NSC-treated group, with 57% fewer mistakes at week 10 compared with pretransplantation. Immunohistological analysis of the graft revealed a good survival of the transplanted cells. A combination of physical and cell therapy therefore produces sub-additive therapeutic effects that are greater than each treatment by itself.

## Extracellular Matrix Hydrogel Injection for the Treatment of Stroke: Time Course Comparison of Hydrogel Retention and Phenotypic Characterization of Invading Cells

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Stroke is the leading cause of adult disability and a significant effort is under way to develop therapies to repair the damaged tissue. The loss of function after stroke is caused by the death of neurons, leaving behind a tissue cavity filled with extracellular fluid (ECF) and cell debris. Biomaterials composed of mammalian extracellular matrix (ECM) promote constructive tissue remodeling with minimal scar formation. At ECM concentrations that have similar rheological properties as brain tissue, the biomaterial exists in a fluid phase at room temperature, while forming hydrogels at body temperature. ECM with different concentrations (0, 3, 4, 8 mg/ml) was injected into the lesion cavity after stroke to support endogenous repair mechanisms. Retention and gelation of the ECM, as well as host cell invasion and phenotype was analyzed at 1, 14 and 90 days post-injection using immunohistochemistry. Complete retention of ECM hydrogel within the cavity occurred at concentrations >3 mg/ml, with extensive diffusion into the host tissue at lower concentrations. A significant host cell invasion into the ECM hydrogel was seen at 1 day post-injection, with an average of over 350,000 cells invading in the 8 mg/ml concentration. As the acute inflammatory response was replaced with an ECM remodeling phase at later time points, there was a significant decrease in the total number of cells invading the biomaterial. Initial invading cells were of a microglia and macrophage phenotype and followed specific trails into the ECM biomaterial along topological features conducive to cell migration. The follow-on cells were neural and oligodendrocyte progenitor cells, which are essential for repopulation of the neural tissue. This characterization demonstrates that an ECM hydrogel can be readily injected and retained within the lesion cavity, while promoting a continued endogenous repair response. A behavioral study is necessary to evaluate the therapeutic efficacy of this approach.

## Phase I Dose-Escalation Study of Neural Stem Cells in Patients with Parkinson's Disease

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Parkinson's disease (PD) is a devastating neurodegenerative disease with progressive degeneration of the nigrostriatal pathway and dopaminergic cells in the substantia nigra pars compacta. There are over 10 million people afflicted with PD and the yearly mortality rate is more than 100,000 worldwide. Unfortunately, none of the available treatment options have the potential to restore the damaged nigrostriatal pathway. We have previously demonstrated in preclinical PD models that intracranial transplantation of human parthenogenetic derived neural stem cells (ISC-hpNSC<sup>®</sup>) is safe, restores nigrostriatal pathway damage and significantly ameliorates PD-like symptoms. We are currently conducting a phase I open-labeled, dose-escalation study investigating the safety and preliminary efficacy of ISC-hpNSC<sup>®</sup> in moderate-to-severe Parkinson's disease patients [ClinicalTrials.gov identifier: NCT02452723]. The clinical trial will evaluate three different dose regimens of 30, 50 and 70 million ISC-hpNSC<sup>®</sup> in 12 patients divided into 3 cohorts of 4 patients each. Currently seven patients from the first and second cohorts have successfully been transplanted with 30 and 50 million ISC-hpNSC<sup>®</sup>. Delivery of ISC-hpNSC<sup>®</sup> to the striatum and substantia nigra went according to plan without intraoperative complications. No serious adverse events associated with ISC-hpNSC<sup>®</sup> or the immunosuppression regimen have been reported. No graft-induced dyskinesia or evidence of tumors, inflammation or infection has been reported. A 6 month interim analysis of <sup>18</sup>F-dopa positron emission tomography (PET) scans and neurological scores from the first cohort will be presented. In summary, interim data shows that administration of ISC-hpNSC<sup>®</sup> is safe and has the potential to repair the nigrostriatal pathway.

## Exosomal Biomarkers in Blood for Neurodegenerative Disease

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Exosomes are extracellular vesicles secreted from all cell types in the body and range in size from 40 to 100 nm. Exosomes carry with them both surface markers that can identify their cell of origin, and also protein, peptide, and RNA cargo which may be used to identify disease processes in a particular cell. They are small enough to escape the blood-brain barrier, and it has been shown that exosomes derived from neurons or glia in the brain can be examined

after purification of blood samples. We have purified neuron-derived exosomes from individuals who are at risk of developing neurodegenerative disorders later in life, to determine whether exosomal cargo could be used to identify specific disease processes before any symptoms develop. In the first study, amyloid and phosphorylated tau (p-tau) assessment in neuron-derived exosomes revealed significant elevations in both of these biomarkers for Alzheimer's disease in children with Down syndrome, more than three decades prior to onset of dementia symptoms. In the second study, which is currently ongoing at the Knobel Institute, we have found significant alterations of dementia-related biomarkers in exosomes isolated from Division 1 athletes suffering from a concussion, and participating in high-impact sports including ice hockey, soccer, and lacrosse. Currently, methods are being refined and validated, using post-mortem brain tissue as well as blood to purify neuronal exosomes. Collectively, this novel biomarker method may be able to detect the earliest signs of pathology in individuals with a genetic or injury-related brain degenerative process.

### **Resolvin E1 Reduces Inflammation and Enhances Memory in the Ts65Dn Mouse Model of Down Syndrome**

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Alzheimer's disease (AD) occurs early in individuals with Down syndrome (DS) and progresses to near uniformity by the age of 60. Chronic inflammation, including microglial activation and elevated proinflammatory cytokines, is a key hallmark of DS-AD and a primary therapeutic target to prevent neuronal degeneration. Inflammation is normally counterregulated by specialized proresolving mediators (SPMs) which bind a special class of conserved G protein-coupled receptors that promote resolution processes. In vivo SPM therapy is in phase II clinical trials but the therapeutic potential to augment chronic brain inflammation remains unexplored. To date, resolution factors in the DS brain are uncharacterized. Since SPMs present a novel class of neuroprotective compounds that could translate towards clinical administration, we sought to explore how administration would affect neuropathological indices in a mouse model of DS. We aimed to quantify the therapeutic potential of Resolvin E1 (RvE1), a potent SPM, in the well-characterized DS mouse model, Ts65Dn and evaluate various components of resolution in post-mortem brains from individuals with DS-AD. At 8 months of age, Resolvin E1

or vehicle was delivered by subcutaneous mini-osmotic pumps for a 30-day period. At 9 months of age, behavioral tasks were employed to assess locomotion, short-term versus long-term object discrimination and cue-based memory performance. SPM-related biosynthetic enzymes, resolution receptors, downstream signaling effectors, and microglia markers were quantified by immunostaining. Proinflammatory cytokines (tumor necrosis factor (TNF)- $\alpha$ , interleukin (IL)-1 $\beta$ , IL-6, IL-12) were quantified with multiplex enzyme-linked immunosorbent assay (ELISA) in serum or by the classical Western blot technique of brain region extracts. Resolution receptors are more highly expressed in pathology-positive post-mortem DS-AD brain sections suggesting a protracted production of SPMs. In our mouse studies, chronic RvE1 treatment significantly enhanced memory behavior tasks while normalizing open field hyperactivity. Chronic RvE1 treatment also reduces peripheral inflammatory cytokines and brain microglial activation in Ts65Dn mice. We measured very specific proteomic responses to RvE1 treatment that suggest a peroxisome proliferator-activated receptor (PPAR) gamma mechanism may underlie neuroprotection conferred by this novel class of compound. Resolution receptor perturbations are correlated with pathological events in post-mortem DS-AD brains. This suggests a potential involvement of resolution processes with chronic inflammation. The positive results from chronic RvE1 treatment in Ts65Dn mice suggest a potentially safe and transferable therapy that could target chronic inflammation.

### **Effects of Traumatic Brain Injury on Measures of Limb and Tongue Function in Rats**

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Traumatic Brain Injury (TBI) can lead to neurological deficits. Testing potential therapeutic approaches in preclinical models of TBI is essential for developing therapies for clinical use. Controlled cortical impact (CCI) is a commonly used model of contusive TBI for studies in rodents. This approach allows for the control of injury severity across animals that are then tested for motor, cognitive, or other effects. As in most preclinical studies involving motor outcomes, most studies using CCI have focused on limb function (e.g. locomotion, gait, or balance). The goal of the current study was to determine the effects of CCI on orolingual motor function in rats by measuring tongue force and tongue motility as rats lick water from a force-sensing disc. Rats were challenged by increasing the distance required to reach the disc and by increasing the force required to

produce more water. We compared these effects to the rotarod and beam walk tests which are widely used and validated in rodent CCI. After collecting pre-injury baseline data for orolingual motor function, rotarod, and beam walking, rats received a unilateral CCI targeting the right sensorimotor cortex. We found that, compared with non-injured sham controls, rats with CCI exhibited immediate deficits on the beam walk and rotarod. With time, beam walk performance normalized but rotarod deficits persisted. Orolingual motor function effects were more subtle than effects on limb function, but tongue motility deficits emerged as task requirements increased. High-resolution *ex vivo* magnetic resonance imaging (MRI) was used to quantify lesion volumes. Results will be discussed in the context of modeling orolingual motor function in preclinical TBI studies.

### **The Tale of the Tail of MANF: ER Calcium Homeostasis as a Therapeutic Target**

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Mesencephalic astrocyte-derived neurotrophic factor (MANF) is an endoplasmic reticulum (ER) stress response protein capable of promoting neuroprotection and neuroregeneration. We conducted structure and function studies of the various protein domains of MANF and found the C-terminal tail (ASARTDL) is necessary and sufficient for ER localization. We also found the tail is mediating secretion in response to the depletion of calcium in the ER. ER calcium stores are important for many cellular functions, such as protein folding, lipid metabolism, and signaling pathways. Disruption of ER calcium homeostasis is implicated in multiple neurological diseases, including stroke, Parkinson's disease, and Alzheimer's disease. Using the tail of MANF, we developed a secreted ER calcium modulated protein (SERCaMP) to monitor ER calcium depletion in both *in vitro* and *in vivo* disease models. We created a *Gaussia* luciferase (GLuc)-ASARTDL SERCaMP reporter and showed that oxygen-glucose deprivation caused ER calcium depletion and decreased cell viability. Treatment with the ryanodine receptor antagonist dantrolene attenuated secretion of our reporter and increased cell viability *in vitro*. Intracranial adeno-associated virus (AAV)-GLuc-SERCaMP-injected rats showed evidence of ER calcium depletion following occlusion of the middle cerebral artery and dantrolene treatment reduced the infarction volume. Lastly, we developed a high throughput screen based on our GLuc-SERCaMP reporter and

identified novel drugs for stabilizing ER calcium and improving recovery from oxygen-glucose deprivation. Our data support a model in which stroke causes ER calcium depletion and suggests that dantrolene can be used to reduce ER calcium depletion and improve the outcome from ischemic damage.

### **Cerebral Aneurysm Healing: Monocyte Chemotactic Protein-1, Interleukin 6 and Osteopontin**

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Cerebral aneurysms (CAs) occur in up to 5% of the population, and can rupture causing 50% mortality and 30% dependency. CAs are treated with surgical clipping or endovascular coiling. Coiled human CAs that have not recurred and are obtained at autopsy have been compared with recurrent CAs, and the successfully treated nonrecurrent CAs are characterized by a sac 'plugged' with tissue ingrowth. These are characteristics of inflammatory wound healing. Based on our preliminary results, the central hypothesis is that monocyte chemotactic protein-1 (MCP-1) is necessary for aneurysm healing, which is directed by cell-specific populations and regulated by downstream mediators to create tissue ingrowth in the aneurysm lumen. We have previously demonstrated that local delivery of MCP-1, via a MCP-1-releasing poly(lactic-co-glycolic acid) (PLGA)-coated coil, promotes intra-aneurysmal tissue healing. Using a murine model of carotid intra-aneurysmal healing, we observed increased expression of interleukin 6 (IL-6) in MCP-1-coil-treated aneurysms and not in control-PLGA-only-treated aneurysms. MCP-1-mediated intra-aneurysmal healing is inhibited in mice given a blocking antibody to IL-6 receptor, and only at early time points. MCP-1-mediated intra-aneurysmal healing is also inhibited by a blocking antibody to osteopontin (OPN). Local delivery of OPN to murine carotid aneurysms via an OPN-releasing coil significantly promotes intra-aneurysmal healing, but an IL-6-releasing coil does not, suggesting IL-6 cannot promote aneurysm healing independent of MCP-1. In the MCP-1-mediated aneurysm healing pathway, OPN expression is dependent on IL-6: inhibition of IL-6 receptor significantly inhibits OPN expression in MCP-1-mediated aneurysm healing. Our findings suggest that IL-6 and OPN are key downstream mediators of MCP-1-mediated intra-aneurysmal healing.

### **Sprouting of the Corticorubral Tract Induced by an Intensive Rehabilitation Contributed to Functional Recovery in Internal Capsule Hemorrhage Rats**

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Capsular stroke causes the disruption of the corticospinal tract and motor dysfunction. Compensative sprouting of the other descending tracts is one of the substrates for functional recovery, which could be induced by rehabilitation. In the present study, we investigated the sprouting of the corticorubral tract (CRT) and the corticoreticular tract (CRt) after internal capsule hemorrhage (ICH) and their contribution towards recovery. Wistar rats were injected with collagenase into the internal capsule, and then biotin dextran amine was injected into the ipsilesional motor cortex at post-ICH day 1 to label the CRT and the CRt. ICH rats were forced to use their impaired forelimb for days 1–8. We found the CRT was significantly increased by forced-limb use on day 12 and 28, but the CRt was not. To block the CRT or the CRt selectively, we next injected lentivirus vectors into the red nucleus (neuron-specific infection of tetracycline responsive element (TRE) and enhanced tetanus neurotoxin (eTeNT) tagged with enhanced green fluorescent protein (EGFP; NeuRet-TRE-EGFP.eTeNT) and reticular formation (neuron-specific infection of murine stem cell virus and Cre recombinase (NeuRet-MSCV-Cre)) and then injected AAV vectors (8 serotype AAV hybrid capsid containing Ca<sup>2+</sup>/calmodulin-dependent protein kinase II promoter linked Tet-On 3G (AAVdj-CaMKII-rtTAV16)/8 serotype AAV hybrid capsid with double-floxed coupled modified human M4 muscarinic receptor designer receptors exclusively activated by designer drugs (DREADD) fused with mCherry (AAVdj-Flex-DIO-hM4D-mcherry)) into the motor cortex. We revealed that the recovered forelimb function was impaired with CRT blockade both in the early-recovery phase (post-ICH days 13–20) and chronic-plateau phase (days 92–99). The data suggest that the CRT is an important pathway for rehab-induced recovery of forelimb function after ICH.

### **Polyphenol Supplementation Reverses Age-Related Microglial Decline via RICTOR-Dependent Rac1/Cdc42 Signaling**

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Microglia are the resident central nervous system (CNS) macrophage, and mediate innate immunity. These cells can become dysfunctional with age, and promote neuronal death that exacerbates neurodegenerative disorders. We have previously described age-related changes in rodent microglia

using mass-spectrometry-based proteomics and identified protein networks that are dysregulated with age including transcriptional regulation, energy metabolism, and cytoskeleton remodeling. We identified a rapamycin-insensitive companion of the mechanistic target of rapamycin (RICTOR), a subunit of the mechanistic target of rapamycin complex 2 (mTORC2), as a significant upstream regulator whose inhibition drives this aging phenotype. In this study, we evaluated the effect of polyphenol supplementation on these networks. We observed a predicted reversal of age-related RICTOR inhibition that accompanied decreased inflammation and actin cytoskeleton remodeling, as well as altered chemotaxis and phagocytosis. We further explored the role RICTOR plays in age-related microglial dysfunction and identified Ras-related C3 botulinum toxin substrate 1 (Rac1) and cell division cycle 42 (Cdc42) as inflammatory mediators and cytoskeleton-remodeling proteins that were downregulated with polyphenol supplementation as possible therapeutic targets. We used label-free mass spectrometry to compare differentially expressed microglial proteins between young (3 mo.) and aged (20 mo.) rats. Ingenuity pathway analysis allowed us to identify signaling networks whose predicted activity may explain differences in experimental groups in our dataset. We then used the same approach to compare microglial proteins from aged animals fed a normal diet and those fed a diet supplemented with NT-020, a proprietary blend of polyphenols including blueberry extract, green tea extract, l-carnosine, and vitamin D-3. To validate our proteomics, we performed a phagocytosis assay, and measured RICTOR, Rac1, and Cdc42, as well as markers for inflammation and microglial homeostasis in highly aggressively proliferating immortalized (HAPI) rat microglial cells co-cultured with lipopolysaccharide (LPS) and NT-020-treated media. Predicted RICTOR inhibition in aged microglia was lost following NT-020 treatment. This loss coincided with an increase in inflammatory mediators, tumor necrosis factor  $\alpha$  (TNF $\alpha$ ) and interleukin 1 $\beta$  (IL-1 $\beta$ ), that we confirmed via the polymerase chain reaction (PCR) and enzyme-linked immunosorbent assay (ELISA). Rac1 and Cdc42 were overexpressed in aged microglia and HAPI cells stimulated with LPS, but downregulated in HAPI cells treated with LPS+NT-020. Phagocytosis was altered compared with controls in HAPI cells after LPS stimulation, but this effect was ameliorated with NT-020 supplementation. Our proteomic analysis identified several cell functions in microglia that become compromised with age and that are predicted to drive inflammation and neurodegeneration. The data revealed protein networks that govern cell growth, cytoskeleton remodeling, and the inflammatory response that converged on RICTOR as a major signaling point. We investigated the role of Rho guanosine triphosphatase (GTPase) activity downstream of RICTOR via Rac1 and Cdc42 in regulating microglial activation and control of phagocytosis. Polyphenol supplementation can attenuate LPS-induced activation of Rac1/Cdc42, and diminish inflammatory gene expression and microglial phagocytosis.

This study highlights the utility of polyphenols as therapeutic agents against age-associated microglial pathology.

## Intra-Arterial Highway to the Brain for Cell Therapy

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We are witnessing an unprecedented explosion of indications, fueled by the rapid progress in the design of endovascular devices, for intra-arterial procedures to treat central nervous system (CNS) disorders. Detachable coils revolutionized the management of intracranial aneurysms, while the introduction of stent retrievers and aspiration catheters redefined the acute stroke care. The intra-arterial neurointerventions are now practiced worldwide at high volumes in a minimally invasive fashion with a low number of complications, thus now they are becoming an attractive way to deliver directly to the brain therapeutic agents such as stem cells, antibodies or small molecules. The recent advances in real-time magnetic resonance imaging (MRI) permit one to deploy intra-arterially administered therapeutic agents in a predictable and precise fashion to maximize therapeutic potential while avoiding adverse effects due to off-target deliveries. The sorting or engineering of stem cells as well as local opening of blood-brain barrier facilitates delivery of therapeutic agents to the brain at the first pass to fully exploit the benefits of intra-arterial route. The intra-arterial route has been shown to be effective in the delivery of stem cells to the brain in large animal models as well as being safe in clinical studies. We do expect that the share of intra-arterial route in delivery of therapeutic agents to the CNS will be growing along with further conceptual and methodological advances in this field.

## Post-Stroke Sonic Hedgehog (SHH) Agonist Treatment Improves Functional Recovery by Enhancing Neurogenesis and Angiogenesis

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Due to the limitation in the treatment window of the recombinant tissue plasminogen activator (rtPA), the development of a delayed treatment for stroke is needed. In this study, we examined the efficacy of delayed post-stroke treatment (post 3–8 days) of the sonic hedgehog pathway agonist (SAG) on

functional recovery and the underlying mechanisms. We evaluated functional recovery at 1 month after stroke using locomotion analysis and Barnes maze test for cognitive function. We utilized a genetically inducible neural stem cell (NSC)-specific reporter mouse line (Cre recombinase fused to a triple mutant form of the human estrogen receptor under a nestin promoter in a Cre-activable yellow fluorescent protein reporter mouse; nestin-CreERT2-R26R-YFP) to label and track their proliferation, survival and differentiation in ischemic brain. Brain tissue damage, angiogenesis and cerebral blood flow recovery was evaluated using magnetic resonance imaging (MRI) techniques and immunostaining. Our results show that delayed treatment of SAG in stroke mice results in enhanced functional recovery both in locomotor function and cognitive function at 1 month after stroke. Further, utilizing the nestin-CreERT2-R26R-YFP mice, we showed that post-stroke SAG treatment increased the number of surviving newly born cells derived from both subventricular zone and subgranular zone (SGZ) NSCs, total surviving doublecortin positive (DCX<sup>+</sup>) neuroblast cells and neurons (neuronal nuclei (NeuN<sup>+</sup>)/YFP<sup>+</sup>) in the ischemic brain. SAG treatment also improved the brain tissue repair in the ischemic region supported by our T2-weighted MRI, cerebral blood flow map by arterial spin labeling and immunohistochemistry (alpha-smooth muscle actin and cluster of differentiation 31 (CD31) immunostaining). These data confirm an important role for the sonic hedgehog pathway in post-stroke brain repair and functional recovery, suggesting a prolonged treatment window for potential treatment strategy to modulate the sonic hedgehog pathway after stroke.

## Involvement of Nuclear Factor- $\kappa$ B Pathway in the Treatment of Spinal Cord Injury

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Spinal cord injury (SCI) is a devastating condition which is characterized by an extended secondary injury phase caused by local inflammation. Nuclear factor- $\kappa$ B (NF- $\kappa$ B) is considered a prototypical proinflammatory signaling molecule, which is activated by cytokines such as tumor necrosis factor  $\alpha$  (TNF $\alpha$ ) and others. Here, we investigated the role of NF- $\kappa$ B after SCI in rats, and determined how anti-inflammatory compounds curcumin or transplantation of spinal neural precursor cells (SPCs) modulate the activation of NF- $\kappa$ B. Rats were given one of the following treatments: curcumin or saline directly at the injured spinal cord, and thereafter daily

intra-peritoneal injection. SPCs were grafted 7 days after SCI. Nuclear translocation of NF- $\kappa$ B p65, and levels of secretory TNF $\alpha$  were determined using immunohistochemistry and multiplex immuno-bead method, respectively. Results demonstrated that the nuclear expression of NF- $\kappa$ B p65 displayed bimodal peaks at days 3 and 28 post-SCI, albeit a higher level was seen at 28 days in the control group treated with saline. Curcumin treatments caused a significant reduction in the density of nuclear NF- $\kappa$ B p65 at all time points as compared with control rats, while TNF $\alpha$  levels decreased at days 1 and 14 after SCI in curcumin-treated rats. Nuclear translocation of NF- $\kappa$ B p65 was significantly decreased at 28 days after SCI in SPC-grafted rats and the levels of TNF $\alpha$  reduced following transplantation of SPC cells at days 10 and 14 after injury which was followed by an increase at 28 days. These results demonstrated that TNF $\alpha$  regulates the NF- $\kappa$ B signaling pathway during the secondary injury phase of SCI. Furthermore, alterations in inflammatory responses produced by, either treatment with pharmacological agents or neural progenitor cell therapy, also appears to regulate NF- $\kappa$ B signaling pathways that could potentially lead to behavioral improvement after SCI.

### **A1-Exosomes Suppress Systemic Inflammation and Neuroinflammation Induced by Lipopolysaccharide in Mice**

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Neuroinflammation is an important component of many diseases of the brain. Recently there has been great interest in the therapeutic potential of the small endometrium-derived vesicles loosely defined as exosomes. We and others previously demonstrated that some preparations of exosomes produced beneficial effects in several models of brain injury. Here we examined the model for inflammation induced by systemic administration of lipopolysaccharide (LPS) to mice. We first established a low dose of LPS that at 2 to 6 hours produced increases of proinflammatory cytokines in multiple tissues, including the brain. We then tested the effects of exosomes that we had previously isolated with a chromatographic protocol from the medium of mesenchymal stem/stromal cells cultured under defined conditions. The exosomes were anti-inflammatory by several criteria and to distinguish them from other exosomes, we referred to them as A1-exosomes. Intravenously administered A1-exosomes suppressed proinflammatory cytokines in multiple tissues, including the hippocampus and cortex. Exosomes

pre-labeled with dye were found in microglia, indicating they had crossed the blood-brain barrier (BBB). In contrast, intravenous administration of 1.2 mg/kg dexamethasone decreased proinflammatory cytokines in the spleen of LPS-treated mice but not in the hippocampus or cortex. The results support previous indications that intravenously administered exosomes may be a more effective therapy for neuroinflammation than drugs and other agents that were tested in the past and that do not effectively cross the BBB.

### **CRISPR-CAS9 Mediated Non-Homologous End Joining Mechanism for Silencing of the Mutant *Huntingtin* Gene in an In Vitro Model of Huntington's Disease**

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Huntington's disease (HD) is a rare autosomal dominant neurodegenerative genetic disorder, resulting in abnormal movements of the limbs, along with cognitive deficits and psychiatric symptoms. The underlying cause of the disease is a genetic mutation of the *Huntingtin* gene (*mHTT*), which codes for the Huntingtin protein (HTT), whereby the polyglutamine (CAG) repeat domain is extended to more than 35 CAG repeats. At present, only palliative treatments are available to alleviate the symptomology of the disease, as a cure or an effective treatment has been elusive. Many studies have shown that embryonic stem cells (ESCs) can delay the progression of HD, putatively by providing a protective environment in the striatum, the region in which neurons are first lost as part of the disease pathology. More recently, transplantation of induced pluripotent stem cells (iPSCs), generated from patient's skin cells, has been proposed as an alternative cell-based therapy for HD. Although allogeneic stem cell transplantation is relatively safe, autologous transplantation provides significantly less immunogenic responses than allogeneic stem cell transplantation. However, since HD is an autosomal dominant neurological disorder, the stem cells generated from the person affected with the HD would carry the heterozygous alleles having the normal *HTT* gene and also the *mHTT*. Since the *mHTT* protein formed from the *mHTT* gene has a toxic gain-of-function property, editing the *mHTT* gene in the stem cells by either silencing the *mHTT* gene or correcting *mHTT* before using it for autologous transplantation, should prove to be more beneficial than utilizing direct autologous

transplantation. The primary goal of this study was to permanently prevent the translation of *mHTT* at a relatively high efficiency. In this study, we are applying clustered regularly interspaced short palindromic repeat (CRISPR)-CRISPR-associated protein 9 (Cas9) to silence the mutant *Huntingtin* gene, that causes HD, in the bone marrow-derived mesenchymal stem cells (BM-MSCs). CRISPR-Cas9 has been revolutionizing the prospects of gene therapy and biotechnology. It has been used widely to create knock-in, knock-out, or to edit the genes, both to investigate the function of a gene, as well as to treat genetic disorders. In order to curb the production of the *mHTT*, two CRISPR-Cas9 plasmids were constructed to target the DNA; one at an untranscription region upstream to the open reading frame (uORF), and the other nicks the DNA at the exon1-intron boundary. Our results suggest that the disruption of uORF through CRISPR-Cas9 has resulted in a 79% decrease in the mutant *mHTT* protein. Also, this study unravels the synchrony between the toxicity exerted by mutant Huntingtin and the expression of mutant Huntingtin protein in the BM-MSCs extracted from YAC128 mice that carry human mutant Huntingtin protein. We have observed that blocking the *mHTT* by CRISPR-Cas9 increased the cell survival of the BM-MSCs, which could in turn provide the means whereby allografts of 'gene-silenced' cells might be safely and effectively used for transplantation into HD patients.

### **Timing Matters: The Impact of Hypothermia on Human Neural Stem Cell Action In Vitro Supports the Need to Find Optimal Timing for Combined Cell-Based Therapy with Hypothermia for Perinatal Hypoxic Ischemic Injury**

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As many as 8 out of every 1000 live births, experiences a lack of oxygen to the brain near the time of delivery, a phenomenon called perinatal hypoxic ischemic injury (HII). If left untreated, up to 65% of these babies may have lifelong neurologic sequelae including cerebral palsy, severe motor, sensory, and cognitive impairment, epilepsy, learning disabilities, and autistic behaviors. Although hypothermia (HT) is the standard-of-care (SOC) in treating HII, it is only marginally effective in moderate cases and ineffective in severe HII. We and others have reported the efficacy and safety of transplanted human neural stem cells (hNSCs) for salvaging injured brain parenchyma (referred to as the penumbra) leading to improved histologic and behavioral outcomes in rodent models of HII. hNSCs help reconstitute damaged neural elements as well as protect endogenous neurons via anti-inflammatory, pro-angiogenic, neurotrophic,

antioxidant, anti-excitotoxic, and other neuroprotective actions. None of these reports, however, evaluated hNSC action under SOC HT conditions. It remains uncertain whether HT antagonizes or complements hNSC function. hNSCs were studied in vitro under normothermic (NT) (37°C) and SOC HT (33.5°C) conditions and evaluated on days 0, 1, 2, 3, and 6. Change in hNSC colony size over time was measured as a marker of proliferation, and migration was assessed using scratch assays. Proliferation and migration were quantified using ImageJ (National Institutes of Health). Measurements for each environment were pooled by day and expressed as means. Proteins produced by NT and HT hNSCs were evaluated on each day by Silver Stain, bicinchoninic acid assay (BCA), immunofluorescence (IF), and Western blot. For IF, we used fluorescent antibodies to  $\beta$ 3-tubulin (an immature neuronal marker) and Ki67 (a marker of cell proliferation). For Western analysis, antibodies to  $\beta$ 3-tubulin and proliferative cell nuclear antigen (PCNA – a marker of cell proliferation) were used. HT decreased the rates of hNSC proliferation and migration ( $p=0.0006$  and  $p<0.0001$ , respectively) compared with NT. The doubling time for NT hNSCs was 5.2 days compared with 21.1 days for HT hNSCs. Microscopic imaging of individual colonies over time was performed. Cells in the NT incubator showed an expansion of the colonies both in number of cells and in production of exploratory lamellipodia. Cells kept in the HT incubator showed minimal change in colony size or production of lamellipodia. HT hNSCs exhibited an altered proteomic profile via Silver Stain and decreased protein abundance by BCA analysis ( $p=0.0004$ ) relative to NT hNSCs. IF of NT hNSCs colonies showed an organized pattern of  $\beta$ -3 tubulin production, a primarily peripheral distribution of Ki67 positive hNSCs, and an even distribution of neuronal cell nuclei (as indicated by 4',6-diamidino-2-phenylindole (DAPI) staining) throughout the colony. This was in marked contrast to the hypothermic hNSCs that had substantial disorganization of both  $\beta$ -3 tubulin and Ki67 as well as an uneven nuclear distribution. Western blot analysis demonstrated no difference in  $\beta$ 3-tubulin presence in NT versus HT cells, however there was a strong trend towards a decrease in markers of cell division in HT compared with NT hNSCs ( $p=0.06$ ). Most of these changes were not apparent until after 3 days of HT. HT significantly altered hNSC proliferation, migration, and protein production in vitro. However, given that SOC HT is instituted for only 3 days following HII and most changes in HT hNSCs were not apparent until after 3 days of exposure, combining these two neuroprotective therapies seems feasible. Alternatively, optimal timing for the introduction of cell-based therapy may be after HT is completed. Coordinating therapies, whether concurrently or in sequence, to improve outcomes must be tested in animal models of HII. Furthermore, our results raise the question of whether endogenous hNSCs, required for repair and regeneration of the neonatal brain after HII, are also affected by HT.

## Hematopoietic Growth Factors Enhance Amyloid Beta Clearance Through Bone Marrow-Derived Monocytes/Macrophages

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Alzheimer's disease (AD), a rapidly growing health problem, has created serious public and personal crises at both medical and financial levels. Developing therapeutic strategies for AD is highly important, as no cure is currently available. There is a growing body of evidence showing that dysfunction of the innate immune system for amyloid beta (A $\beta$ ) clearance is crucially involved in cerebral A $\beta$  deposition and AD progression. Our earlier study has demonstrated that a 12-day treatment of combined two hematopoietic growth factors, stem cell factor (SCF) and granulocyte colony-stimulating factor (G-CSF) (SCF+G-CSF), results in reduced cerebral A $\beta$  load at 9 months post-treatment in amyloid precursor protein/presenilin 1 (APP/PS1) mice. It remains unclear, however, how the SCF+G-CSF treatment leads to cerebral A $\beta$  reduction. This study aimed to clarify whether the SCF+G-CSF-reduced cerebral A $\beta$  load through enhancing A $\beta$  clearance, and the role of bone marrow-derived monocytes/macrophages in SCF+G-CSF-reduced cerebral A $\beta$  accumulation in APP/PS1 mice. Using live brain imaging, we scanned the brain and examined A $\beta$  deposits before SCF+G-CSF treatment and 7 days post-treatment in 13-month-old APP/PS1 mice. We found that methoxy-XO4-labeled plaques in the brain were significantly decreased in SCF+G-CSF-treated mice compared with non-treated controls, suggesting that SCF+G-CSF enhances A $\beta$  clearance in the brains of APP/PS1 mice. To track bone marrow-derived cells, the bone marrow of mice expressing enhanced green fluorescent protein (GFP) under the direction of the human ubiquitin C promoter (UBC-GFP mice) was transplanted into APP/PS1 mice 6 months before SCF+G-CSF treatment. At day 12 of SCF+G-CSF treatment, ionized calcium-binding adapter molecule 1 positive (Iba1<sup>+</sup>)/GFP<sup>+</sup> cells were significantly increased in the brains of APP/PS1 mice, and the association of Iba1<sup>+</sup>/GFP<sup>+</sup> cells with A $\beta$  plaques was also significantly increased. Flow cytometry data further validated the increases of bone marrow-derived Iba1<sup>+</sup> cells in the brain and blood of SCF+G-CSF-treated APP/PS1 mice. In addition, our *in vitro* studies revealed that the ability of bone marrow-derived Iba1<sup>+</sup> cells in engulfing aggregated A $\beta$  was significantly increased by SCF+G-CSF. These findings suggest that bone marrow-derived monocytes/macrophages play an important role in SCF+G-CSF-enhanced A $\beta$  clearance. This study sheds light on the contribution of hematopoietic growth factors in restricting pathological progression of AD.

## Glial Enriched Progenitors: A Novel Cell-Based Therapeutic for White Matter Stroke

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White matter stroke (WMS) occurs in deep penetrating blood vessels in the brain and includes a spectrum of diseases from small infarcts to more diffuse areas of damage. Subcortical WMS constitutes up to 30% of all stroke subtypes. There have been no studies of specific cell transplants in neural repair in WMS. The different cellular constituents of white matter mean that WMS, unlike large artery stroke, damages primarily astrocytes, axons, oligodendrocytes, and myelin, we therefore hypothesized that a more astrocyte-based therapy is ideally suited for brain repair after WMS. Brief changes in oxygen tension permanently alter the fate commitment of induced pluripotent stem cells (iPSCs). This effect is mediated by hypoxia-inducible factor 1, and can be mimicked by prolyl hydroxylase inhibition, such as with deferoxamine (DFX). Brief treatment of iPSC-derived neural progenitor cells (iPSC-NPCs) with DFX permanently biases differentiation so that a substantial percentage of the cells differentiate into astrocytes. This process allows rapid and efficient production of a new astrocyte-based line of iPSC-derived glial enriched progenitors (iPSC-GEPs), ideally suited for brain repair after WMS that allows scaling of this process for a clinical application. In this study, iPSC-GEPs were tested in a model of subcortical white matter stroke that mimics aspects of vascular dementia. These cells were transplanted at a late or subacute stage after the infarct. iPSC-GEPs migrate widely in the injured brain and stimulate oligodendrocyte precursor cell (OPC) proliferation and differentiation, myelination of damaged brain tissue and the formation of cortical connections after stroke. iPSC-GEPs promote recovery of neurological deficits in white matter stroke. Importantly, this recovery is more substantial and complete compared with other iPSC types, including the parent line for iPSC-GEPs.

## Modulation of Receptor Protein Tyrosine Phosphatase $\sigma$ Promotes Neuroregeneration and Functional Recovery After Experimental Stroke

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Stroke leads to permanent cognitive, sensory, and motor deficits in patients. Therapeutic interventions that target the repair of stroke-induced damage that will allow for a longer treatment window after stroke onset are urgently needed. The two main cellular processes of neural repair after stroke have been described: post-stroke axonal sprouting and post-stroke neurogenesis. Understanding how these endogenous cellular mechanisms contribute to the repair of the post-stroke brain will help us develop novel therapeutic interventions. Inhibitory chondroitin sulfate proteoglycans (CSPGs) are increased within the glial scar and perineuronal net after ischemic brain injury, which might play a critical role in blocking neuroblast migration, axonal regrowth and sprouting. Protein tyrosine phosphatase  $\sigma$  (PTP  $\sigma$ ) has been identified as a major receptor for inhibitory glycosylated side chains of CSPGs. Recently, a novel small peptide mimetic of the PTP  $\sigma$  wedge region named ISP (intracellular sigma peptide) was generated and has the capacity to specifically target PTP  $\sigma$  and relieve CSPG inhibition in models of spinal cord injury, spinal root avulsion and myocardial infarction. Here, we aimed to determine whether post-stroke ISP treatment could improve functional recovery in a mouse model of cortical ischemia. C57BL/6 mice underwent transient intraluminal filament middle cerebral artery occlusion (tMCAO) and were then subcutaneously injected with ISP (30 mg/kg/day) or vehicle daily for 4 weeks beginning 24 h or 7 days post-tMCAO. Motor and sensorimotor functions were measured prior to MCAO and at 1, 2, 3 and 4 weeks post-stroke using locomotion analysis and the adhesion removal test. Cognitive function was also assessed with the Barnes maze test at 4 weeks post-stroke. We found that continuous ISP treatment starting at 24 hours post-stroke improves both survival and long-term functional recovery after stroke and 7-day post-stroke treatment improves functional recovery. Correlated with the improved behavior, immunohistochemical analysis revealed that post-stroke ISP treatment increased the total number of newly generated neuroblast cells migrating towards the peri-infarct area. We also found a robust sprouting/regeneration of serotonergic axons around the stroke peri-infarct and even into the lesion core. These results suggest that inhibition of PTP  $\sigma$ /CSPG signaling by ISP stimulates functional recovery after stroke via promoting neuroblast migration and axonal growth. Pharmacological targeting of the PTP  $\sigma$ /CSPGs interaction is therefore a potential novel therapeutic strategy for stroke treatment.

### Arginine-Sensing-Induced mTORC1 Activation Affects Tauopathies

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Tauopathies including Alzheimer's disease (AD) consist of age-associated neurodegenerative diseases for which no disease-modifying treatments exist. Our group has uncovered a unique interaction between arginine metabolism and tauopathies. Arginine metabolism affects multiple biological processes that show considerable influence upon tau pathology. We demonstrated in cells and animal models of tauopathy, the benefits of increasing arginase 1 (Arg1) in reducing many aspects of the tau phenotype (hallmarks comprised of tau effects). Several seminal findings showed lysosomal and cytoplasmic arginine sensors that modulate the mechanistic target of rapamycin complex 1 (mTORC1) signaling pathway. The first identified arginine sensor is solute carrier family 38A9 (SLC38A9), a lysosomal amino acid transporter that signals arginine sufficiency to mTORC1 within the lysosome. The second arginine sensor is a cytoplasmic regulator known as cytosolic arginine sensor for mTORC1 subunit 1 (CASTOR1). Other key studies revealed that G protein coupled receptor family C, group 6 member A (GPCR6A) binds arginine and may serve as an extracellular arginine sensor. Our data indicated increased activation of the arginine-sensing mTORC1 pathway in human AD brains and transgenic mouse model of tauopathies. We discovered that arginine producing and metabolizing enzymes, arginine sensors (SLC38A9, CASTOR1, GPCR6A), and mTORC1 complexes all increased in the hippocampus of AD patients compared with control age-matched brains. We also found that tau increased total arginine levels in the mouse tauopathy brains by 35%, increased basal levels of extracellular arginine and arginine release following neuronal stimulation. Furthermore, genetic reduction of SLC38A9 and GPCR6A inhibited normal mTORC1 signaling pathways, activated autophagy and reduced total tau protein level in cellular models. We posit that tauopathies cause impaired arginine metabolism and uncoupling of arginine-sensing mTORC1 signaling, which leads to hyper-mTORC1 activation creating a positive feed forward loop to augment the tau phenotype. Therefore, arginine sensors can become novel therapeutic targets to modify tauopathies.

### iPSC Technology: Breaking New Ground

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The discovery of induced pluripotent stem cells (iPSCs) has challenged existing paradigms, and revolutionized the fields of developmental biology and regenerative medicine over the past decade. Not only has iPSC research provided fundamental insights into basic biology, but it has also forged advances in unexpected areas, such as human disease modeling, drug development, and precision medicine. The essence of iPSC technology lies in the ability to reprogram, via defined factors, somatic cells from patients with effectively any disorder, to ‘embryonic-like’ pluripotent stem cells. These patient-derived pluripotent cells can then be differentiated into a variety of mature cell types, to potentially recapitulate phenotypes of not only monogenic diseases, but also late-onset polygenic diseases (e.g. Parkinson’s disease, Alzheimer’s disease, and schizophrenia). Moreover, the establishment of such disease- and patient-relevant phenotypes would provide powerful human platforms for finding therapies through candidate drug testing or high throughput screening. The next decade promises to significantly propel iPSC technology through improved directed differentiation methods, and development of organoid culture systems, that will push the horizons of our knowledge to ultimately help tackle complex neurological diseases. This seminar will review the basics of iPSC technology, its applications and limitations, as well as the future opportunities that it offers.

### **A9 Dopamine Neuron Differentiation from Monkey Induced Pluripotent Stem Cells and Somatic Cell Nuclear Transfer**

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Patient-derived stem cells may provide a strategy for the treatment of many diseases including Parkinson’s disease (PD), a degenerative disease characterized by the loss of A9 dopaminergic neurons in the substantia nigra pars compacta. Although numerous in vitro studies have reported successful generation of dopamine neurons derived from embryonic stem cells (mouse, human and nonhuman primates), many of these species are suboptimal for PD modeling or unavailable for transplantation experiments. Here we report the generation of A9-like neurons for African green monkey (AGM), a nonhuman primate that closely mimics PD signs in humans. We generated two different types of stem cell lines: somatic cell nuclear transfer (SCNT) lines from 11 monkeys and induced pluripotent stem cell (iPSC) lines from 2 adult monkeys using fibroblasts. We show that all the SCNT and iPSC lines express markers associated with pluripotency, including octamer-binding transcription factor 3/4 (Oct3/4), NANOG, stage-specific embryonic antigen 4

(SSEA-4), and TRA-1-60, and do not express markers for differentiated cells. Adapting human neural induction protocols to primate cell lines poses significant challenges, and the success of protocols used to derive dopamine neurons from human embryonic stem cells (hESCs) vary greatly. Using a protocol modified from Kirkeby et al., we show our lines are capable of in vitro differentiation towards A9 dopaminergic neurons. Immunofluorescence analysis revealed positive staining for tyrosine hydroxylase (TH) and forkhead box A2 (FOXA2). Western analysis shows expression of key neuronal markers in mature A9 neurons (LIM homeobox transcription factor 1 alpha; LMX1A) and absence of the stem cell pluripotency marker TRA-1-60. Qualitative real-time polymerase chain reaction (qPCR) analysis indicates that during in vitro differentiation towards A9 neurons, AGM and hESC lines express neuronal markers at comparable time points. These results indicate that AGM SCNT and iPSC lines are capable of expressing specific A9 dopaminergic neuron markers and may represent functional and transplantable A9 neurons for further studies in monkeys.

### **Gene Therapy Modalities**

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Gene therapy, regardless of the means by which it is performed, is classically associated with the treatment of the disease. For instance, the delivery of a corrected protein to correct the toxic loss of a mutated protein, is a common means to treat a disorder. Nevertheless, gene therapy is an incredibly valuable tool in the neuroscience laboratory. Gene therapy can be used to manipulate neural activity in specific circuits, to measure neurological and physiological processes, to model disease, and many other possibilities. This seminar will discuss common modalities by which gene therapy is being used in the research laboratories, and the different questions that can be addressed using gene therapy.

### **ESC-Derived Oligodendrocyte Progenitor Cells (AST-OPC1): Clinical Update and Preclinical Progress in Cervical Spinal Cord Injury**

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AST-OPC1 is a population of early-stage oligodendrocyte progenitor cells (OPCs) that is differentiated from human embryonic stem cells (hESCs) using the H1 cell line. The AST-OPC1 differentiation process is commercially scalable, compatible with current good manufacturing practices, and the resulting OPC population has been approved by the

United States Food and Drug Administration for early phase clinical testing in spinal cord injury (SCI). Studies of AST-OPC1 in rodent models of SCI provide evidence of its therapeutic benefit when transplanted directly into the injured spinal cord approximately 1 week after injury and have indicated that AST-OPC1 can act via multiple therapeutic mechanisms to promote CNS repair and augment functional recovery. In this presentation, we will discuss recent preclinical studies that explore AST-OPC1's cellular composition, potency, and multi-faceted mechanisms of action. An update on the ongoing cervical SCI clinical trial will be provided, and future directions of this candidate cell therapy will be discussed.

### **Viral Vector-Mediated $\alpha$ -Synuclein Overexpression Rat Models of Parkinsonian and Cerebellar Variants of Multiple System Atrophy**

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Multiple system atrophy (MSA) is a rare, neurodegenerative disorder with an uncertain etiology and pathophysiology. MSA can be stratified into a Parkinsonian variant (MSA-P) characterized by striatonigral degeneration with associated Parkinsonian-like motor features, and a cerebellar variant (MSA-C) characterized by olivopontocerebellar atrophy with associated ataxia. MSA is a unique synucleinopathy, where  $\alpha$ -synuclein accumulates preferentially in oligodendrocytes. MSA is believed to be a primary oligodendroglialopathy in which  $\alpha$ -synuclein aggregation is thought to elicit dysfunction in oligodendrocytes, causing disruption in myelin and reduced neurotrophic support leading to secondary neurodegeneration. Traditional animal modeling of MSA relies on transgenic murine models in which human  $\alpha$ -synuclein is overexpressed using different oligodendrocyte-specific promoters. In this study, we sought

to develop novel viral vector-mediated overexpression models of both MSA-P and MSA-C to establish more clinically relevant models for future use as platforms for drug discovery.  $\alpha$ -synuclein or green fluorescent protein (GFP) was overexpressed in oligodendrocytes using a recently developed, novel oligotrophic adeno-associated virus vector, Olig001. Sprague-Dawley rats were injected in the striatum to model MSA-P and in the pontine nucleus and middle cerebellar peduncle to model MSA-C. Histological analysis showed 94–97% of the GFP-positive cells colocalizing with oligodendroglial marker Olig2. There was little coexpression in neurons (2.9–4.7%) or astrocytes (0.18–0.49%), indicating the highly oligodendrocyte-specific tropism of this vector *in vivo*. Widespread  $\alpha$ -synuclein accumulation was seen throughout the injection areas, which were resistant to Proteinase K digestion, indicating the formation of insoluble inclusions. Loss of myelin was observed in white matter regions containing  $\alpha$ -synuclein expression. Unbiased stereological counts indicate a ~20% loss of neurons in the striatum of MSA-P rats. Taken together, our data indicates the establishment of novel animal models of the Parkinsonian and cerebellar variants of MSA, recapitulating key aspects of the disease. Long-term studies are underway to evaluate the progression of pathology and development of motor symptoms.

### **Conserved Mechanisms Underlying Benefit of MultiStem Cell Therapy for Neurological Injury and Disease**

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Over the past 20 years, cellular therapies have progressed into the clinic for the treatment of neurological disease and injury, however, an understanding of the mechanisms of benefit following cell infusion has remained incomplete. Athersys is developing MultiStem<sup>®</sup>, a unique allogeneic cell therapy, for the treatment of multiple indications. To evaluate potential conserved mechanisms of action underlying MultiStem treatment we have performed focused preclinical animal studies in models of central nervous system (CNS) injury and disease. Based on cumulative results, we hypothesize that MultiStem infusion alters the innate immune response directly via interactions with splenocytes. Detailed biodistribution studies have revealed that MultiStem cells home primarily to the spleen in an injury-dependent fashion, reside in the marginal zone and likely interact with splenocytes prior to efflux. Further, MultiStem treatment reduces both systemic inflammation and neuro-inflammation, as demonstrated by a reduction of proinflammatory gene changes, circulating proinflammatory cytokines, and monocytes-macrophages entering the nervous system. We have observed a consistent Th2 type response following MultiStem infusion, consisting of increased T regulatory

cells and ‘alternatively’ activated (M2) macrophage/microglial phenotypes. Our completed phase II randomized double-blinded placebo-controlled clinical trial of MultiStem in acute ischemic stroke has provided support of these preclinical findings. Intravenous delivery of 1.2 billion cells, 24–36 hours following stroke onset provided sustained significant functional improvements at 1 year, but also significantly reduced global proinflammatory cytokine levels and circulating T-cells in the blood. Our studies have identified conserved mechanisms underlying immunomodulation leading to neuroprotection and recovery following MultiStem therapy.

### Neurodegeneration and Neuroprotection in a Nonhuman Primate Model of Parkinsonian Cardiac Dysautonomia

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Cardiac dysautonomia is a common nonmotor symptom of Parkinson’s disease associated with loss of sympathetic innervation to the heart and decreased plasma catecholamines. Disease-modifying strategies are not available, and biomarkers are lacking. Systemic administration of the catecholaminergic neurotoxin 6-hydroxydopamine (6-OHDA) recapitulates this loss of cardiac sympathetic innervation and circulating catecholamines. We recently used positron emission tomography (PET) imaging in 10 6-OHDA-treated (50 mg/kg intravenously) adult, male rhesus monkeys to visualize and quantify cardiac sympathetic neurodegeneration, increased inflammation, and oxidative stress following the neurotoxin, which were attenuated in pioglitazone-treated animals ( $n=5$ ; 5 mg/kg) compared with placebo ( $n=5$ ). Here we report post-mortem characterization of heart and adrenal tissue in these animals compared with age- and sex-matched normal controls ( $n=5$ ). Tissues were collected following paraformaldehyde (PFA) perfusion, blocked in paraffin, sectioned at 5  $\mu\text{m}$ , immunostained, and immunoreactivity (-ir) quantified as the area above threshold (%AAT) in the National Institutes of Health, ImageJ. In the heart, immunohistochemistry was performed for sympathetic innervation (tyrosine hydroxylase; TH) and inflammation (human leukocyte antigen-DR; HLA-DR) in three layers (base to apex) and four regions (septal, anterior, lateral, inferior) of the left

ventricle. 6-OHDA induced a significant loss of cardiac nerve bundle TH-ir in all left ventricle levels and regions in both placebo- and pioglitazone-treated animals compared with healthy controls ( $p<0.001$ ); no differences were found between placebo and pioglitazone groups. A repeated measures correlation across cardiac regions and levels was observed between TH-ir and in vivo PET data for sympathetic innervation 12 weeks post-6-OHDA ( $r=0.442$ ,  $p<0.000001$ ). HLA-DR-ir was greater in the pioglitazone group than normal controls when evaluated semi-quantitatively in cardiac nerve bundles ( $p<0.01$ ) and around vessels ( $p<0.01$ ). Additionally, perivascular HLA-DR-ir was elevated in the cardiac base relative to apex, similar to PET findings. Catecholamine production in the adrenal medulla was analyzed by TH-ir and aromatic amino acid decarboxylase (AADC)-ir quantification. TH-ir %AAT was reduced in placebo animals (53.6%) compared with controls (90.5%;  $p<0.0001$ ), and was preserved in the pioglitazone group (78.9%) compared with placebo ( $p<0.01$ ). AADC-ir %AAT in the adrenal medulla confirmed these findings, with a significant difference between placebo (50.0%) and control (86.7%) animals ( $p<0.01$ ), but not between pioglitazone (69.0%) and control. High performance liquid chromatography (HPLC) for plasma norepinephrine showed significantly higher percent loss from baseline to 1 week post-6-OHDA in placebo (69.1% loss) compared with pioglitazone-treated (57.4% loss) animals ( $p=0.016$ ). Overall, these results validate in vivo findings of 6-OHDA-associated cardiac sympathetic denervation and demonstrate the ability of pioglitazone to preserve enzymes critical for catecholamine production in the adrenal medulla.

### Somatic Transgenesis Modeling in the Brain Using Different Promoters to Target Cell Type-Specific Gene Expression

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Neurodegenerative diseases affect different areas in the brain; therefore, targeting different cell types will be an advantageous strategy for further research. The Chakrabarty and Golde laboratories have proposed an experiment that will evaluate the efficacy of cell-specific promoters to target different cell types in the mouse brain. This experiment will be performed by using self-complementary adeno-associated virus (scAAV), to circumvent the need for DNA synthesis after injection, and AAV capsid 6 that is triply mutated at Y731F/Y705F/T492 V (TM6). TM6 has been shown to effectively permeate microglial cells, one of the most difficult cell types to permeate, so we set out to test each cell-specific promoter with TM6 to evaluate if the capsid

effectively targets other cell types. The following different specific cell types were tested: neuron-specific (microtubule-associated protein 2 (MAP2), Ca<sup>2+</sup>/calmodulin-dependent protein kinase II (CaMKII), neuron-specific enolase, nestin and synapsin), astrocyte-specific (glial fibrillary acidic protein (GFAP), GFAP-104 and brain lipid-binding protein (BLBP)) and oligodendrocyte-specific (myelin basic protein (MBP)). Each recombinant AAV genome was self-complementary and expressed a reporter gene, enhanced green fluorescent protein (EGFP), from a cell type-specific promoter and was packaged in the TM6 capsid. AAV preparations were intraventricularly injected into the subventricular area of neonatal wild-type mice. Mice brains were harvested 15 days and 30 days later. These brains were fixed in formalin, paraffin-embedded and expression of EGFP was tested in different cell types using immunohistochemical techniques. We observed that all of the neuronal promoters resulted in neuron-specific expression of EGFP, except the nestin promoter which also targeted astrocyte cells. The astrocyte promoter-induced expression of EGFP was tightly regulated, resulting in primarily astrocyte-specific expression of EGFP. The oligodendrocyte promoters, however, either did not result in any expression or showed scant expression from non-specific cell types. This exhaustive analysis has thus yielded a tool-kit that can be used for understanding gene function in different cells or delivering cell-specific therapeutic techniques in the brain.

### Local Action of Monocyte Chemoattractant Protein-1 and Osteopontin in Murine Aneurysm Healing

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Asymptomatic cerebral aneurysms occur in up to 5% of adults. In aneurysm therapy, though complication rates are lower for endovascular coiling than for operative clipping, aneurysm recurrence is a limitation of coiling. Recurrence is attributed to recanalization of the aneurysm neck following coil embolization, whereas the goal is to establish durable occlusion from the parent artery. Coiling therapy is limited by lack of mechanistic knowledge regarding aneurysm healing. In our murine aneurysm model, sustained local elution of monocyte chemoattractant protein-1 (MCP-1; also known as C-C motif chemokine ligand 2; CCL2) increases tissue ingrowth into the aneurysm sac which requires osteopontin (OPN)-mediated signaling. Local MCP-1 activity is necessary for murine aneurysm healing. OPN mediates healing by reparative (M2) macrophage interaction with lymphocytes and platelets, by cluster of differentiation 44 (CD44) or

integrin pathways. Carotid aneurysms are created in wild-type (WT), MCP-1 knock-out (KO) or C-C motif chemokine receptor 2 (CCR2) KO mice using our established model. MCP-1-eluting, OPN-eluting, or control poly(lactic-co-glycolic acid) (PLGA)-coated platinum coils are implanted into the aneurysm sac. Systemic neutralizing antibody is applied to inhibit MCP-1 or OPN pathways. Cytokine levels of OPN-coiled aneurysm lysate are assayed in the early days of aneurysm healing. Coiled aneurysm tissue is measured for % tissue ingrowth into the aneurysm lumen and immunohistochemistry for effector cell populations. With MCP-1-coiling, systemic depletion of MCP-1 or CCR2 by neutralization (0.8% or 9.2%) or KO (11% or 4.6%) attenuates luminal ingrowth versus WT (56%,  $p < 0.0001$ ). M2 macrophage-stained area is increased in WT (7.4%) versus MCP-1/CCR2 KO (1.4% or 1.6%) or inhibitor (1.4% or 1.5%) groups. Systemic MCP-1 administration reveals no difference in ingrowth versus vehicle. Cytokine arrays at post-OPN coiling days 1, 3, and 7 reveal upregulation of numerous cytokines including platelet factor 4 (PF4) and interleukin 17 (IL-17) versus control. Local delivery of MCP-1 is critical for murine aneurysm healing. OPN may further activate lymphocyte subsets and platelets to promote aneurysm healing, which merits future study.

### Insulin-Like Growth Factor-2, an Increasing Factor in Neonatal White Matter Injury Brain, Promotes the Differentiation of Oligodendrocyte Progenitor Cells In Vitro

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A rat model of neonatal white matter injury (NWMI) was made by hypoxia-ischemia at postnatal day 3 (P3). This model had fewer oligodendrocyte (OL) progenitor cells (OPCs), and exhibited disorganization of OL development in layers II/III of the sensorimotor cortex without apparent neuronal loss, as well as mild hindlimb dysfunction with imbalanced motor coordination in adulthood. Alterations of dendrite morphology and electrical responses in the cortex were observed before the rearrangement of the motor map in the model. Green fluorescent protein (GFP)-positive OPCs grafted into the corpus callosum at P5 can survive at least for 3 weeks and differentiate to adenomatous polyposis coli (APC or CC-1) positive cells accompanied by myelination. To know what kinds of factors are related to the differentiation of grafted OPCs, analysis of gene expression at P5 of neonatal WMI model was performed. Among the 98 factors increased, we focused on insulin-like growth factor (IGF)-2, and investigated the role of IGF-2 on cultured OPCs. OPCs obtained from mixed glial culture, were plated on poly-L-lysine (PLL)-coated slide glass at a density of 5000/cm<sup>2</sup>,

expanded with fibroblast growth factor 2 (FGF-2) and platelet-derived growth factor (PDGF) for 4 days and allowed to differentiate for 6 days with ciliary neurotrophic factor (CNTF) and 3,5,3'-triiodothyronine (T3). IGF-2 (100 ng/ml) was applied to OPCs from the start of the differentiation, followed by fixation at 2 or 6 days later. Serial expressions of the following immunological markers on OPCs were first confirmed: neural glial antigen 2 (NG2), PDGF receptor A (PDGFR $\alpha$ ), CC-1 and 2',3'-cyclic-nucleotide 3'-phosphodiesterase (CNPase). It was found that the proportion of NG2-positive cells and PDGFR $\alpha$ -positive cells were significantly decreased while the proportion of CC1-positive cells and CNPase-positive cells were increased on the second day. A similar tendency was also observed on day 6 of differentiation. These data suggest that IGF-2, a factor upregulated in NWM brain, promotes the differentiation of OPCs, which is more prominent in the presence of CNTF and T3.

### Schwann Cell Transplantation Alters the Innate Immune Response After Spinal Cord Injury

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Schwann cell (SC) transplantation has shown potential as a therapeutic strategy for spinal cord injury (SCI) treatment. Currently, United States Food and Drug Administration-approved phase I clinical trials seek to determine the safety of SC transplantation in humans with subacute and chronic SCI. SCs are the myelinating glia of the peripheral nervous system (PNS) and are known to play a critical role in repair after injury. In the PNS, SC-mediated repair occurs in the presence of macrophage recruitment from the peripheral circulation and an upregulation of both pro- and anti-inflammatory mediators. However, the interaction between SCs and the activities of the innate immune response during central nervous system injury and repair remains poorly understood. The goal of our study was to assess if SCs, when implanted subacutely in an animal model of incomplete thoracic SCI, were able to regulate the phenotypic state of microglia and macrophages within the lesion environment. Using fluorescence-activated cell sorting (FACS) analysis and immunohistochemistry, we found that transplanted SCs drive a specific anti-inflammatory low level of induced nitric

oxide synthase (iNOS<sup>LOW</sup>) phenotypic state, in activated immune cells, without altering the expression of other proinflammatory markers like cyclooxygenase 2 (COX2) and cluster of differentiation 38 (CD38), or enhancing the levels of characteristic anti-inflammatory markers, such as arginase 1 (ARG-1) and CD206. In addition, while the SC graft showed less CD68 positive (ED1<sup>+</sup>) or ionized calcium-binding adapter molecule 1<sup>+</sup> (Iba1<sup>+</sup>) expression compared with lesion-only controls, both markers were elevated in adjacent tissue surrounding the transplants along with increased coexpression of the scavenger receptor CD163. In sum, these findings have revealed that SCs, within the injured spinal cord milieu, can drive alternative phenotypic states of activated immune cells of the innate immune response and these alterations could play a key role in their reparative action.

### The Difference Between Activity and Function: Utilizing Mouse Models and hiPSCs to Elucidate the Electrophysiology of Bipolar Disorder

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Bipolar disorder (BD) is a neuropsychiatric disease that impacts 2.6% of the adult population, and is characterized by oscillations in depressive and manic behavior. BD is the most fatal psychiatric disease due to a high suicide rate, and little is known regarding its underlying pathology. Currently there is no therapy that is both safe and efficacious for treating BD, which is a critical unmet need. Utilizing transgenic mouse models, we recently demonstrated collapsin response mediator protein-2 (CRMP2) plays an integral role in BD's molecular pathology; to the best of our knowledge, our study is the first to provide mechanistic insight into the molecular pathology of BD. How CRMP2 mediates BD has yet to be elucidated. Employing CRMP2 transgenic mice as models for BD, we have discovered CRMP2 activity impacts neuronal electrophysiology, structure, and proteomics. Interestingly, many of the aberrations found in the transgenic CRMP2 neurons superficially appear counterintuitive, but under further examination expose the complexity of how neuronal networks function. Specifically, BD-like transgenic CRMP2 neurons appear to have hyperactive calcium activity, while having less neuronal-network signaling. Collectively, these works begin to illuminate long sought-after insights in BD pathology, and offer new targets for future BD therapeutics.

## **Stem Cell Factor and Granulocyte Colony-Stimulating Factor Promote Brain Repair and Cognitive Function Through VEGF-Mediated Angiogenesis in a Mouse Model of CADASIL**

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Cerebral autosomal dominant arteriopathy with subcortical infarct and leukoencephalopathy (CADASIL), a NOTCH3 gene mutation-induced cerebral small vascular disease, is characterized by progressive degeneration of vascular smooth muscle cells (VSMCs) in cerebral small arteries, leading to ischemic stroke and vascular dementia. There is currently no treatment that can slow the progression of CADASIL. We have recently demonstrated the efficacy of combining two hematopoietic growth factors, stem cell factor and granulocyte colony-stimulating factor (SCF+G-CSF) in improving cognitive function and increasing cerebrovascular density (angiogenesis) in a transgenic mouse model of CADASIL (TgNotch3R90C). The aim of this study was to determine how SCF+G-CSF promoted angiogenesis and whether SCF+G-CSF-enhanced angiogenesis was involved in brain repair and cognitive improvement in TgNotch3R90C mice. To examine whether SCF+G-CSF-promoted angiogenesis is mediated by vascular endothelial growth factor (VEGF), Avastin, a drug for inhibiting angiogenesis by neutralizing the biologic activity of VEGF, was given before SCF+G-CSF treatment. Water maze testing results revealed that the SCF+G-CSF-improved spatial learning and memory in TgNotch3R90C mice was prevented by Avastin pretreatment. In addition, reduced cerebrovascular densities in TgNotch3R90C mice were restored by SCF+G-CSF treatment, whereas the SCF+G-CSF-enhanced angiogenesis was completely eliminated by Avastin. Moreover, Avastin also blocked the SCF+G-CSF-increased neural network rewiring (microtubule-associated protein 2 (MAP2), neurofilament marker SMI312, and growth-associated protein 43 (GAP43) immunostaining), neurogenesis (doublecortin immunostaining) and synaptogenesis (polysynaptic density 95 (PSD95) and synaptophysin immunostaining) in the brains of TgNotch3R90C mice. Furthermore, Western blot data showed that VEGF expression was significantly decreased in cultured cerebral VSMCs of TgNotch3R90C mice and the whole brain of TgNotch3R90C mice compared with the age-matched wild-type mice. SCF+G-CSF treatment enhanced the VEGF expression in both the cultured VSMCs and brain tissue of TgNotch3R90C mice. These findings suggest that SCF+G-CSF-increased VEGF may play a key role in enhancing cerebral angiogenesis, which is required for promoting brain repair and cognitive function in a mouse model of

CADASIL. This study sheds light on how hematopoietic growth factors restrict CADASIL pathology.

## **Cerebral Chemokine Receptor CCR5 Contributes to Postischemic Brain Protection**

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The chemokine (C-C motif) receptor 5 (CCR5) is a G protein-coupled receptor highly expressed on T-cells and macrophages. It has been well studied that CCR5 is involved in the recruitment of immune cells in both physiological and pathological conditions. Emerging evidence has revealed that CCR5 is also expressed in the brain and plays an important role in the central nervous system. However, the role of CCR5 in ischemic brain injury remains unclear. In this study, we have determined whether hematogenous or cerebral CCR5 is involved in brain damage after ischemia. Bone marrow chimeras were created in both wild-type (WT) and CCR5<sup>-/-</sup> mice (3-month-old male). After hematopoietic reconstitution at 1 month post-bone marrow transplantation, permanent cerebral ischemia was performed by permanent occlusion of the middle cerebral artery (MCAO). At 48 hours after MCAO, cerebral infarct volume was measured by triphenyl tetrazolium chloride staining. In an additional experiment, mice were examined for neurological deficits starting 1 week after MCAO and were sacrificed at 2 months post-ischemia. Brain sections were processed for histological and immunohistochemical analyses. We found that CCR5<sup>-/-</sup> mice transplanted with WT bone marrow had larger infarcts than those of WT mice with WT bone marrow transplantation. Importantly, the infarct volume of CCR5<sup>-/-</sup> mice that received WT bone marrow transplantation was similar to those of mice lacking CCR5 both in the brain and in the hematogenous cells, suggesting that CCR5 in the host brain is involved in the brain damage after ischemia. Sensorimotor function (tape removal test) and motor coordination (rotarod test) were significantly decreased in CCR5<sup>-/-</sup> mice that received either WT or CCR5<sup>-/-</sup> bone marrow transplantation. At 2 months after MCAO, the infarct cavity was still significantly enlarged in CCR5<sup>-/-</sup> mice that received either WT or CCR5<sup>-/-</sup> bone marrow transplantation as compared with the WT mice. In addition, the number of Fluoro-Jade C-positive degenerative neurons in the peri-infarct area was significantly increased in the CCR5<sup>-/-</sup> mice. We also observed that microtubule-associated protein 2 (MAP2) positive dendrites were significantly decreased in the peri-infarct area of CCR5<sup>-/-</sup> mice treated with either WT or CCR5<sup>-/-</sup> bone marrow. These data suggest that cerebral CCR5 is required for neuroprotection in ischemic brain injury. This study offers new insight into a novel role of CCR5 in stroke, which would provide a new target for stroke therapy.

## Combined Stem Cell Factor and Granulocyte Colony-Stimulating Factor Promote Corticospinal Tract Sprouting and Improve Neurological Function in the Chronic Phase of Traumatic Brain Injury

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Traumatic brain injury (TBI) is a major cause of long-term disability among children and adults. To date, evidence-based treatment for TBI recovery, especially in the chronic phase, is not yet available. Our previous studies have shown that combined stem cell factor (SCF) and granulocyte colony-stimulating factor (G-CSF) can enhance cerebral neural network remodeling and improve neurological function recovery in the chronic phase of stroke. However, the efficacy of the combined SCF and G-CSF (SCF+G-CSF) to enhance TBI recovery in the chronic phase remains unexplored. In this study, we used a controlled cortical impact model of TBI to determine the effectiveness of a single or repeated SCF+G-CSF treatment on central nervous system (CNS) repair in the chronic phase of TBI. At 3 months post-TBI, mice were randomly divided into three groups: a vehicle control group, a single SCF+G-CSF treatment group, and a repeated SCF+G-CSF treatment group. Age-matched mice without TBI served as sham controls. SCF+G-CSF were subcutaneously injected for 7 consecutive days (single treatment) or the 7-day treatment was repeated a total of three times with a 4-week interval between each treatment (repeated treatment). Neurobehavioral testing revealed that repeated treatment significantly improved recovery of spatial learning and memory (water maze test) and sensorimotor function (adhesive tape removal test). The TBI mice that received a single SCF+G-CSF treatment showed a trend towards improved sensorimotor function ( $p=0.069$ ). Using the approach of tracking corticospinal tract pathways by intracortical injections of biotinylated dextran amine in the contralesional hemisphere, we observed that the contralateral corticospinal tract sprouting to the lesion side of 5–7 cervical spinal segments was significantly increased in TBI mice that received the single treatment and repeated treatment of SCF+G-CSF while the repeated treatment showed superior enhancement effects in sprouting of corticospinal tract fibers as compared with the single treatment. Moreover, Sholl analysis of the sprouting axons further confirmed that both the single treatment and repeated treatment of SCF+G-CSF significantly increased sprouting axons when compared with the vehicle controls. The repeated SCF+G-CSF treatment-enhanced axon sprouting was much greater than the single treatment group. Confocal imaging also revealed that the newly generated axons formed synapses with other neurons in the cervical spine. Furthermore, the number of sprouting axons was positively correlated with sensorimotor function recovery. These findings suggest that combined

SCF and G-CSF treatment in the chronic phase of TBI promotes corticospinal tract sprouting and improves neurological function recovery. This study provides a potential novel therapy for chronic TBI recovery and reveals a possible mechanism underlying SCF+G-CSF treatment-enhanced TBI recovery.

## The Role of eIF5A Hypusination on TDP-43 Accumulation and Stress Granule Formation in Frontotemporal Dementia TDP-43 Proteinopathy

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Transactive response (Tar) DNA Binding Protein-43 (TDP-43) is a heterogeneous ribonuclear protein and is linked to frontotemporal dementia (FTD) pathology. FTD refers to a group of disorders resulting in progressive brain atrophy involving the frontal and/or temporal lobe. One major feature of FTD neuropathology is nucleocytoplasmic shuttling of TDP-43 and inclusion formation in the cytoplasm. Recently, accumulation of TDP-43 protein in stress granules (SGs) has been reported to be the surrogate feature of FTD pathology. Interestingly, the eukaryotic translation initiation factor 5A (eIF5A), is a crucial translation factor with linkage to nucleocytoplasmic transport, mRNA stability and SG formation. eIF5A is the only known protein undergoing a post-translation modification known as hypusine, eliciting therefore activation of eIF5A (eIF5A<sup>hypk50</sup>). We found a correlation between the levels of eIF5A<sup>hypk50</sup> and TDP-43 pathology in the TDP-43 transgenic mouse model. Moreover, we found increased SG formation in this model, as measured by expression levels of T-cell-restricted intracellular antigen-1 (TIA-1; SG marker). By immunoprecipitation, we demonstrate a direct interaction of TDP-43 with eIF5A<sup>hypk50</sup> proteins, suggesting that eIF5A<sup>hypk50</sup> binds to and promotes TDP-43 accumulation in SGs and the cytoplasm. Indeed, our group is the first to report that pharmacological or small interfering RNA (siRNA) inhibition of hypusination reduces cytoplasmic TDP-43 levels and its accumulation in SGs, suggesting that modification in the eIF5A pathway beneficially reduces TDP-43 pathology. Strategies to investigate the exact mechanism by which eIF5A<sup>hypk50</sup> exerts its effects on TDP-43 pathology both in vitro and in animal models is currently under investigation. Preliminary genetic studies have implicated eIF5A<sup>hypk50</sup> in impairing RNA metabolism of TDP-43 target molecules, suggesting a TDP-43 loss-of-function. Overall, we reveal eIF5A<sup>hypk50</sup> as a novel and plausible therapeutic target in TDP-43 proteinopathy disorders.

## Gene Expression Changes and Chronic Responses in a Repetitive Mild TBI Mouse Model

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The incidence of traumatic brain injury (TBI) is estimated at 0.5% per year worldwide, with a much higher frequency among service personnel and athletes. The majority of TBIs are mild; however, even mild injuries can result in deleterious cognitive effects, especially if these injuries are repetitive in nature. There is no current effective treatment for these types of injuries. In an effort to better understand the underlying mechanisms of injury, and how to treat it, we tested mice receiving closed head injuries once per week for 5 weeks along with potentially synergistic treatment by tert-butylhydroquinone (tBHQ), a nuclear factor erythroid 2-related factor (Nrf2) antioxidant pathway activator, and pioglitazone, a peroxisome proliferator-activated receptor (PPAR)- $\gamma$  agonist. At acute and chronic times, we evaluated gene expression, cognitive changes, and immunohistochemistry for microglial changes (ionized calcium-binding adapter molecule 1; Iba1) and lipid peroxidation (4-hydroxynonenal). mRNA samples from the ipsilateral hippocampi 1 day post-injury were evaluated with Affymetrix GeneChip Arrays. Our initial examination (four groups,  $n=6$  per group) indicated that genes displayed a variety of expression patterns. For example, dysregulations after injuries alone included upregulation of G protein-coupled receptor 3 (*Gpr3*) and downregulation of aminolevulinic acid synthase (*Alas2*). After injuries, the transcription factor modulation caused elevation of cysteine-rich secretory protein LCCL domain-containing 2 (*Crispld2*) and lowering of erythroid differentiation regulator 1 (*Erd1*) expression. Some genes that were decreased by the injury were increased by the treatment, e.g. sex determining region Y (SRY)-box 3 (*Sox3*), and certain genes, like ankyrin repeat and suppressor of cytokine signaling box-containing 6 (*Asb6*), were induced by the injury and reduced by the treatment. We have also shown that object recognition memory is impaired 2 months following injury and that this is ameliorated by treatment. Through these approaches, we hope to better define inflammatory responsive transcription factor signaling pathways and identify factors that could be targeted to produce neuroprotection and improve outcomes for TBI patients.

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## Brain Slice Culture Models of CNS Targeted Gene Expression

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We sought to establish ex vivo models for future experiments by employing the use of organotypic brain slice cultures (BSCs). BSC models can be used to study aspects of Alzheimer's disease (AD) through three-dimensional systems and can be used to observe cellular and molecular processes of the brain in real-time over long periods in culture. The BSCs of neonatal mice contain the cortex and hippocampus and are harvested from p8 or p9 mice. The slices are 350  $\mu\text{m}$  thick and are transduced on day 0 in culture with recombinant adeno-associated viruses (rAAVs) to deliver fluorescent proteins with different neuronal and nonneuronal promoters and mutated and native capsid serotypes. We have used our BSCs in combination with rAAVs to study gene expression of AD-relevant genes and the development of proteinopathies, but they show potential to study other aspects of AD such as neuroinflammation and synaptic and network properties. The rAAVs were used to transduce the BSCs and show great efficiency since initially they cause mild immune responses, infect both nondividing and dividing cells, and lack pathogenicity, but show no toxicity. By using different promoters, rAAVs can target specific transduction of neurons, astrocytes, oligodendrocytes, and microglia, and in conjunction with certain capsids, show varying efficiencies and infectivities. To produce rAAVs, the microscale production method for rAAV was used where HEK293 T-cells grown in a 6-well plate format are transfected with plasmid of interest/AAV helper plasmid with polyethylenimine (PEI) and the media produced transduces the BSCs. This microscale production method requires minimal incubator space, is cost efficient, requires small amounts of plasmid of interest, and enables one to harvest, produce, and transduce on the same day. Using the BSC models and rAAVs, we were able to determine what capsids and promoters are most effective and efficient in successfully transducing specific brain cells relevant to AD ex vivo, which is necessary to further understand and develop gene therapy. These models could enable streamlining of preclinical experiments and may be used to further understand

mechanisms contributing to AD and other neurodegenerative diseases.

## Endoplasmic Reticulum Located Trophic Factor CDNF Protects and Maintains Dopamine Neurons

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In Parkinson's disease (PD), dopamine (DA) neurons located in the substantia nigra (SN) degenerate and die. Since all current therapies are symptomatic, our attempt is to develop novel disease-modifying therapies for PD. Glial cell line-derived neurotrophic factor (GDNF) and its homologous protein neurturin can protect and repair DA neurons in animal models of PD, but the results of their phase II clinical trials with PD patients have given controversial results. Our group has discovered a new endoplasmic reticulum (ER)-located neurotrophic factor (NTF)-cerebral dopamine neurotrophic factor (CDNF). We have solved the crystal and solution nuclear magnetic resonance structure of CDNF and its homologous protein mesencephalic astrocyte-derived neurotrophic factor (MANF) and found that they differ from other known NTFs. We have demonstrated that CDNF can protect and repair midbrain DA neurons in rodent 6-hydroxydopamine (6-OHDA) and 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) models of PD at least as efficiently as GDNF. Thus, CDNF acts like the 'conventional' NTFs promoting cell survival and functional recovery of midbrain circuits. However, differently from other NTFs, CDNF is mainly located in the ER, where it regulates ER stress and unfolded protein response (UPR) pathways. In nonhuman primate models of PD, CDNF protects and repairs dopamine neurons, and regulates ER stress more efficiently than GDNF. These results indicate that CDNF may prove to be an efficient disease-modifying therapy for PD. To understand the role of CDNF in mammals we created CDNF knock-out mice (*Cdnf*<sup>-/-</sup>). CDNF-deficient mice have a normal lifespan, but surprisingly develop an age-dependent loss of enteric neurons and constipation, similar to PD patients. There is also an age-dependent amphetamine-induced hyperactivity in *Cdnf*<sup>-/-</sup> mice that most probably is the result of aberrant dopamine transporter function.

## CRISPR/Cas9 Technology. What is the Big Deal?

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The ability to modify the genome holds enormous potential for biotechnology and biomedical applications. To facilitate

this, a number of technologies have been developed over the years; the most efficient of which are 'designer nucleases'. These engineered proteins consist of a catalytic domain of an endonuclease coupled with a DNA binding domain, allowing you to introduce a desired modification at a specific genomic location. The newest class of these customizable nucleases, CRISPR/ Cas9 (Clustered Regular Interspaced Short Palindromic Repeats/CRISPR-associated protein 9), was developed from the adaptive immune system of bacteria. In this approach, the effector domain – the protein Cas9 – is targeted to the desired loci by a short guide RNA with complementarity to the DNA sequence of interest, therefore, multiple custom targeting reagents can be easily designed by standard molecular biology techniques. Furthermore, the nuclease domain of the Cas9 protein can be mutated to be adapted for applications beyond DNA editing, such as epigenetic modulation or DNA labeling. Considering its remarkable ease, efficiency, and versatility, it is no surprise that CRISPR/Cas9 technology has been described as 'the biggest biotech discovery of the century'. This seminar will review the basics of the technology, as well as potential applications and limitations, including a detailed review of the workflow for putting CRISPR into practice.

## Differentiation and Characterization of Isogenic Cells from African Green Monkey for Potential Transplantation

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Major aspects of idiopathic Parkinson's disease (PD) are due to a depletion of dopaminergic neurons in the substantia nigra for which cell replacement therapy might ameliorate signs and symptoms associated with the disease. Published evidence and preliminary data in rodents and nonhuman primates support the hypothesis that dopamine (DA) neuron replacement strategy can achieve modest success. However, a major limitation in the effectiveness of DA-ergic neuron transplantation in humans is that (1) only a relatively small fraction of transplanted cells survive or retain their neural characteristics and (2) all human studies thus far have been done with allografts using different protocols for immunosuppression. Since the contribution of innate immunity in cell replacement strategies is poorly understood, it is difficult to determine if cell survival and differentiation limitations post-transplantation are due to inefficient differentiation methods, allograft issues, or unintended effects of long-term immunosuppression. To circumvent immune compatibility issues, patient-derived induced

pluripotent somatic cells (iPSCs) and somatic cell nuclear transfer (SCNT) have emerged as candidates to produce viable replacement dopamine neurons but have not been extensively studied in nonhuman primates. Here we present data for the derivation of 10 SCNT and 2 iPSC lines reprogrammed from the adult fibroblasts of African green monkeys. Our analysis shows normal karyotype, expression of pluripotency markers and teratoma formation for these lines. We differentiated both lines to A9 subtype dopamine neurons and characterized them based on quantitative real-time polymerase chain reaction (qPCR) analysis, immunocytochemistry (ICC), DA release and fluorescence-activated cell sorting (FACS) analysis. We injected four of the SCNT-derived lines into six monkeys and showed green fluorescent protein (GFP) expression and cell survival for up to 3 months. The successful derivation and initial characterization of iPSC and SCNT lines will prove useful in studies examining the contribution of innate immunity to cell survival and functionality post-transplantation when matched or not matched with their fibroblast donors.

### The HSP90 Activator AHA1 Promotes Pathogenic Tau

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Pathogenic tau is the hallmark protein in diseases known as tauopathies, the most common one being Alzheimer's disease (AD). Previous studies have shown that heat shock protein 90 (HSP90) and its cochaperones can regulate tau pathogenicity. The activator of HSP90 adenine triphosphatase (ATPase) homolog 1 (AHA1) is a cochaperone of HSP90 that stimulates its ATPase activity. Our lab has shown that AHA1 is able to dramatically increase the production of aggregated, toxic tau species. AHA1 colocalized with tau pathology in human brain tissue and this association positively correlated with AD progression. Overexpression of AHA1 in the rTg4510 tau transgenic mouse model promoted the formation of both insoluble and oligomeric tau, which led to both neuronal loss and cognitive deficits. Drugs targeted at inhibiting HSP90 have been a popular therapeutic treatment in tauopathies recently, and while these inhibitors do lead to reductions in toxic tau species, most HSP90 inhibitors are not blood-brain barrier-permeable and can present associated toxicities. We hypothesized that targeting AHA1, which stimulates the ATPase activity of HSP90, could be a more direct way of reducing pathogenic tau. Therefore, we screened several novel AHA1 inhibitors and found that some were able to reduce insoluble tau *in vitro*. Overall, these data suggest that AHA1 is able to modulate pathogenic tau

species through its interaction with HSP90 and could offer an excellent therapeutic target for AD and other tauopathies.

### LISPRO Mitigates Alzheimer-Like Cognitive- And Neuropsychiatric-Behavioral Deficits in APP/PS1 Mice

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Alzheimer's disease (AD) is characterized by progressive decline of cognitive function with associated neuropsychiatric symptoms including weight loss, anxiety, agitation, depression, irritability, and aggressiveness. However, no therapeutic interventions have yet been shown to delay progression of the disease. Recently, we have shown that LISPRO (LP), an ionic cocrystal of lithium salicylate and proline, has a better therapeutic profile than lithium carbonate in terms of safety and efficacy in ameliorating AD pathology in cell culture and transgenic mouse models. The present study was designed to evaluate and compare the efficacy of LP with lithium carbonate (Li<sub>2</sub>CO<sub>3</sub>; LC) and lithium salicylate (LS) in ameliorating cognitive impairments and associated neuropsychiatric symptoms, including depression, anxiety, irritability, and impaired locomotor function, in amyloid precursor protein/presenilin 1 (APP/PS1) mice, a commonly used transgenic model of AD. Female APP/PS1 mice at 4 months of age were orally treated with LP, LS, or LC for 9 months at 2.25 mmol lithium/kg/day followed by determination of body weight, growth of internal organs and cognitive and non-cognitive behavior. Untreated age-matched non-transgenic mice (B6C3F1/J) served as controls. No significant differences in the growth of body weight, brain, heart, lung, spleen, liver or kidney were found between lithium treated- and untreated cohorts. LP treatment produced superior improvement of cognitive function compared with untreated APP/PS1 mice, as shown by a lower escape latency during training and probe trial of the Morris water maze test and longer contextual freezing time during the fear conditioning test. LP treatment also reduced depression-like behavior, as assessed by tail suspension test, and irritability, as assessed by the touch escape test. However, lithium treatment did not alter anxiety or locomotor activity as assessed by open field, elevated plus maze or accelerated rotarod tests. Our findings suggest that LISPRO showed superior prevention of cognitive impairment, depression, and irritability in female APP/PS1 mice

compared with lithium carbonate, supporting the potential of this lithium formulation for the treatment of AD.

## The Development of a Neural Stem Cell Therapy for Chronic Disability After Stroke

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Chronic disability after stroke represents a major unmet neurologic need. ReNeuron's human neural stem cell (hNSC) therapy using its proprietary CTX cell line, currently progressing through clinical studies, is pioneering a unique treatment. The Pilot Investigation of Stem Cells in Stroke phase I trial conducted in Glasgow, Scotland published in August 2016, showed no safety concerns and some promising signs of efficacy. A UK single-arm phase II multicenter trial in patients with stable upper-limb paresis showed positive improvements in patients across a range of motor function tests and disability scales up to 12 months post-treatment, which has led to Investigational New Drug approval for a pivotal randomized controlled trial of CTX in disabled stroke patients in the United States. This trial will begin recruitment in the second quarter of this year, with Dr Sean Savitz (University of Texas, USA) as PI. CTX (or CTX0E03) is a manufactured, conditionally immortalized and clonal hNSC line generated with our c-Myc and modified estrogen receptor fusion protein (c-mycERTAM) technology. The cell line was originally isolated in 2003 and a substantial amount of characterization as well as quality, safety, biological activity, and animal efficacy data has been generated since then. The c-mycERTAM technology has enabled CTX to be manufactured at large scale under current Good Manufacturing Practice (cGMP) conditions, ensuring sufficient supply to meet the demands of research, clinical development and, eventually, the market. CTX has key pro-angiogenic, pro-neurogenic, and immunomodulatory characteristics; these are mechanistically important in functional recovery post-stroke. In this talk, I will chart the progress of CTX cell therapy both in preclinical stroke models and phase I and II clinical trials. In collaborative preclinical studies, I will show the versatility of CTX in responding to different host environments to adapt its regenerative capabilities.

## PAMAM Dendrimers Cross the Blood-Brain Barrier When Injected Systemically via Tail Vein In Vivo and Use of Dendrimers as a Vehicle to Deliver Large Coding and Noncoding Plasmids In Vitro

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Dendrimers are 3-dimensional nanoparticles that are highly branched having multiple applications in the field of biomedicine. Previous evidence shows that the conventional G4 polyamidoamine (PAMAM) dendrimers having 100% amine surface (G4-NH<sub>2</sub>) are highly toxic in vitro and in vivo due to their highly positive-charged surface. Therefore, there is a need to modify and synthesize a new form of dendrimers having an overall less positive charge. We have synthesized dendrimers having only 10% of the surface covered with NH<sub>2</sub> and 90% of the surface covered with hydroxyl groups (-OH; known as G4-90/10) thereby the total number of positive charge being less than G4-NH<sub>2</sub>. These dendrimers were found to be less toxic than the G4-NH<sub>2</sub>. Our previous study showed that these modified dendrimers are taken up the cells (neurons and different types of stem cells) in vitro, and by neurons and glial cells in vivo. Our previous study also showed that the G4-90/10 dendrimers can cross the blood-brain barrier (BBB) when injected systemically via the carotid artery in C57BL/6 J mice. However, multiple injections via carotid artery is not feasible in rodents. Therefore, to overcome this issue, and still have the dendrimers cross the BBB, we administered dendrimers via tail vein injection multiple times at different doses. Our results showed that the dendrimers cross the BBB when injected via tail vein in C67BL/6 J mice and are taken up by neurons and glial cells. Analysis of dendrimers in peripheral organs such as lungs, liver, spleen, and kidneys showed that the dendrimers were present in the kidney cells as well at higher amounts compared with the lungs, liver, and spleen. This proves that the remaining dendrimers which are not taken up by the brain cells are excreted from the biological system, thereby not causing any adverse effects, as seen in other types of nanoparticles that get retained in the biological system. Dendrimers are known to have anti-inflammatory properties and have the capacity to carry drugs/biomolecules. Therefore, conjugating the drugs/biomolecules with the dendrimers could be a future aspect of delivering cargo systemically across the brain by crossing the BBB. The G4-90/10 dendrimers are capable of forming complexes with plasmid DNA of various sizes. We have shown that these dendrimers can deliver large coding and noncoding plasmids (having a reporter gene) in mesenchymal stem cells (MSCs) in vitro. Since our major interest lies in using brain-derived neurotrophic factor (BDNF)-based therapy for Huntington's disease, we have also delivered hBDNF using dendrimers in MSCs and quantified the amount of BDNF using

enzyme-linked immunosorbent assay (ELISA) following delivery of the plasmid. Our data has shown that the amount of BDNF produced from the MSCs that received the dendrimer-delivered plasmid was higher than the control MSCs which did not receive the plasmid. Our research findings showed that (1) the dendrimers alone can cross the BBB when injected via the tail vein, and are taken up by neurons and glial cells; (2) the dendrimers can deliver coding and noncoding plasmids of various sizes in vitro and (3) the delivered plasmid containing the gene of interest (such as BDNF) results in secretion of the protein, showing that the plasmids remain intact and functional following complexing with the dendrimer and delivery. The future aspect involved delivering various plasmids using dendrimers in vivo.

### **Biodistribution of Glial Progenitors Injected to the 3D Bioprinted Model of Piglet Cerebral Ventricular System Depends on Infusion Speed, Volume of Cell Suspension and Iron Oxide Labeling**

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White matter damage persists in hypoxic-ischemic newborns treated with hypothermia. We have previously shown that intraventricular delivery of human glial progenitors (GPs) at the neonatal stage is largely capable of replacing host abnormal glia and rescuing a lifespan of dysmyelinated mice. However, the small size of the murine brain does not allow investigation of the transplantation challenges related to high-volume ventricles and long transport distances for stem cells in human brain. Our long-term goal is to study the potential benefit of complete glia replacement in a large animal (piglet) model of neonatal hypoxia-ischemia. The cerebral ventricles would be an attractive gateway to introduce cells to vast brain areas, and so we investigated the potential variables that could maximize the biodistribution of injected GPs within the ventricular system while minimizing outflow to the subarachnoid space. A custom-designed, 3D-printed model of the piglet ventricular system was used in our study. We found that lower injection speed and lower volumes are better suited to minimize outflow outside the ventricles, while having little effect on the distribution within the ventricular system. More importantly, we found that iron oxide (Molday ION Rhodamine B, Bio-PAL) labeling changes the rheological properties of GP suspension such that, even at high speeds and high volumes,

outflow beyond the ventricular system was reduced whereas more cells were displaced longer distances from the injection site. The viability of non-labeled and labeled cells was comparable. The macro view of the injected iron oxide-labeled cells suggested formation of clumps or a kind of hydrogel during a passage through a catheter. To summarize, we have found that the infusion speed, volume of cell suspension, and iron oxide labeling strongly influence the biodistribution of GPs injected through a catheter in a model of piglet cerebral ventricles.

### **AAV-Mediated Silencing of Striatal CaV1.3 Calcium Channels Can Prevent and Reverse Expression of Levodopa-induced Dyskinesia**

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Striatal voltage-dependent, L-type, alpha 1D subunit calcium channels (CaV1.3) are a target of interest for prevention of levodopa-induced dyskinesias (LIDs) in individuals with Parkinson's disease (PD). Previous studies have shown that voltage-dependent, L-type, alpha 1C subunit calcium channel (CaV1.2)/1.3 channel antagonists can reduce expression of LID in Parkinsonian rats, however, this effect is partial and lost over time. The limitation in scope and loss of protection over time are thought to be related to pharmacological limitation of currently available drugs. To provide unequivocal target validation we developed a recombinant adeno-associated virus (rAAV)-CaV1.3-short hairpin RNA (shRNA) to provide continuous, high potency, target-selective, mRNA-level silencing of striatal CaV1.3 channels. For the LID Prevention Study, adult male Sprague-Dawley (SD) rats received a unilateral intrastriatal injection of rAAV-CaV1.3-shRNA ( $N=11$ ) or a scrambled shRNA control, rAAV-Scr-shRNA ( $N=9$ ). At 1 week post-vector, rats were rendered unilaterally Parkinsonian with intranigral 6-hydroxydopamine (6-OHDA) and 2 weeks post-lesion began daily (M-Fr) treatment with escalating doses of levodopa (low=6 mg/kg; moderate=9 mg/kg; high=12 mg/kg; and supratherapeutic=18 mg/kg; 2 weeks/dose; +12 mg/kg benzerazide for all doses). For a LID Reversal Study, SD rats were unilaterally lesioned with 6-OHDA and primed with daily 12 mg/kg levodopa for 3 weeks to *establish stable LID prior to CaV1.3 silencing*. Rats displaying LID received unilateral intrastriatal injection of rAAV-CaV1.3-shRNA ( $N=11$ ) or rAAV-Scr-shRNA ( $N=12$ ); 96 h post-vector they

resumed daily levodopa (12 mg/kg) for 2 months. The results revealed that mRNA-level silencing of striatal CaV1.3 channels: (1) *prior to the introduction of levodopa* can completely prevent induction of LID, and the antidyskinetic benefit persists long-term (2 months) and with high doses of levodopa; (2) in rats with *already established LID* can ameliorate these behaviors, however a 7-day ‘drug holiday’ appears beneficial and/or necessary. Importantly, striatal CaV1.3 silencing does not impair motor behaviors in response to low dose levodopa. These data suggest that genetic silencing of striatal CaV1.3 channels may be a useful antidyskinetic clinical therapy in the PD.

### Soluble CX3CL1 Rescues Cognitive Deficits in CX3CL1 Knock-Out Mice

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Fractalkine (chemokine C-X3-C motif ligand 1; CX3CL1) is a chemokine expressed predominately by neurons that mediate communication between neurons and microglia. CX3CL1 possesses two isoforms, a full-length membrane-bound form and a soluble form, generated by cleaving membrane-bound CX3CL1. Recently, the idea that these two isoforms may display differential activities within the central nervous system (CNS) has garnered increasing attention, but has not been extensively explored. By regulating microglial activity, CX3CL1 can effectively mitigate the damaging effects of chronic inflammation within the brain, a state that plays a major role in aging. Microglial activity is critical for establishing and refining neural circuits in both the developing CNS and the adult brain. Levels of soluble CX3CL1 decrease with aging, which could lead to enhanced inflammation, deficits in synaptic remodeling, and subsequent declines in cognition. Here, we assessed the consequences of CX3CL1 knock-out on the cognitive behavior of mice at different ages. These mice displayed impaired long-term retention of a contextual fear conditioning task at both 3 months and 15 months of age. Moreover, at 3 months of age, CX3CL1<sup>-/-</sup> mice tested on the Barnes maze learned the location of an escape hole at a similar rate as wild-type controls, but displayed an altered search pattern in a subsequent probe trial, spending less time in the target zone than their wild-type counterparts. Cognitive impairments correlated with altered synaptic plasticity and impaired long-term

potentiation, as well as decrease in neurogenesis within the hippocampus. Treating CX3CL1<sup>-/-</sup> mice with a viral vector expressing the soluble form of CX3CL1 at 2 months of age partially rescued the deleterious effects of CX3CL1 knock-out when mice were assessed at 3 months of age in the Barnes maze task, but appeared to negatively impact long-term memory retention in the contextual fear conditioning task.

### Bilateral Transplantation of Human Fetal Retinal Pigment Epithelial Cells Attached to Microcarriers into the Shell of the Nucleus Accumbens of Cocaine Self-Administering Rats Protects Against Drug-Seeking

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Chronic high dose exposure to recreational stimulant drugs leads to damage to the ‘reward pathway’ that originates in the ventral tegmental area (VTA) and terminates in the nucleus accumbens (NAc). This damage is associated with loss of continuous dopaminergic stimulation and it causes dopamine dysregulation syndrome (DDS) that is characterized by risky drug-seeking behavior. Bilateral transplants of human fetal retinal pigment epithelial cells (hFRPECs) attached to microcarriers into the NAc, rescued cocaine self-administering rats with a history of high drug-taking from drug-seeking after a period of abstinence. Excellent survival of hFRPEC grafts was seen in all grafted rats. Tyrosine hydroxylase positive (TH<sup>+</sup>) cell bodies in the VTA were better preserved ( $p < 0.035$ ) in transplanted animals compared with controls suggesting that these grafts prevent damage to the VTA-NAc pathway. To verify underlying mechanisms, we investigated if cocaine-induced neuronal death in fetal mesencephalic (FVM) primary cultures and tested whether cocaine-induced damage can be prevented by hFRPEC-secreted factors. E13.5 FVM cultures were treated with increasing concentrations of cocaine to demonstrate dose-responsive loss of TH<sup>+</sup> neurons. Pretreatment or simultaneous treatment with hFRPEC culture supernatant 24 h with cocaine exposure significantly improved survival of TH<sup>+</sup> neurons. Our results suggest that NAc hFRPEC grafts permit retrograde transport of graft-secreted factors to enhance VTA neuronal survival. Thus, hFRPEC grafts may be an attractive therapeutic option for severe, chronic, conventional treatment-resistant neurostimulant drug addictions.

## Using Mouse Organotypic Spinal Cord Cultures to Validate New Self-Complimentary AAVs to Target Central Nervous System Cells by Using AAV6 Triple Tyrosine Mutant (T492V-Y70SF-Y731F)

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One of the primary limitations of using dissociated in vitro brain cell cultures is that it is difficult to understand and study cell-to-cell interactions. One avenue to study these interactions is by utilizing in vivo animal models; however, traditional animal models are costly and labor intensive to maintain. The use of ex vivo organotypic spinal cord slice cultures (SSCs) presents a viable intermediate model between cell and animal models, as it preserves the in situ cell architecture, making it useful for neurodegenerative, pharmacological, and tissue regeneration studies. Recombinant adeno-associated viruses (rAAVs) are nonpathogenic viruses that are useful in gene therapy applications as they are highly infectious and persist in human cells. Typically, rAAVs are capable of transducing all cell types in the spinal cord (neurons, astrocytes, and Schwann cells) except for microglia. AAV6 triple mutant T492V-Y70SF-Y731F (TM6), is one such rAAV that can transduce microglia with a self-complementary AAV vector (scAAV) that transduces faster, more efficiently, and with greater persistence than a single stranded AAV. We transduced p10 mouse SSCs using scAAV with the TM6 capsid and packaged with a plasmid that expresses hGFP (humanized green fluorescent protein). The universal promoter chicken B-actin was used to target expression of hGFP to all spinal cord cells, while cell-specific promoters were used to target expression to individual cell types. Neurons were targeted by using Ca<sup>2+</sup>/calmodulin-dependent protein kinase II (CamKII), glutamate decarboxylase 67 (GAD67), microtubule-associated protein 2 (MAP2), and synapsin (SYN) promoters. Motor neurons were targeted by using the homeobox HB9 promoter. Schwann cells were targeted by using myelin basic protein (MBP) and myelin oligodendrocyte glycoprotein promoters. Astrocytes were targeted by using the glial fibrillary acidic protein 104 (GFAP104), brain lipid-binding

protein (BLBP), and sodium-dependent glutamate/aspartate transporter 1 (GLAST) promoters. Microglia were targeted using the cluster of differentiation 68 (CD68) and epidermal growth factor module-containing, mucin-like hormone receptor (F4/80) promoters. Ependyma cells were targeted using the nestin promoter. The potential universal herpes simplex virus type 1 thymidine kinase (HSV1-TK) 66 bp long minimal promoter was also evaluated. By utilizing SSCs in concert with scAAVs, we can more rapidly screen clinically significant capsid/promoter combinations.

## Graft-Host Synaptic Connectivity Can be Optogenetically or Chemogenetically Inhibited to Eliminate Graft-Induced Dyskinesias Without Losing Anti-Parkinsonian Benefits

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Striatal fetal ventral mesencephalic (FVM) and other dopaminergic transplants are under investigation as experimental therapy in Parkinson's disease (PD). However, such graft recipients are at risk of graft-induced dyskinesias (GIDs). We tested the efficacy of optogenetic and chemogenetic inhibition of striatal FVM transplants to overcome GID without losing graft-induced anti-Parkinsonian benefits. The denervated striatum of hemiparkinsonian rats were injected with adeno-associated virus 2 elongation factor 1 alpha promoter, mCherry tagged, internal ribosome entry site, wheat germ agglutinin Cre recombinase (AAV2Efla-mCherry-IRES-WGACre) 3 weeks prior to transplantation of E13.5 mouse FVM cells transfected with either AAV5Efla, double-floxed inverse open reading frame, halorhodopsin, enhanced yellow fluorescent protein (AAV5Efla-DIO-eNpHR3.0-EYFP) or AAV8-human synapsin I, DIO, inhibitory modified human M4 muscarinic receptor, mCherry (AAV8-hSyn-DIO-hM4Di-mCherry). Thus, synaptic connectivity between the graft and the host was obligate for the expression of eNpHR3.0-EYFP or hM4Di-mCherry. Activation of eNpHR3.0 using a 590 nm light or hM4Di with clozapine-N-oxide (CNO)/clozapine caused completely reversible loss of graft-derived behavioral benefits ( $p < 0.05$ ) at 3, 8 and 12 weeks post-transplantation. Control grafted animals did not show any loss of graft function in response to laser or CNO/clozapine exposure. In transplanted animals, 5-hydroxytryptamine 6 (5HT6) receptor agonist ST1936-induced GID was eliminated by both optogenetic and chemogenetic inhibition ( $p < 0.05$ ) without any

loss of anti-Parkinsonian benefits. All grafted animals had excellent survival of large numbers of striatal tyrosine hydroxylase (TH) positive FVM neurons that co-expressed eNpHR/EYFP or hM4Di/mCherry confirming extensive synaptic connections with host medium spiny neurons. These preclinical experiments provide proof-of-principle that optogenetic or chemogenetic modulation of dopaminergic grafts can be safely incorporated into dopaminergic grafts to overcome GID without losing graft-induced anti-Parkinsonian benefits.

## LRRK2 Genomic Editing in Common Marmoset Stem Cells

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Leucine-rich repeat kinase 2 (LRRK2) G2019 S is the most common monogenic mutation associated with Parkinson's disease (PD). Located in the kinase domain of LRRK2, G2019 S facilitates substrate access to the kinase, thus increasing the catalytic rate of the enzyme. G2019 S patient-derived dopaminergic (DAergic) neurons present increased activated caspase-3, simplification of neurites and cell body accumulation of  $\alpha$ -synuclein. In an effort to validate the common marmoset (Cj) monkey as a candidate species for genomically modeling PD, we are investigating in vitro cell modeling strategies. LRRK2 inhibitors in G2019 S patient-derived DAergic neurons have been reported to largely restore a wild-type (WT) phenotype. However, removing LRRK2 leads to autophagy-related phenotypes in mice. Therefore, we hypothesized that truncated LRRK2 (tLRRK2) would present WT morphology, although with dysfunction in autophagy and endoplasmic reticulum (ER)-related homeostasis. Utilizing a WT clustered regularly interspaced short palindromic repeat (CRISPR)-associated protein 9 (Cas9) complexed with a guide RNA species that recognizes the 6055 genomic site for G2019 S, we successfully isolated a biallelic tLRRK2 cell line from our marmoset embryonic stem cell line (Cj-ESCs). After the tLRRK2 clone was expanded, we administered a dual-mothers against decapentaplegic (SMAD) inhibition protocol that our group previously validated for differentiating marmoset stem cells to neurons. The WT parental cell line was differentiated in parallel as a control for phenotypic comparison. The ImageJ

(National Institutes of Health) plugin NeuriteQuant was used for objective quantification of neurite complexity. tLRRK2 in neurons had no effect on average neurite length, branches per cell, neurites per cell, branches per neurite length, and overall neurite length per cell when compared with WT controls. Preliminary quantitative real-time polymerase chain reaction (qPCR) for ER stress and autophagy-related genes binding immunoglobulin protein (*BiP* or glucose-regulated protein, 78 kDa; *GRP78*), CCAAT/enhancer binding protein (C/EBP) homologous protein (*CHOP*), autophagy-related 7 (*ATG7*), and sequestome 1 (*P62*) indicated similar levels of expression at d30 between WT and tLRRK2. However, at d42, *CHOP* expression was higher in tLRRK2 cells while *ATG7* was higher in WT cells. Immunohistochemistry revealed a nonuniform cell body localization of the mitochondrial marker translocase of outer mitochondrial membrane 20 (TOMM20) in tLRRK2 neurons. We then performed a 2',7'-dichlorodihydrofluorescein diacetate (H<sub>2</sub>DCF-DA) reactive oxygen species assay in the pluripotent WT and tLRRK2 cells; no difference at baseline, or after hydrogen peroxidase (H<sub>2</sub>O<sub>2</sub>) burden, was observed. To investigate the feasibility of genomically editing the G2019 S mutation in marmoset-derived stem cells, we optimized and combined our CRISPR/Cas9 system with a repair template to accomplish the more challenging task of single nucleotide editing. We successfully isolated G2019 S clonal lines from both Cj-ESCs and marmoset induced pluripotent stem cells. Off-target analysis and next-generation sequencing validated site-directed precision as well as purity of the isolated clones respectively. Our results demonstrate that genomic editing of the LRRK2 gene can be successfully accomplished in common marmoset-derived pluripotent cells, and tLRRK2 may be associated with altered gene expression and TOMM20 cellular distribution.

## Role of CDNF in SOD1-G93A Mouse Model of Amyotrophic Lateral Sclerosis

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Neurotrophic factors (NTFs) regulate the development, maintenance and plasticity of the nervous system (Airaksinen & Saarma, 2002) and in adult animals protect and repair injured neurons. Several NTFs promote the survival of motoneurons (MNs) in vitro and in vivo, thus being possible drug candidates for amyotrophic lateral sclerosis (ALS). Among them, the novel cerebral dopamine neurotrophic factor

(CDNF) seems particularly promising, since it is highly expressed in muscle tissues, spreads better than other NTFs in the brain and rescues only degenerating neurons. Furthermore, CDNF is crucially involved in the regulation of the endoplasmic reticulum (ER) stress, which plays an important role in the pathophysiology of ALS. Here we show that a single intraventricular (i.c.v.) injection of human recombinant CDNF can significantly postpone the appearance of clinical symptoms, improve motor coordination and increase lifespan in the G93A mutant superoxide dismutase 1 (SOD1-G93A) mouse model of ALS. CDNF treatment can prevent the death of MNs compared with controls and CDNF also preserves neuromuscular junctions (NMJs) in the studied gastrocnemius muscle. We also found upregulation of the mRNA levels of unfolded protein response (UPR) genes such as glucose-regulated protein, 78 kDa (*Grp78*) and CCAAT/enhancer binding protein (C/EBP) homologous protein (*Chop*) in the SOD1-G93A model, whereas their levels were reduced in CDNF-treated animals. Therefore, our results strongly suggest that CDNF has a protective effect in the SOD1-G93A mouse model of ALS, promoting the survival of MNs and the preservation of NMJs. The decrease in UPR gene mRNA levels after CDNF treatment also suggests the intriguing possibility that CDNF might rescue MNs by regulating the ER stress response.

### The Effect of Low Dose Carbon Monoxide on Adult Neurogenesis in Injured Mammalian Spinal Cord

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We previously reported that non-toxic low dose carbon monoxide (CO) inhalation dose-dependently protected epicenter motor neurons and white matter in a rat model of T9-10 moderate compression spinal cord injury (SCI). CO treatment resulted in significantly improved hindlimb function mainly through preserving neural tissue and antagonizing secondary inflammatory or oxidative damages. In a subsequent replication study, ventilatory exposure of 500 ppm CO, the most potent dose identified before, was applied to SCI rats, starting 4 hours post-injury (p.i.) and thereafter 1 h CO inhalation per day for 12 consecutive days. In addition to reconfirming CO's therapeutic benefits on locomotion, neuroprotection and neuroinflammation,

the effect of CO on adult neurogenesis derived from the central canal ependymal cells was investigated, to test our hypothesis that CO could activate neural stem cells (NSCs) via the heme oxygenase 1 p38 mitogen-activated protein kinase-vascular endothelial growth factor (HO1-p38 MAPK-VEGF) and phosphorylated neuronal nitric oxide synthase (p-nNOS) pathways. We uncovered that there were differentially manifested neurogenic outcomes in the lesion epicenter and lumbar enlargement, respectively, in the CO-treated spinal cords that were discernibly different from those of the control group that was exposed to room air. The impact was detectable at 5–6 weeks after CO treatment (i.e. 7–8 weeks p.i.), indicating a possible role of CO-induced neurogenesis in promoting long-term neural repair. Compared with the control epicenters where newborn neural progenitors contributed to augmenting glial scar formation, the mean scale of reactive astrogliosis was significantly reduced in the CO-treated spinal cords. Importantly, we observed the presence of immature neurons in Rexed laminae (RL) IV–VIII in the CO-ventilated groups only. Since the quantities of CO-mediated preservation of cholinergic and gamma-aminobutyric acid (GABA)ergic interneurons (Ins) in the same RL zones were positively correlated with the levels of hindlimb locomotor improvement, our data suggested that besides its efficacy observed in treating acute SCI, non-toxic low dose CO may also be an effective therapeutic to recover function and/or ameliorate inflammatory complications (e.g. neuropathic pain, etc.) for chronic SCI.

### Multiple Intra-Arterial Dosing of Mesenchymal Stem Cells Reduces Ischemic Brain Injury in a Rat Stroke Model

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Cell therapy is emerging as a promising novel therapy for ischemic stroke. Intra-arterial (IA) mesenchymal stem cell (MSC) delivery in ischemic stroke has a high potential for clinical translation. Recently, we demonstrated safety and efficacy of IA delivery of MSCs at 24 h in a reversible middle cerebral artery occlusion (rMCAo) rodent model.

Given the trophic mechanism of action in cell therapy for stroke, a second dose of cells may be beneficial. However, it is unclear if a second IA-MSc administration is safe and efficacious. Therefore, we aimed to evaluate administration of two doses of IA-MSCs in the rodent ischemic stroke model. Female ovariectomized Sprague-Dawley rats were exposed to MCAo for 90 min. Rats were treated with IA-MSCs ( $1 \times 10^5$  cells) or phosphate-buffered saline (PBS) at 1 and 6 days (1D–6D) after MCAo. To test neurological and motor function, the standardized neurobehavioral test battery and the rotarod test were performed. The mean duration (in seconds) on the device was recorded from three rotarod measurements. The rats were tested at 7, 15, and 30 days after MCAo. Rats were sacrificed at 30 days for infarct volume measurement using histology. There was no neurological worsening or mortality seen in either treatment group. We observed significant reduction in infarct volume in 1D-6D MSCs group ( $21 \pm 15 \text{ mm}^3$ ;  $n=5$ ) compared with the PBS-treated group ( $86 \pm 19 \text{ mm}^3$ ;  $n=8$ ,  $p<0.05$ ). The 1D-6D MSCs group also showed non-significant improvement in rotarod test results ( $16.8 \pm 5.8\%$  versus  $7.9 \pm 3.4\%$ ,  $p=0.075$ ) at 30 days and neurological scores ( $5.6 \pm 1.2$  versus  $7.75 \pm 0.6$ ,  $p=0.15$ ) at 15 days. Dual dosing of IA-MSCs at 1D-6D post rMCAo is safe and reduces ischemic brain injury in female rats, with a trend towards functional improvement.

### RNA-Seq and Histological Characterization of Human Peripheral Nerve Tissue for Use in Brain Grafts for the Treatment of Parkinson's Disease

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Currently two clinical trials [ClinicalTrials.gov identifiers: NCT01833364 and NCT02369003] are underway which feature the implantation of a peripheral nerve autograft to the brain (targeted to the substantia nigra) in combination with Deep Brain Stimulation (DBS) for the treatment of patients with Parkinson's disease. As of 8 January 2018, 46 patients have received a graft. This nerve tissue is harvested from the sural nerve, a cutaneous sensory nerve located in the lateral ankle, from patients undergoing DBS surgery. The tissue receives a conditioning injury in situ 2 weeks prior to grafting. This study aims to characterize the effect of this conditioning, as well as the state of the nerve graft tissue immediately prior to implantation. Two sural nerve tissue samples (pre-conditioned and post-conditioned) per patient were collected from six patients during DBS surgeries 14 days apart. RNA sequencing (RNA-seq) was used to

measure absolute and relative levels of gene transcripts in the pre-conditioned and post-conditioned nerve tissue. These findings were supplemented by histology of the nerve tissue. The results of these experiments show: (1) Consistent similarity within the pre-conditioned and post-conditioned group transcriptomes; (2) Consistent changes between the pre-conditioned and post-conditioned group transcriptomes; (3) Increased transcript levels related to nerve repair, growth factor production, and immune cell migration pathways; (4) Decreased transcript levels related to myelin production pathways, consistent with the repair Schwann cell phenotype. All results are statistically significant ( $p<0.05$ ). These findings suggest that the nerve graft tissue implanted in human patients has a pro-regenerative phenotype which has the potential to alter the course of neurodegeneration in the brain. In addition, some preliminary clinical data of the patients' Unified Parkinson's Disease Rating Scale (UPDRS) score will be presented.

### Neurophysiological Approaches for the Study of Neuropharmacological and Genetic Manipulations

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Neurophysiology is a branch of electrophysiology which studies the flow of ions in neuronal tissues, as well as the communication and signaling pathways within individual neurons, local neuronal networks, and anatomically distinct neuronal systems. Neurophysiology encompasses both the electrical and optical recording techniques that allow for the measurement of this ion flux (i.e. changes in current flow) and changes in potential differences (voltage potentials) across neuronal cell membranes separating the extracellular and intracellular neuronal compartments. Studies in reduced preparations such as brain slices focus on the examination of the electrophysiological properties of single neurons or the effects of neuromodulators such as monoamines, peptides, and others, on the passive membrane properties, synaptic integration, and neuronal output of the cells of interest. In vivo neurophysiology is useful for solving systems neuroscience level questions and determining how pharmacodynamic drug effects and genetic manipulations performed in intact animals act on the neuronal and network levels. This seminar will detail neurophysiological approaches for studying the impact of neuropharmacological and genetic manipulations with a focus on in vivo recording techniques. I will also describe in detail various approaches which can be combined with the above recording techniques for local drug delivery (e.g. reverse dialysis, microinjection, and intracellular application).

## Standards for Deriving Nonhuman Primate Induced Pluripotent Stem Cells

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Our limited understanding of the cues controlling proliferation, differentiation and maturation of induced pluripotent stem cells (iPSCs) and their ability to establish functional connections or integrate into host tissue after cell grafting remains a significant bottleneck in the development of stem cell-based therapies. Standardized and well-defined stable iPSC lines and animal model resources will play a critical role in supporting efforts in the scientific community and industry to overcome this hurdle and to move forward with this therapeutic strategy for clinical use. However, qualified nonhuman primate (NHP) iPSC lines from various NHP species for studying transplantation in various NHP disease models are currently lacking. iPSCs have virtually an unlimited capacity to provide defined populations of all cell types of the body. However, the use of well-characterized and validated NHP iPSCs is rare. This contributes to variability and discrepancies between laboratories. Thus, establishing standards for deriving a high quality validated NHP iPSC lines is of critical need. We use the non-integrative episomal vector system to generate iPSCs from NHP skin biopsies. Taking advantage of our genomic resources, we have validated these iPSC lines by confirming pluripotency and propensity to differentiate into all three germ layers (ectoderm, mesoderm and endoderm) according to established standards and measurable limits for a set of marker genes. The iPSC lines were characterized in vitro and morphologically in vivo, cryopreserved, stored and made available for distribution to scientists through our online biological materials request form. NHP-iPSC-based species-specific cell types will open the door to test transformative developmental, regenerative or evolutionary hypotheses.

## Melanoma-Derived Exosomes Induce Inflammatory Microenvironments: Possible Involvement in Brain Metastases

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Brain metastases are secondary brain tumors that can occur through release of cancer cells from the primary tumors via hematogenous and lymphatic vessels of the circulatory system. Compared with other non-central nervous system (non-CNS) systemic cancers, melanoma has the highest tendency to metastasize to the brain and shows brain metastasis rates above 40% in malignant melanoma patients. Despite the aggressive progress of melanoma with high morbidity, mortality, and poor prognosis, however, the metastasis mechanism has not been clearly elucidated. Accumulating evidence has suggested that cancer-derived exosomes can enter into the lymphatic/blood vessels and then rapidly traffic to other distal organs. Also, recent results suggest that the involvement of the exosomes in metastasis is at least partially explained by their delivery components including cytokines, proteins, lipids, saccharides, and RNAs that induce a favorable microenvironment for cancer cells. In our melanoma B16F10 grafting experiments, we found that proinflammatory cytokines, tumor necrosis factor alpha (TNF- $\alpha$ ), and interleukin 6 (IL-6) levels, were significantly increased in the brain at 10 days after subcutaneous inoculation of the cancer cells. We also found that, compared with control (no treatment), the exosomes chromatographically isolated from the culture media of melanoma cells induce the enhanced level of TNF- $\alpha$  and IL-6 in the brain ( $p < 0.05$ ) as well as other peripheral organs at 2 h or 6 h after intravenous injection of melanoma exosomes. Our results may provide that molecular and cellular aspects for therapeutic intervention with brain metastatic processes of melanoma.

## Intraventricular Implantation of Human Mesenchymal Stem Cell 3D Aggregates as Regeneration Center for Ischemic Stroke Treatment

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Human mesenchymal stem cells (hMSCs) have become a potential candidate for cell therapy in stroke treatment due to their trophic effect, immunomodulation and lineage-specific differentiation. However, reduced cell survival and therapeutic functions after transplantation are the major barriers for clinical application of hMSCs in stroke patients. Moreover, replicative expansion of hMSCs is required to meet clinical scale-up, but this is known to result in cellular aging with reduced cellular properties and therapeutic potency. To enhance hMSC therapeutic efficacy,

preconditioning hMSCs has shown promising results in the middle cerebral artery occlusion (MCAO) rat model in our previous studies. Here, we report the direct delivery of three-dimensional (3D) hMSC aggregates as a 'regeneration center' for sustained immunomodulation and trophic effects in stroke rats. hMSCs labeled with micrometer-sized iron oxide (MPIO) particles spontaneously formed 3D aggregates on ultra-low adherent surface. Instead of intra-arterial injection with single hMSC suspension, hMSC aggregates (diameter: ~400  $\mu\text{m}$ ; 5000 cells/aggregate) were intraventricularly implanted into the cortex or lateral ventricle of MCAO rats. The influence of MPIO on hMSC aggregation and the effects of aggregation on hMSC secretion of immunomodulatory, pro-angiogenic, and antiapoptotic cytokines were assessed in vitro. To map temporal changes, ex vivo magnetic resonance imaging (MRI) was performed at 11.75 T. To monitor the in vivo lifespan for transplanted hMSC aggregates, serial in vivo MRI at 21.1 T was performed to acquire images of cell migration and localization in the cerebrum. Preliminary results showed hMSC aggregates retained the MRI signals at very low concentrations of MPIO, with reduced size and enhanced migration. In vivo MRI demonstrated penetration into the contralateral and ventricle of transplanted hMSC aggregates. We are currently evaluating the effects of aggregate transplantation on lesion recovery based on  $^1\text{H}$  and  $^{23}\text{Na}$  signal as evidence of the influx of extracellular water and disruption of ionic homeostasis. Neurological function recovery is also being evaluated by behavioral tests.

### The Dynamics of Microglial Polarization in Experimental Subarachnoid Hemorrhage

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Subarachnoid hemorrhage (SAH) is a devastating stroke type. Microglia, considered as resident immune cells, play a critical role in the neuroinflammatory response after SAH. Microglia are capable of M1/M2 two-dimensional polarization after activation. The classic M1 phenotype is related to the tissue injury, while the alternative M2 phenotype tends towards tissue repair. This study aims to unravel the polarization-specific dynamic of microglia after SAH in a murine model. SAH was induced by endovascular perforation on C57BL/6 mice. Immunofluorescence and quantitative real-time reverse transcriptase polymerase chain reaction (qRT-PCR) were employed to characterize the profile of microglial polarization on day 1, day 3, day 5, and day 10. The SAH mice showed subarachnoidal bleeding around the circle of Willis and SAH-like symptoms. Microglia showed the M1-predominant phenotype at the early phase, then gradually transformed into the M2 phenotype. The functional microglial polarization advanced along with the morphological changes through their ramified to an amoeboid shape. The bipolar microglia appeared through the

morphological transformation with the colocalization of M1/M2 markers. Consistently, the M1-related proinflammatory cytokines (interleukin 6 (IL-6) and tumor necrosis factor (TNF)- $\alpha$ ) showed an increasing expression in the early phase, while M2-related anti-inflammatory cytokine (IL-4 and transforming growth factor (TGF)- $\beta$ ) gradually upregulated during the delayed phase. The endovascular perforated SAH model mimics the ruptured aneurysm, which is similar to the clinical patient. Microglia demonstrated an early activation after SAH. The dynamic of microglial morphological changes and polarization was largely function-related. The temporal phenotype changes implied a neuroinflammatory response and the pathophysiologic process of restoration upon the hemorrhage attack. This study contributes to the comprehensive understanding of SAH pathogenesis, and provides the novel therapeutic target of microglia for SAH patients.

### Transplantation of Neural Progenitor Cells and V2a Interneurons into the Injured Cervical Spinal Cord

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While there is growing interest in the use of transplanted neural progenitor cells (NPCs) to repair the injured spinal cord, the identities of cellular components that most effectively contribute to repair and recovery remain unclear. With a focus on cervical spinal cord injury (SCI) and the resulting respiratory deficits, we recently identified a population of spinal interneurons (SpINs) that contribute to anatomical plasticity post-SCI. These SpINs are a glutamatergic class of ventrally-derived interneurons (Ins) that are known to mediate locomotor and respiratory functions within the uninjured spinal cord – the V2a. Building upon this discovery, the present work tests the hypothesis that transplantation of NPCs enriched with V2a INs can contribute to neural networks that promote repair and enhance respiratory plasticity after cervical SCI. Adult female rats (~250 g) received a lateralized, mid-cervical contusion injury (Infinite Horizon Impactor; 200 kilodyne), which disrupts the phrenic motor circuit controlling the diaphragm, the primary respiratory muscle. Cultured NPCs (comprised of neuronal and glial restricted progenitor cells) derived from the embryonic (E13-14) rat spinal cord were enriched with stem

cell-derived, Ceh-10 homeodomain-containing homolog (Chx10)-driven, V2a INs and allowed to aggregate for 2 days in vitro. These cellular aggregates (~120 micron diameter) were then transplanted into the lesion epicenter (500,000 cells) 1 week post-injury. Anatomical and functional analyses were performed 1 month following transplantation. Immunohistochemical analysis revealed donor cell survival, differentiation and integration with the injured host phrenic circuitry. NPC-derived glia migrated both rostral and caudal to transplant epicenter, and donor V2a INs extended neurites both rostrally (up to 8.6 mm) and caudally (up to 7.5 mm) as measured from the edge of the transplant. A proportion

(12.2%) of V2a donor cells expressed neuronal marker neuronal nuclei (NeuN), while NPCs yielded both NeuN-positive neurons and glial fibrillary acidic protein (GFAP)-positive glia. Functional diaphragm electromyography demonstrated increased diaphragm output and enhanced ability to respond to respiratory challenges (hypoxia, hypercapnia) 1 month following transplant, compared with injured, vehicle-treated animals. These ongoing studies not only test the efficacy of a promising therapeutic strategy, but also offer insight into the neuronal phenotypes that can be effective for neural transplantation to repair injured neural circuits.