MSCRF

# MARYLAND
## STEM CELL RESEARCH FUND

# ANNUAL REPORT

2020

# Contents

# Maryland Stem Cell Research Commission

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# The COVID-19 Challenge

2020 has been an unprecedented year for all of us and the regenerative medicine field is no exception. At MSCRF, in response to COVID-19 challenges, we stepped up our efforts to support our community and continue to advance science. We modified our review and funding processes to virtual or socially distanced formats. We supported each active project with necessary modifications for successful completion or to enable researchers/companies to pivot, to provide rapid response and technology development to address COVID-19. We organized numerous virtual networking events for our researchers and companies to build and expand scientific collaboration and to stay connected during these difficult times. We also expanded our global presence and industry engagement by collaborating with multiple national and international organizations to allow Maryland-based researchers and companies to participate in scientific meetings for free or at a discounted rate. We expanded our daily news updates on our website home page (www.mscrf.org) and App to include not just regenerative medicine updates, but also the latest information on Covid-19. In addition, we funded two new proposals to address Acute Respiratory Distress Syndrome (ARDS), a prevalent COVID-19 complication that results in fatalities. One of these ARDS projects, a clinical trial, is currently recruiting patients in Maryland to evaluate this treatment. It is important to note that mesenchymal stem cell treatments were amongst the first-approved clinical trials for reducing fatality by COVID-related complications. This rapid approval of cell therapy in the fight against COVID-19 is due to the visionary investment in stem cell research and the immense progress we have already made in this field in understanding these cells and their safety profile. This knowledge and progress positioned us to readily fight this and perhaps the next unforeseen challenge. There are several ongoing cell therapy clinical trials, related to COVID-19, in MD institutions and we are ready to enroll additional trials if needed.

Beyond progress and resilience, our community is thriving in the face of this pandemic. Our awardees, companies and research institutes advanced innovation and accelerated cures. They developed new partnerships and collaborations, and created new opportunities. Our companies continued to grow and participated in conferences around the globe (virtually). We have witnessed unprecedented scientific collaboration, and 2020 presented us with a record year for regenerative medicine and advanced therapy financing $15.9 billion through Q3[1]. Globally, there were 1,109 ongoing regenerative medicine clinical trials, with several targeting more prevalent diseases such as cancer, cardiovascular disease, diabetes, central nervous system disorders and infectious disease including COVID-19[1].

Without doubt, the pandemic has underscored the importance of funding research and innovation and how dependent we are as a society on medical research to deliver cures. We have seen the success of our Maryland-based vaccine industry and how it allowed us to help the world develop and manufacture COVID-19 vaccines, and our rapidly growing regenerative medicine industry is poised to be next in line to have this impact.

*1. Informa/Alliance for Regenerative Medicine*

# Creating a New Industry in Maryland

To advance the research and discoveries into the market we must enable an industry to commercialize these technologies, manufacture products and make them accessible to patients. We work with our faculty and companies to help them succeed and advance their technologies. Our efforts have stimulated the creation and growth of this industry in Maryland. We create value by providing critical support for early-stage high-risk/high-reward scientific discoveries, de-risking these innovations with key milestone-based grants, enabling company formation and novel product development, supporting each project and scientist with guidance and resources to meet their unique needs, and creating a collaborative community to accelerate cures. Regenerative medicine is thriving in Maryland, our research continues to accelerate at a rapid pace, and our companies continue to grow. Some of the companies in our portfolio are highlighted below. For more information on all our portfolio companies, visit https://www.mscrf.org/portfolio-companies.

LIFESPROUT

**LifeSprout, Inc.** founded with technology licensed from Johns Hopkins University, closed a $28.5 million Series A financing. The company will be using proceeds to support clinical development of novel therapeutic products from its Regenerative Matrix platform.

RoosterBio®

**RoosterBio, Inc.** following their Series B raise last year, had the first patient dosed under a newly awarded Investigational New Drug (IND) application using their CliniControl™ products, in addition to product launches, CRADA, partnerships and international expansion.

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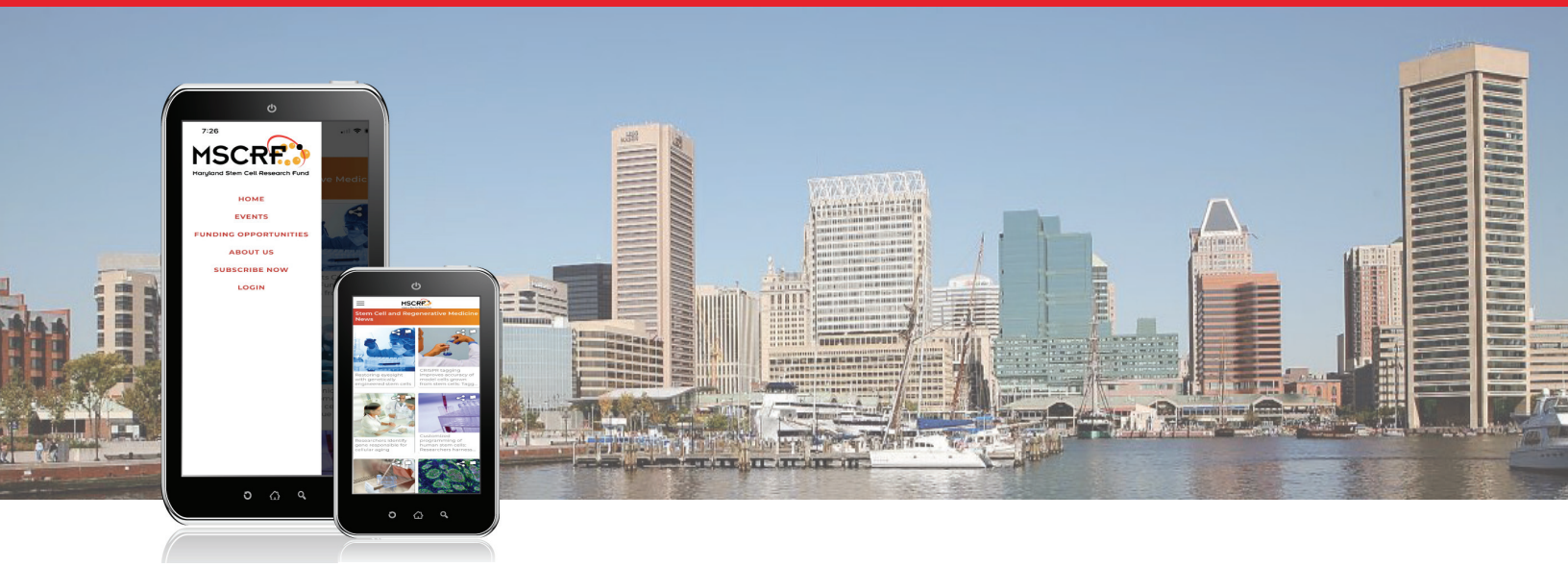
VITA Therapeutics

**Vita Therapeutics, Inc.**, a Johns Hopkins University spinout, announced that it received orphan drug designation (ODD) from the U.S. Food and Drug Administration (FDA) for VTA-110, a novel regenerative therapy for the treatment of Duchenne Muscular Dystrophy (DMD).

NeoProgen

**NeoProgen, Inc.**, a University of Maryland Baltimore company, announced a strategic partnership with Aspire Health Science for development and manufacturing of their cell-based therapy.

In addition, two of our portfolio companies (Vita Therapeutics, Inc. and Theradaptive Inc.) made the Maryland Future 20 List, selected by the Department of Commerce and announced by the Governor. The selection was based on a variety of factors, including innovation, future growth potential, the company's Maryland story, and "wow" factor. You can read more about the new companies that we funded this year in the Commercialization section of this report.




ANDROID APP ON Google play

Download on the App Store

## Connect with us on the MSCRF Mobile App

Our unique MSCRF App is available in the iOS and Google app stores. Keep up with the latest on our funding opportunities, events and daily news on cutting-edge research and findings from around the world. Our App serves as a one-stop place for our community.

## Meetings Go Virtual

Due to COVID-19, all regional, national and international conferences changed their platform to go virtual in 2020. Although at first we thought this was a challenge, we soon realized that this was a great opportunity. It allowed us, our awardees and the community to participate in many more meetings and to present and interact with the global industry from the comfort of our own homes. This unprecedented access spurred scientific collaboration and innovation. Our proposal review meetings also changed to a virtual format, and we were able to make some modifications and still have an engaging review process.


## MSCRF Website

The Maryland Stem Cell Research Fund (MSCRF) remains committed to identifying and fostering cutting-edge research and innovation in the field of regenerative medicine in Maryland. Our Accelerating Cures initiative comprises programs that help transition human stem cell-based technologies from the bench to the bedside as well as mechanisms to build and grow stem cell companies in Maryland. Check out our website to learn more about us, our mission, our strong community of innovative stem cell scientists and companies, and to keep up with regenerative medicine news from around the world!

www.MSCRF.org

@MD_Stem_Cell

# MSCRF Funding Opportunities

Our six programs are designed to catalyze innovation and sequentially transition the most promising discoveries from the labs where the invention occurred, to the clinic where they will be offered to patients. There is an urgent need to accelerate these cures across diverse therapeutic areas in this field.


## Discovery Program

The MSCRF Discovery grant supports faculty developing innovative hypotheses or models to advance the stem cell field. This program welcomes groundbreaking, high risk/high reward ideas with minimal feasibility data. Applicants to these grants may request up to $345,000 over up to 24 months.

## Launch Program

The MSCRF Launch grant supports new and new-to-the-field faculty to bring novel thought and orthogonal expertise to the regenerative medicine field to develop innovative solutions to emerging challenges. Applicants to these grants may request up to $345,000 over up to 24 months.

## Validation Program

The MSCRF Validation grant supports faculty with IP for promising human stem cell technologies, interested in transitioning these discoveries to the commercial sector, where they can be developed into products, services, or cures. This program enables faculty to meet critical milestones towards commercialization of innovative stem cell technologies through technology validation, market assessment, and the creation of University start-up companies in Maryland. Applicants to these grants may request up to $230,000 over up to 18 months.

ACCELERATING CURES

## Post-Doctoral Fellowship Program

The MSCRF Post-Doctoral Fellowship grant supports exceptional post-doctoral fellows who wish to conduct human stem cell research in academia or in industry in the State of Maryland. Applicants to these grants may request up to $130,000 over up to 24 months.

## Commercialization Program

The MSCRF Commercialization grant supports the transition of promising technologies having significant commercial potential from Universities, where they were discovered, to the commercial sector, where they can be developed into products and services that meet identified market needs. Applicants to these grants may request up to $270,000 over up to 12 months.

## Clinical Program

The MSCRF Clinical grant supports organizations that wish to conduct clinical trials in the State of Maryland using human stem cells to advance medical therapies. Applicants to these grants may request up to $650,000 over up to 24 months.

# Accelerating Cures

## Launching Research Careers

In 2020, we created a program aimed at helping new or new-to-the-field faculty bring novel thought and expertise to the field of human stem cell research. The program is for research grants to develop innovative solutions to emerging challenges in the field, but which have limited or no preliminary data supporting the application. We received 14 applications for this new program and were able to fund four of them. We hope to help these faculty launch their stem cell research careers and innovative projects. You can read more about these projects in the Launch section of this report.

## MSCRF 2020 Awards

**$8.2M**
**Program Funds**

Committed $8,241,439 towards accelerating research and cures through all 6 of the MSCRF Programs

**8**
**Organizations**

Awarded 8 different world-class research institutes and companies to advance the field in Maryland

**27**
**Grants Awarded**

Funded 27 innovative projects from early-stage research to clinical trials to accelerate stem cell research and cures in Maryland

**27**
**Disease Indications**

Funded projects address a wide range of disease indications including COVID-19, Diabetes, Stroke, Heart disease, Cancer, Alzheimer's disease, Parkinson's disease, and more

# Advancing Medical Research & Treatments in Maryland

During calendar year 2020, we had six active programs. We received 87 applications in two separate funding cycles, and we funded 27 new awards with $8,241,439 in 8 different research institutes and companies. We supported projects addressing a wide range of disease indications including psychiatric, neurological and neurodegenerative diseases, heart and blood disorders, autoimmune conditions, aging-related disorders as well as research advancing an understanding of human development and genetics. We identify and fund the most innovative research that may provide new treatments for several prevalent, chronic diseases as well as for rare diseases that then serve as a platform to expand therapies to other areas. As an indication of the scientific collaboration that we have driven, we received several collaborative proposals, including partnerships between for-profit Maryland companies and academic institutions as well as projects between various Maryland research institutions. Such synergistic partnerships help us accelerate discoveries towards products and clinical trials so they can reach patients sooner.

The Maryland Stem Cell Research Fund is also an economic engine for the State, creating jobs and generating new revenue. MSCRF grants support researchers, physicians, lab technicians, and students as well as scientists and executives at stem cell companies. We are creating the next generation of workforce to grow the regenerative medicine industry and advance therapies.

Many of the research grants we have funded to date study stem cells in the context of specific diseases including heart disease, cancer, stroke, chronic lower respiratory disease, diabetes, Alzheimer's disease and flu/pneumonia. It is important to note that these conditions account for the top 10 leading causes of death in Maryland[2]. Resulting therapies improve the health of many Marylanders and also reduce health care costs for the state. State funding has already allowed Maryland scientists and companies to advance the use of stem cells all the way to the clinic. It is imperative that we continue to advance research and treatments for all diseases affecting Marylanders with the added understanding that several of the listed pre-existing conditions cause greater risk of serious illness from COVID-19 and the knowledge that the virus targets multiple organs and systems in our bodies, not just the lungs. Despite the immense progress we've made, our work is far from over and we, at MSCRF, continue to bring the stem cell community together with a sense of urgency and a common purpose to advance biological science and improve human health.


*2. Centers for Disease Control and Prevention/National Center for Health Statistics*

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**Launch** Program

Discovery

Validation

Commercialization

Clinical

# 2020 Annual Report

# Grant Awards

**Xia Feng, M.S., Ph.D.**
Johns Hopkins University
Award Amount: $344,697
Disease Target: Huntington Disease

## An Isogenic HiPSC-Derived Cortical-Striatal Co-Culture System to Investigate Transneuronal Propagation of Mutant Huntingtin in HD

Huntington disease (HD) is an autosomal dominant, progressive neurodegenerative disorder, characterized by primary dysfunction and death of striatal medium spiny neurons and cortical pyramidal neurons. There is as yet no effective treatment for this devastating disorder. HD is recognized as a model with known genetic mutation (a CAG repeat expansion in the huntingtin (HTT) gene) for other more common neurodegenerative diseases with more complex genetics, such as Parkinson disease and Alzheimer disease. Similar to other mutant proteins related to neuro-degenerative disorders, the mutant HTT (mHTT) protein is prone to aggregation and gains cytotoxicity, leading to aberrant function and regulation of multiple cellular processes. The mHTT also shows propagation capability from cell to cell, displaying prion-like characteristics. This project aims at defining specific molecular mechanisms underlying transneuronal propagation of mHTT and its contribution to HD pathology using an isogenic human induced pluripotent stem cell (hiPSC)-derived cortical-striatal co-culture system.

Our specific aims for this project will be as follows:
Specific Aim 1: we will use the CRISPR-Cas9 system to develop a novel isogenic hiPSC model of HD by manipulating the CAG repeat length in the endogenous HTT gene as well as tagging its 5-end with coding sequences of green or red fluorescent proteins. We will differentiate the isogenic hiPSC lines into disease-relevant cortical and striatal neuron-like cells, and use these cells to examine neuron-type-specific toxicity induced by mHTT.

Specific Aim 2: we will build up a cortical-striatal co-culture system, and use co-culture with same or mixed genotypes of HTT to assess cell and non-cell autonomous toxicity and to investigate molecular mechanisms underlying transneuronal propagation of mHTT. The mechanistic insights we gain into disease-relevant, neuron-type-specific, cell- and non-cell-autonomous toxicity and transneuronal propagation of mHTT using a cortical-striatal co-culture system derived from the proposed isogenic hiPSC model may lead to new design and development of therapeutic strategies for the intervention of HD and other related neurodegenerative diseases.

**Tae In Kam, Ph.D.**
Johns Hopkins University
Award Amount: $345,000
Disease Target: Alzheimer's and Parkinson's Diseases

## Different Mode of Cell Death in Neurodegeneration

The worlds aging population is expected to increase the prevalent cases of neurodegenerative diseases. Alzheimers disease (AD) and related dementias and Parkinsons disease (PD) and related disorders accounting for the majority of those afflicted and the greatest economic burden. Currently, however, medical treatment for these diseases only focuses on symptomatic therapy and there is no disease modifying therapy yet available. Despite their enormous diversity in pathological and clinical phenotypes, most neurodegenerative diseases share common features, which are the accumulation of protein aggregates and neuronal cell death. The vast majority of research has focused on the neurotoxicity induced by the pathologic proteins that are thought to drive the disease process, aggregated amyloid-beta (Ab) and Tau in AD and aggregated alpha-synuclein (a-syn) in PD. Neuronal death is an important feature of a variety of neurodegeneration and critically important in the development of disease and progressive loss of function, but the type of neuronal death has been not fully understood. Disease-prone protein aggregation contribute to neurodegeneration, and must be understood and controlled if each disease process is to be selectively tempered or stopped. Recently we discovered that different type of cell death pathways are separated and have their own functional role in pathogenic conditions, but not cross talk with each other. We also found that activation of poly (ADP-ribose) (PAR) polymerase 1 (PARP-1) dependent cell death (parthanatos) is the primary driver of pathologic a-syn neurodegeneration and that PAR levels are increased in the cerebrospinal fluid (CSF) and brains of PD patients. In preliminary studies from mouse primary cultures using specific cell death inhibitors, we have found that parthanatos is the only type of cell death caused by pathologic a-syn in PD models, while pathologic Abinduced cell death is mostly combined with parthanatos and apoptosis. Taken together, we suggest an important role of PARP-1 in the mediation and acceleration of neurodegeneration as a common mechanism of neurodegenerative diseases. Human stem cell research offers extraordinary opportunity to study the diseaseselective cell death caused by aggregated protein in neurodegeneration. These studies will provide a platform for the discovery of new therapeutic approaches to limit the contribution of the common or different mode of cell death in human disease. The major goals of this project are to determine if pathologic proteins in neurodegenerative diseases activate distinct cell death pathways in the human setting, and to provide a platform to screen for key target and agents that can block the specific neuronal death in brain diseases. Selective blocking cell death would be expected to have disease modifying effects in different neurodegenerative diseases including PD and AD.

**Byoung Chol Oh, Ph.D.**
Johns Hopkins University
Award Amount: $299,765
Disease Target: Peripheral Nerve Injury, Hand Transplantation

## Human iPSC-Derived EGFR+ Functional Schwann Cells to Enhance Nerve Regeneration and Improve Functional Outcomes in VCA

Vascularized composite allotransplantation (VCA) holds much promise to improve the quality of life for our civilian who suffer from devastating injuries such as severe and irreplaceable tissue loss or amputations. Transplantation is currently the only treatment option to fully restore missing limbs with functional and anatomical equivalents by replacing like-with-like tissue. Recent advances in microsurgical techniques and immunosuppressive protocols have enabled wider application of VCA with highly encouraging immunologic and aesthetic results. However, the overall success of VCA is dictated by the pace and quality of nerve regeneration. Following transplantation, the recipients peripheral nerve axons must regenerate into the graft so as to innervate the transplanted muscle and skin. This process allows the recipient to establish motor control over and receive sensory input from the graft. Without adequate innervation, a transplanted graft remains inanimate and insensate and provides little if any benefit to the recipient. Over time, a lack of innervation will result in progressive, permanent atrophy within the graft, rendering it useless. The importance of adequate graft innervation applies to all types of VCA; in upper extremity transplantation, meaningful hand function is dependent on the recipients motor and sensory axons reaching the intrinsic muscles and distal skin of the transplanted hand; in facial transplantation, graft innervation is necessary for everything from facial expression to preventing drooling. Substantial advances have been made in the enhancement of nerve regeneration across gaps through the use of conduits and acellular nerve grafts. However, very few therapeutic approaches have been successfully studied in primary end-to-end nerve repairs, which is the preferred and most commonly used method of repair in VCA. Therefore, the objective of this proposal is to develop a novel stem cell-based therapy utilizing human iPSC-derived Schwann cells to enhance nerve regeneration and improve functional outcomes in reconstructive transplantation. In VCA, the slow pace and the requirement for nerve regeneration over long distances to regain full motor and sensory function is still prohibitive to expand the indication for VCA to full arm or lower extremity transplantation. The overall hypothesis of this proposal is that human iPSC-Schwann cells will significantly increase the pace and degree of nerve regeneration after end-to-end injuries in VCA and thus improve functional outcomes. In experimental approaches, we plan to expand human Schwann cells without losing myelination capability and to investigate the interaction of donor and host Schwann cell toward remyelination. Moreover, we propose to demonstrate enhanced functional recovery following peripheral nerve transection and repair following syngeneic forelimb transplantation as well as allogeneic transplantation to investigate the neuro-regenerative potential of Schwann cells in a setting that recapitulates the clinical scenario of VCA using unrelated cadaveric donor.

**Sashank Reddy, M.D., Ph.D.**
Johns Hopkins University
Award Amount: $345,000
Disease Target: Initial - Craniofacial Microsomia

## Regenerative Cell Therapies for Soft Tissue Restoration

Devastating soft tissue losses from cancer surgery, trauma, congenital malformation, and inflammatory diseases are a common occurrence in clinical medicine. For breast cancer alone, more than 100,000 women undergo reconstructive surgery in the United States every year. Losses of soft tissues like skin, fat, and muscle throughout the body compromise patient form and reduce function. Current strategies for soft tissue reconstruction consist of: (1) autologous solutions in which soft tissues from one part of the body are moved to an area of deficiency and (2) prosthetic-based reconstructions. Both have significant drawbacks. Autologous reconstructions involve major surgery, can cause donor site morbidities, and transferred tissues do not always survive in their new locations. Prosthetic implant-based reconstruction is plagued with issues including device failure, fibrosis, infection, capsular contracture, or even the development of prosthetic-associated malignancies such as anaplastic large cell lymphoma.1 Given these limitations, there is a major need for minimally invasive solutions that promote the body's intrinsic regenerative capacity while minimizing donor site morbidity. Recently, transfer of autologous adipose-derived stem cells (ADSCs) and adipocytes has been used for soft tissue reconstruction. This procedure called lipotransfer has been a major advance in reconstructive surgery. Yet as currently practiced, lipotransfer is hampered by serious limitations. Most notably, a significant portion of grafted tissue fails to survive leading to unpredictable results and a requirement for repeat procedures.11-13 We have developed a clinically-translatable synthetic tissue substitute called the nanofiber-hydrogel complex (NHC) that promotes survival and functional integration of mesenchymal stem cells such as ADSCs and their derivatives.18 Here we propose a regenerative cell therapy approach to soft tissue reconstruction that combines adipose-derived stem cells (ADSCs) with NHC for durable and natural restoration. In the studies proposed here, we will explore the relationship between these biomimetic materials, host immune response, and embedded stem cells to define the optimal ADSC/NHC combination to support soft tissue regeneration. These studies will provide the foundational understanding for planned clinical trials for patients with soft tissue losses. Our approach is readily translatable owing to its reliance on constituent biomaterials with a long history of safe use in medical devices coupled with use of autologous cells. ADSCs are an abundant, versatile, and easily accessible autologous cell source for regenerative medicine, as they can be harvested through liposuction. Prior studies with NHC alone have shown promise in restoring small soft tissue defects18, paving the way for more far reaching solutions through the addition of autologous stem cells. The investigators have met with FDA and conducted preclinical testing and manufacturing studies on NHC, affording a wealth of data that can be used in support of translational studies for ADSC/NHC combinations. In the studies proposed herein, we will engineer NHC variants optimized for cell therapy, discover rules governing stem cell survival and differentiation on these materials, and explore the interplay between transferred stem cells and the incipient pro-regenerative inflammatory response. Our ultimate goal is to develop the first true regenerative cell therapy for orphan and common causes of soft tissue loss.

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Launch **Discovery** Program Validation Commercialization Clinical

# 2020 Annual Report

# Grant Awards

**Kenneth Boheler, Ph.D.**
Johns Hopkins University
Award Amount: $345,000
Disease Target: Vascular Diseases

## Chemoproteomic Immunophenotyping of Human Pluripotent Stem Cell Derived Vascular Smooth Muscle Cells

Human pluripotent (i.e., embryonic and induced) stem cell (hPSC) derived vascular smooth muscle cells (vSMCs) are of considerable interest for the study of human vascular development, modeling of vascular diseases, and regenerative medicine. While significant advances in the differentiation and purification of hPSC-derived vSMCs have been achieved, the lack of specific protein or genetic markers that distinguish both among phenotypically distinct vSMC cell subtypes in mixed cultures and between vSMCs and non-SMCs is a major limitation to progress in this field. This limitation is particularly problematic for studies involving polymorphic hiPSC lines, where interline variabilities affect both the quality and composition of in vitro differentiated vSMC cultures and the phenotypes of these cells. One proven method to overcome issues of heterogeneity is through immunophenotyping and isolation of marker-defined cell types by fluorescence-activated cell sorting (FACS). Currently, known surface proteins suitable for sorting vSMCs with defined functional phenotypes are extremely limited. To discover new and improved marker panels, we have labelled, captured and identified cell surface glycoproteins using state of the art chemoproteomic and mass spectrometry (MS) techniques. These pilot experiments have led to the identification of 13 targets that appear to be restricted to vSMCs (when compared with the cell surface protein atlas (CSPA)). Additional targets have been putatively identified that are restricted to early, synthetic vSMCs. Based on our existing data, we propose to develop commercially (while maintaining IP) two sets of monoclonal antibodies (mAbs) for vSMC restricted targets. The MS output, which experimentally confirms protein orientation and identifies cell surface accessible epitopes present in the extracellular (EC) space, will be used to guide the development of the new mAbs. We also propose to test and validate markers for the selective sorting of synthetic versus contractile vSMCs and for assessing interline variability. For this, we propose to examine the surfaceomes of both synthetic and contractile vSMCs generated from 6-10 hPSC lines (normal and diseased) and from primary human aorta SMCs. This broad-based assessment of vSMCs is now feasible because we have established in the laboratory a new state-of-the-art micro-Cell Surface Capture (mCSC) approach, which requires 10-fold fewer cells than our original protocol. As marker panels are established and Abs are validated for sorting vSMCs with defined phenotypes, molecular, biochemical and bioengineering approaches will be used to validate the phenotypes of sorted cells across 2-3 hPSC lines. At the completion of this study, the improved isolation and characterization of human vSMCs with defined phenotypes (synthetic versus contractile) will positively impact the field and overcome the current lack of a specific protein or genetic marker that distinguish vSMC cell types in mixed cultures. Once published, the data and reagents generated in this study will not only improve the isolation and characterization of vSMCs from heterogeneous cultures for basic science, the reagents produced may be apt for drug repurposing and for regenerative medicine approaches involving hPSC-derived vSMCs.

**Jeff Bulte, M.S., Ph.D.**
Johns Hopkins University
Award Amount: $339,297
Disease Target: Stroke

## Developing MPI for Tracer-Based, Non-Radioactive, and Quantitative Whole-Body Imaging of Cell Delivery

The use of stem cells and progenitors for cell replacement therapy and tissue restoration is a sought-after approach for correction of damaged tissue. When such therapies are pursued in patients, it would be highly desirable to have non-invasive cell tracking techniques available that can report longitudinally on the local and whole-body distribution of transplanted cells, in order to better understand their fate in vivo and in particular to optimize dosing and delivery as part of personalized therapies. In this proposal, we aim to develop magnetic particle imaging (MPI) in conjunction with magnetic resonance imaging (MRI) to answer several basic questions associated with the efficacy and safety of cell therapy. These are two complementary techniques, where MPI can report on the local and whole-body distribution of administered cells in an absolute quantitative manner, whereas MRI provides supporting anatomical information and can report on real-time homing and immediate retention of cells. We propose to use human glial-restricted progenitor cells (hGRPs) as an example therapeutic cell type, as these cells have been extensively used in several pre-clinical animal models, have been approved for clinical use, and we have ample experience using them. We have chosen an ischemic stroke model (middle cerebral artery occlusion or MCAO) as an example of a target disease, in which loss of axonal myelin is one of the key events leading to permanent debilitation. This MPI/MRI two-pronged approach of imaging the same cell (labeled with superparamagnetic iron oxide particles or SPIO for both MPI and MRI) will be applied for intraarterially injected hGRPs with and without VLA-4 transfection where VLA-4/VCAM-1 docking has been shown to result in diapedesis and increased targeting to the brain parenchyma. If successful, this example MPI application of tracking (transfected) hGRPs in stroke may encourage the use of MPI to interrogate the fate of other cells and other disease scenarios in vivo.

**Alan Friedman, M.D.**
Johns Hopkins University
Award Amount: $345,000
Disease Target: Cancer

**Yingli Fu, M.S., Ph.D.**
Johns Hopkins University
Award Amount: $345,000
Disease Target: Diabetes

## Human iPSC-Derived NF-kB p50-Deficient Myeloid Cell Immunotherapy for Cancer

Multiple solid tumors grow slower in mice lacking NF-kB p50, and adoptive transfer of immature myeloid cells lacking p50, after a dose of myelo-depleting 5-fluorouracil, slows the growth of murine prostate cancer, pancreatic ductal carcinoma, and neuroblastoma. We have developed methods for efficient p50 gene editing in human marrow myeloid progenitors and for their expansion in serum-free media. As a more feasible and cost-effective means to obtain sufficient numbers of p50-IMC for clinical translation, including repeated cycles of treatment, we herein propose to develop methods for p50 gene editing of human iPSC and their optimal differentiation and expansion into p50-IMC. In doing so we will utilize stromal-activated, myeloid progenitor-derived iPSC that have minimal epigenetic abnormalities, will convert such lines to the „nave" state using LIF-3i if this proves advantageous, will compare lines for off-target edits and their functional consequences, and will evaluate their ability to form M1-polarized tumor myeloid cells in tumor-bearing immune-deficient mice. In addition, we will evaluate the utility of heterologous p50-IMC in murine models, with the view towards using „off-the-shelf" iPSC-derived p50-IMC should heterologous p50-IMC reduce tumor growth as effectively as autologous p50-IMC. Upon completion of these studies we anticipate initiating clinical trials evaluating the safety and efficacy of iPSC-derived p50-IMC in patients with a broad range of malignancies. As we observe cooperation between p50-IMC and anti-PD-1 antibody in the treatment of murine pancreatic ductal carcinoma, we also intend to explore addition of T cell checkpoint inhibitory antibodies to p50-IMC in the clinical context.

Immunotherapy has emerged over the past decade as a key weapon against cancer. We have developed a novel immunotherapy utilizing immature myeloid cells lacking NF-kB p50 (p50-IMC), which activates T cells to inhibit tumor growth. p50-IMC have the potential to be effective in patients with a broad range of malignancies. The goal of this proposal is to develop iPSC as an effective source of p50-IMC. While p50-IMC can be derived from patient marrow or blood, we anticipate that gene editing of autologous iPSC will more effectively provide sufficient p50-IMC for multiple rounds of therapy. We also propose murine studies to determine the efficacy of heterologous p50-IMC in an effort to validate use of „off-the-shelf" iPSC-derived p50-IMC, which would be even more cost-effective and convenient to provide patients. As we find cooperation between p50-IMC and T cell checkpoint inhibition in our murine pancreatic cancer model, these two immunotherapies may cooperatively activate anti-tumor T cell activity, as we anticipate evaluating also in clinical trials.

## Engineering Stem Cell Microenvironment for Image-Guided Therapeutic Intervention

Type I diabetes mellitus (T1D) is a devastating T-cell mediated autoimmune disease that results in the destruction of insulin-producing beta cells in the pancreas and subsequent hyperglycemia, for which no cure exists. Current treatment with exogenous insulin injections, although lifesaving, cannot replicate the level of feedback control afforded by naturally occurring functional beta cells, and is inefficient to prevent chronic complications. Thus, novel therapies to provide long-term and dynamic glucose control via minimally invasive beta cell delivery would significantly improve T1D health care. The discovery of human induced pluripotent stem cells (hiPSCs) and their ability to differentiate into functional beta cells offers significant potential to overcome the shortage of human beta cells, while immunoisolating 3D constructs that are amenable for injection can be engineered using biocompatible materials to deliver hiPSC-derived beta cells. The goal of the present proposal is to develop an off-the-shelf, injectable, hiPSC-derived beta cell therapeutic for image-guided T1D treatment using novel bioprinted 3D constructs. As such, we propose an integrated approach in which insulin-responsive, functional beta cells derived from hiPSCs will be printed with biocompatible materials to create an imaging-visible, immunoprotective matrix that can be delivered via minimally invasive, routine delivery routes, such as s.c. or i.p.

Specific Aims (1): To test the hypothesis that perfluorinated, fibrin-alginate microcapsules can be printed for single hiPSC-beta cells using a custom-made, fully automated, piezoelectric bioprinting system. In this aim, we will establish the most promising bioprinting formulations for imaging-guided hiPSC-derived beta cell delivery to enhance cell viability and imaging visibility.

Specific Aim (2): To test the hypothesis that bioprinted fibrin-alginate microcapsules improve single beta cell survival and function over conventional alginate microcapsules in vitro and in vivo and enables image-guided, minimally invasive delivery and longitudinal tracking. In this aim, we will determine the effect of bioprinting on beta cell function in vitro and analyze the kinetics of glucose normalization by bioprinted hiPSC-beta cells in diabetic animals. The proposed study brings a unique combination of expertise in type I diabetes management, stem cell encapsulation, imaging-guided delivery, biomaterials and 3D bioprinting to address the critical challenges in engineering and delivering beta cell-based therapeutics. Successful completion of the proposed activities will have vast ramification for hiPSC-derived beta cell therapy by creating a off-the-shelf cellular product that can be precisely delivered via minimally invasive s.c. or i.p. injections for T1D treatment, which is aligned with the mission of Maryland Stem Cell Research Fund.

**Muthukumar Gunasekaran, Ph.D.**
University of Maryland, Baltimore
Award Amount: $345,000
Disease Target: Myocardial Infarction

## A Novel Mechanism of Immune Evasion by Human Neonatal Cardiac Progenitor Cells

Stem cell transplantation is an effective treatment option for myocardial infarction (MI). However, identification of potential stem cell type, which rescues the infarcted myocardium is still under investigation. Further, immune response to transplanted stem cells reduces its regenerative potential in stem cell therapy. Recent findings from our group demonstrated that neonatal cardiac progenitor cells (nCPCs) showed superior efficacy in repairing the injured heart compared to other stem cells types mesenchymal stem cells (MSCs), cardiosphere derived cells (CDCs) and umbilical cord (blood) in an ischemic immune deficient rodent MI model. However, the therapeutic effects of nCPCs to evade the immune response by molecular signaling pathways, essential for cardiac repair remains uncharacterized and critical for its clinical success. Our preliminary data supports that CPCs evade from the immune response by novel mechanism. Controlling CD47 levels in CPCs, critical for evading the innate immune surveillance by binding to signal regulatory protein- (SIRP) on macrophages which inhibits the macrophage phagocytic activity. Therefore, we hypothesize that CD47 to be the key molecule upregulated in nCPCs, and responsible for their immune evasion leading to increased cell retention and restoration of cardiac function in an immune-competent rat MI model. Furthermore, HSF1 and microRNAs such as miR-133a and miR-34a regulate CD47 expression in CPCs. Our hypotheses will be achieved by, 1). Determine HSF1-CD47 axis mediated immune evasion by CPCs 2). Demonstrate miR-133a and miR-34a regulates CD47 expression in CPCs. This study is the first step towards optimization of nCPCs for allogeneic stem cell therapy in clinical settings for its superior cardiac regeneration efficacy in the infarcted myocardium compared to other stem cells. In the future, we will contend that nCPCs will be utilized for therapeutic application or used as an adjunct to surgical intervention for patients with myocardial infarction to provide game-changing outcomes and thus change their life expectancy. We believe our focus on nCPCs will lead to innovative avenues to identify novel mechanism of immune evasion, hence paving yet another milestones in regenerative medicine.

**Xiaofeng Jia, B.M., Ph.D.**
University of Maryland, Baltimore
Award Amount: $345,000
Disease Target: Peripheral Nerve Injury

## Developing Human iPSC-Derived Exosome Therapy to Improve Recovery after Peripheral Nerve Injury

Approximately 360,000 people suffer from upper extremity peripheral nerve injury (PNI) in the United States of America each year. PNI often results in poor functional recovery and subsequently impaired quality of life for the patient. Currently, many approaches to improve peripheral nerve regeneration have not surpassed the gold standard‘ autograft procedures. However, autografts are not ideal due to limited graft source availability and the morbidity of a second surgical incision. Supported by the 2013 and 2018 MSCRF awards, our previous studies have shown that human neural crest stem cells (NCSCs) promote sciatic nerve regeneration with axonal regrowth and myelin formation. Despite significant progress, tumourigenicity and immunogenicity have long been a challenge for stem cell therapy and hinder clinical applications. Increasing evidence suggests that exosome therapy represents a novel cell-free treatment with compelling advantages over whole cell therapy by their parent cells. Our preliminary data showed exosomes from NCSCs improve recovery after nerve crush injury. Objectives/Hypothesis: With the long-term goal to develop and advance the exosome-based therapeutic approach to ultimately improve the patients quality of life, we will test the hypotheses: (1) NCSC exosome transplantation will improve nerve regeneration in vivo, possibly through facilitating differentiation into Schwann cells (SCs) and/or upregulation of growth factors; (2) Compared to NCSC transplantation, exosome therapy will lessen the immune responses with similar therapeutic effect; (3) microRNAs (miRNAs) is the key molecular target in exosomes to promote peripheral nerve regeneration, and modulation of miRNA cargo can further improve outcomes after nerve injury. Specific Aim 1: Establish NCSC derived exosomes therapy to improve outcomes after PNI. Specific Aim 2: Compare the immune responses and therapeutic effects between exosome and NCSC therapies. Specific Aim 3: Further enhance therapeutic effects of exosome therapy by modulating miRNA cargo to promote peripheral nerve regeneration. Study Design: This proposal will investigate the effect of NCSC derived exosome therapy in peripheral nerve crush injury and nerve defect repair animal models via histopathological, electrophysiological and functional outcome measurements. Innovation and significance: For the first time, we will not only develop exosome therapy but also further enhance the therapeutic effect by modulating miRNA cargo, the signaling vehicle, to promote nerve regeneration after PNI. We are the first to target immunogenicity and autotomy by comparing the rodents treated with exosomes and their parent cells. We have developed a novel cell modification technology with less invasive sources of stem cells and a more desirable source of SCs for transplant therapies. Our project combines the expert knowledge of interdisciplinary investigators to execute this clinically motivated, translational experimental design with systematic in vivo measurements. The proposed research aims to provide a novel cell-based therapeutic approach to promote nerve regeneration. The specific design and motivation of our technology and protocols allow for straightforward translation to a clinical setting. Further development will enable commercialization towards a point of service product. It will develop optimal treatment protocols for clinicians treating patients with nerve injury. Success in this project would greatly enhance the surgical repair of nerve injuries with better functional outcomes.

**Tami Kingsbury, Ph.D.**
University of Maryland, Baltimore
Award Amount: $345,000
Disease Target: Red Blood Cell Disorders

## Leveraging Novel SIX1 Transcription Factor Network Interactions to Stimulate Human Erythropoiesis

Defects in erythropoiesis lead to anemias and blood malignancies. Elucidating novel mechanisms regulating red blood cell (RBC) formation from hematopoietic stem-progenitor cells (HSPCs) has the potential to provide new approaches to stimulate erythropoiesis in patients to reduce transfusion dependency and to support the development of improved methods for in vitro expansion of RBCs for clinical applications. We recently demonstrated that the developmental transcription factor SIX1 is a positive regulator of human erythropoiesis. Overexpression of SIX1 in primary peripheral blood HPSCs or CD34 positive TF1 erythroleukemia cells used to model early stage erythropoiesis triggered erythroid differentiation. Conversely, CRISPR mediated SIX1 knockout reduced the ability of erythropoietin (EPO) to stimulate erythropoiesis. SIX1 BioID proximity labeling performed in TF1 cells revealed GATA1 was part of the SIX1 proximal interactome. Further work demonstrated that SIX1 associates with GATA1 and stimulates GATA1 mediated gene expression. Consistent with these observations, we showed that SIX1-stimulated erythropoiesis required the master erythroid transcription factor GATA1, but not the EPO receptor. Thus, SIX1-GATA1 interaction is a novel regulatory mechanism functioning in human hematopoietic cells that may provide a method to stimulate erythropoiesis downstream of ineffective EPO signaling. The work outlined in this proposal seeks to address gaps in our knowledge needed to translate this exciting discovery into novel approaches to treat red blood cell disorders or enhance red blood cell formation in vitro for utilization in the clinic or development of drug delivery sytems.

**Aim 1:** We will determine the cellular mechanisms by which SIX1 increases erythropoiesis and cell stages impacted by SIX1 manipulation using gain and loss of function approaches combined with analysis of cell proliferation, cell death, and cell lineage specification, as determined by immunophenotyping, single cell RNA sequencing and colony formation assays. SIX1 mutant alleles selectively disrupting interaction with its co-activator EYA or co-repressor TLE, will determine whether SIX1 gene activation and/or repression mediates erythroid effects. SIX1 mutants incapable of binding GATA1 will determine reveal GATA1 dependent vs independent functions of SIX1 in erythropoiesis. Importantly, we will extend our initial findings on early stage erythropoiesis to late stage erythropoiesis to determine the consequences of SIX1 manipulation of terminal erythroid differentiation, assessing red blood cell yields, blood group antigen expression, hemoglobin profiles and immunomodulatory function.

**Aim 2:** We will perform CRISPR knockout screening to functionally identify additional members of the SIX1 proximal interactome required for SIX1 or EPO-stimulated erythropoiesis. In addition to GATA1, and multiple known GATA1 interacting factors, the SIX1 interactome contains 56 factors not previously implicated in erythropoiesis. Novel erythropoietic factors identified within the SIX1 interactome will provide new molecular insight into normal and defective human erythropoiesis and reveal additional novel targets for erythropoiesis stimulating agents.

**Hanseok Ko, M.S., Ph.D.**
Johns Hopkins University
Award Amount: $345,000
Disease Target: Parkinson's Disease

## Targeting an Activated Microglia/Astrocyte Axis for Reducing Neurodegeneration

Parkinsons disease (PD) pathophysiology is a complex cascade of protein interactions and molecular pathway activation that is associated with pathologic -synuclein (-syn) folding and subsequent neuronal cell death. There is growing recognition that one of the critical steps in neurodegeneration in PD is neuroinflammation, characterized by activation of microglia and astrocytes. Recently, we described a subtype of reactive astrocytes, A1 astrocytes, that we observed in various human neurodegenerative diseases including PD. We found that activation of microglia leads to the conversion of beneficial A2 astrocytes into toxic A1 type astrocyte via secretion of IL-1alpha, TNF-alpha, and C1q (reported as A1 astrocyte inducer). Building on these findings in primary murine neuronal cultures, we discovered that -syn preformed fibrils (PFF), which mimic pathologic -syn in PD, activate microglia to induce A1 astrocyte formation and neuronal death in vitro. We observed evidence for microglial activation and A1 astrocyte formation that correlates with neurobehavioral deficits in mouse models of PD. However, the mechanisms underlying the activated microglia/astrocyte axis in the mediation and acceleration of the neurodegeneration in PD remains unclear. As such, an important role for the activated microglia/astrocyte axis in PD needs to be studied in great depth. Importantly, our preliminary study indicates that RIPK2 (receptor-interacting serine/threonine-protein kinase 2) is active in microglia in PD brain and genetic depletion and pharmacological inhibition of RIPK2 inhibit the microglia activation, A1 astrocytes conversion and neuronal toxicity due to -syn aggregates in murine cells suggesting RIPK2s role in the activated microglia/astrocyte axis in PD pathogenesis.

**Specific Aim 1:** We will further extend and confirm the findings in human cells using control and PD iPSC lines derived human microglia with or without RIPK2 depletion, astrocytes, and dopaminergic (DA) neurons that may afford us a more predictive model of human brain. Furthermore, we will identify and characterize the down-stream pathway associated with RIPK2 activation in neuroinflammation due to -syn aggregates by employing proximity-dependent biotin identification (BioID) of RIPK2 from control and PD iPSC lines derived microglia.

**Specific Aim 2:** We will explore the role of a newly devolved RIPK2 inhibitor, CMPD0673 with highly potent and blood brain barrier (BBB) permeable on the microglia activation, A1 astrocytes conversion and neuronal toxicity due to -syn aggregates in control and PD iPSC lines derived human microglia, astrocyte and dopaminergic neurons. This study enables us to uncover the role of RIPK2 activation on microglia activation, A1astrocytes conversion and DA neurodegeneration due to -syn aggregates in human cells. Also, this investigation will also provide new insights into the pathogenesis in PD and leads to promising novel therapeutic agent for the treatment of PD and related -synucleinopathies.

**Vassilis Koliatsos, M.D.**
Johns Hopkins University
Award Amount: $345,000
Disease Target: TBI, AD, PD

## A Stem Cell-Derived Human Neuron Model To Explore Treatments of Axonopathies

Axonal pathology and axonal transport impairments are key pathogenic mechanisms in several neurological disorders including traumatic brain injury, peripheral neuropathies, glaucoma, and select neurodegenerative diseases. Together, these conditions affect a substantial portion of the US population, impair quality of life, have high mortality and, to a large extent, have no satisfactory treatments. In all previous disorders, axons undergo a series of molecular and cellular changes that have been best characterized in simple axotomy models and are known as Wallerian degeneration. Our proposal is inspired by recent exciting work in our laboratory in models of traumatic brain injury and engages novel technologies developed by our collaborators to create an in vitro platform of axonopathy/Wallerian degeneration in human neurons useful for both mechanistic studies and drug discovery. This axonopathy platform is a significant addition to in vivo models because it obviates daunting challenges related to the intricate anatomy of axons and complex inflammatory and other tissue responses to injury and, in addition, utilizes human nerve cells. Here we compartmentalize neuronal cell bodies and axons and allow room for the potential addition of astrocytes, oligodendrocytes and microglial cells to study pathology at a desired level of complexity. Perhaps more importantly, the proposed platform paves the way for future use of induced pluripotent stem cells from patients where mechanisms and treatments of disease can be explored at a higher level of precision, at the population and individual level. Aim 1, we establish the platform by combining compartmentalized microfluidic device technology that ensures the separation of axons from cell bodies and a rapid neuronal differentiation technology that allows the efficient conversion of human embryonic stem cells to neurons inside the devices with minimal perikaryal contamination of the axonal compartment. Axons are then subjected to different insults to address different patterns of axonal degeneration applicable to diverse neurological diseases. In particular we establish models of mechanical injury (axotomy and graded crush injury) relating to TBI and spinal cord injury; neurotoxic injury (treatment with the tubulin polymerization inhibitor vincristine) pertinent to chemotherapy-induced peripheral neuropathy; and treatment with hydrogen peroxide that models oxidative stress-related axonal degeneration. Following injury, axons are analyzed with unbiased methodologies to assess and quantify patterns of degeneration. Aim 2, we use the humanized axonopathy platform to explore select mechanisms of Wallerian degeneration based on our promising published and pilot work. We specifically focus on two molecular pathways, one centered on Sterile alpha and heat/armadillo motifs-containing protein 1 (SARM1) and the other related to the activation of Mitogen-activated protein kinases (MAPKs). This work will not only yield important information about mechanisms, but also sharpen molecular targeting for drug discovery. In Aim 3, we follow on molecular leads from work in Aim 2 and block SARM1- and MAPK-associated signals in the platform with small molecules with which we have considerable experience. This work will serve to confirm the molecular findings from Aim 2 but also validate the platform as a bioassay system for screening novel compounds that may eventually be used as drugs for traumatic and other axonopathies. We are already in talks with the Hopkins Drug Discovery Program for future screening of small-molecule libraries and further translational work using our platform. In conclusion, we propose the establishment and characterization of a novel human axonopathy platform designed as a versatile tool to model a variety of axonal degenerative conditions, explore mechanisms, and develop drugs.

**Linda Resar, M.D.**
Johns Hopkins University
Award Amount: $345,000
Disease Target: Osteoporosis and Aging

## Developing Stem Cell Therapy for Bone Loss and Fractures with Aging

The goal of our collaborative project is to develop stem cell technology to enhance bone formation for patients with fractures caused by bone loss or osteoporosis with aging. Our focus is High Mobility Group A (HMGA1) chromatin remodeling proteins. The Resar laboratory pioneered studies demonstrating that HMGA1 proteins are key epigenetic regulators in adult stem cells while Drs. Wan & Xian are international leaders in mesenchymal stem cell (MSC) biology and bone formation (osteogenesis). With aging, there is a global loss of stem cell function, including decreases in the regenerative capacity of MSC within the bone marrow. Bone loss (osteoporosis or OP) occurs because bone resorbed by osteoclasts is not fully restored with bone deposited by osteoblasts. Since osteoblasts require constant replenishment from multipotent bone marrow MSCs, an insufficient supply of osteoblasts from MSCs is central to age-related OP. Importantly, systemic infusion of MSCs does not improve osteogenesis due to poor delivery and impaired differentiation to bone. Here, we focus on the role of HMGA1 as a key factor in osteogenic potential of MSCs. Rigorous prior research indicating that HMGA1 is a critical factor for adult stem cell (ASC) function includes the following published work and new, unpublished findings: 1) HMGA1 is a key epigenetic regulator that maintains ASC number and function within intestinal epithelium (Nature Comm, 2017; Cancer Res, 2018). 2) HMGA1 acts as a molecular switch by flipping on Wnt signaling genes required for stem cell function and homeostasis, including genes important in bone formation. 3) More recently, we discovered that mice deficient in Hmga1 develop premature aging with accelerated kyphosis and osteoporosis. 4) RNA sequencing (RNAseq) data from mouse embryonic fibroblasts (MEFs) indicates that Hmga1 represses senescence genes (Cdkn2A) while activating genes required for osteoblast differentiation. 5) Lrp6 encodes a Wnt co-receptor essential for osteogenesis by maintaining MSC survival and osteoblast differentiation (Bone Res, 2014 & 2018). 6) Cellular senescence depletes the MSC pool, thus impairing osteogenesis (Nature Comm, 2018). 7) HMGA1 levels decrease in diverse tissue-specific stem cells with aging in both mice and humans. Together, these intriguing results support the following hypotheses: 1) HMGA1 is required for bone forming potential from MSC, 2) HMGA1 deficiency in MSC with advancing age promotes bone loss by disrupting key epigenetic networks, and, 3) Maintaining HMGA1 function will rejuvenate MSCs, improve bone formation, and restore bone mass by enhancing MSC osteogenic potential and antagonizing senescence. Specific Aims: To test this, we propose to harness our unique human cell-based and murine models with genetic manipulations in HMGA1 in the following Aims: 1) To dissect the function of HMGA1 in MSC maintenance, osteogenic potential, and bone mass, and, 2) To develop stem cell technology for bone formation from MSC using CRISPR-based activation (CRISPRa) to enhance HMGA1 transcriptional networks and epigenetic reprogramming. Impact: These innovative studies will not only provide insight into MSC function, bone formation, and aging, but could also reveal new therapies to foster osteogenesis, treat fractures, and even prevent bone loss and osteoporosis with aging.

**Jeffrey Rothstein, M.D., Ph.D.**
Johns Hopkins University
Award Amount: $345,000
Disease Target: Dementia, ALS, Alzheimers, FTD

## CHMP7 in the Initiation of Nuclear Pore Injury in C9orf72 ALS/FTD and Sporadic ALS

The motor neuron disease Amyotrophic Lateral Sclerosis (ALS) and the second most common form of dementia, Frontotemporal Dementia (FTD), comprise a spectrum of fatal neurodegenerative diseases. Although clinically two distinct diseases, multiple genetic loci including an intronic GGGGCC (G4C2) hexanucleotide repeat expansion (HRE) in the C9orf72 gene, have been linked to ALS and FTD. The C9orf72 HRE is the most common cause of both familial and sporadic ALS accounting for ~40% and ~8% of patients respectively. Overall, only about 10% of ALS cases are familial with the remaining 90% being sporadic in nature. Despite >20 genetic causes of ALS having been identified, the molecular mechanisms underlying disease pathogenesis remain poorly understood. Defects in nucleocytoplasmic transport and the nuclear pore complex itself have recently emerged as a prominent pathomechanism underlying multiple neurodegenerative diseases including C9orf72 ALS/FTD, sALS, Alzheimers Disease, and Huntingtons Disease. However, little is known about the nature of the injury to the nuclear pore complex and its individual nucleoporin components themselves. Using induced pluripotent stem cell derived spinal neurons and postmortem human tissue, we have now amassed substantial data that loss of the transmembrane nucleoporin POM121 from the nuclear envelope and nuclear pore complexes initiates a pathological cascade impacting nuclear pore complex composition, function, and cellular survival. Notably, loss of POM121 is mediated by pathologic G4C2 repeat RNA and not DPRs or loss of C9ORF72 protein. Given that POM121 is not mislocalized or aggregated in the cytoplasm, we hypothesized that POM121 and subsequently altered nucleoporins are aberrantly degraded in the early stages of C9orf72 ALS/FTD pathogenesis. Indeed, our new preliminary data suggests that the degradation of POM121 is initiated by nuclear accumulation of CHMP7. Nuclear CHMP7 has previously been shown to activate ESCRT-III mediated degradation of nuclear pore complexes and nuclear envelope components during nuclear pore surveillance and homeostasis. Our preliminary data suggest that the G4C2 repeat RNA mediated aberrant activation of this pathway is responsible for nuclear pore injury in C9orf72 ALS/FTD, making CHMP7 an attractive therapeutic target in neurodegeneration. Prevention of CHMP7mediated nuclear injury could prove to be a powerful therapy for thee earliest event in ALS and FTD. Importantly- this research program can only be carried out properly using human iPS derived neurons.

**Elias Zambidis, M.D., Ph.D.**
Johns Hopkins University
Award Amount: $345,000
Disease Target: Diabetes, Vascular Disease

## Universal Donor Naive Vascular Progenitors for Regenerative Medicine

Human vasculature arises during development from prolific embryonic vascular progenitors (VP) that differentiate into endothelial progenitor cells (EPC) and pericytes. In the adult, circulating EPC and pericytes are rare, limited in their multi-potency and expansion, and functionally defective in diabetes. Our group first established that prolific CD31+CD146+CXCR4+ embryonic VP could be generated from patient-specific conventional hiPSC and utilized to repair damaged adult retinal blood vessels in a murine ischemia/reperfusion (I/R) model (1-3). We also reported a new class of tankyrase/PARP inhibitor-regulated nave hiPSC (N-hiPSC) with improved epigenetic plasticity (4-6). These novel N-hiPSC displayed enhanced multi-lineage differentiation potential, elimination of interline variability, and erasure of donor epigenetic memory bias. The therapeutic potential of these novel N-hiPSC has not been evaluated by any group. However, we recently reported that the functionality of embryonic VP generated from both normal and diseased conventional hiPSC were significantly improved following reversion to this tankyrase/PARP inhibitor-regulated nave epiblast-like state (7). Conventional, primed diabetic hiPSC (DhiPSC) were reprogrammed from type-1 diabetic donor fibroblasts and stably reverted to nave DhiPSC (N-DhiPSC) Nave diabetic VP (N-DVP) differentiated from N-DhiPSC expanded more efficiently, possessed more stable genomic integrity, and displayed higher in vitro vascular functionality than primed diabetic VP (DVP) generated from isogenic conventional DhiPSC. Moreover, N-DVP survived, migrated, and engrafted in vivo into the deep vasculature of the neural retinal layers with significantly higher efficiencies than isogenic primed DVP in a murine model of ischemic retinopathy. Analyses of CpG DNA methylation and histone configurations at developmental promoters of N-hiPSC revealed tight lineage-specific gene expression and a de-repressed nave epiblast-like epigenetic state. Although nave VP (N-VP) with improved functionalities may have wide impact for vascular regenerative medicine, their broad clinical application via patient-specific approaches faces important challenges. For example, the labor and cost of screening individual hiPSC lines for high-quality clones makes patient-specific therapies inaccessible in a cost-conscious health care system. As an alternative, global efforts have begun to develop HLA-homozygous iPSC banks, including from inventories of clinical-grade HLA-typed cord blood (CB) banks. However, clinical bone marrow transplantation (BMT) provides important paradigms to facilitate such hiPSC bank therapies. For example, the existing infrastructure of BMT routinely leverages partially HLA-matched, or haplo-identical HLA-matched hematopoietic stem cells, along with post-transplant systemic immune suppression to cure a multitude of hematopoietic disorders. We (8,9) and others (10-16) have proposed the adoption of tolerance induction paradigms to broaden hiPSC therapies to a wider number of individuals via use of HLA-defined Universal Donor hiPSC lines. We propose that HLA-homozygous Universal donor N-hiPSC with improved, versatile multi-lineage differentiation potential, and more efficient erasure of disease-associated epigenetic lesions can significantly advance the goals of regenerative medicine. Herein, we advance these concepts by testing the pre-clinical functionality of N-hiPSC-derived N-VP for rescuing vision loss in a humanized ischemic retinopathy animal model, using a patient-specific approach. Secondly, we begin pilot efforts to test the feasibility of generating clinical-grade, HLA-defined Universal Donor tankyrase/PARP inhibitor-regulated N-hiPSC (UTIRN-iPSC) from existing blood bank repositories for future expanded application of these improved human stem cells.

MSCRF
Maryland Stem Cell Research Fund


# 2020 Annual Report

# Grant Awards

**Chulan Kwon, M.S., Ph.D.**
Johns Hopkins University
Award Amount: $230,000
Disease Target: Alzheimer's Disease

## Engraftment of Human iPSC Derived Neural Progenitors in Mouse for Developing Treatments for Alzheimers Disease

We have developed a technology that can quickly mature human iPSC (hiPSCs)-derived cells in vivo through bioincubation. As a next step, we will validate this technology for use as in vivo model to serve the market of preclinical Alzheimer's Disease (AD) research. AD is the most common cause of dementia and billions of dollars are spent on AD therapeutics and diagnostics research annually. However, the lack of faithful preclinical models for AD has been a long-standing roadblock for developing treatments for the disease and no disease-modifying therapy has been developed. Mouse models, while widely used in preclinical AD research, are poor predictors of whether an experimental treatment will be successful in clinical trials, largely because the evolutionary divergence between mice and humans, especially in the central nervous system. Using the bioincubation technology, we will create novel mouse models that carry mature human neurons derived from AD patient hiPSCs, providing a faithful preclinical animal model for AD treatment development.

The bioincubation technology is based on engrafting hiPSC-derived progenitor cells inside the corresponding organs of a neonatal animal host. In this process, the host organ promotes maturation in hiPSC-derived progenitor cells as it goes through post-embryonic developmental stages, typically in a much-accelerated manner compared to humans. Thus, phenotypes of late-onset diseases such as AD are expected to be recapitulated much quicker in an animal host using patient-derived hiPSCs. In the proposed MSCRF project, we will adapt the bioincubation technology to engraft mice with human AD patient-derived cells in the brain and validate their use as novel preclinical AD models. Specifically, we will optimize methods to engraft hiPSC-derived neural progenitor cells (NPCs) into the neonatal mouse brain, reproducibly generate adult mice carrying patient hiPSC-derived neural tissue and use these models to confirm and characterize specific AD-related tissue-level phenotypes. Successful completion of the proposed project will enable future manufacture process development for generation of these novel preclinical animal models and support the commercialization of this technology as a drug development platform serving the AD preclinical research market.

MSCRF
Maryland Stem Cell Research Fund


# 2020 Annual Report

# Grant Awards

**Douglas Falk, M.S.**
Vita Therapeutics, Inc.
Award Amount: $300,000
Disease Target: Limb-Girdle Muscular Dystrophy

**Amir Saberi, Ph.D.**
Domicell, Inc.
Award Amount: $268,180
Disease Target: Acute Respiratory Distress Syndrome

## Satellite Stem Cell Therapy for Limb-Girdle Muscular Dystrophy

Muscular dystrophy is a group of 30+ diseases that is characterized by loss of muscle mass referred to as muscle wasting. A common form of muscular dystrophy is Limb-girdle muscular dystrophy (LGMD), which is a diverse subgroup of genetic disorders that usually manifests in the proximal muscles around the hips and shoulders. There are approximately 4000 patients in the US alone and approximately 80-100 new patients identified every year. The onset and progression of LGMD varies and can manifest during adulthood as well as during childhood. In most cases, childhood onset of LGMD progress more rapidly and is associated with early disability such as difficulty climbing stairs and walking resulting in many patients being wheelchair-bound. Thus, LGMD is related to a poor quality of life and currently no curable treatments exist. Consequently, developing effective treatments for these degenerative disorders are in high demand. At Vita Therapeutics, we are developing an allogeneic long-term muscle stem cell-based therapy to repair and replace damaged muscle tissue. We have demonstrated that transplanted human muscle stem cells behave like endogenous satellite cells as they engraft, repair and reside in a quiescent state in the basal lamina between the myofibers to regulate homeostasis and provide long-term regeneration in mouse injury- and genetic muscular dystrophy models. The current proposal aims to validate the technology in a pig model and to determine optimal dosing needed for effective repair of large muscle groups, an essential step for commercialization of this technology. To do this, we have set the following specific goals with quantifiable milestones: To determine dosing, engraftment and regeneration by quantitative analyses of (1) myofiber fusion, (2) human dystrophin levels, (3) numbers of human satellite cells residing under the sarcolemma in the basal lamina (niche) between the myofibers, and (4) inflammatory signaling and leucocyte infiltration following treatment.

## Intravascular Bioreactor Delivery of Anti-Inflammatory Paracrine Factors to Treat Acute Respiratory Distress Syndrome

COVID-19 inflicts high mortality in patients who develop severe pneumonia and acute respiratory distress syndrome (ARDS). A hallmark of ARDS is rampant maladaptive inflammation, the so-called "cytokine storm", resulting in hyperinflammatory vascular injury, multi-organ failure, and often death. Stem cell therapy is being investigated in clinical trials to treat ARDS; in particular, mesenchymal stem cells (MSCs) are thought to be able to modulate maladaptive inflammation to a large extent through release of secreted factors. Sustained, optimized delivery of stem cell secreted factors to the lungs and other organs remains a major hurdle, however, largely due to rapid elimination of administered cells following delivery to the patient, leaving little time (typically minutes to hours) to exert beneficial effects. Domicell's Stem Cell Implantable Bioreactor (SCIB) is a novel cell delivery platform that overcomes these limitations and enables sustained delivery of therapeutic factors produced and released on demand by stem cells housed in a protected environment in the patient. The SCIB's proprietary selectively permeable cell chamber protects contained cells from washout and immune clearance while allowing sustained delivery of beneficial secreted therapeutic factors tailored to the stage of tissue injury and inflammation. SCIB-based MSC therapy was safe and showed efficacy in limiting adverse heart remodeling and inflammation after myocardial infarction ("heart attack") in a large animal model. In light of the COVID-19 pandemic, and the exaggerated inflammation and ARDS seen in critically ill COVID-19 patients, we seek to assess the efficacy of SCIB-based MSC therapy to suppress maladaptive inflammation and lung injury in a clinically relevant sheep model of ARDS. The results, leveraged with the SCIB's existing large animal safety data, will pave the way for rapid translation to early-phase clinical trials to bring SCIB technology to critically ill COVID-19 patients, and those with other conditions complicated by ARDS and cytokine storm.

**Bhanu Telugu, Ph.D.**
RenOVAte Biosciences, Inc.
Award Amount: $269,500
Disease Target: Hepatic Insufficiency

## Generating Human Liver in Pigs: Meeting a Growing Unmet Need

In the United States alone, more than 123,000 men, women and children currently need lifesaving organ transplants (https://optn.transplant.hrsa.gov/ ). Every 10 minutes another name is added to the national organ transplant waiting list. Sadly, an average of 22 people die each day due to lack of available life-saving organ, with the numbers expected to increase every year. The same is true for patients on liver transplantation waitlist. In the United States, there are an average of 12,000 patients waiting for a liver transplantation at any given time. The ability to generate exogenous organs in pig for transplantation into humans (xenotransplantation) is considered as one of the sources to bridge this shortfall. Pig is already being used for xenotransplanation studies as the size of the animal, organs and physiology are similar to humans. Several tissues from pigs (heart valves, bladder, cornea, etc) are already being used or in advanced stages of product development for transplantation into humans. The main goal of our Company is to generate organs of endodermal origin, in this case liver from donor progenitor cells called extraembryonic endodermal cells or XEN cells established from patient- specific stem cells using pig as a bio-incubator. Following technical validation, this will provide a pathway for revenue generation by providing "on-demand" source of transplantation ready hepatic cells for cellular therapies and will plug-in into the associated technologies such as organ-on chips, 3D- printing, and pharmaceutical applications in the short-term. In the long-term, the goal is to generate immune-compatible transplantation ready solid organs for transplantation.

There exist closely related alternative methodologies including the humanization of livers in FRG (FAH-deficient mice)11, 12; uPA-transgenic mice13, etc., where human hepatoblasts are transplanted into murine liver14, 15. Following transplantation, the human hepatocytes populate the liver and the associated death of mouse hepatocytes allow for 70% of the liver being humanized. Similar efforts are currently underway in pigs. Conceptually, our approach will allow for 100% of the hepatocytes to be of human origin. Other competing technologies including cellular therapies16-18, extra-corporal devices (Bio-articifical liver, etc)19-21, 3-D printing22, etc., do exist that offer a more humane and safer alternative. However, the current bottleneck for these technologies is the availability of unlimited supply of on-demand "functional" human hepatocytes. Competing technologies, such as iPS cell-based differentiation into hepatocytes have not matured yet and cannot replicate the full- spectrum of hepatocyte functionality, and supply of primary hepatocytes from cadaver are in short supply. In summary, our technology and approach are conceptually and technologically innovative and will bridge a critical gap in the availability of primary human hepatocytes for transplantation.

MSCRF
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# 2020 Annual Report

# Grant Awards

**Luis Garza, M.D., Ph.D.**
Johns Hopkins University
Award Amount: $750,000
Disease Target: Amputees with Limb Loss

## Autologous Volar Fibroblast Injection Into the Stump Site of Amputees

Stem cell therapy holds great promise in medicine, but faces hurdles to define basic parameters such as ideal schedule, ideal dose, evidence of long-term engraftment, and evidence of long-term tissue alteration. The skin is an ideal model system to test these variables given the accessibility and low morbidity for intervention, but the results hopefully will be generalizable to the field of regenerative medicine. To begin to address these questions in the pursuit of a defined clinical goal, we have been using a fibroblast cell therapy to try to help the more than 2 million amputees in the US. Many improvements have been made in prosthetics design, but amputees still underuse prosthetics or abandon their use altogether. To enhance prosthetic use, we propose to imbue normal palmo-plantar (VOLAR) skin features such as pressure adaptation to the non-volar stump site of amputees. Our solution hinges on the ability of mesenchyme (fibroblasts) to influence epithelial (keratinocyte) gene expression. Since 2013, we have been working under a CBER IND to test how ectopic volar fibroblasts can alter skin function in normal healthy volunteers. With MSCRF Clinical program funding, our ongoing results after injecting volar fibroblasts versus vehicle in each of more than 30 normal human subjects nonvolar skin show promising and significant changes. We first tested for histologic endpoints known to be greater in native volar skin to see if these were enhanced in injected non-volar skin. Greater KRT9 expression, higher epidermal thickness, larger keratinocyte cytoplasmic size, and longer collagen length are markers of volar skin. We find that these are ectopically increased in nonvolar skin after volar fibroblast injection ($n=31$, $p<0.03$; $n=31$, $p<0.005$; $n=29$, $p<0.007$; $n=11$ $p<0.04$ respectively), even after 5 months. Excitingly, in scRNA seq data we can also see a persistent ectopic cell population after 5 months. Finally, both sc and bulk RNA seq demonstrates gene ontology categories of Organ Development ($n=3$, $p<3*10-4$) and Morphogenesis ($n=3$, $p<8*10-6$). In physical tests with a durometer, we find increased physical firmness at the site of volar fibroblast injections. The long-term engraftment of these cells and tissue changes of our model create a robust platform to test concepts of stem cell therapy towards the development of a new therapeutic. The above successful data motivate us to apply for a second round of MSCRF funding for a phase 2 study in actual amputees. For matching funds, we are already awarded a phase 2 grant from the Department of Defense. However, we are now concurrently applying to the NIH for an RMIP grant (Regenerative Medicine Innovation Project) that also itself requires matching funds. To build on those grants, we propose to add more subjects where we inject the entire stump rather than a small test area, and also include novel noninvasive techniques to test the success of our therapy, in an effort to validate these evaluation tools for a larger phase 3 study.

**Anthony Oliva, Ph.D.**
Longeveron, LLC.
Award Amount: $650,000
Disease Target: Acute Respiratory Distress syndrome (ARDS)

## The RECOVER Trial: Longeveron Mesenchymal Stem Cells (LMSCs) for ARDS due to COVID-19 and Flu

Acute respiratory distress syndrome (ARDS) is a devastating consequence of infection with SARS- CoV-2, which has led to the coronavirus disease 2019 (COVID-19) pandemic with a high mortality rate [1-4]. Symptoms onset can take over a week after exposure, with rapid progression to ARDS thereafter [5, 6]. Such severely affected patients can have resting respiratory rates exceeding 30 breaths per minute, C reactive protein (CRP) levels >30 mg/L (reference range <3mg/L), and blood oxygen saturation below 93% [5]. Computed tomography (CT) typically reveals rapidly developing subpleural ground-glass opacities (GGOs) in these patients, with potential development of long-term fibrosis [3, 5, 7-9]. Acute inflammation resulting in a cytokine storm is a primary factor in COVID-19-induced ARDS, and results in disruption of the pulmonary endothelial and epithelial barriers [10, 11]. ARDS can also result from other infectious agents via similar mechanisms, including seasonal influenza virus. There are no approved treatments or preventions for COVID-19, several promising treatments have failed in clinical trials, and a potential vaccine is likely a year away. As an RNA virus, there is strong potential for SARS-CoV-2 to mutate into new strains for which vaccines under development may be ineffective. Furthermore, the most highly vulnerable patients to COVID-19 often respond poorly to vaccines. Thus, there is an urgent need to develop symptomatic treatments that can be of general benefit to highly vulnerable patients to COVID-19, or whatever the next pandemic may be. Allogeneic mesenchymal stem cells (MSCs) present a highly attractive therapeutic candidate for ARDS due to their pleiotropic mechanisms of action. MSCs have powerful anti-inflammatory properties without leading to toxic immunosuppression, and thus can potentially treat virally-induced cytokine storms and detrimental inflammation to the pulmonary endothelial and epithelial barriers. MSCs also have potential to reduce fibrosis, improve immune function, and promote intrinsic regenerative responses [12-20]. Longeveron Mesenchymal Stem Cells (LMSCs) are a proprietary formulation of allogeneic MSCs backed by over a decade of pre-clinical and early clinical research, and currently under evaluation in several Phase 1 and 2 clinical trials. We will conduct a Phase IIa trial to evaluate the safety and efficacy of LMSCs to treat ARDS due to COVID-19 or influenza virus infection. The premise of this study is that LMSCs can be a disease- modifying intervention by decreasing ARDS severity and duration, and improve both short- and long- term clinically outcomes, including survival. The rigor of this study comes from its double-blinding, placebo-control, appropriate powering and stopping rules, and evaluation of endpoints in multiple domains. This study is under an FDA-cleared IND and CMC section , is IRB- approved, and is poised to begin patient enrollment immediately.

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# Post-Doctoral Fellowship Program

2020
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GRANT AWARDS

**Ji Young (Julie) Choi, Ph.D.**
University of Maryland, College Park
Mentor: John Fisher, Ph.D.
Award Amount: $130,000
Disease Target: Osteoarthritis

## Engineering the Human Joint Microenvironment to Understand the Mechanism of Cellular Senescence and Stem Cell Aging

Cellular senescence is a critical mechanism that contributes to age-related cellular phenotypes and diseases. Physiological aging in human is linked to cellular aging which is associated with oxidative stress, genetic instability, telomere shortening, and mitochondrial dysfunction. This proposal investigates the bioengineering approach to convey mechanistic study on age-related changes due to cellular aging and senescence in human joint tissue that lead to degenerative bone disease such as osteoarthritis (OA). Although past studies noted that accumulation of senescent cells in cartilage causes early onset of OA in response to joint injury or aging, less is known about the interaction of senescent cells with neighboring cells within the local and distant tissues that forms articular cartilage and synovial joint.

Here, we propose two major aims:
Specific Aim 1: Develop stem cell-based platform to recreate a biomimetic multilayer cellular niches of the healthy human joint.

Specific Aim 2: Investigate the effect of senescent hMSC- chondrocytes (CHD) on neighboring synoviocytes (HFLS) and hMSC-osteoblasts/osteocytes (OB/OC) within the 3D joint tissue model of age-related OA. The study explores the threshold level of senescent CHD that negatively influence the differentiation capacity of local stem cells into osteogenic lineage, and develop detrimental cellular function in HFLS within in vitro OA joint model.

To understand the cellular interactions between senescent CHD and adjacent cells, our initial goal is to design 3D printed system to co-culture gradient of three different cell types that are encapsulated in hydrogel construct for tri-culture bioreactor (TCB). The impact of senescent cells will be evaluated by several cell-based assays to assess the expression of inflammatory chemokines and cytokines produced by HFLS, and analyze osteogenesis potential by transcriptional & protein markers. Overall, this study will demonstrate the mechanistic role of senescent cells in altering cellular microenvironment of human joint, and potential advancement in identifying the targeted therapeutic strategy for age-related OA.

**Laura D'Ignazio, Ph.D.**
Lieber Institute for Brain Development
Mentor: Jennifer Erwin, Ph.D.
Award Amount: $130,000
Disease Target: X-linked Dystonia Parkinsonism

## Dissecting the Pathological Molecular Mechanisms Underlying X-Linked Dystonia-Parkinsonism (XDP) with Patient iPSC-Derived Striatal Organoids

X-linked Dystonia-Parkinsonism (XDP) is a fatal adult-onset Mendelian neurodegenerative disorder. Neuropathological analyses of XDP postmortem brain samples detected a progressive loss of the medium spiny neurons (MSNs) within the striatum, but the reasons underlying this degeneration remains unknown. Conventional genetics analysis identified a founder haplotype consisting of seven variants mapping on the X chromosome, including a SINE-VNTR-Alu (SVA) retrotransposon insertion. These variants cluster within or around the non-coding region of human TAF1 gene. TAF1 (TATA- binding-protein (TBP)-associated factor 1) is a subunit of the TFIID complex, essential component of the transcriptional machinery. Although XDP neurodegeneration causes are not clearly defined yet, defective TAF1 RNA splicing, aberrant intron retention, and transcriptional alterations driven by the presence of the SVA in TAF1 intronic region are associated with cellular models of the disease. However, these molecular associations are only present in XDP fibroblasts, blood RNA and neural progenitors derived from induced pluripotent stem cells (iPSC). Unfortunately, all in vitro models based on monolayer neuronal cultures failed to identify molecular features associated with the disease in neurons. Therefore, any potential therapeutic target remains unknown. Here, we propose that a three- dimensional (3D) cellular model of XDP iPSCs differentiated into striatal brain organoids will better mimic the complexity of XDP brains, shedding light on specific pathological mechanisms affecting XDP MSNs. We posit that the SVA insertion into the intronic region of TAF1 modifies the methylation status of the region, altering TAF1 transcription or splicing. We hypothesize that XDP SVA hexamer arrays form toxic RNA aggregates in neuronal cells. Since neuroinflammation is a common feature across neurodegenerative disorders, we will explore the activation of key inflammatory mediators in neuronal cells and astrocytes in 3D striatal cellular models. We aim to decipher their involvement in the detrimental activation of inflammation, and consequent neurodegeneration mechanisms associated with XDP pathology.

Specific Aim 1: We will determine the expression of TAF1 isoforms in XDP iPSC-derived striatal organoid models.

Specific Aim 2: We will investigate the contribution of SVA to the etiology of the disease, by evaluating how SVA insertion changes the methylation status of the region in vicinity to TAF1 gene, and by monitoring the accumulation of toxic SVA-derived transcripts or single-stranded DNA in the different XDP 3D cellular models.

Specific Aim 3: Furthermore, we will assess the contribution of neuronal and glia cells in the induction of neuroinflammation mechanisms, likely associated to the neuronal damage found in XDP MSNs.

**Dipankar Dutta, Ph.D.**
University of Maryland, College Park
Mentor: Steven Jay, Ph.D.
Award Amount: $130,000
Disease Target: Spinal Cord Injury

## Engineering Potency and Delivery of Oligodendrocyte-derived Extracellular Vesicles for Spinal Cord Injury

Stem cell transplantation has shown promise in animal models of spinal cord injury (SCI) where transplant-mediated regulation of the signaling milieu in host tissues (rather than direct integration) is a key regenerative mechanism. Extracellular vesicles (EVs) – nanoscale particles secreted by nearly all cell types – have thus gained acute interest due to their bioactive cargo that retain the potential to recapitulate the regenerative the properties of their parent stem cells, while mitigating some concerns related to cell therapies . Specifically, EVs derived from mesenchymal stem cells (MSCs) have been linked to anti-inflammatory and angiogenic effects in many applications, including SCI. However, they have not been shown to directly induce robust remyelination and axonal plasticity in the CNS, critical determinants of long-term prognosis after SCI. The overarching hypothesis for this proposal states that EVs derived from oligodendrocyte precursor cells (OPCs) and their differentiated oligodendrocyte (OL) phenotypes, the only myelinating cells of the CNS, have CNS-specific therapeutic potency for SCI. Although OPC differentiation to give rise to mature (myelinating) OLs is well understood, OPC/OL-derived EVs as a function of parent cell phenotype is not known. The first aim of this proposal will isolate EVs from defined populations of induced-pluripotent stem cell (iPSC)-derived OPC/OLs and characterize their CNS-specific therapeutic bioactivity relative to EVs isolated from MSC- and iPSC-derived neural progenitor/stem cells (NPSCs). The second aim of this proposal will develop a novel platform for sustained EV release from fibrin hydrogels to probe the efficacy of EV controlled release in an in vivo model of rodent SCI. OPC/OLs play critical roles in diverse CNS injury/disease conditions (stroke, multiple sclerosis, Alzheimer's disease, etc.). OPC biology, differentiation and heterogeneity is well characterized both in vitro and in vivo from the perspective of cell morphology, function, genomics and proteomics. The dynamic gene regulatory mechanisms that underlie OPC differentiation have also received significant attention. Moreover, studies regarding EV biogenesis and modulating EV bioactivity have seen an exponential rise in interest over the previous 15 years spurred by the potential of cell-free therapies. However, OPC/OL-derived EVs are scarcely characterized in literature even though the oligodendroglial lineage of cells play critical roles in diverse CNS injury/disease conditions (multiple sclerosis, stroke, Alzheimer's disease, etc.). Completion of the aims set forth in the current proposal will significantly enhance the mission of Maryland Stem Cell Research fund because this proposal will:

Aim (1): Contribute to a better understanding of whether known phenotypic changes in iPSC-derived OPC/OLs at different stages of differentiation plays a role in the bioactivity of their EVs.

Aim (2): Characterize a novel bifunctional fusion protein (expected to function in a modular fashion with EVs from any cell type) to achieve EV sustained release for diverse applications.

Aim (3): Determine the therapeutic efficacy of sustained bioavailability (relative to bolus injections) of EVs with CNS-specific regenerative potency in a in vivo rodent model of SCI.

**Su Bin Lim, Ph.D.**
Johns Hopkins University
Mentor: Valina Dawson, Ph.D.
Award Amount: $130,000
Disease Target: Parkinson's Disease

## RNA Velocity of Single Nuclei in Parkinson Disease

Parkinson's Disease (PD) is prion-like disorder characterized by the spread of pathologic α-Synuclien (α-syn) from cell to cell. It is thus clinically pertinent to identify modulators of α-syn transmission and aggregation as potential disease-modifying or neuroprotective targets. Yet, the mechanism underlying α-syn PFFs triggered aggregation of endogenous α-syn - and the effect of PD-causing mutations on this process - is still largely unexplored particularly at the single-cell or single-nuclei resolution. Consequently, it remains unclear whether such genetic variants can be targeted for therapeutic interventions.

While most PD is idiopathic, genetic models of PD have provided deep insights into the more common sporadic form of the disease, uncovering novel candidate biomarkers. Mutations in the leucine-rich repeat kinase 2 (LRRK2) gene are the most common cause of late onset PD, with similar clinical features and neurochemical changes in idiopathic PD including α-syn pathology.

In this proposal, I aim to address the aforementioned gaps by leveraging in vitro model of PFF-induced α-syn aggregation and single nuclei RNA-sequencing (HiF snRNA-seq), coupled with the newly established concept of RNA velocity, using human-induced pluripotent stem cells (hiPS)-derived dopamine neurons from PD patients with LRRK2 mutations. Since the role of G2019S mutation in LRRK2 on α-syn uptake, transport, aggregation and neurodegeneration are being elucidated, and the deletion of LRRK2 has been reported to rescue α-syn PFF-induced pathology in mouse models and human neurons, it is hypothesized that LRRK2 modifies α-syn pathology, with potential clinical implications for LRRK2 inhibitors, which are currently in clinical trials.

Unlike bulk-cell profiling, sc/snRNA-seq analyses enable a more holistic interrogation of distinct cell subpopulations and cellular trajectories through pseudotime analysis, revealing temporal and evolutionary processes defining PD. Here, the concept of 'RNA velocity' will be applied to HiF snRNA-seq data, in combination with subclustering, to provide insights into the transcriptional dynamics of different cellular states and PFF- induced α-syn pathology related to LRRK2 mutations. Supported by exciting preliminary data, and through high-level cross-disciplinary collaborations, I aim to achieve a deep molecular understanding of the effect of PD- causing mutation on α-syn PFF-induced pathology at the single-nuclei level. The proposed research will shed light on how early biological changes in transcriptomic profiles influence later disease phenotype through the study of molecular trajectories and transition status. In addition to unbiased classification of cell types and states, the study will enable construction of systems biology models that predict the behavior of degenerating cells during dynamic processes.

**Seong Hyun Park, Ph.D.**
Johns Hopkins University
Mentor: Gabsang Lee, Ph.D.
Award Amount: $130,000
Disease Target: Charcot–Marie Tooth Diseases

## Human iPSC-Derived Myelinating Schwann Cell-Based Disease Modeling and Efficient Differentiation Study

Charcot-Marie-Tooth (CMT) disease is the most common and incurable genetic disorder of the peripheral nervous system (PNS), disrupting myelin structures and leading to permanent neuron loss, significant pain, and morbidity. While there is some capacity for remyelination and regeneration of the PNS, recovery is often incomplete, and patients are left with life-long debilitating symptoms. The current rodent models of CMT have provided valuable information, but there are no humanized models using patients' genetic mutations and no effective treatments to prevent demyelination or to enhance remyelination, partly because of an incomplete understanding of the genetic and molecular details of PNS remyelination and a lack of a robust human Schwann cell model. These barriers to our understanding of myelination in the PNS can be overcome with new approaches to derive human Schwann cells and in vitro myelination. Therefore, we propose to establish a new cellular model for CMTs, using human induced pluripotent stem cells. Our "CMT in a dish" model will be beneficial to identify of each CMT disease cell types, develop new pharmacological drugs and to realize future cell replacement therapy. In this proposal, we will enhance the efficiency of the in vitro myelination of human Schwann cell culture system and identify the molecular pathology of differentiated CMT hiPSCs.

**Aim 1**: In silico analysis of CMT diseases (ISAC) for molecular phenotyping of CMT1A, 1B and 1X in an hiPSC-based Schwann cell culture model: We will differentiate multiple hiPSC lines of CMT1A, CMT type 1B (CMT1B), and CMT type 1X (CMT1X) with the myelinating culture and identify the transcriptional phenotypes in single cell levels.

**Aim 2**: Enhance human Schwann cell myelination using pharmacological invention: We will optimize our current in vitro myelination protocol (in a 96-well plate format) via a compound screening with a small molecule library containing ~160 chemical modifiers of multiple signaling pathways.

Our study will enable us to develop a hiPSC-based platform for a high throughput screening, a new humanized and patient-specific myelination model of CMT, and a new strategy to isolate competent human Schwann cells for pharmacological rescue and cell replacement therapy in future.


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