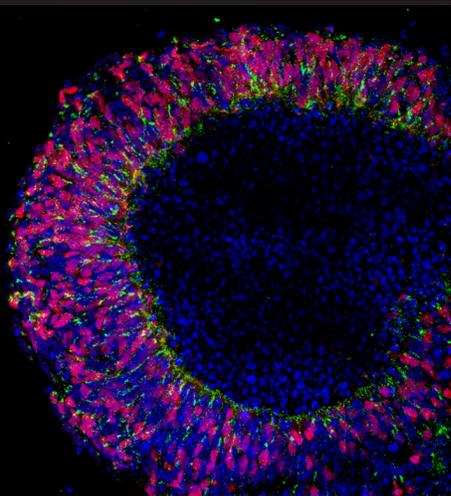


MSCRF 

2018

Maryland

STEM CELL RESEARCH FUND



Annual Report

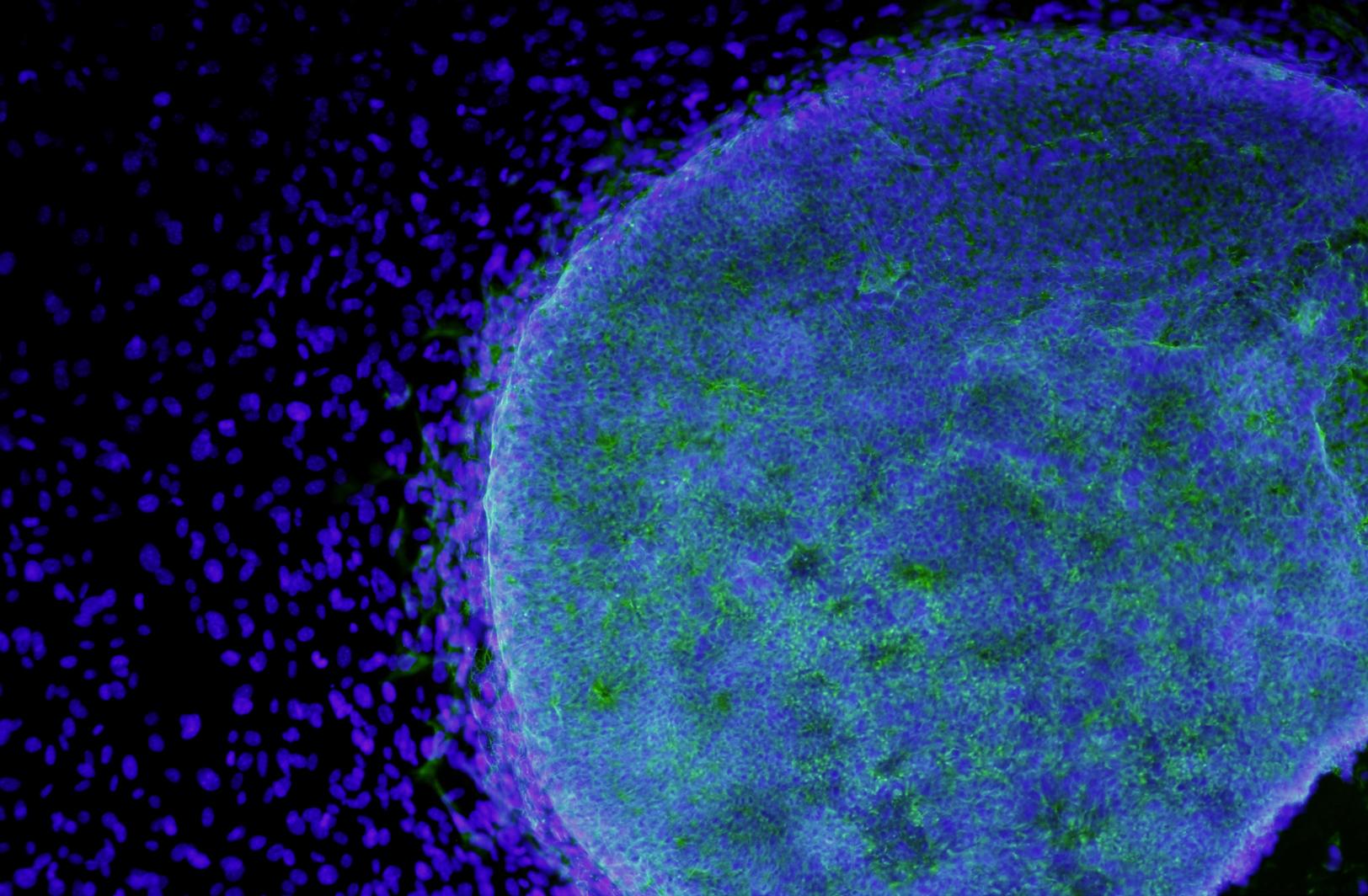


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Clinical Grant Awards:

Luis Garza, M.D., Ph.D.
[Johns Hopkins University](#)
 Stem Cell Therapy to Convert Stump Skin to Palmo-Plantar Skin in Amputees

Anthony Oliva, Ph.D.
[Longeveron, LLC](#)
 Longeveron Mesenchymal Stem Cells (LMSCs) to Improve Vaccine Response in Aging Frailty

Commercialization Grant Awards:

Luis Alvarez, Ph.D.
[Theradaptive, Inc.](#)
 Development of a Biphasic MSC Delivery System for the Repair of Osteochondral Defects

Validation Grant Awards:

Warren Grayson, Ph.D.
[Johns Hopkins University](#)
 Oxygen-Delivering BiO2-Bone Scaffolds for Regenerating Vascularized Craniofacial Bone

Tonya Webb, Ph.D.
[University of Maryland, Baltimore](#)
 Convertible Natural Killer T cells for Immunotherapy

Discovery Grant Awards:

Peter Calabresi, M.D.
[Johns Hopkins University](#)
 Transcriptional and Functional Profiling of iPSC Derived A1 Astrocytes from People with Multiple Sclerosis

Samarjit Das, Ph.D.
[Johns Hopkins University](#)
 Design and Use of Human Pluripotent Stem Cell Bioreactors in Myocardial Preservation

Valina Dawson, Ph.D.
[Johns Hopkins University](#)
 Neurotoxic Astrocytes in Neurodegeneration

Ricardo Feldman, Ph.D.
[University of Maryland, Baltimore](#)
 Targeting a Novel Lysosomal Sphingolipid-Sensing Mechanism for Reversal of GBA1-Associated Neurodegeneration

Xiaofeng Jia, M.D., Ph.D.
[University of Maryland, Baltimore](#)
 Novel 3D Bioprinted Scaffolds to Promote Neural Crest Stem Cell Mediated Nerve Regeneration

Min Jung Kim, Ph.D.
[University of Maryland, Baltimore](#)
 Reducing RAB GTPase14 to Drive In-Vitro Human Erythropoiesis

Gabsang Lee, Ph.D.
[Johns Hopkins University](#)
 Optically-Induced VEGF Activation in Human Endothelial Cells

Discovery Grant Awards (Cont'd):

Rachana Mishra, Ph.D.
[University of Maryland, Baltimore](#)
 Mesenchymal Stem Cells Preserve Right Ventricular Function in a Neonatal Swine Model of Pressure Overload by Releasing GDF15 and miR-132 Enriched Exosomes

Satoru Otsuru, M.D., Ph.D.
[University of Maryland, Baltimore](#)
 Developing MSC-derived Extracellular Vesicle Therapy for Osteogenesis Imperfecta

Pankaj Pasricha, M.D.
[Johns Hopkins University](#)
 Identification and Isolation of the Human Enteric Neural Stem Cell: Laying Foundation for Curing Achalasia

Hilary Vernon, M.D., Ph.D.
[Johns Hopkins University](#)
 Development of an iPSC Derived Cellular Model of Barth Syndrome: Towards Novel Therapeutic Discovery

Jiou Wang, M.D.
[Johns Hopkins University](#)
 A Novel 3D Microphysiological Brain Model for Studying Neurodegenerative Disease ALS/FTD

Post-Doctoral Fellowship Grant Awards:

Adriana Blazeski, Ph.D.
[Johns Hopkins University](#)
 Mentor: **Leslie Tung, Ph.D.**
 Study of Arrhythmogenic Cardiomyopathy Using a Syncytial Model of hiPSCs

Muthukumar Gunasekaran, Ph.D.
[University of Maryland, Baltimore](#)
 Mentor: **Sunjay Kaushal, M.D., Ph.D.**
 Donor Derived Exosomes as Non-Invasive Serum Biomarker for Immune Rejection Following Human Neonatal Cardiac Progenitor Cell Transplantation

Minseong Kim, Ph.D.
[Johns Hopkins University](#)
 Mentor: **Gabsang Lee, Ph.D.**
 Modeling of Parkinsons Disease using PD-Patients iPSCs-Derived Dopaminergic Neurons with Optical Controllable Alpha-Synuclein

Mehreen Kouser, Ph.D.
[Johns Hopkins University](#)
 Mentor: **Jeff W.M. Bulte, M.S., Ph.D.**
 Manganese-Enhanced MRI For Interrogating Astrocyte Replacement in A Mouse Model of ALS

Su Chan Lee, Ph.D.
[Johns Hopkins University](#)
 Mentor: **Gabsang Lee, Ph.D.**
 Generation of Cancer Progression Models through New Optogenetic Tool to Control p53 in iPSCs

Seungman Park, Ph.D.
[Johns Hopkins University](#)
 Mentor: **Yun Chen, Ph.D.**
 Functional Property Evaluation of iPSC-Derived Cardiac Tissues for Optimized Heart Disease Treatment

Wei Zhu, Ph.D.
[Johns Hopkins University](#)
 Mentor: **Jeff W.M. Bulte, M.S., Ph.D.**
 3D Vascularized Hydrogel Scaffold to Carry Stem Cells for Traumatic Brain Injury Repair

2019 MSCRF Awards: (First Funding Cycle)**Commercialization Grant Awards:****Emily English, Ph.D.**[Gemstone Biotherapeutics, LLC](#)

Stem Cell Loaded Extracellular Matrix Replacement Scaffolds for Skin Regeneration in Burns

Validation Grant Awards:**Sharon Gerecht, Ph.D.**[Johns Hopkins University](#)

Swine Study of Patient-Specific Small-Diameter Tissue Engineered Vascular Grafts

Completed 2017 Awards:**Ha Nam Nguyen, Ph.D.**[3Dnatics, Inc.](#)Commercialization Award
Engineering Human Pluripotent Stem Cell-Derived Brain Organoids for Drug Screening and Toxicity Testing**Linhong Li, Ph.D.**[MaxCyte, Inc.](#)Commercialization Award
Translational Development of Gene-Corrected Hematopoietic Stem Cells as Treatment for Sickle Cell Disease (SCD)**William Rust, Ph.D.**[Seraxis, Inc.](#)Commercialization Award
Long-term Function of Stem Cell Grafts for Insulin-Dependent Diabetes**Chengkang Zhang, Ph.D.**[Propagenix, Inc.](#)Commercialization Award
Building Commercial Path for EpiX™ Technology - Breakthrough in Expanding and Utilizing Tissue Resident Stem Cells**COMPLETED 2016 AWARDS:****Christopher Chiang, Ph.D.**[TheraCord, LLC.](#)Investigator Initiated Award
TheraCord Cord Blood Collection Device**Miguel Flores-Bellver, Ph.D.**[Johns Hopkins University, School of Medicine](#)Post-Doctoral Fellowship Award
Mentor: Maria Valeria Canto-Soler, Ph.D.
3D Neural Retinal/RPE Complex from Human iPS cells: a Novel Age-related Macular Degeneration System**Ziyuan Guo, Ph.D.**[Johns Hopkins University, School of Medicine](#)Post-Doctoral Fellowship Award
Mentor: Hongjun Song, Ph.D.
Investigating Cellular Mechanisms Underlying NF1-Associated Cognitive Impairments using iPSCs**Hyunhee Kim, Ph.D.**[Johns Hopkins University, School of Medicine](#)Post-Doctoral Fellowship Award
Mentor: Valina Dawson, Ph.D.
Parthanatos in Parkinson's Disease**Tami Kingsbury, Ph.D.**[University of Maryland, Baltimore](#)Exploratory Award
Eyes Absent-1 (EYA1) as a Novel Hematopoietic Stem-Progenitor Cell Regulator**Chinmoy Sarkar, Ph.D.**[University of Maryland, Baltimore](#)Exploratory Award
Neuronal Differentiation of iPS Cells by Autophagy Induction in Oxidative Environment to Treat TBI



Maryland Stem Cell Research Commission

David Mosser, Ph.D. – Chair

(Appointed by the University System of Maryland)

Department of Cell Biology and Molecular Genetics, University of Maryland, College Park.

Debra Mathews, Ph.D., MA - Vice Chair

(Appointed by Johns Hopkins University)

Assistant Director for Science Programs, Johns Hopkins Berman Institute of Bioethics; Associate Professor, Dept. of Pediatrics, Johns Hopkins School of Medicine.

Scott Bailey, Ph.D.

(Appointed by Johns Hopkins University)

Associate Professor; Biochemistry and Molecular Biology, Johns Hopkins Bloomberg School of Public Health; Johns Hopkins School of Medicine

Rachel Brewster, Ph.D.

(Appointed by the University System of Maryland)

Associate Professor; Biological Sciences University of Maryland, Baltimore County

Margaret Conn Himelfarb

(Appointed by the Governor)

Health Advisory Board and Institutional Review Board, Johns Hopkins Bloomberg School of Public Health; Embryonic Stem Cell Research Oversight Committee, Johns Hopkins School of Medicine.

Diane Hoffmann, M.S., J.D.

(Appointed by the University System of Maryland)

Professor of Law, Director Law & Health Care Program, University of Maryland School of Law

Haig H. Kazazian, Jr., M.D.

(Appointed by Johns Hopkins University)

Professor of Pediatrics McKusick-Nathans Institute of Genetic Medicine

Linda Powers, J.D.

(Appointed by the President of the Senate)

Managing Director of Toucan Capital, Early & Active Supporter of Biotech Companies

Rabbi Avram I. Reisner, Ph.D.

(Appointed by the Governor)

Rabbi of Congregation Chevrei Tzedek, Baltimore, Maryland.

Ira Schwartz, Esq.

Senior Assistant Attorney General & Counsel to the Maryland Technology Development Corporation (TEDCO)

Curt Van Tassell, Ph.D.

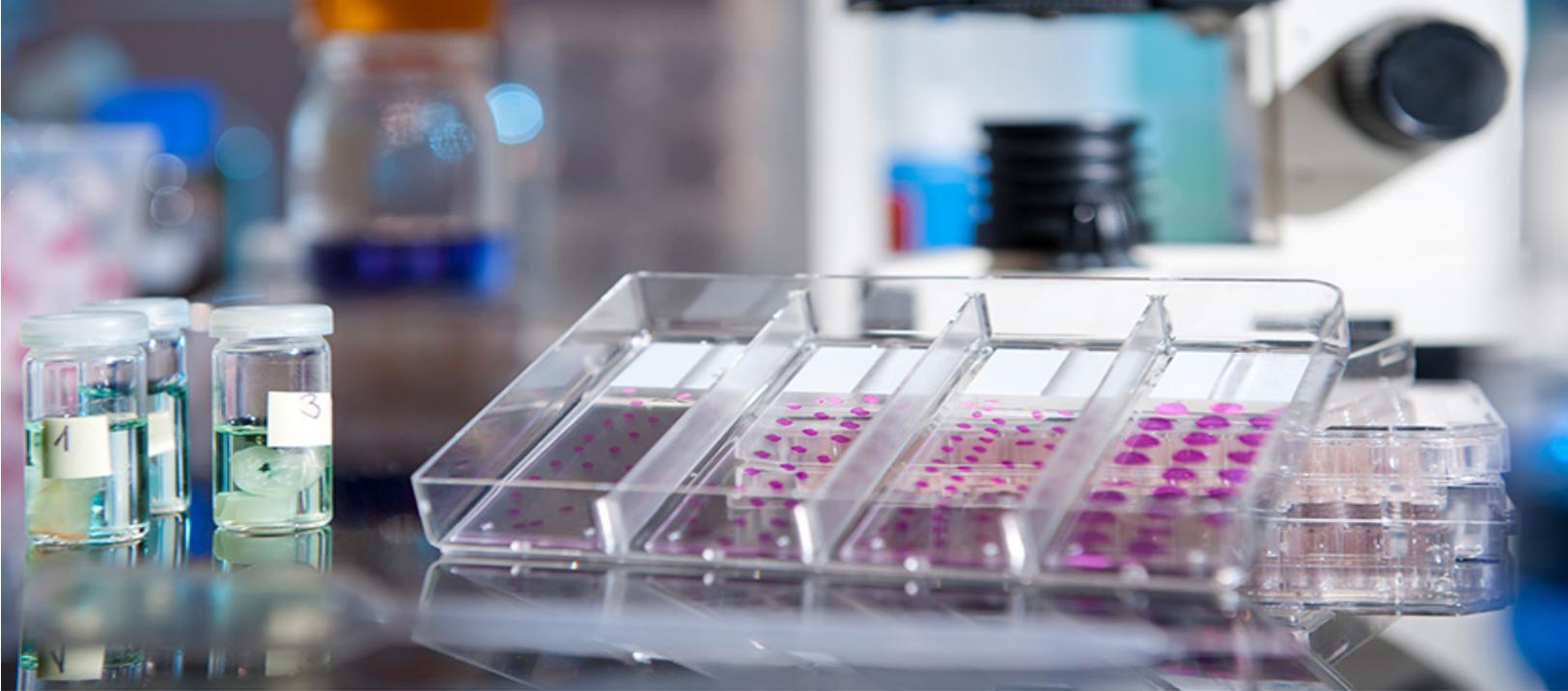
(Appointed by the Speaker of the House of Delegates)

Research Geneticist, USDA-ARS, Beltsville, MD

Bowen P. Weisheit, Jr.

(Appointed by the Governor)

Patient Advocate; Board member of the Maryland Chapter of Cystic Fibrosis Foundation; & Attorney, Law Office of Bowen Weisheit, Jr.



New Hope for Regenerative Medicine

In December 2016, the US Congress passed the 21st Century Cures Act and within it the new Regenerative Medicine Advanced Therapy (RMAT) focus. The legislation provides the federal government with critical tools and resources to advance biomedical research across the spectrum, from basic research studies to advanced clinical trials of promising new therapies. Additionally, the Cures Act provides \$30 million in multiyear funding to highly innovative scientific initiatives in regenerative medicine.

The Cures Act is also designed to help accelerate medical product development and bring innovation and advances rapidly and more efficiently to patients in need. The US FDA recently approved 16 new cell therapy products¹ which led to a flurry of M&A activities, amongst them the acquisition of Kite Pharmaceutical by Gilead (\$11.9 billion) and the acquisition of Juno by Celgene (\$9 billion).



Opportunities in Cell & Gene Therapy in Maryland

We, in Maryland, are at the forefront of the cell therapy market and have all the right assets² to take the lead in this emerging industry, in particular, to fill the critical need of manufacturing. This is evident from the recent moves by leading companies such as Kite Pharmaceutical (partnering with the National Cancer Institute³) and Autolus (a UK company) to Montgomery County.⁴

Discussions in recent industry focused meetings and conferences⁵ have all highlighted the manufacturing challenges faced by the field, especially by international companies looking to enter the US market; according to the Alliance of Regenerative Medicine (ARM) about half the companies are outside the North America region.⁶

1. <https://www.fda.gov/biologicsbloodvaccines/cellulargenetherapyproducts/approvedproducts/default.htm> ; 2. Maryland Stem Cell Research Fund (MSCRF) proprietary information; 3. <https://www.gilead.com/news/press-releases/2018/5/kite-announces-new-worldwide-facilities-and-expanded-collaboration-with-national-cancer-institute-to-support-cell-therapy-pipeline> ; 4. <https://biobuzz.io/> ; 5. <https://alliancerm.org/arm-event/meetingonthemesa/>; <http://www.phacilitate-leaders-world.com/>; <http://expo.tedco.md/> ; 6. <https://alliancerm.org/publication/q2-2018-data-report/>

Mission Focused

We are staying focused and on course with our mission - to develop new medical strategies for the prevention, diagnosis, treatment and cure of human diseases, injuries and conditions through human stem cells. We hold the charge of ensuring that these programs under the Accelerating Cures initiative remain relevant for the next 20 years and that we increase our impact and reach. We are surrounded by research institutes that are second to no other region and it is our mission to create an environment that accelerates these assets and discoveries to deliver on the promise of cures. We continue to operate our five key programs. Additionally, we expanded our support through key market research data and also organize meetings, conferences and seminars for our researchers.

“Develop new medical strategies for the prevention, diagnosis, treatment and cure of human diseases, injuries and conditions through human stem cells.”



Enhance Research and Innovation

Accelerating Cures starts with support for research and innovation at a very basic level of understanding the cell processes that are enabling the stem cells to differentiate, proliferate and regenerate human tissue. We continue to support the research through our Validation Program and work with our researchers side by side, leading and guiding them as they develop a commercialization plan, validate their technologies and are ready for the next step.



During the last year we have partnered with many groups and provided several meetings, seminars, conferences and events to our community. We also partnered with global organizations and promoted Maryland to companies considering relocation from other parts of the world.

Some of our events in the past year include:

- 

Maryland Stem Cell Symposium – held in conjunction with TEDCO’s Entrepreneur EXPO. This event attracted 1,000 people to participate in 12 hours of science and technology discussions.
- 

First Cardiac Regenerative Symposium for Congenital Heart Disease - a daylong session by world leaders on advances and challenges in cardiovascular research and surgery.
- 

We also supported a few of the University of Maryland Stem Cell Center seminars as well as the grand opening of the new Cell Therapy Center.
- 

In late May we hosted a panel discussion on Regenerative Medicine: Opportunities and Challenges in partnership with American Gene Technologies in Rockville.
- 

We continue to partner with Biotrac to offer crucial hands on training and other relevant courses to scientists in the stem cell field.



This is a great time to be a Marylander and an amazing time to be a stem cell researcher in Maryland. We continue to support our community on many levels and through great partnerships with leading universities, companies and organizations. Together we can accelerate our research into products and treatments that will revolutionize medicine.



New Look Same Great Programs

After 10 years our website is finally getting a new look with updated information about the research that we support as well as current news and innovation in the stem cell /cell therapy field from around the world. This new user-friendly website is up and running and will be a great tool to educate, grow and support our community. The new home page will serve as a news for stem cell, cell therapy and regenerative medicine news and our partners and researchers would be able to both read and contribute information.



Building Great Companies

Over the past year we actively worked with our portfolio companies helping them grow and succeed in this rapidly progressing field. We awarded some new companies and welcomed several others to our community in MD. We remain committed to supporting the growth of our portfolio companies and facilitate their progress into the clinic and help them establish themselves with additional funds and employees. We have dedicated section on our website to these companies in our portfolio. We also provide our companies an opportunity for enhanced media coverage through our strategic partnerships. Some examples of such media can be seen on the media section of our website as well as on our social media channels.



CLINICAL RESEARCH GRANT AWARDS



Luis Garza, M.D., Ph.D.

Johns Hopkins University

Award Amount: \$650,000

Disease Target: Amputations

Anthony Oliva, Ph.D.

Longeveron, LLC.

Award Amount: \$750,000

Disease Target: Aging Frailty

Stem Cell Therapy to Convert Stump Skin to Palmo-Plantar Skin in Amputees

Cellular therapy holds great promise in medicine. This grant will employ autologous fibroblasts stem cells in an attempt to help more than 1.7 million (1 out of every 200) people in the US who have had amputations. While improvements in prosthetics exist, their use is still dramatically limited by pain and skin-breakdown at the stump. Our long-term goal is to convert the skin at the stump permanently to volar type (palmo-plantar) skin. Volar epidermis is markedly thicker than all other skin and uniquely expresses Keratin 9 (KRT9) that makes it more friction- and irritant-resistant. If the skin at the stump is thick volar type skin then the use of prosthetics will be enhanced dramatically. The markets for this technology are likely to be even broader and include preemptive modification of pressure points in wheelchair and bed-bound patients to prevent pressure ulcers (\$8.5 billion dollar annual costs). We propose the method to convert skin identity from non-volar to volar will be the injection of volar fibroblast stem cells, and our endpoint will be KRT9 given that it is pressure responsive, provides structural support, and the most unique volar skin gene. We already have full IRB and FDA IND approval (CBER IND #15658), and registration on ClinicalTrials.gov (NCT01964859) for a human clinical trial to test the ability of fibroblast stem cells to convert the skin identity from non-volar to volar skin. We have enrolled more than 20 subjects where we are testing on buttocks skin for proof of concept. We have tested multiple variables and show-- particularly after mild wounding to prepare the recipient site--statistically significant induction of KRT9 in our human subjects where we have injected 10 million volar fibroblasts compared to injections of non-volar fibroblasts or vehicle (n=3, p<0.05). We also induce other genes found in volar skin such as KRT6 and AKR1B10. Finally, we have showed architectural changes (the increase of cytoplasmic area of keratinocytes) as in volar skin. These results prove the feasibility and eventual clinical potential of this product. As we plan for phase 2 and 3 trials we are encountering two challenges which this grant will address. The *first* is that manufacturing costs are high and although we have discovered a dose ceiling where the therapy is ineffective (30 million), we have not discovered the dose floor. What is the minimum dose we can use to minimize side effects and manufacturing costs? The *second* is our end users (amputees and their caregivers, prosthetists) resist the idea of using mild wounding at the stump site to improve engraftment/identity conversion. Can we find a replacement for wounding? We will use Department of Defense grant funding to analyze how wounding enhances engraftment and KRT9 induction to provide matching funds for the following work suggested by our animal data: After an initial dose of fewer cells, test if 2 follow-up weekly injections of vehicle only (freezing media) will enhance engraftment/KRT9 induction to substitute for mild wounding and allow for lower dosages of cells.

Longeveron Mesenchymal Stem Cells (LMSCs) to Improve Vaccine Response in Aging Frailty

Aging frailty is characterized by the progressive physiologic decline in multiple organ systems, leading to increased vulnerability to disease, comorbidity, and mortality. This includes the age-related decline of the immune system, termed immunosenescence. Immunosenescence results in diminished responsiveness to antigen challenges such as vaccinations, and increases vulnerability to infection and associated complications such as opportunistic infections, increased hospitalization rate, and death. Aging Frailty and immunosenescence appear driven, at least in part, by a chronic pro-inflammatory state, called inflammaging. Therefore, decreasing this pro-inflammatory state could potentially improve immune functioning. In this phase 1/2 clinical trial, we are investigating the potential efficacy of Longeveron-produced allogeneic mesenchymal stem cells (LMSCs) improve immune-response to influenza vaccine in Aging Frailty subjects. LMSCs are a proprietary formulation of mesenchymal stem cells, which are multipotent cells that have powerful anti-inflammatory properties, and support intrinsic repair and regenerative mechanisms. LMSCs are also immunoprivileged, and thus offer promise as an "off-the-shelf" allogeneic therapeutic. This study entails 3 phases. First was a Run-In Phase to demonstrate provisional safety and tolerability. All subjects of this phase have been treated with LMSCs and vaccinated against influenza. Safety was demonstrated, and the Data Monitoring Committee (DMC) recommended the remainder of the trial proceed without modification. The second phase (Pilot Phase) was designed to evaluate the optimal time-interval between LMSC-treatment and influenza vaccination. All subjects of this Phase have been treated with LMSCs and vaccinated against influenza. Safety was again demonstrated. Preliminary efficacy data also support the hypothesis that LMSCs can improve immune response and immunosenescence in Aging Frailty subjects. The third phase of this study is a Placebo-Controlled, Double-Blinded, Randomized Phase designed to evaluate efficacy. This phase is currently enrolling. We anticipate that the results of this study will demonstrate that LMSC therapy is an efficacious adjuvant therapy that provides significant advantages over influenza vaccine alone. We also anticipate that these results should broadly translate across a spectrum of diseases by restoring back towards normal the functioning of the immune system.



COMMERCIALIZATION GRANT AWARDS



Luis Alvarez, Ph.D.

Theradaptive, Inc.
2018 Commercialization Award
Award Amount: \$299,005
Disease Target: Cartilage

Emily English, Ph.D.

Gemstone Biotherapeutics, LLC
2019 Commercialization Award (1st Round)
Award Amount: \$299,998
Disease Target: Severe burns

Development of a Biphasic MSC Delivery System for the Repair of Osteochondral Defects

Theradaptive has developed a novel tunable delivery platform, ConForma® that can control both the dose and timing of release of a protein therapeutic at the site of injury. ConForma® offers endless versatility in that (1) it is mechanically tunable due to the particle size ranging from nano particles to millimeter granules, (2) it supports the delivery of a wide variety of small molecules, peptides and proteins, and (3) it has demonstrated an excellent targeted release performance in a canine model. With the Maryland Stem Cell Research Fund (MSCRF) Commercialization Grant, we can extend the utility of the current ConForma® composite by: (1) optimizing the delivery of MSCs in the biphasic matrix, and (2) validating the key components and competitive advantages of ConForma's MSC delivery matrix compared to the market benchmark for focal cartilage defects. The incorporation of a subpopulation of MSCCD29+ with high chondrogenic potential into ConForma® delivery platform offers a breakthrough technology in reconstructive medicine. With Theradaptive's new MSCCD29+ readily seeded onto ConForma, orthopedic surgeons can for the first time offer patients a potent, viable and regenerative restoration for focal cartilage defects without the need for marrow isolation.

Stem Cell Loaded Extracellular Matrix Replacement Scaffolds for Skin Regeneration in Burns

Skin wound healing is a complex process involving three overlapping phases: inflammation, proliferation, and remodeling, and stem cell niches in the skin play a role in all phases of healing. Mesenchymal stem cells enhance cutaneous healing by modulating the inflammatory response, promoting cytokine secretion, and stimulating cell differentiation. However, commercialization of stem cell-based therapies has been slow because of technical challenges associated with manufacturing, storing, and delivering cell-based therapies for clinical use. Gemstone Biotherapeutics is developing and commercializing its Extracellular Matrix Replacement (EMR) for regenerative medicine applications, with the goal of commercializing products that offers scar-free skin regeneration for acute and chronic wounds. The EMR is a UV-curable synthetic material that promotes skin regeneration in preclinical third-degree burn and excisional wound models. Because it is a synthetic material with tunable manufacturing parameters, the EMR technology can be customized to address specific pathologies by the addition of drugs, biologics, or cells to the material. We propose to optimize the EMR for delivery of viable and scalable RoosterBio hMSCs to third degree burns, an indication for which there are few effective therapeutic approaches. This approach will combine the regenerative capacities of both hMSCs and the EMR, leveraging established manufacturing and regulatory pathways for the product components. The resultant stem cell-loaded EMR will comprise a new regenerative medicine product offering in Gemstone Bios portfolio, building on the core EMR technology, which was developed at and licensed from the Johns Hopkins University Whiting School of Engineering, and leveraging the unique capability of RoosterBios scalable and cGMP manufacturable xeno-free human MSC technology.

The background of the page is a composite image. The upper portion shows a close-up of a blue, cylindrical microscope objective lens with a light blue ring near its tip, positioned just above a glass slide. The lower portion shows a grid of petri dishes containing various bacterial cultures. The dishes are filled with media of different colors: green, pink, orange, and yellow. Some dishes show distinct bacterial growth patterns, such as streaks and colonies.

DISCOVERY GRANT AWARDS

Peter Calabresi, M.D.

Johns Hopkins University (JHU)

Award Amount: \$345,000

Disease Target: Multiple Sclerosis (MS)

Samarjit Das, Ph.D.

Johns Hopkins University (JHU)

Award Amount: \$318,615

Disease Target: Heart

Transcriptional and Functional Profiling of iPSC Derived A1 Astrocytes from People with Multiple Sclerosis

Multiple sclerosis (MS) is a disabling neurological disease characterized by demyelination, gliosis, and neurodegeneration in the central nervous system (CNS). There is known genetic susceptibility, with 240 gene variants being associated with risk of developing the disease, but the course of the disease and severity of disability is highly heterogeneous. Further, it is unclear why certain patients respond well to therapies targeting peripheral immune cells, while others develop the progressive form of the disease and are refractory to presently available immunotherapies. The absence of approved therapies for progressive MS is partly due to a lack of knowledge regarding pathogenesis of this form of the disease. The mechanisms underlying progressive MS likely involve phenotypic changes in subsets of microglia (M1 and M2) and astroglia (A1 and A2) that result in failed endogenous remyelination and neurotoxicity. Our ability to interrogate the biological responses of the glia is markedly hindered by limited access to human tissues, especially during periods of ongoing CNS injury rather than years later, at post mortem examination. We propose to generate MS patient-derived astrocytes from existing iPSC lines. We will determine whether patient-specific gene variants predispose to neurotoxic A1 astrocyte profiles. We will also examine the transcriptomic profile of astrocytes from different patients and controls before and after exposure to different inflammatory cytokines. Taken together, our data may help to elucidate molecular pathways involved in A1 astrocyte formation and to identify therapeutic targets to develop novel treatments for this untreatable form of MS, as well as perhaps other diseases characterized by A1 astrocytes and neurodegeneration (e.g. ALS, Parkinson's, and Alzheimer's Disease).

Design and Use of Human Pluripotent Stem Cell Bioreactors in Myocardial Preservation

Human induced pluripotent stem cells (hiPSCs) hold great promise for myocardial preservation after acute myocardial infarction (AMI), but are susceptible to various concerns. For instance, when iPSCs were injected into mice following myocardial ischaemia (MI), or in the AMI model, the results showed beneficial effects in terms of cardiac contractility. In contrast, a higher incidence of tumorigenesis was observed when iPSC injection was performed in rat hearts. Additionally, poor survival and engraftment coupled with inadequate cardiac commitment of the adoptively transferred hiPSCs diminishes the improvement in cardiac function. Recently, we and others have demonstrated an important and underappreciated mechanism of paracrine cell-cell communication involving exosomal transfer, and its subsequent functional impact on recipient cells. Exosomes enriched in proteins, mRNAs, and miRNAs characteristic of parental hiPSCs represent a potential approach for myocardial preservation after MI. hiPSCs have the ability to produce extracellular vesicles (EVs), including exosomes; however, their effect in the context of the heart is unknown. We have calculated that hiPSCs release approximately 2000 EVs/cell/hour, including almost 70-75% of the EVs with an average 122 nm in size. The common tetraspanin markers CD9, CD63 and CD81 by these EVs, which we termed hiPSC-secreted exosomes (hiPSC-exo). We have developed a novel strategy to isolate hiPSC-exo from the ultracentrifugated (UC) fraction of cell culture media using Fluorescence-activated Exosome Sorting (FAEs). We then incubated the CD81+ EVs in the culture media of human cardiomyocytes (iPSC differentiated cardiomyocytes, hiPSC-CM) (1X10⁶ hiPSC-exo /10⁵ cells). Our preliminary data suggests that the uptake of iPSCs-exo protects the hiPSC-CM against oxidative stress by reducing apoptotic cell death, and by improving cardiac contractility. Bioanalyzer data suggests that the majority of the total RNA within hiPSC-exo are small RNAs, such as miRNAs. Thus, we propose to characterize the miRNA-enriched hiPSC-exo and show its cardioprotective role. We hypothesize that miRNAs found within hiPSC-exo can be internalized by cardiomyocytes, and exert protective effects on the heart against oxidative stress. We will test this hypothesis through two specific aims. Aim 1. To validate the miRNA(s) present within hiPSC-exo and identify the specific miRNA(s) which offer cardioprotection. Using RNA-Seq for small RNAs, we have identified the 13 most abundant miRNAs inside the hiPSC-exo. Thus, we hypothesize that all or a subset of these 13 miRNAs compartmentalized in the hiPSC-exo play a cardioprotective role. We will validate these miRNAs in the hiPSC-CM, and subsequently identify which miRNA(s) offer cardioprotection. Aim 2. To determine the effect of hiPSC-exo on the repair of ischemic myocardium. We hypothesize that hiPSC-exo contents, including miR-206, are transferred into cardiomyocytes, and can protect the heart from I/R injury. We are proposing to study hiPSC-exo cardioprotection in response to I/R injury with an in vitro model, and an in vivo model by ligating the left anterior descending coronary artery. These studies will help us to understand the mechanism by which hiPSC-exo protects the heart from common cardiac injuries, such as ischemia/reperfusion injury. Ultimately, a better understanding of the underlying mechanisms may lead to the development of innovative new therapies for heart disease.

Valina Dawson, Ph.D.

Johns Hopkins University (JHU)

Award Amount: \$344,999

Disease Target: Neurodegenerative Diseases

Ricardo Feldman, Ph.D.

University of Maryland, Baltimore

Award Amount: \$345,000

Disease Target: LSD's

Neurotoxic Astrocytes in Neurodegeneration

As the world population ages, the alarm has been raised for a “sliver tsunami” of a global epidemic of people suffering from neurodegenerative diseases. There is grave concern that in the coming years the sharp increase in patients suffering from neurodegenerative diseases will overwhelm the current health care systems. Alzheimer’s disease (AD) and related dementias and Parkinson’s disease (PD) and related disorders accounting for the majority of those afflicted and the greatest economic burden. Delaying the onset of disease would have tremendous beneficial impact. Currently, medical treatment for these diseases focuses on symptomatic therapy. There is no disease modifying therapy yet available. 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 and Tau in AD and aggregated alpha-synuclein in PD. Fewer studies have explored another common pathologic element across neurodegenerative diseases, the reactive astrocyte. Normal astrocytes provide trophic support for neurons, promote functional synapses, and other homeostatic maintenance activities. This changes in the setting of brain injury or disease where the genetic profile of astrocytes changes and the astrocytes become reactive. While widely observed through the change in morphology and expression of proteins, how astrocytes become reactive nor the actions of reactive astrocytes, is not been well understood. Recently we discovered that microglia are responsible for the conversion of normal (A2) astrocytes into reactive (A1) astrocytes that promote the death of neurons. We found abundant A1 astrocytes in postmortem tissue from various human neurodegenerative diseases including Alzheimer’s, Huntington’s and Parkinson’s disease, amyotrophic lateral sclerosis and multiple sclerosis. In preliminary studies in mouse microglial, astrocyte and cortical cultures we have found that aggregated amyloid-beta or alpha-synuclein can trigger microglial activation and conversion of A2 to A1 astrocytes which results in subsequent neuronal death supporting the notion that reactive A1 astrocytes participate in, and accelerate the progression of neurodegenerative diseases. Human stem cell research offers extraordinary opportunity to study this process in human systems to determine the role of the microglia-astrocyte axis in neurodegeneration. Specific Aim 1: Does pathologic alpha-synuclein, amyloid-beta or Tau activate microglia? Specific Aim 2: Do A2 Astrocytes convert to A1 Astrocytes? Specific Aim 3: Do A1 Astrocytes secrete a neurotoxin? The major goals of this project are to determine if human microglia activated by pathologic proteins direct the conversion of A2 to A1 astrocytes that then direct neurodegeneration in the human setting, and to provide a platform to screen for agents that can block the conversion of A2 to A1 astrocytes or that can block the neurotoxic molecule(s) produced by A1 astrocytes. Blocking reactive astrocytes would be expected to have disease modifying effects in many neurodegenerative diseases including PD and AD.

Targeting a Novel Lysosomal Sphingolipid-Sensing Mechanism for Reversal of GBA1-Associated Neurodegeneration

Mutations in GBA1 cause Gaucher disease (GD) and are the highest known risk factor for Parkinson’s disease (PD). GBA1 encodes the lysosomal enzyme glucocerebrosidase (GCase). GD is an autosomal recessive disorder characterized by visceral abnormalities, and in 20% of cases with severe mutations there is fatal neurodegeneration. Mono-allelic GBA1 mutations do not cause neuronopathy, but 7% of individuals with PD are carriers of GBA1 mutations. GCase catalyzes the hydrolysis of glucosylceramide (GluCer), one of the first steps in the biosynthesis of complex sphingolipids. Mutations in enzymes of sphingolipid metabolism are the cause of >70 lysosomal storage disorders. Most sphingolipidoses cause neurodegeneration, indicating that sphingolipid balance is essential for neuronal survival. Brains from patients with severe GCase deficiency have highly elevated levels of glucosylsphingosine (GluSph), a neurotoxic metabolite of GluCer. Using iPSC-derived neurons harboring GBA1 mutations we found that GCase deficiency causes lysosomal depletion through mTOR hyperactivation and TFEB destabilization, leading to neuronal cell death. Inhibition of mTOR by Torin1 rescued lysosomal biogenesis. Significantly, incubation of WT neuronal cells with GluSph recapitulated the mTOR hyperactivation and lysosomal depletion caused by GCase deficiency, identifying a novel link between sphingolipid imbalance, mTOR activation, lysosomal dysfunction, and neurodegeneration. Incubation of neuronal cells harboring GCase mutations with glucosylceramide synthetase (GCS) inhibitors that inhibit the biosynthesis of GluCer, reversed mTOR hyperactivation and rescued lysosomal biogenesis. Restoration of sphingolipid balance in sphingolipidoses is a major therapeutic goal, not only to treat GD and GBA1-associated PD but also other neurodegenerative diseases where there is GCase deficiency. The FDA has recently approved a glucosylceramide synthetase (GCS) inhibitor for the treatment of type 1 GD. While this compound does not cross the blood-brain-barrier, more recent ones that are brain-penetrant gave promising results in animal models. We hypothesize that the lysosomal mTORC1 complex is not only a nutrient and amino acid sensor, but also a sphingolipid sensor. We postulate that neurotoxic sphingolipids cause hyperactivation of mTOR and destabilization of TFEB, resulting in lysosomal depletion and neuronal cell death. In this application we will identify the molecular components of the mTOR-based sphingolipid sensing machinery, and use this system to evaluate the therapeutic efficacy of new GCS inhibitors. In Aim 1, we will identify the molecular targets of neurotoxic sphingolipids. Using iPSC-derived neurons from WT subjects, we will uncover the mechanisms by which GluSph deregulates major determinants of lysosomal function (e.g. mTOR, TFEB) and how this leads to neuronal cell death. In Aim 2, we will use iPSC-derived neurons harboring GBA1 mutations to identify the molecular mechanisms by which GCS inhibitors reverse GBA1-associated neurodegeneration. We will determine whether new brain-penetrant GCS inhibitors can prevent mTOR hyperactivation, lysosomal depletion, alpha-synuclein aggregation and neuronal cell death. As GCase deficiency is not only caused by GBA1 mutations but is also found in other neurodegenerative conditions and during aging, the proposed work will lead to earlier diagnosis and new treatments for a spectrum of neurological disorders. The work proposed is based on a novel concept and will lead to clinical trials for new GCS inhibitors.

Xiaofeng Jia, M.D., Ph.D.

University of Maryland, Baltimore

Award Amount: \$345,000

Disease Target: Nerve Injury

Minjung Kim, Ph.D.

University of Maryland, Baltimore

Award Amount: \$345,000

Disease Target: Blood Disorders

Novel 3D Bioprinted Scaffolds to Promote Neural Crest Stem Cell Mediated Nerve Regeneration

Background: Approximately 360,000 people suffer from upper extremity peripheral nerve injury in the USA each year. Peripheral nerve injury often results in poor functional recovery and subsequent impaired quality of life for the patient. Severe injury leads to a large gap separating the severed nerve stumps. Currently, many approaches to improve peripheral nerve regeneration have not surpassed the 'gold standard' set by autograft procedures. However, autografts are not ideal due to limited graft source availability and the morbidity of a second surgical incision. Recently, stem cell therapy has garnered attention as a possible improvement in the repair of injuries to the peripheral nervous system. Improvement in robotics-based manufacturing approaches have advanced conventional printing technology to 3D bioprinting, which enables the design of multifunctional tissue scaffolds for peripheral nerve regeneration applications. **Objectives/Hypothesis:** With the long-term goal to ultimately help improve the patient's quality of life, we will test the hypotheses: (1) Transplantation of neural crest stem cells (NCSCs) will improve regeneration possibly through facilitating differentiation into SCs and/or upregulation of growth factors; (2) 3D printing of bifurcated multi-gradient (3D-BMG) nerve scaffold (NS) will promote further differentiation of transplanted NCSCs into SCs and/or upregulation of growth factors, thus improving nerve regeneration and functional recovery. (3) 3D-BMG NS will promote proper motor and sensory pathway specification, contributing to enhanced functional recovery. **Specific Aims:** 1. Optimize novel 3D-BMG NS for nerve regeneration and evaluate the effect on transplanted NCSCs in functional recovery. 2. Identify the role of 3D-BMG NS and NCSCs on motor and sensory pathway specification. 3. Evaluate and compare the effect of NCSC-loaded 3D-BMG NS in immunocompetent condition. **Study Design:** This proposal will investigate the effect of NCSC therapy delivered by 3D-BMG NS in a peripheral nerve defect repair animal model via histopathological, electrophysiological and functional outcome measurements. **Innovation and significance:** For the first time, an anatomical 3D printed NS, 3D-BMG NS, was developed to aid the repair of complicated nerve injuries. Furthermore, we have developed a novel cell modification technology with less invasive sources of stem cells and more desirable source of SCs for transplant therapies. Additionally, 3D-BMG NS enables further research of growth factors specified regeneration pathways. 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. Thus, success in this project would greatly enhance the surgical repair of nerve injuries and offer better functional outcomes. It will help in developing optimal treatment protocols for orthopaedic surgeons specializing in nerve repair or for clinicians treating patients with nerve injury

Reducing RAB GTPase14 to Drive In-Vitro Human Erythropoiesis

Generation of mature and maturing erythroid cells via in vitro culture of hematopoietic stem-progenitor cells (HSPCs) is under investigation as an alternative to blood donation^{1,2}, but clinical use of in vitro-cultured erythroid cells is still challenged by extensive technical/logistical factors and low erythroid cell yields³⁻⁵. Indeed, we believe that coordinated manipulation of several erythroid regulatory molecules will be required to enhance current protocols for sufficient in vitro erythropoiesis to produce the huge numbers of mature/maturing erythroid cells needed for clinical transfusions^{6,7}. Previously, we utilized miR-144 and miR-451 to identify RAB GTPase14 (RAB14) as a novel endogenous physiologic inhibitor of human erythropoiesis⁸. In Prelim Results, RAB14 knockdown (KD) in the TF1 human erythropoietic cell line increased the frequency and total number of erythroid (CD34-/CD71hi/CD235ahi) cells with elevated levels of GATA1, beta-/gamma-hemoglobins (Hbs), erythropoietin receptor (EPOR) and phosphorylated-STAT5 (p-STAT5) (and reduced GATA2 level), in the presence of either erythropoietin (EPO) or granulocyte-macrophage colony-stimulating factor (GM-CSF). Similarly, RAB14KD in primary human mobilized blood CD34+ HSPCs increased the frequency and total number of erythroid cells, in the presence of EPO or GM-CSF. This suggests that RAB14KD might enhance HSPC-derived in vitro erythropoiesis independently of exogenous EPO and beyond the levels obtained with erythropoiesis-stimulatory cytokine cocktails in current use^{2,5}. RAB GTPases (RABs) are the largest RAS-related GTPase superfamily, and RABs play major roles in vesicle trafficking and receptor recycling⁹. In classic transferrin receptor-recycling, RAB14 functions in the intermediate recycling endosome compartment, between RAB5-dependent early endosomes and RAB11-dependent recycling endosomes⁹. Endosomes may also serve as intracellular signaling platforms where internalized receptors may be concentrated and dimerized to signal without ligand^{10,11}. RAB14 has been reported to function as a positive regulator of AKT signaling to increase the proliferation of cancer cells^{12,13}. In contrast, a recent loss-of-function (LOF) screen found that RAB35 regulates AKT phosphorylation via ligand-independent signaling by mis-localized PDGFR in signaling endosomes of cancer cells, and this same screen implicated RAB14 as a negative regulator of AKT activation¹¹. Thus, RAB14 may be a positive or negative regulator, depending on cell type. In this proposal, we plan to investigate the cellular and molecular mechanisms of RAB14 as a novel erythropoietic signaling regulator, in order to propose new therapeutic strategies to enhance in vitro expansion of erythroid cells for clinical use. In Aim 1, we will confirm that increased erythropoiesis results from RAB14 knockout (KO) in HUDEP2 cells by CRISPR/Cas9 gene-editing technology. We will determine if RAB14KO-mediated increased erythropoiesis is due to increased cell proliferation and/or reduced cell death. In addition, we will determine whether RAB14 affects early (ie BFU-E and/or CFU-E progenitors) and/or late (ie morphologically maturing erythroid precursors and enucleating/enucleate cells) erythroid stages. In Aim 2, we will determine whether RAB14 regulates erythropoiesis by modulating erythroid cytokine receptor trafficking and signaling. Based on Prelim Results, we will first study EPOR/JAK/STAT signaling. As we reveal the upstream and downstream molecules and pathways involved in RAB14-mediated erythropoiesis, we may identify opportunities to intervene translationally to further enhance in vitro generation of mature/maturing erythroid cells for clinical transfusion products.

Gabsang Lee, Ph.D.

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Award Amount: \$345,000

Disease Target: Vasculopathy

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Award Amount: \$345,000

Disease Target: HLHS

Optically-Induced VEGF Activation in Human Endothelial Cells

VEGF is a major angiogenic factor that undergoes transcriptional and post-transcriptional induction by hypoxia and has an important role in important clinical conditions, such as, atherosclerosis, collateral cerebral circulation, cerebral edema, neuroprotection, neurogenesis, cerebral angiogenesis, postischemic brain repair, postischemic vascular repair, and stroke therapeutics. Most studies on VEGF have been conducted with recombinant proteins and/or viral delivery, providing ample information for decades, however, recombinant protein-based and virus-mediated approaches cause uneven and uncontrolled distribution, resulting in heterogeneous and unsynchronized signaling. To fully realize the therapeutic promise of VEGF in numerous ischemic conditions and improve clinical outcomes of the patients, it is imperative to develop an innovative approach to control VEGF signaling with great temporal and spatial precision to ensure a long-term protective effect. To enhance angiogenic potential of stem cells in vitro and in vivo, we propose to establish photoactivatable VEGF in hPSCs to see if optical VEGF activation is sufficient to achieve EC fate determination and if this can enhance their recruitment to ischemic limb. The following are our Specific Aims to establish an 'opto-VEGF' system in human motor neurons. First, we will generate opto-VEGF human embryonic stem cell (hESC) and induced pluripotent stem cell (hiPSC) lines via CRISPR/Cas9-based gene targeting system. Opto-VEGF hESC/hiPSC line will be differentiated into EC lineage with optimized illumination conditions. Second, via transplantation of hESC/hiPSC-derived ECs in ischemic mouse model, we will test the opto-VEGF system can enhance therapeutic outcomes including hPSC-derived EC (hPSC-EC) survival and vascularization in our established ischemic mice models. Our proposed experiments will address fundamental biological questions and have a wide-range of applications. Our results should lead to the development of a potential therapeutic cure for various ischemic conditions, by delivering a long-term opto-VEGF activation with precise temporal and spatial control. This project is expected to lead to substantial advancements in our understanding of neovascularization as well as provide unique tools for the in vivo protection of human ECs.

Mesenchymal Stem Cells Preserve Right Ventricular Function in a Neonatal Swine Model of Pressure Overload

One of the most severe forms of congenital heart disease (CHD) is hypoplastic left heart syndrome (HLHS). HLHS patients show a serious lack of left sided heart structure development, including mitral and aortic valve stenosis and hypoplasia of the left ventricle. The only viable option for the failing RV is heart transplantation; however survival after heart transplantation is poor after HLHS surgical staged palliation. Due to high morbidity and mortality in HLHS and pediatric heart failure patients, new treatment options need to be explored that will remodel/regenerate the RV myocardium. There is no animal model for HLHS. The best available approach is to replicate the physiologic and tissue changes seen in HLHS patients into a RV pressure overload model in the swine. The swine myocardium resembles the intrinsic cardiomyocyte characteristics of the human myocardium, related to the number of binucleated and mononucleated cardiomyocytes. The goal of this project is to improve the myocardial function in HLHS and CHD patients by using mesenchymal stem cells (MSCs) treatment. Manipulating stem cells ex vivo has been shown to be feasible and useful method for increasing stem cell functional abilities by increasing cellular numbers. Determining the MSCs mechanism for positive RV remodeling is critical for the eventual success of this therapy. We hypothesize that injected MSCs decrease miR-21 levels which results in secreting GDF15 enriched exosomes during pressure over load conditions. In addition, pressure overload triggers the MSCs to stimulate miR-132 levels which are released in the MSC derived exosomes. Then, GDF15-miR-132 enriched exosomes are up taken by the cardiomyocytes and this leads to positive cardiac remodeling response, critical for RV functional recovery. Successful completion of the project will provide a deeper understanding of the mechanism of how MSCs recover the myocardium, not only in preclinical animal models but in HLHS patients. Additionally, this project may have clinical implications for all diseases-both pediatric and adults-where stem cell may be used as a viable therapeutic strategy. Using our swine model system of RV dysfunction, we discovered the positive RV remodeling abilities of MSCs, which were critical for securing FDA-approval for our ELPIS trial. This important discovery demonstrated for the first time that MSCs have the unique abilities to adapt to the pressure overload myocardial stress. More importantly, MSCs positively remodeled the RV myocardium, a different biological and physiological myocardium than the left ventricle (LV) myocardium where all previous myocardial infarction studies have been performed. This discovery then led us to examine the different pathways that are triggered during the RV remodeling by the MSCs. In Aim1, we will first determine the importance of the GDF15-miR-21 pathway to control the upstream production of GDF15 enriched exosomes derived from injected MSCs during pressure overload conditions. In Aim2, we will determine the downstream miR-132-Smad7 signaling pathway, which is activated in cardiomyocytes by the enriched GDF15-miR-21 exosomes. We will also test the hypothesis that MSC derived exosomes are released into the serum of treated HLHS patients and correlate the levels to RV functional improvements.

Satoru Otsuru, M.D., Ph.D.

University of Maryland, Baltimore

Award Amount: \$336,410

Disease Target: Osteogenesis

Pankaj Pasricha, M.D.

Johns Hopkins University

Award Amount: \$344,817

Disease Target: Achalasia

Developing MSC-Derived Extracellular Vesicle Therapy for Osteogenesis Imperfecta

Mesenchymal stem/stromal cell (MSC) therapy has been clinically and preclinically investigated for various disease conditions. Their therapeutic mechanism has not been fully elucidated; however, accumulating data indicate that MSCs rarely make direct contribution to tissue regeneration or restoration but indirectly stimulate tissue repair by secreting trophic factors which activate residual recipient cells. Given that MSC therapy has several limitations such as 1) potential transformation into malignant cells, 2) limited cell doses that can be injected due to their large size, and 3) frequent failure of cryopreserved MSCs providing therapeutic effects, it is critical to develop cell-free substitutes for MSC therapy through identification of the trophic factors, which bypass these limitations. Osteogenesis imperfecta (OI) is a genetic bone disorder often characterized by fragile bones and short stature. Anti-anabolic drugs such as bisphosphonates have been effective for the prevention of fractures. However, no treatments are available to correct growth deficiency in OI. Even growth hormone has limited ability to improve this growth impairment in OI. It has been shown that systemic MSC infusion stimulates longitudinal bone growth in children with OI. We are investigating this mechanism of action using animal models and found that infusion of mouse MSCs (mMSCs) induced the production of a serum factor that enhances chondrocyte proliferation in the growth plate, resulting in bone growth. Consecutive studies revealed that extracellular vesicles (EVs) derived from mMSCs stimulated longitudinal bone growth equivalent to parental cell infusion. Based on these findings, we hypothesize that EVs mediate MSC-stimulated bone growth and that EV-based therapy can replace MSC therapy for OI. This hypothesis will be tested via the following aims: Aim1: To establish the effective regimen of hMSC-derived EV therapy for OI using a mouse model carrying the same mutation found in OI patients. To implement EV therapy in OI, we will determine optimal doses and frequency of hMSC-derived EV infusion that improve bone growth most efficiently. We will also test whether the EVs derived from hMSCs isolated from OI patients also possess therapeutic activity. Specifically, using EVs from healthy donor- or OI patient-derived hMSCs, we will compare three different doses of EVs as well as three injections per week with one injection per week on bone growth in OI mice. Aim 2: To determine the protocol that supports high-yield, clinical grade and scalable EV production for OI therapy. Large-scale manufacturing of EVs from hMSCs is a critical issue translating EV therapy to patients. Our preliminary study indicates that highly proliferative hMSCs release more EVs. Thus, we will define the culture conditions which enhance hMSC proliferation and increase EVs production. We've specifically, established a local collaboration with RoosterBio who have developed a xenogenic-free, clinical grade culture system for rapid cell expansion of hMSCs. We will compare our conventional culture media containing fetal bovine serum with the clinically relevant xenogenic-free high performance media from RoosterBio for EV production. We will examine the quantity and quality of EVs released into the culture media, and verify whether the isolated EVs maintain or increase the therapeutic activity compared to the control EVs obtained from our conventional culture system. Impact of the proposed studies: Completion of the proposed experiments will provide solid evidence for efficacy of hMSC-derived EVs for OI therapy, leading to the development of novel cell-free EV therapy. Moreover, the outcome from Aim1 will determine an optimized regimen of EV therapy for OI. Aim2 will provide the efficient MSC culture conditions to harvest EVs. Given that we'll be using clinical grade reagents, our findings can be rapidly translated into Good Manufacturing Practices (GMP) qualified processes and Phase 1 studies of EV therapy for OI.

Identification and Isolation of the Human Enteric Neural Stem Cell: Laying Foundation for Curing Achalasia

It is estimated that 25 million Americans have what have come to be known as motility or "functional" disorders of the gastrointestinal tract, such as achalasia, gastroparesis, irritable bowel syndrome, slow transit constipation, functional dyspepsia and gastro-esophageal reflux (GERD). Many of these disorders are brought about by a dysfunction of the enteric nervous system (ENS) which is an autonomous nervous system contained entirely within the wall of the gastrointestinal tract and consists of two concentric networks of neurons and glia, the submucosal and myenteric plexi that regulate secretion and motility. In our laboratory we have been studying neurogenesis in the ENS for several years and recently shown (Kulkarni et al PNAS 2017, attached to this proposal package) that despite evidence of a significant rate of steady state neuronal apoptosis, neuronal number is maintained at a constant level in health. These efforts have culminated in the identification of the murine enteric neural stem cell (ENSC) with demonstration of its ability to constantly generate new neurons in the adult gut. The next key scientific milestone that needs to be achieved for translating the potential of ENSC into both disease modelling as well as transplantation-mediated cures is the isolation of the adult human ENSC that can facilitate their isolation from gastrointestinal tissue. Our recent studies in mice provide convincing evidence that that ENSC are located within myenteric ganglia and express both Nestin and p75NTR, but not the pan-glial marker Sox10. Since then we have also screened two hundred surface marker candidates that provide us a very good roadmap to finding the optimal combination to isolate ENSC from the human gut using flow cytometry. With this in mind, and using our knowledge of the adult murine ENSC biology, we propose to perform identification and characterization of the surface marker profile of the adult human ENSC for their targeted isolation from resected human tissue as well as characterization of their proliferation and neurogenic potential both in vitro, as well as in vivo in immune-deficient mice models. This surface marker identification of human ENSC and their targeted isolation and characterization of neurogenic potential would not only be an innovative first in understanding the basic biology of these readily accessible population of neural stem cells, but also will have potentially very significant clinical implications by providing the first ever platform for using stem cell-mediated therapies that would aim to cure gut motility disorders such as achalasia.

Hilary Vernon, M.D., Ph.D.

Johns Hopkins University

Award Amount: \$330,491

Disease Target: Barth Syndrome

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University of Maryland, Baltimore

Award Amount: \$345,000

Disease Target: ALS/FTD

Development of an iPSC Derived Cellular Model of Barth Syndrome: Towards Novel Therapeutic Discovery

Barth Syndrome (BTHS) is an X-linked genetic condition caused by defects in tafazzin (TAZ), which encodes for a transacylase involved in the final remodeling step of cardiolipin (CL), a phospholipid localized to the inner mitochondrial membrane. Deficiency of TAZ results in abnormal CL content, including an accumulation of immature monolysocardiolipin (MLCL) and a reduction of mature CL, and significant mitochondrial dysfunction. Clinically, BTHS is characterized by early onset cardiac dysfunction, intermittent neutropenia, growth defects, and skeletal myopathy. The burden of morbidity and mortality is high, and disease-specific treatments are not available. We have taken a multi-faceted approach to the clinical care, clinical research, translational research, and development of novel therapeutics in BTHS. With the integration of each aspect of disease discovery, we aim to streamline bedside-to-bench and bench-to-bedside discovery. Our approach includes implementation of a multidisciplinary clinic for individuals with BTHS the Kennedy Krieger Institute in Baltimore, an IRB approved longitudinal and cross-sectional natural history studies, an IRB approved biobank at Johns Hopkins University for biological materials (plasma, urine, whole blood, cells) from affected individuals, and establishment of model cell lines (patient derived lymphoblastoid cell lines, and fibroblast lines, HEK-293 edited cell lines). We have also established collaborations with multi-disciplinary academic teams, family organizations including the Barth Syndrome Foundation, and industry partners. Through this work, we have identified several major roadblocks limiting novel therapeutic discovery in BTHS. Firstly, available BTHS models have significant limitations. For example, a germline mouse model of BTHS has never been achieved, and the current available model is a doxycycline inducible, short-hairpin TAZ knockdown mouse. This mouse model displays an altered CL profile, cardiac abnormalities and skeletal muscle disease. However, research groups have applied different doxycycline induction strategies, which limits comparison across publications. Furthermore, doxycycline alters mitochondrial function, and likely confounds mitochondrial pathology. Secondly, there are major knowledge gaps in the basic pathophysiology of BTHS that limit the availability of identification of secondary treatment targets. These gaps include: (a) it is not known whether the absence of CL, the accumulation of MLCL, or a combination of the two is detrimental to mitochondrial function (b) it is not known why some tissues are affected by CL abnormalities to a greater extent than others (i.e. heart vs. brain) (c) is not known which aspects of mitochondrial function are directly correlated to the degree of TAZ dysfunction. We applied the accumulated knowledge gained from our multi-disciplinary studies to select a cross-section of informative TAZ variants associated with distinct clinical and biochemical endophenotypes. We will build iPSC-based cellular models of these TAZ variants in order to solve key questions about the mitochondrial pathology of different tissue lineages in BTHS, and provide foundational information towards the development of novel clinical monitoring tools and novel therapeutic targets. Once established this will be an invaluable tool for the BTHS research community and for the wider community of scientists studying CL defects.

A Novel 3D Microphysiological Brain Model for Studying Neurodegenerative Disease ALS/FTD

We seek to build a miniature brain model using human stem cells to better mimic human conditions and develop treatments for a set of common neurodegenerative diseases. Nucleotide repeat elements, including microsatellites and short tandem repeats, are common in eukaryotic genomes. Recently, a hexanucleotide repeat expansion (HRE), (GGGGCC)_n, in a noncoding region of C9orf72 was linked to the neurodegenerative diseases amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD). ALS is characterized by a loss of motor neurons. Approximately 90% of ALS cases are sporadic, with the other ~10% having a family history; the C9orf72 HRE represents the most common genetic cause of both familial and sporadic ALS. FTD is characterized by degeneration of the frontal and temporal lobes of the brain and is the second most common type of dementia in people younger than 65. The C9orf72 HRE is one of the most common genetic causes of FTD. Increasing evidence suggests that ALS and FTD are two related diseases in a continuous clinical spectrum, and there is a possibility that the C9orf72 HRE also contributes to Alzheimer's disease. However, the pathogenic mechanism underlying the diseases associated with the C9orf72 HRE remains unknown, and our understanding of non-coding repeats in the context of human disease is still in its infancy. The best model to study C9orf72 HRE-initiated pathogenesis to date is that based on induced pluripotent stem cells (iPSC) derived from patients carrying the long repeat expansion. Using the stem cell model system, we have discovered a mechanism that provides a molecular cascade coupling C9orf72 HRE nucleic acid structures to cellular defects and human pathology. We are the first to show that the DNA of the C9orf72 HRE adopts non-conventional secondary structures such as G-quadruplexes and R-loops, which lie at the root of the C9orf72-linked ALS/FTD. In short, these studies define the molecular defects of the C9orf72-linked ALS/FTD at its root, and lay a foundation for further studies of diverging pathogenic cascades. However, the traditional 2D monolayer cell cultures fail to take into account the 3D natural environments of neurons and glia in the intact brain, thus providing a less optimal system to model human brain diseases and test potential therapeutics. Recently, our co-investigators have made significant advances in developing 3D brain models using human iPSCs. These microphysiological systems provide much improved and reliable models to study neuronal and glial functions during development and diseases. Combining our long-standing interests in neurodegenerative diseases with our recent success in applying iPSC models for studying ALS pathogenesis, we are at a unique position to develop a novel 3D mini-brain culture model system to study the ALS disease pathways and potential therapeutic interventions. In this project, we plan to focus on establishing a mini-brain model from the patient-derived iPSCs to uncover previously unknown aspects of the pathophysiology and to test potential therapeutic agents for ALS/FTD associated with the C9orf72 hexanucleotide repeat. The proposed work will provide a new technological platform for understanding the neurodegenerative diseases associated with nucleotide repeat expansions such as the C9orf72 HRE. Upon completion, we expect to build the first mini-brain model for studying ALS/FTD that could help unravel new features of the molecular basis of the disease and identify potential therapeutic strategies. This study will have the potential to open new avenues of research for neurodegenerative diseases and have a wide impact on medical research and technology development.



VALIDATION GRANT AWARDS



Warren Grayson, Ph.D.

Johns Hopkins University

Award Amount: \$230,000

Disease Target: Bone Defects

Tonya Webb, Ph.D.

University of Maryland, Baltimore

Award Amount: \$230,000

Disease Target: Cancer

**Oxygen-Delivering BiO₂-Bone Scaffolds
for Regenerating Vascularized Craniofacial Bone**

Each year there are 200,000 surgeries involving the use of bone grafts and implants for facial bone reconstruction of injuries caused by cancer, trauma, or congenital disorders. Of these, there remains a subset of geometrically complex defects for which there is no satisfactory solution. Treatment of these large facial bone defects has additional, unique challenges due to the complex, three-dimensional geometry of the bone. In order to stabilize patients with these deformities, surgeons have been forced to rely on expensive stopgap technologies that do not recoup quality of life or suitably resolve the defect, and patients are often burdened with long-term complications. To address this limitation, we propose the use of porous, biodegradable, patient-specific scaffolds, which provide immediate mechanical integrity for calvarial or facial defects and facilitate tissue regeneration. Clinical-grade polycaprolactone (PCL; DURECT) is a biodegradable, FDA-approved polymer which is used clinically and confers little risk to the patient. It will be combined with Bio-Oss® – clinical-grade bovine decellularized bone matrix – obtained via collaborations with Geistlich Pharma, and oxygen-delivering ‘microtanks’, which are hollow microspheres that can provide controlled delivery of oxygen to cells seeded into the scaffold upon transplantation into the defect. This mixture will be 3D-printed to create anatomically shaped BiO₂-Bone scaffolds customized to patient’s defects. We have demonstrated (i) capacity to 3D-print anatomically shaped scaffolds, (ii) desirable osteoconductivity and osteoinductivity of scaffolds and bone regeneration in murine models of critical-sized calvarial defects when used in conjunction with adipose-derived stem cells, and (iii) capacity of polyvinyl alcohol (PVA) microtanks to maintain prolonged cell survival in anoxic environments. These technologies are uniquely suited to facilitate tailored reconstruction of craniofacial bone defects, enable prolonged cell survival and tissue assembly following transplantation, and promote vascular and bone regeneration at the defect sites.

**Convertible Natural Killer T-Cells
for Immunotherapy**

Adoptive immunotherapy is one strategy used to enhance immune responses in cancer patients. Treatments utilizing engineered T cells have the potential to offer long-term protection, through memory, but are directed only towards cancer cells expressing the tumor antigen for which they were designed. This therapy is therefore limited by the possibility of tumor escape – a tumor can down regulate the antigen to which the immunotherapy is directed. An alternate cellular strategy in development utilizes natural killer (NK) cells. NK cells directly lyse cancer cells, and produce cytokines and chemokines that can activate other components of the immune system. Here, we propose to examine a third type of cellular therapy based on Natural killer T (NKT) cells. NKT cells can activate both NK cells and classical T cells. Their dual function is ideal for cancer treatment, because NKT cell-based therapy offers the possibility of inducing an initial cytotoxic tumor response, and also activating the adaptive immune system to produce tumor-directed cytotoxic T cells (CTL) with long-lived memory. We have developed a novel method to generate NKT cells from human adult stem cells. We hypothesize that these stem cell-derived “convertible” NKT cells will exert potent anti-tumor responses. Thus, the goal of this project is to validate our method of generating NKT cells from adult stem cells and further characterization of their effector functions. The information gained in the proposed studies will aid in the development of a novel immunotherapy, specifically the generation of universal NKT cells, that can be used for the treatment of cancer.

Sharon Gerecht, Ph.D.

Johns Hopkins University
2019 Validation Award (1st Round)
Award Amount: \$230,000
Disease Target: Arterial Bypass Surgery

Swine Study of Patient-Specific Small-Diameter Tissue Engineered Vascular Grafts

Autologous and synthetic polymer small-diameter grafts used for arterial bypass surgery are insufficient and often lead to morbidity and mortality. Despite the need for and extensive literature on small-diameter tissue engineered vascular grafts (sTEVGs), the main hurdles to clinical translation seem to be twofold: (1) The lack of natural material that can sustain aortic pressure, and ultimately completely degrade while being replaced by the native tissue; (2). Advanced solutions, such as de-cellularized tissue grafts or fully cellularized grafts, are relatively expensive and require several months of preparation, thus limiting translatability and appeal to investors. Development of a material and a process that would enable the creation of a natural polymer based, fully functional sTEVG that requires a short fabrication process would address this compelling clinical unmet need. With the support of an MSCRF Validation grant, we have recently generated perfusable sTEVGs and shown their ability to support aortic flow for 24 weeks in small animals.

Here we propose to advance our technology to large animal studies to validate the grafts toward treatment of coronary artery diseases and pediatric congenital cardiovascular defects. Our team includes a vascular bioengineer, biomaterial scientist, and a cardiac surgeon with clinical and research experience in vascular grafts. We request funds to achieve these specific milestones: (1) Scaling up sTEVG fabrication from small animals to a porcine model and (2) Implantations in a porcine model. We will examine both off-the-shelf acellular and patient-specific endothelialized sTEVG. Progress will be evaluated with the team at JHTV to ensure comprehensive and long-term development of the technology towards regulatory approvals. Given the success of our studies in small animals (24 weeks in vivo), we anticipate that with the support of the MSCRF, we will validate patient-specific endothelialized sTEVGs in a porcine model and be able to decisively move towards sTEVG commercialization.



POST-DOCTORAL FELLOWSHIP GRANT AWARDS



Adriana Blazeski, Ph.D.

Johns Hopkins University

Mentor: Leslie Tung, Ph.D.

Award Amount: \$130,000

Disease Target: Arrhythmogenic Cardiomyopathy (AC)

Muthukumar Gunasekaran, Ph.D.

University of Maryland, Baltimore

Mentor: Sunjay Kaushal, M.D., Ph.D.

Award Amount: \$130,000

Disease Target: Myocardial Infarction (MI)

Study of Arrhythmogenic Cardiomyopathy Using a Syncytial Model of hiPSCs

Heart disease remains the leading cause of death in the United States, accounting for more than 23% (or nearly 600,000) of deaths in 2011. The prevalence of cardiovascular disease, which affected more than 36% of adults above the age of 20 in the period from 2011 to 2014, is associated with tremendous costs to the healthcare system, to the tune of over \$300 billion dollars annually. Because of this, there is great incentive to advance our understanding of a variety of cardiac diseases, including sudden cardiac death (SCD), and to develop new therapeutic approaches. This proposal is aimed at studying the leading cause of SCD in young adults, arrhythmogenic cardiomyopathy (AC). Along with frequent arrhythmias that can lead to SCD, AC is characterized by distinct histopathological features: right ventricular enlargement, loss of myocytes accompanied by inflammatory infiltrates, and replacement of lost myocardium with fibrous and fatty tissue that progresses from the subepicardium to the endocardium, leading to wall thinning and aneurysmal dilatation in the “triangle of dysplasia” region of the heart. Disease progression has been described in 4 phases: 1) early concealed phase of the disease, prior to the presence of gross structural abnormalities, during which SCD can occur, 2) presence of arrhythmias, 3) early heart failure, and 4) end-stage heart failure. Genetic mutations have been identified in up to 60% of AC patients. A majority of these mutations affect the proteins of desmosomes, cell-cell junctional complexes at the intercalated disc that span the intracellular and extracellular space of CMs, linking them through intermediate filaments and N-cadherin and relaying tensile force during contraction. Human induced pluripotent stem cellX-derived cardiomyocytes (hiPSC-CMs) are a promising source for modeling the pathogenic mechanisms of AC, and several studies have demonstrated the ability of these cells to reflect a disease phenotype. Studies with hiPSC-CMs have been able recapitulate certain aspects of AC, including decreased expression of desmosomal structures and appearance of intercellular lipid droplets. However, in order to develop new diagnosis and treatment strategies, syncytial models are needed to understand how genetically-encoded mutations of the desmosomes that interconnect cells lead to aberrant electrical conduction and arrhythmias. Further, models of AC that can translate findings to patient diagnosis and treatment will require the ability to recapitulate the chronic nature of this disease and its progression over time. To this end, this proposal uses a novel approach, the engineered heart slice (EHS), to make a syncytial preparation of AC hiPSC-CMs. EHS incorporate decellularized myocardial matrix, organize hiPSC-CMs into tissue-like constructs, and can be maintained long-term in culture.

Donor Derived Exosomes as Non-Invasive Serum Biomarker for Immune Rejection Following Human Neonatal Cardiac Progenitor Cell Transplantation

Cardiac stem cell transplantation is a proven effective treatment for myocardial infarction (MI) as per various clinical trials using different kind of stem cells (1, 2). Recent studies have showed exosomes derived from human neonatal cardiac progenitor cells (c-kit+ nCPCs) improve MI recovery (3-5). Though role of exosomes in myocardial regeneration has recently been highlighted, immune response to transplanted nCPCs exosomes in MI rats remains unknown as the experiments so far were conducted in immune-compromised rats or mice (6). In this application, we propose that exosomes will provide significant new information not only regarding the mechanisms for immune rejection of transplanted nCPCs, but also may serve as a potential biomarker. We have recently obtained two Investigational New Drugs (INDs) from the FDA to use c-kit+ CPCs in Hypoplastic Left Heart Syndrome patients. Studies have demonstrated that infiltration of inflammatory cells in to the infarcted myocardium induces tissue damage and expansion of infarction (7). In this study we will measure antibodies (Abs) developed against mismatched donor HLA and cardiac associated antigens (SAGs: Myosin, Myo; Troponin, TnT) in human nCPCs transplanted rat MI model. We will determine whether the immune response to transplanted nCPCs reduces its cardiac regeneration potential. Additionally, studies showed that exosomes from donor origin induce allogeneic T cell response following solid organ transplantation (8-10). My recent study also demonstrated that sera derived exosomes from lung transplant recipients with acute and chronic rejection showed presence of mismatched donor HLA, lung associated self-antigens (Collagen-V and K-alpha 1 Tubulin) and immunoregulatory miRNA, but not in stable LTxR (11). Therefore, in this proposed study, we will investigate the kinetics of exosomes released at different time points to determine differentially expressed protein, immune-regulatory mRNA and miRNA changes during the recovery of MI in rat model. Our study will also determine exosomes isolated from nCPCs invitro are immunogenic in rat MI model. Preliminary finding from our group shows mesenchymal stem cells transplantation with immune suppressive regimen cyclosporine A increases cardiomyocyte regeneration in rat and pig MI model. Therefore, we will demonstrate cyclosporine mediated immune suppression will enhance the cardiac regeneration of transplanted nCPCs. Serial sera sample analysis will be performed to determine donor derived exosomal protein, mRNA and miRNA, which can be used as non-invasive biomarkers prior to diagnosis of immune rejection. Our proposed study is novel and innovative due to the identification of novel mechanism mediated by donor exosomes and its intrinsic active biomolecules induces immune rejection of transplanted nCPC in MI model. Further, we demonstrate the biomarker potential of donor-derived exosomes by developing noninvasive biomarker platform for monitoring immune rejection of nCPCs. Strategies to minimize immune response against transplanted nCPCs will enhance the therapeutic applications of nCPCs to recover myocardial infarction.

Minseong Kim, Ph.D.

Johns Hopkins University
Mentor: Gabsang Lee, Ph.D.
Award Amount: \$130,000
Disease Target: Parkinsons Disease

Mehreen Kouser, Ph.D.

Johns Hopkins University
Mentor: Jeff W.M. Bulte, M.S., Ph.D.
Award Amount: \$130,000
Disease Target: ALS

Modeling of Parkinsons Disease Using PD-Patients iPSCs-Derived Dopaminergic Neurons with Optical Controllable Alpha-Synuclein

Parkinson's disease (PD) is one of the most common and an incurable neurodegenerative disorder with a broad range of motor and non-motor symptoms, results from a specific dopaminergic neuronal cell death in the substantia nigra pars compacta (SNpc). Since PD places a significant socioeconomic burden on our healthcare system and devastates the individual patient's life, establishment of a novel model system to study the pathogenesis of PD will be important. Misfolded α -synuclein becomes insoluble and aggregates to form intracellular inclusion bodies called Lewy bodies, and the Lewy body formation with neuronal loss is a most general pathology of PD. However, because the process of α -syn aggregation and Lewy body formation takes a significantly long time, it is extremely challenging to induce such α -syn aggregation in human neurons in vivo or in vitro. To address this issue we have developed a new synthetic biological technique to optically control the aggregation of α -syn with great efficiency in an exceptionally short window of time (within hours, based on our preliminary data) by using interactions of photo-activatable proteins. Using this model system, we will characterize the PD-related cellular and molecular phenotypes, from α -syn aggregation to neuronal cell death. In addition, we propose to carry out a screening to identify the chemical compound(s) that inhibit the aggregation of α -syn or/and neuronal cell death. Our proposed studies will be beneficial to model PD with human neurons, and build a new screening platform to develop a therapeutic intervention. We expect our opto- α -syn to exhibit qualitatively similar properties to those found in Lewy bodies in PD patients; we will look for an opportunity to access human samples to use as controls and compare our results to previously published systematic genetic⁴⁷⁻⁵⁰ and proteomic analysis. We have included the SNCA triplication PD-hiPSC line for its higher probability to express α -syn, but, as an alternative, we will consider using another PD-hiPSC line carrying the A53T mutation, which shows more aggressive phenotypes in mouse studies⁶⁰⁻⁶². As we are aware of clonal variation among hiPSC clones⁶³⁻⁶⁵, we will use multiple clones for proposed experiments. To prevent any off-target effect, we will use second generation of the Cas9 nuclease (CPF1) showed enhanced levels of specificity or fidelity^{66,67}. Because there may be unexpected side effects in other cell types due to the delayed dissociation time between the CRY2PHR proteins in the PD-specific hiPSCs. If we see such issues, we will use other light-sensing actuator modules that have a low dissociation constant, such as the LOV module.²⁶⁻²⁸

Manganese-Enhanced MRI for Interrogating Astrocyte Replacement in A Mouse Model of ALS

Amyotrophic Lateral Sclerosis (ALS) is a devastating disease with near 100% mortality. An important role has been assigned to (over)activated astrocytes. Given the few available treatment options, stem cell therapy is now actively being pursued as a new treatment paradigm, aimed at either immunomodulation (using mesenchymal stem cells - MSCs), astrocyte replacement (using glial progenitors), or motor neuron restoration, with several clinical trials currently in progress. Our goal is to better understand the mechanisms underlying astrocyte activation that creates a toxic environment for motor neurons causing their death. We aim to replace the host glial-restricted progenitors (GRPs) to preserve the neuronal environment using intraspinal transplantation. In order to improve survival of the transplanted cells, we will also co-transplant MSCs. We aim to develop novel imaging biomarkers that can report not only on the fate of transplanted cells but also on changes in the ALS host environment. To this end, we aim to interrogate 1) the in vivo survival of co-transplanted GRPs/MSCs using conventional bioluminescent imaging (BLI) and the disease outcome using time of onset, animal survival, and behavioral measurements; 2) the activation state of host cells in vivo using manganese-enhanced magnetic resonance imaging MRI (MEMRI). Five transgenic SOD1 and five age-matched wild type cohorts of animals will be enrolled in these studies. Luciferase-transfected and hGRPs and MSCs will be co-transplanted at day 60, i.e., about 30 days before onset of the disease. Animals will undergo immunosuppression and are monitored weekly for weight, behavioral scores, and end survival. Randomized groups will undergo BLI and MEMRI at 1 day, and 1, 2, and 3 months after transplantation up until approximately 150 days. Immunohistological analysis of neuroinflammation, motor neuron degeneration, astrocyte pathology, and grafted cell survival will be used to validate the imaging findings and to compare the potential therapeutic benefit of hGRP and hMSC co-transplantation. In summary, we propose an innovative approach to developing an efficient cell therapy in ALS, and elucidate the fate of transplanted cells using different imaging modalities. Upon completion of this study, we anticipate: (i) establishing a new approach for cell therapy in ALS; (ii) generating crucial insight into the long-term efficacy and safety of this therapy; and (iii) better understanding the role of Mn²⁺ in the activation of host astrocytes which may serve as an early imaging biomarker for ALS. In the future, this new approach may be applicable to the treatment of other motor neuron diseases as well.

Su Chan Lee, Ph.D.

Johns Hopkins University
Mentor: Gabsang Lee, Ph.D.
Award Amount: \$130,000
Disease Target: Cancer

Seungman Park, Ph.D.

Johns Hopkins University
Mentor: Yun Chen, Ph.D.
Award Amount: \$130,000
Disease Target: Heart Failure

Generation of Cancer Progression Models through New Optogenetic Tool to Control p53 in iPSCs

This proposal of a two-year post-doctoral fellowship is to study on establishing a new cancer model to optically induce p53 aggregation in human induced pluripotent cells (iPSCs). p53 is a tumor suppressor protein and transcriptional regulator, regulating cell cycle arrest and apoptosis under stressful conditions such as hypoxia and starvation. Mutant p53 proteins play an important role in cancer research because they are commonly detected in various types of cancer. Previous researchers reported that p53 was aggregated in various cancers, causing acceleration of abnormal cell proliferation in cancer cells. One of limitations of p53 research is the lack of a proper human cellular model with spatial and temporal control over p53 aggregation during cancer progression. Therefore, we are in hope to develop a human cell model to optically aggregate p53 with our novel optogenetic technique in order to understand p53 aggregation in multiple cancer progression. Our specific aims are to induce p53 aggregation by light and to characterize phenotypes in neural stem cells (for brain tumor) and enteric organoids (for colon cancer) derived from human iPSCs. Our proposed studies will elucidate the role of aggregated p53 during tumorigenesis and present a new drug screening platform. Although advanced cancer diagnosis methods and anti-cancer drugs have been developed and applied in the medical field, resulting in increased survival rates, patients who have aggressive or metastatic cancer still suffer death worldwide. Chemotherapy and targeted therapy can only extend their lives from a few months to a few years. Even worse, long-term treatment with anti-cancer drugs could cause to development of anti-cancer drug resistance. Many scientists have studied functions and mechanisms of mutant p53. Their previous research revealed functions of mutant p53 in fully differentiated cancer cells. Various mutant p53 constructs transfected into different cancer cell lines, resulting in the discovery of different functions such as increased invasion, migration, proliferation, propagation of cell cycle, anti-cancer compounds resistance, and avoidance of cell death in previous cancer studies. However, these previous results are inappropriate for determining the function and the mechanism of mutant p53 during the cancer progression. Moreover, there are no drugs targeting or abrogating mutant p53 in clinical usage although a lot of mutant p53 targeted drugs have been synthesized and developed. For these reasons, cancer progression studies using iPSCs are essential for understanding how to initiate and progress cancers from normal cells. Based on the results of this project, we will understand the cancer progression mechanism of mutant p53 by controlling opto-mutant p53 protein spatially and temporally. With the confirmed results from aim1, and 2, we could apply our opto-p53 system to various xenograft and knock-in in vitro and in vivo models. It will be to reinforce our understanding of cancer progression studies. Moreover, our opto-p53 screening platform in induced pluripotent stem cells will be used to identify small molecules that inhibit or reverse the p53 aggregation.

Functional Property Evaluation of iPSC-Derived Cardiac Tissues for Optimized Heart Disease Treatment

Heart failures (HFs) is the leading cause of death in the United States. Currently, the most effective way to treat HF is organ transplantation. However, this method has limitations due to lack of organ donors, immune responses to the transplanted organ, and high costs. Recently, tissue engineering approaches are being widely explored to address the limitations. Among those, cardiac patch is a promising replacement for the damaged tissues. Stem cell-based cardiac patches and acellular patches consisting of ECM proteins were shown to effectively resuscitate injured myocardium in animal models [1, 2]. In addition, 3D bioprinting, where cells and ECM proteins are organized in specific geometry and dimensions to yield functional cardiac patch warrants a potential solution to treat HFs [3]. Nonetheless, two major obstacles described in the following remain to be surmounted before engineered tissues can be used as a general treatment option. First, direct mapping of mechanical force and stress over 3D cardiac tissues is yet to be developed, as opposed to its 2D counterparts, despite that 3D cardiac tissues are more physiologically representative and can be readily applied clinically. Second, for vascularization, though methods employing bioprinting [4], microfluidics [5], and vasculogenesis [6] have been proposed, physiologically mimetic vascularization of the engineered cardiac tissues are yet to be established in a reproducible, systematic and standardized manner. I envision to overcome these two major challenges by an integrated solution. First, I will develop a force/stress measurement technique using particle dynamics and finite element method (FEM) to map 3D forces generated by the human induced pluripotent stem cells (hiPSCs) - derived cardiac tissue in precision. Second, I will introduce biomimetic engineered vasculature by modifying established bioprinting protocols and validate that the vascularized tissue is mechanically optimized for clinical use by the quantitative assessment method developed in the first part of the proposal.

Wei Zhu, Ph.D.

Johns Hopkins University

Mentor: Jeff W.M. Bulte, M.S., Ph.D.

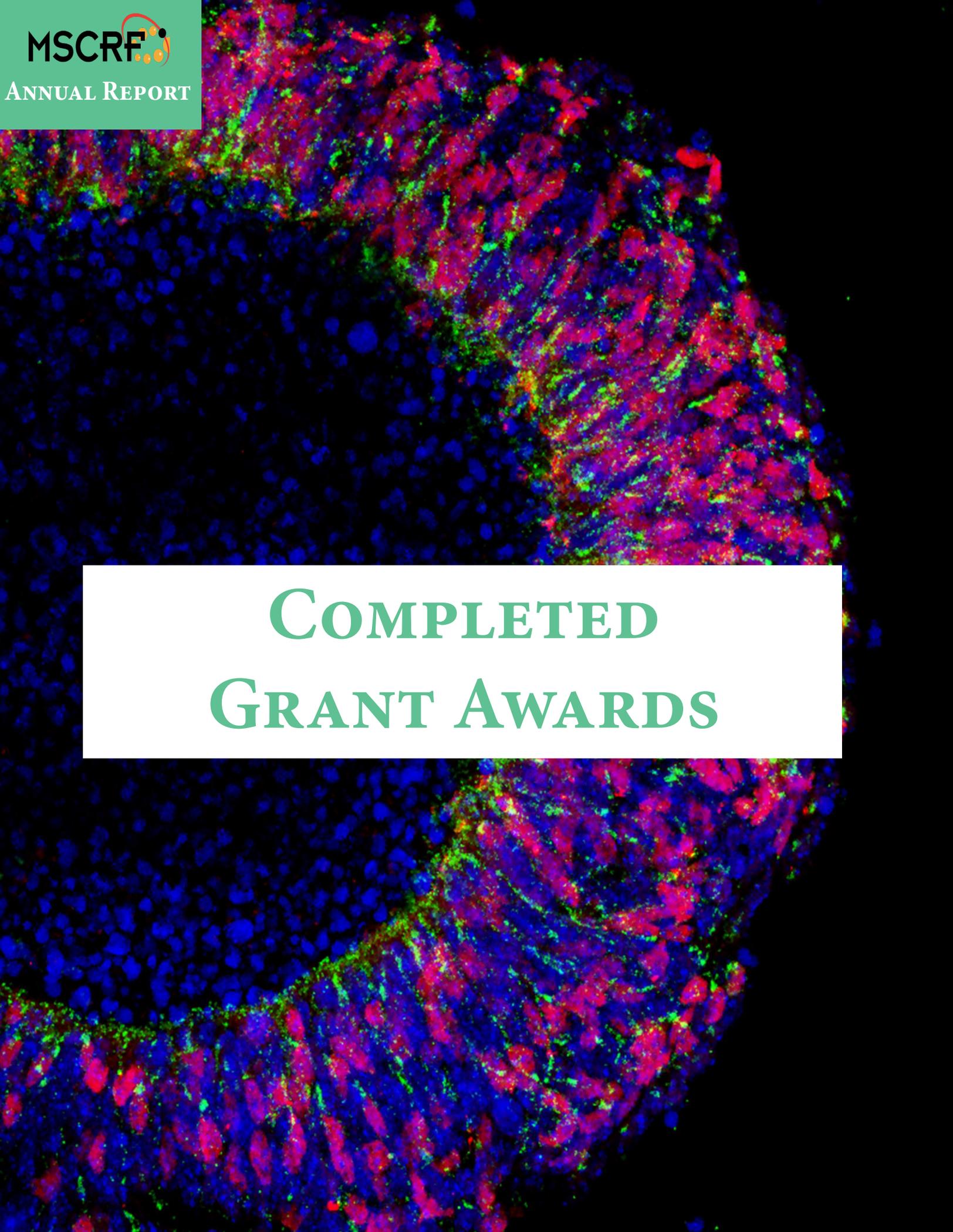
Award Amount: \$130,000

Disease Target: Traumatic Brain Injury (TBI)

3-D Vascularized Hydrogel Scaffold to Carry Stem Cells for Traumatic Brain Injury Repair

Traumatic brain injury (TBI) and the challenge to develop effective treatment. TBI is a primary medical problem that results in morbidity and mortality. It contributes to around 30% of all deaths from injury [4]. The major causes of TBI are motor vehicle accidents, falls, and struck by/against events, including collision, contact sports, and assaults [5]. In the USA, the estimated incidence is 200 per 100,000 people [6], and the annual burden has been over 60 billion dollars, based upon year 2000 dollars [7]. Surviving the TBI may still result in suffering from persistent physical, behavioral/emotional, and psychosocial changes, which are mainly attributable to the brain tissue damage and neuronal cell death [7]. Current treatments focus only on preserving the healthy tissue that remains after injury, as opposed to seeking to regenerate the damaged tissue and regrow the lost cells [8]. Transplantation of neural stem cells (NSCs) has demonstrated notable advantages in promoting regeneration and functional recovery after TBI [9-12]. However, directly injecting stem cells into the TBI cavity leads to substantial cell loss, whereas carrying and retaining the transplanted cells with scaffolds is usually associated with low cell viability due to the hypoxia and nutrient-deficient microenvironment inside the scaffolds [13, 14]. Development of a novel vascularized scaffold. In this project, we aim to develop a vascularized neural scaffold that can create a cell-favorable environment for human NSCs to reside and repair TBI. To this end, a gelatin-based hydrogel will be prepared, composed of a thiol-modified gelatin (Gelin-S) and a methacrylamide-modified gelatin (GelMA). Gelin-S and GelMA can become gelled in the absence of any initiators, which avoids the introduction of agents toxic to cells and allows for in situ injection of a hydrogel for minimally invasive surgery. To address the problem of low cell viability, we will engineer a vascular-like structure in the neural scaffold to maintain the metabolic functions of encapsulated hNSCs for high cell survival.

Specifically, the proposed work will concentrate on the following two aims: Aim 1. Assess the vascularization and neurogenesis in the gelatin-based hydrogel in vitro. The injectable hydrogel will be developed by cross-linking Gelin-S and GelMA. Growth factor carriers will be fabricated via an emulsion method. The ratio of the two hydrogel components (Gelin-S and GelMA), growth factor concentration, and cell density will be screened to achieve the formulation that has the optimal cell viability, vascularization, and neurogenesis in vitro. Aim 2. Evaluate the brain regeneration capacity of the neural scaffold in vivo. A mouse model of TBI will be used to assess the brain regeneration capacity of our neural scaffold using the optimal formulation obtained in Aim 1. The hydrogel-carrying hNSCs, vascular cells, and growth factor will be transplanted into the TBI cavity of immune-deficient mice. Brain regeneration and functional recovery will be evaluated. Innovation: Delivering hNSCs with scaffolds for TBI repair is limited by poor cell viability because of the hypoxia and nutrition-deficient environment at the defective site. We will engineer vascular-like structures in the gelatin-based hydrogel scaffold to maintain the metabolic functions of encapsulated hNSCs. To induce vascularization, microspheres loaded with angiogenic growth factor will be incorporated into the hydrogel scaffold. The microspheres can maintain a more sustained release of growth factor when compared to growth factor supplement alone. Our new vascularized neural scaffold would be applicable for the treatments of TBI and this methodology will also benefit the whole field of neurological disorder therapeutics.



**COMPLETED
GRANT AWARDS**

Ha Nam Nguyen, Ph.D.

3Dynamics, Inc.

2017 Commercialization Award

Award Amount: \$300,000

Disease Target: Alzheimer's Disease

Linhong Li, Ph.D.

MaxCyte, Inc.

2017 Commercialization Award

Award Amount: \$300,000

Disease Target: Sickle Cell Disease

Engineering Human Pluripotent Stem Cell-Derived Brain Organoids for Drug Screening & Toxicity Testing

The quest to find novel therapeutics for neurological disorders has been hindered by the lack of access to live human brain samples and relevant experimental models. Currently, roughly 0.1% of drugs that show promise in preclinical testing make it to Phase 1 clinical trials, and 90% of those drugs go on to fail FDA approval. One of the reasons for such a high failure rate is that standard animal and two-dimensional cell culture models cannot fully recapitulate genuine disease phenotypes and are not good enough predictors of how drugs will work in humans. Progress in three-dimensional (3D) tissue engineering offers a promising new platform that may be the key in accelerating and improving current drug developmental trend. These 3D tissues or organoids are differentiated from human pluripotent stem cells (hPSCs) to resemble specific parts of the human brain, which include architecture composition and physiology. Thus, the aims of this project were 1) to scale-up 3D brain region-specific organoids for drug screening and toxicity testing and 2) to commercialize 3D brain organoids and 3D cell culture bioreactors. We launched 3Dynamics Inc. in September of 2017 and, currently, the company has leased lab and office spaces in Fastforward Bioincubator, Baltimore City. We have successfully developed a method to scale up midbrain organoids (10,000+). We have tested and shown that brain organoids can model Parkinson's disease and can serve as ideal platform for compound and toxicity testing. Furthermore, we have obtained clients and partnerships in Pharma, National Institute of Health, and academia. Human brain organoids derived from hPSCs can model human diseases (e.g., Alzheimer's disease) that are not amenable in other systems, such as animals, monolayer cell culture, and other cell-free systems. For this reason, brain organoids can be used to further understand disease mechanisms and develop specific assays for targeted drug screening and toxicity testing. A more accurate human brain organoid disease model and drug screening platform can lead to discoveries of novel medicines for intractable neurological diseases and can reduce human suffering and potentially save lives.

Translational Development of Gene-Corrected Hematopoietic Stem Cells as Treatment for Sickle Cell Disease (SCD)

MaxCyte is a biotech company based in Gaithersburg, Maryland with successful track record in translational development of cellular therapeutics. MaxCyte's cell loading platform technology, enables 40+ engineered cell therapy products for therapy of multiple unmet needs in oncology, genetic diseases, cardio-pulmonary diseases, and regenerative medicine applications, of which 15+ are in human clinical trials, and 1 is in commercial market in Japan. Sickle cell disease (SCD) is a genetic disease of c.20A>T mutation in β -globin gene. There is no cure for most patients. It is our goal to find cure for this disease by using gene correction approach. In this one year grant, we proposed to develop a process to correct the A>T mutation in the first exon of the β -globin gene in plerixafor-mobilized peripheral hematopoietic stem cells (HSC) from SCD patient. Specifically, we used an EBV-transformed B cell line derived from the patient with SCD to screen the CRISPR guide RNA (gRNA) for the efficient correction of the mutation assayed by targeted deep sequencing. Considering the limited availability of SCD HSC, the successful correction procedure was first translated to converse health HSC into SCD mutation, by using a newly designed gRNA with one nucleotide change targeting to the same site in health HSC as that of the mutation in SCD. This new gRNA could be used to make a A to T converse, just an opposite case of fixing the mutation by making T to A correction. The developed correction procedure could converse the health HSC into SCD HSC at 20-40% rate assayed by targeted deep sequencing (N=3). Then, the correction procedure was translated to correct SCD HSC in vitro and validated by the recovery of the adult wild type β hemoglobin expression after the in vitro 17d differentiation of HSC into erythroid. It was found that the correction procedure is consistency in gene correction (N=3), could reach as high as 30-40% in the assumed biallelic correction, and does not decrease with the 17d differentiation. Considering the corrected cells could have both single allelic and biallelic correction for individual cell, the cellular correction efficiency could be about 1.5 fold higher, reaching to 50-60%. Furthermore, as assayed by hemoglobin gel electrophoresis and HPLC, the efficient correction results in wild type adult β -hemoglobin expression to a level as high as 92% among all hemoglobin, when the fetal hemoglobin induced by in vitro differentiation procedure is ignored. After the gene correction could reach to the therapeutic level in vitro, the IND-enabling study of animal engraftment and scale up becomes a task in reality. The efficient SCD gene correction and the high wild type adult β -hemoglobin expression after gene correction have motivated MaxCyte and NHLBI, NIH to enter into a Collaborative Research and Development Agreement (CRADA) to further translate this finding into clinical. We are currently ready to perform IND-enabling study of animal engraftment and scale up. Based on clinical data from allogeneic transplantation outcomes, 20% level of engraftment of single allelic correction in human subjects has been established as being clinically 'curative' for SCD patients. Our long-term engraftment outcomes in animals with the similar gene correction approach in CGD could reach 70% of gene correction rate after long-term engraftment relative to that without going through animal engraftment, the current 50-60% cellular correction efficiency of SCD in vitro and the high percentage of 90% of wild type adult β -hemoglobin expression after differentiation from the corrected SCD HSC to erythroid give us the hope to be optimistic that the gene correction procedure developed by the support of this grant may reach to the curative window for curing SCD patients. These results have been presented and published in peer-reviewed scientific community. We will continue to translate this finding to clinical with our collaborator Drs. John Tisdale in NHLBI and Harry Malech in NIAID, NIH under a CRADA between MaxCyte and NHLBI, NIH established through the work supported by this grant.

William Rust, Ph.D.

Seraxis, Inc.
2017 Commercialization Award
Award Amount: \$298,960
Disease Target: Diabetes

Chengkang Zhang, Ph.D.

Propagenix, Inc.
2017 Commercialization Award
Award Amount: \$300,000
Disease Target: Cystic Fibrosis (CF)

Long-term Function of Stem Cell Grafts for Insulin-Dependent Diabetes

Insulin-dependent diabetes can be cured in rodent models by the implant of human insulin-producing cells encapsulated within devices that protect the cells from the host immune system. These promising pre-clinical results have stimulated the launch of SERAXIS, INC, a cell-replacement therapy company targeting type 1 diabetes. To transform the preclinical concept to a clinically validated and practical therapy, the cell/device combination must be demonstrated to be safe, stable and implantable without encumbering the patient. The goal of this project is to evaluate the impact of a macro-device surgically implanted to the omentum of rats on the long-term stability of that organ. The purpose is to show that engraftment to the omentum enables the device to associate with the host vasculature without initiating scar formation, tumorigenesis, or other safety risks to the patient. The long-term implant of the Seraxis device to the omentum of diabetic rodents did not stimulate genetic or cellular alteration to the omentum that is known to initiate or contribute to cancer. The implant did cause structural alteration to the omentum Seraxis, Inc. www.seraxis.com that was evident by the proximity of blood vessels to the implant and the deposition of collagen. These alterations were not suggestive of safety risk to the animals. Further studies evaluating the safety of the implants on the remaining organ systems are ongoing. In total, these studies encompass a major step towards the regulatory acceptance of the proposed therapy for testing in human patients. These results comprise completion of originally stated aims of the project.

Building Commercial Path for EpiX™ Technology - A Breakthrough in Expanding and Utilizing Tissue-Resident Stem Cells

With the support of MSCRF commercialization grant, we successfully built the commercial path for the EpiX™ technology – a breakthrough in culturing tissue-resident epithelial stem and progenitor cells from diverse tissues for studying epithelial cell biology in vitro and unleashing their potential for regenerative cell therapy. In this project, our work aimed at 1) illustrating the unparalleled competitive advantage of EpiX technology over conventional methods using human airway epithelial cells as an example; 2) demonstrating the utility of EpiX technology for personalized medicine applications by expanding nasal epithelial cells out of minimal-invasive nasal brushing samples obtained from cystic fibrosis patients; and 3) scaling up EpiX medium production from bench scale to lot size of 100 liters, to provide support for moving forward with full commercialization. In AIM 1, we verified that the EpiX medium supported the expansion of primary airway epithelial cells from two different donors for over 50 population doublings, while retaining consistent functionality as assessed by various molecular and cellular characterization assays by the end of 1Q 2018. This is a significant improvement over current practice, and allows researchers to generate billion-fold more airway epithelial cells for their research study than using the convention methods. In AIM 2, we tested a personalized medicine approach for cystic fibrosis patients by using patient-derived nasal epithelial cells for functional in vitro drug response evaluation assay. We successfully generated 50–100 millions of nasal epithelial cells using brushing samples from 7 adolescent CF patients (4 males and 3 females, 7/7, 100% success), and tested their responses to Ivacaftor and Lumacaftor using the Ussing chamber assay, a “gold standard” drug efficacy evaluation assay for CFTR-directed therapeutics. The results revealed distinct responses towards the same drug despite that these patients share the same CFTR mutation. In AIM 3, we have successfully established the production SOPs and QC specifications for the EpiX medium, including analytical and functional quality tests and specifications such as standard pH, sterility, osmolality, endotoxin level and support of cell growth and differentiation. So far, we have manufactured 4 lots of EpiX medium at a scale of 100 liters and delivered high-quality, reproducible products for many researchers working with airway epithelial cells, including international customers

Christopher Chiang, Ph.D.

TheraCord, LLC.

2016 Investigator Initiated Award

Award Amount: \$466,325

Disease Target: Leukemias, Lymphomas, Anemias, Cerebral Palsy

Miguel Flores-Bellver, Ph.D.

Johns Hopkins University

2016 Post-Doctoral Fellowship Award

Award Amount: \$110,000

Disease Target: Age-related Macular Degeneration

TheraCord Cord Blood Collection Device

TheraCord is developing a technology to boost the stem cell yields of umbilical cord blood collection in order to increase the utility of this stem cell source in Hematopoietic transplants for adults and to lower costs of establishing and maintaining a national registry of cord blood units for transplant. The goals of TheraCord's MSCRF project included the following: Assessment of Maternal Cell Contamination detected in cord blood collected by placental perfusion, a point of regulatory concern for this technology. Results: Utilizing Fluorescent In-situ hybridization, Sensitivities up to 0.001% were established. Perfusion by TheraCord's protocol was not found to have resulted in maternal cell contamination of cord blood. Evaluation and optimization of cell separation technique to ensure the collection technology works with existing processing equipment and methods. Results: Cell recovery rates for both nucleated cells and CD34+ cells were not impacted by dilution in CPD anticoagulant solution as a part of placental perfusion method of collection while utilizing Prepacyte-CB method of processing. Also accomplished during the period, TheraCord connected with approximately 50% of domestic public cord blood banks, providing key data to guide research and commercialization. TheraCord is working with TEDCO mdPACE program, with a pre-submission anticipated in September, 2018. TheraCord has also translated a component of its perfusion technology into a stand-alone accessory product for the conventional cord blood collection bag, allowing for an immediate cost reduction in the cord place industry and providing funding for further development. Product is anticipated to launch Q1 2019. This project de-risks and further develops the TheraCord technology. TheraCord anticipates to increase the typical cell yield of cord blood collection by approximately 40-50%, resulting in 3 benefits: First, the number of collections that reach the minimum Total Nucleated Cell (TNC) to be transplanted into an adult (70kg, 17.5x10⁸ TNC) are anticipated to increase from approximately 4% to 20%. This is important, as approximately 90% of disease occurrence treated with HSPC Transplant occurs in adults. Second, higher cell dose is correlated to better outcomes and may compensate for the negative effects of imperfect HLA Matching. Doubling cell dosage for 5/6 HLA Matched transplants results in roughly a doubled 3-year rate of disease-free survival. Doubling of dose in transplants with 2+ HLA mismatches (36% of adult transplants) results in approximately 30-40% reduction in 3-year transplant-related mortality and 20% reduction in engraftment time. Lastly, raising cord blood cell yields also has a profound economic impact on public cord blood banking. Public cord blood banks struggle with unsustainable costs associated with banking unsellable (due to low cell yield) units in the inventory. In 2012, units >17.5x10⁸ TNC, made up more than 50% of CBU sold, yet made up less than 8% of bank inventory. To reduce the cost of banking low TNC units, threshold for banking has continuously risen – from 6x10⁸ TNC in 1996 to 12.5x10⁸ TNC in 2014 with 60%-80% of all collections presently being discarded. A 50% boost to yield is anticipated to allow a cord blood bank to either collect 5-fold more units >17.5x10⁸ TNC, or to reduce collect the same number of >17.5x10⁸ units with a 60% decrease in non-overhead costs.

3D Neural Retinal/RPE Complex from Human iPS cells: a Novel Age-related Macular Degeneration Systems

Age-related Macular Degeneration (AMD) ranks third as a cause of blindness (after cataract and glaucoma) in the world, and is the most common cause of blindness in the United States. Unfortunately, AMD remains incurable. A critical limiting factor preventing effective treatments for the initial stages of AMD is our poor understanding of its early pathophysiologic events, and the lack of appropriate models for its study. An in vitro "eyecup" derived from stem cells composed of all of the cellular elements of the neural retina and retinal pigment epithelium (RPE) could help to rapidly advance our understanding of the etiology of AMD. Thus, our goal was to develop the first human mini-retina model consisting of a 3D neural retina and associated RPE (3DNR/RPE complex) derived from human iPS cells and capable of recreating the physical and functional interactions occurring between photoreceptors and RPE during the early stages of AMD. The main aims of the project were: to determine the extent to which the maturation level of the 3DNR and/or RPE influences the establishment of native-like physical interactions between photoreceptors and RPE (SA1); and to determine the extent to which our 3DNR/RPE complex recapitulates early events of AMD (SA2). Milestone 1a and 1b: Determine the optimum maturation-stage required for the establishment of native-like photoreceptor/RPE physical interactions, and confirm photoreceptor/RPE functional association. Our originally proposed strategy was based on generating a 3DNR/RPE complex by means of a specially designed hydrogel microwell-based culture system that allows "coating" cellular aggregates (in this case the 3DNR) with a second cell type (RPE). However, our results demonstrated that even though RPE cells were capable of attaching to the surface of the 3DNR, they did not form a monolayer but rather developed clumps independently of the maturation stage. In order to overcome this issue, we pursued the proposed alternative approach using a transwell-based culture system. Briefly, we established and characterized a novel methodology for deriving RPE monolayer cultures similarly to the approach generally followed for primary RPE cultures. These RPE monolayers show a similar behavior to that observed in human primary RPE cultures and follow a reproducible timeline of maturation. Building upon this success, we then co-cultured 3DNRs and RPE monolayers at different stages of maturation and evaluated cellular composition and laminar organization of the neural retina, morphological and molecular differentiation of photoreceptors, morphological and molecular maturation of RPE. In all cases, 3DNRs successfully attached to the RPE monolayer after 1 week, achieving close physical association between photoreceptors and the underlying RPE cells. We are currently evaluating the photoreceptor/RPE functional association by using a "chimeric" 3DNR/RPE combining 3DNRs expressing a GFP reporter with RPE cells that will allow us to understand better the interaction between both retinal tissues. In order to establish an "in vitro" system able to mimic early events of AMD, we proposed to generate a transgenic 3DNR/RPE complex containing Nrf2 knockout RPE (hiPSC-RPENrf2^{-/-}) since Nrf2-deficiency in the RPE leads to an AMD-like phenotype. Milestone 2a: Generation and validation of hiPSC-Nrf2^{-/-} cell lines. We have successfully generated five Nrf2^{-/-}-transgenic hiPS cell lines and we are currently in the process of functionally validating these cell lines. The goals of this project are highly translational, with a great potential to lead to the development of different therapeutic strategies for AMD. Our 3D Neural Retina/RPE complex from human iPS cells would signify an important breakthrough because it would provide an innovative, highly versatile model to study the cellular interactions between photoreceptors and RPE involved in the early stages of AMD, as well as to test potential therapeutic interventions for this disease.

Ziyuan Guo, Ph.D.

Johns Hopkins University
2016 Post-Doctoral Fellowship Award
Award Amount: \$110,000
Disease Target: Neurofibromatosis Type 1(NF1)

Hyunhee Kim, Ph.D.

Johns Hopkins University
2016 Post-Doctoral Fellowship Award
Award Amount: \$110,000
Disease Target: Parkinson's Disease

Investigating Cellular Mechanisms Underlying NF1-Associated Cognitive Impairments Using iPSCs

Aim 1: To differentiate NF1-specific iPSCs into forebrain cortical neurons and characterize synaptic function of neurons with NF1 mutations: (1-1) To differentiate NF1-iPSCs into forebrain cortical neurons and characterize neuronal differentiation. I have established a robust protocol to differentiate NF1-iPSCs into forebrain cortical neurons. I have begun to characterize control and patient-specific iPSC-derived neurons through immunohistochemistry at several time points. (1-2) To characterize synaptic function of neurons with NF1 mutations. I have examined sodium and potassium channel function with whole cell recordings of neurons with NF1 mutations at 2 and 4 weeks post-differentiation. I have begun recording spontaneous excitatory postsynaptic currents (EPSCs) and inhibitory postsynaptic currents (IPSCs) in these neurons at 4 and 6 weeks. I will perform immunohistochemistry to analyze synaptic markers such as SV2, vGlut1 and vGat at the same time points. **Aim 2:** To generate whole genome transcriptomes via RNA-seq to determine key dysregulated genes by NF1 mutations in cortical neurons. (2-1) To compare transcriptomes of patient and control cortical neurons derived from iPSCs with bulk-RNA-seq analysis. I have cultured both normal and patient neurons for bulk RNA-seq. I have started the library preparation for sequencing. (2-2) To compare neuronal subtype specific transcriptomes of patient and control neurons with single-cell RNA-seq analysis. I have started to prepare samples for in silico cell sorting and am optimizing the protocol for single-cell sequencing. (2-3) To validate transcriptomes data by qPCR and at the protein level. I will perform qPCR and Western blots to validate the sequencing results after completing Aim 2-2.

Parthanatos in Parkinson's Disease

Parkinson's disease (PD) is the second most common neurodegenerative disorder, affecting the lives of 1% of population who are 60 years old and older. Many individuals with PD do not carry mutations related to PD, however, exhibit a common feature of producing misfolded α -synuclein proteins. In this study, we differentiated human embryonic stem cells (hESC) to neurons and induce idiopathic PD-like features by α -synuclein fibril treatment. Although the underlying cause of progressive neuronal loss in PD is still debatable, our laboratory has accumulated compelling evidences that the neuronal loss takes place through a specific cell death pathway called parthanatos through rodent studies. Thus, we investigated if parthanatos takes place in human sporadic models of PD by developing human neurons from embryonic stem cells, inducing PD-like phenotypes in these neurons and examining the activation of PARP1 and its upstream/downstream proteins that play important roles in this cell death pathways. **Findings:** Human cortical neurons (HCN) were developed from human embryonic stem cells using RONA method that we recently published (*Xu et al., 2016; Sci. Transl. Med.*). We found that α -synuclein PFF treatment on HCN induces mitochondrial ROS production, cell death and PAR formation. PARP-1 inhibitors (*Olaparib, ABT 888, and AG 014699*), which are clinically used in cancer treatment, significantly reduced cell death and PAR formation, suggesting that cell death in human cortical neurons by α -synuclein PFF is due to activation of PARP-1 (parthanatos). The dramatic increase of pathological phospho-serine 129 of alpha-synuclein in response to PFF treatment was significantly decreased by PARP-1 inhibition. C-abl kinase, which is implicated in tumorigenesis, is recently discovered to be upstream of PARP-1. Nilotinib, a c-abl inhibitor being tested in anti-cancer clinical trial, was effective in reducing cell death, PAR formation and c-abl activation following PFF treatment. Nuclear translocation of AIMP2, AIF and MIF is a hallmark of parthanatos. These molecular players progressively move into the nucleus in response to PFF treatment. Pre-treatment with nilotinib significantly reduces the translocation of AIMP2. Our lab previously reported that Lag-3 is a receptor responsible for α -synuclein PFF uptake in neurons (*Mao et al., 2016 Science*). Lag-3 neutralization antibody, which is similar to antibodies now being tested in anti-cancer clinical trials, reduced the endocytosis, inhibited cell death, and significantly decreased accumulation of α -synuclein PFF in the PFF-treated neurons, suggesting Lag-3 inhibition may be a potential therapy for sporadic parkinsonism. **Impact on Public Health:** The findings in this study will be critical in development of a novel therapy for PD treatment, as PARP1 inhibitors, C-abl inhibitors, and Lag-neutralization antibodies are currently clinically tested for its safety in the field of oncology. Thus, the result of this study will indicate whether these inhibitors should be advanced toward PD clinical trials.

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2016 Exploratory Award

Award Amount: \$230,000

Disease Target: Hematopoietic Disorders, Bone Marrow Transplants

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2016 Exploratory Award

Award Amount: \$230,000

Disease Target: Traumatic Brain Injury

Eyes Absent-1 (EYA1) as a Novel Hematopoietic Stem-Progenitor Cell Regulator

The major goal of this project was to translate our preliminary findings implicating the conserved PAXSIX- EYA-DACH transcriptional gene network in controlling stem/progenitor cells of the hematopoietic system of the model system *Drosophila* to human hematopoietic stem-progenitor cells (HSPCs). Using gain of function studies, we have shown that overexpression of EYA1 or its binding partners SIX1, SIX2 or DACH1 stimulates erythroid differentiation of CD34+ human erythroleukemia cells used as an in vitro model for early stages of erythropoiesis. Overexpression of PSEDN members reduced cell growth and stimulated the differentiation of cells, as determined by gain of erythroid and loss of stem cell surface marker expression and stimulation of hemoglobin expression. Enforced SIX expression in primary HSPCs similarly resulted in growth disadvantage and stimulated hemoglobin gene expression. Thus, in contrast to prediction that enforced expression of these genes would inhibit cell differentiation, our results suggest these genes can drive cell differentiation. For loss of function studies, we generated CRISPR/Cas9 engineered cell lines for inducible knockdown or standard knockout. To date, no overt phenotypes have been observed, potentially due to functional compensation provided by the expression of multiple EYA and SIX family members. Using biotin proximity labeling and co-immunoprecipitation assays, we demonstrated the novel finding that SIX proteins could interact with GATA1, the master regulator of erythropoiesis. Reporter gene assays further revealed that SIX proteins could also stimulate GATA1-mediated gene expression. Our findings reveal a novel functional and physical interaction between two central developmental pathways conserved across taxa, that function in diverse tissues and organs to regulate stem-progenitor cell proliferation, specification and differentiation. Our findings could therefore have broad impact on stem cell and developmental biology, potentially providing the basis for a novel mechanism by which GATA and SIX proteins contribute to stem cell biology during normal and malignant development, including how SIX1 and GATA1 each function to enhance stem cell phenotypes and EMT in breast cancer. The first manuscript derived from this work is in preparation for August submission. In conjunction with this submission, we will be discussing potential patent disclosure for the protein interaction. Products. We have generated CRISPR engineered cell lines as well as lentivirus expression constructs for human EYA1, SIX1, SIX2 and DACH1 and CRISPR constructs for knockdown and knockout. We have also generated an RNA Seq dataset for SIX overexpression. Based in part on the experience gained in this project, the PI has initiated a CRISPR Core Services in conjunction with the UMGCC Translational Core Laboratory to facilitate utilization of CRISPR technology by researchers on campus that is open and available on a fee/use basis to researchers beyond UMB. The preliminary data obtained during this project also directed resulted in acquisition of an ACS IRG pilot grant for MJ Kim.

Neuronal Differentiation of iPSC Cells by Autophagy Induction in Oxidative Environment to Treat TBI

TBI is one of the major causes of death and disability among young adults as well as in aged population. It is associated with severe and progressive neurodegeneration that has no available effective treatment to prevent neuronal loss after injury. In this study we aimed to replenish lost neurons by transplanting neuronal stem cells. However highly oxidative environment of the injured area is not conducive for survival and proper neuronal differentiation of NSCs. Since autophagy, a cellular degradative process provides neuroprotective function and promotes neuronal differentiation of NSCs we hypothesized that activation of autophagy in NSCs will help them to survive and differentiate into neurons in the oxidative and inflammatory environment of injured cortices of mice. Accordingly, we enhanced autophagy in NSCs using both chemical and genetic approach. We first tested our hypothesis in vitro in iPSC-NSCs. We transduced these cells with FIP200, an autophagy protein that is essential for the induction of autophagy, then allowed them to differentiate under oxidative environment. We observed increased differentiation into neurons in cells transduced with FIP200 as compared to vector-transduced iPSC-NSCs which showed increased astrogliosis under the oxidative environment. This result clearly suggests that autophagy upregulation might be beneficial for neuronal differentiation of iPSC-NSCs in the oxidative and inflammatory environment of injured cortex. Thus, we planned to transplant iPSC-NSCs in the injured cortex after activating autophagy. We first used genetic approach to enhance autophagy in these cells by overexpressing lentiviral FIP200 or transcription factor TFEB which regulates transcription of autophagy genes. But unfortunately, we could not achieve good transduction efficiency with either of these genes. This led us to take chemical approach to enhance autophagy in the transplanted cells. Accordingly, we transplanted iPSC-NSCs transduced with red fluorescent protein (RFP) into the mouse brains at day 7 after controlled cortical impact induced TBI, then treated the animals with either vehicle or rapamycin every day for 7 days. We detected markedly higher levels of RFP positive iPSC-NSCs in the cortices of TBI mice treated with rapamycin as compared to mice treated with vehicle when we visualized the cortical brain section under fluorescent microscope. Transplanted iPSC-NSCs were found to be localized mainly near the injury site, however some cells were detected in the inner cortical layers in rapamycin treated mice. We also observed increased level of neuronal differentiation of transplanted cells in the cortices of mice treated with rapamycin as compared to mice treated with vehicle. Additionally, enhanced levels of autophagy were detected in transplanted iPSC-NSCs in rapamycin treated mice, suggesting that autophagy upregulation in iPSC-NSCs in mice treated with rapamycin is protective and helps them to differentiate into neurons in the oxidative environment of injured brain. Finally, we assessed the cognitive function in the injured mice transplanted with iPSC-NSCs using Y-maze, which measures the spatial memory. We found significant improvement in spatial memory in injured mice transplanted with iPSC-NSCs as compared to TBI mice in which iPSC-NSCs were not transplanted. However, we detected very slight improvement in spatial memory in iPSC-NSC transplanted TBI mice which were treated with rapamycin as compared with vehicle treated mice. We planned to extend this study to perform additional behavioral tests with larger sample size to make proper assessment of cognitive function in iPSC-NSC transplanted TBI mice treated with or without rapamycin. Taken together, our data demonstrate that autophagy upregulation is beneficial in enhancing survival and inducing neuronal differentiation of iPSC-NSCs in injured cortices and may improve cognitive function in TBI mice. This study clearly indicates therapeutic potential for neural stem cell transplantation along with autophagy upregulation for TBI.

