MARYLAND STEM CELL RESEARCH FUND

Annual Report

2022

MSCRF

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The Maryland Stem Cell Research Fund

Our Mission

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

We strive to improve human health by advancing innovative cell-based research, treatments and cures to benefit patients with unmet medical needs.

About Us

The Maryland Stem Cell Research Fund (MSCRF) is focused on identifying and fostering cutting-edge research and innovation in the field of regenerative medicine in Maryland. Our Accelerating Cures initiative comprises programs that help transition human stem cell-based technologies from the bench to the bedside as well as mechanisms to build and grow stem cell companies in Maryland. MSCRF has supported over 500 projects to accelerate stem cell-based research, commercialization, and cures, in addition to building a collaborative stem cell community in our region. Learn more about us at www.mscrf.org.

Maryland Stem Cell Research Commission

Diane Hoffmann, M.S., J.D. (Chair)

Appointed by the University System of Maryland
Professor of Law, Director Law & Health Care Program, University of Maryland School of Law

Scott Bailey, Ph.D. (Vice Chair)

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. (Vice Chair)

Appointed by the University System of Maryland
Associate Professor; Biological Sciences University of Maryland, Baltimore County

Ira Schwartz, Esq.

Attorney General's Designee
General Counsel, MD Technology Development Corporation

Mary Armanios, M.D.

Appointed by Johns Hopkins University
Professor of Oncology and Genetic Medicine; Director, Telomere Center at Johns Hopkins; Associate Director for Education and Training, Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins

Margaret Conn Himelfarb, MPH

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

Debra Mathews, Ph.D., M.A.

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

David Mosser, Ph.D.

Appointed by the University System of Maryland
Department of Cell Biology & Molecular Genetics, University of Maryland, College Park.

Barbara Nsiah, Ph.D.

Appointed by the President of the Senate
Director, Tissue Systems; United Therapeutics

Linda Powers, J.D.

Appointed by the President of the Senate
Managing Director of Toucan Capital, Early & Active Supporter of Biotech Companies

Rabbi Avram Reisner, Ph.D.

Appointed by the Governor
Rabbi of Congregation Chevrei Tzedek, Baltimore, Maryland

Curt Van Tassell, Ph.D.

Appointed by the Speaker of the House of Delegates
Research Geneticist, USDA-ARS, Beltsville, MD

MSCRF and the Regenerative Medicine Sector Soar to New Heights in 2022

2022 marked a year of tremendous growth, success, and synergy for MSCRF as well as for the regenerative medicine sector. As we returned to our new normal, we continued to innovate and accelerate the pace of scientific advances. The regenerative medicine sector continued the momentum from the previous year and steadily made progress as we celebrated more clinical milestones and regulatory approvals. It was a notable year for the approval of new gene therapies to treat rare diseases. We continued to see advances and approvals of Chimeric Antigen Receptor T-cell (CAR-T) therapies, which are also becoming earlier-line treatment options. With over two thousand clinical trials ongoing worldwide, there continues to be excitement around the treatment paradigm that this industry offers. While investment in the sector was not at the record-breaking pace of the past two years, **our portfolio companies were amongst those who completed successful funding raises in 2022. Thanks to the support of the Governor and the Maryland General Assembly, MSCRF funding levels more than doubled to $20.5 Million,** the highest in over a decade. Maryland was among very few states that made a visionary investment in an emerging regenerative medicine field in 2006 and the renewed commitment this year is a testimony to our leadership, stewardship, and to the dedication and hard work of the scientists and companies we support.

The Maryland Stem Cell Fund

MSCRF was established by the Governor and the Maryland General Assembly through the Maryland Stem Cell Research Act of 2006 during the 2006 General Assembly Session. The purpose of the Fund is to promote state-funded, scientifically meritorious stem cell research and cures through grants and loans to public and private entities in the state.

This year, at MSCRF, we celebrated 15 years since our first grants were awarded. With over 500 grants and almost $200 Million committed to stem cell research and commercialization, we have shaped and advanced this field in Maryland. **We also celebrated 5 years of our accelerating cures initiative.** This initiative originally comprised five programs aimed at de-risking and transitioning the promising research we had funded for the previous decade into companies, products, and clinical trials. We subsequently introduced a sixth program to support faculty early in their career paths or those transitioning into the field, so we could nurture novel, orthogonal ideas and technologies to solve emerging challenges in the industry.

5 Years of Accelerating Cures Initiative

25 ORGANIZATIONS

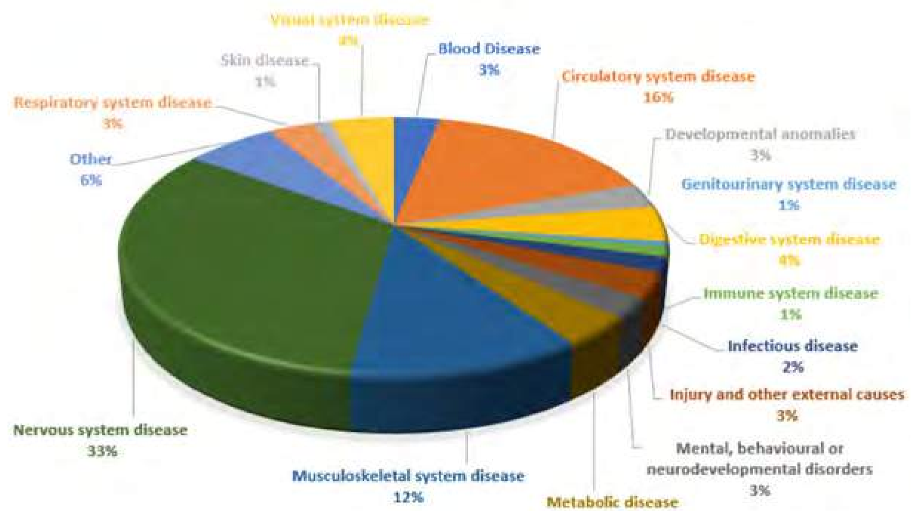
165 AWARDS

In 5 years, we have invested over $48M in 165 awards to 25 different organizations.

$48,377,683 Total Committed Funding to Awardees

Disease Indications Funded

The work we have supported with our accelerating cures initiative span several disease areas, with a majority of projects advancing research targeting the nervous system, circulatory system and musculoskeletal system diseases.



Source: MSCRF Disease Classification

We have moved over a hundred technologies toward validation, into companies and clinical trials and have supported the creation and/or growth of companies through **26 research/product development and clinical trial grants** in the past 5 years (compared to 12 in the previous ten years). Notably, most of the companies in our portfolio have raised follow-on funding and several are either in clinical trials or are scheduled to commence one next year. This represents the sustainable success of our program and exemplifies our mission.

100 We have transitioned over one hundred technologies

Our efforts, in identifying and investing in the right ideas early, as well as in building an ecosystem to support it, has further catalyzed innovation and the emergence and growth of numerous life science companies in the region. This industry, that we have helped build in Maryland, stimulates the economic activity in the state by attracting private investment and by recruiting and retaining a talented workforce in the region.

Products development and clinical trial grants **26**

At MSCRF, we continually improve our programs to address key gaps that impede progress in the field. This year we are proud to launch our Manufacturing Assistance program (page 10).

1 MSCRF Launched a New Manufacturing Assistance Program

Over the years, we have identified and fostered technologies that will result in the next medical breakthroughs, and we have supported the creation of numerous companies and clinical trials to advance these discoveries to the patient. However, producing these treatments at scale remains a key challenge in the industry, especially for early-stage companies. It has been incredibly important to us to support our companies on this journey as they move towards clinical trials and to provide the resources necessary to make these treatments available and accessible to patients. This program will enable GMP production of cell therapy products in Maryland and will serve to accelerate and de-risk the commercialization of these therapies. In addition, it will help us build a diverse and skilled advanced therapy workforce in the region. The long-term impact of this program is crucial to the field, the state and most importantly the patients.

In 2022, we funded 36 new awards with over $11 Million, addressing a wide range of medical conditions including cancer, stroke, liver disease, eye disease, heart disease, diabetes, blood disorders and neurological conditions amongst others (page 12). Our companies have persevered through challenges to raise follow-on funds, create partnerships, and have advanced their research and clinical programs to bring us one step closer to delivering therapies to the public (page 14). Explore the highlights in the year in review section.

36 New awards in 2022 to awardees

Beyond funding, we serve as Maryland's connective tissue and drive collaborations between our scientists, our many research institutes/universities, our companies as well as with various industry stakeholders so that we can accelerate cures together. Collaboration has been central to MSCRF's success. Building on last year's immense success and thought leadership, our initiatives this year focused on championing and empowering our scientists and companies to take our place on the global stage. We leveraged our industry alliances, visibility, and leadership to empower our awardees with the unique resources, expertise, connections and support they each needed to accelerate their research programs. We created opportunities to share knowledge and drive collaborative science (pages 14-15).

As part of our commitment to serve as a champion for women in stem cell research, we spearheaded a celebrating women in science initiative to bring together, empower and shine a light on some of our incredible women leaders and their groundbreaking research (page 17).

We have accelerated progress by awarding grants for transformative scientific research that can advance the regenerative medicine field, by fostering interdisciplinary scientific and global collaborations and by building a strong community through our outreach and engagement activities.

MSCRF's mission and the cutting-edge science that we support, drives investment in the industry and importantly, delivers a human and societal impact beyond the economic impact (pages 18-34). Read about some of the research we've supported this year (pages 35-62). Resulting therapies will improve the health of many Marylanders and also reduce health care costs for the state.

Our leadership has built a vibrant regenerative medicine ecosystem in Maryland, helped advance innovative scientific ideas with capital, coaching and connections, created meaningful partnerships and investment opportunities, and trained the future workforce in the field. We were honored to be recognized for our efforts with the 2022 stem cell and regenerative medicine action award (page 14).

We are grateful each day to lead this community and to create a culture of excellence, collaboration and innovation that has enabled us to advance groundbreaking research forward and improve human health.

It has been a privilege to lead this organization to new heights and with our historic levels of funding and a rapidly maturing field, we hope to accelerate even more cures together and positively impact the scientists and communities we serve.

Sincerely,

Amritha Jaishankar

Amritha Jaishankar, PhD
Executive Director, Maryland Stem Cell Research Fund

Diane Hoffman

Diane Hoffmann, M.S., J.D.
Chair, Maryland Stem Cell Research Commission

2022

Year In Review

ACCELERATING CURES

Our Programs

Our seven programs are designed to catalyze innovation and sequentially transition the most promising discoveries from the labs where the invention occurred, to the clinic where they will be offered to patients. Research we identify and support here in Maryland will have local, national and global impact and will help patients worldwide.

During calendar year 2022, we had six active programs and we funded 36 new awards with over $11 Million.

We supported projects addressing a wide range of medical conditions including cancer, stroke, liver disease, eye disease, heart disease, diabetes, blood disorders and neurological conditions including Alzheimer's disease amongst others.

We have fostered research and innovation through our university-based programs, where we've identified and supported the high risk-high reward ideas that rarely receive other investments, yet are often the basis for the next medical breakthroughs.

We have moved this research to Validation, Commercialization, and Clinical Trials, where we've been able to create value by de-risking these technologies, building stem cell companies, and advancing cures.

MSCRF has established a strong track record in identifying and supporting cutting-edge stem cell research, commercialization and clinical trials through our unique funding programs that are tailored to advance a stem cell-based discovery from the lab where it occurred, to the clinic where it can reach patients.

Meeting the Demand for Manufacturing

In 2022, MSCRF launched a seventh funding program, the Manufacturing Assistance Program. The program is designed to help growth-stage stem cell companies build their own manufacturing capabilities, which is a critical need, particularly among the Fund's portfolio companies that have reached this stage in their development.

New

Manufacturing Assistance Program

"This program will provide initial resources to enable GMP production of cell therapy products in Maryland. This will help our companies advance their therapies to patients sooner and in a more cost-effective way, whilst simultaneously creating and retaining an advanced therapy manufacturing workforce in our region,"

Amritha Jaishankar, PhD
Executive Director
MD Stem Cell Research Fund

Doug Falk, MS
Chief Executive Officer (CEO)
Vita Therapeutics

"As the cell therapy industry continues to grow, the demand and price for GMP facilities continue to rise. The establishment of the MSCRF's Manufacturing Assistance Program will enable growing biotechnology companies like Vita Therapeutics to begin the process of owning their own clinical scale facilities which could yield significant financial savings in the long term. We're very excited about the opportunity to access this program in the future," stated Doug Falk, CEO of Vita Therapeutics, one of the MSCRF's portfolio companies.

New

Manufacturing Assistance

The Manufacturing Assistance Program supports Maryland-based stem cell therapy companies to build or acquire modular manufacturing facilities, prefabricated clean rooms, closed systems, or similar manufacturing platforms to enable GMP production of cell therapy products in Maryland. 1:1 match of non-state money is required. Applicants for these grants may request up to $1,000,000 for a duration of up to 24 months.

Discovery

The MSCRF Discovery grant is intended to support stem cell investigators with innovative research ideas that differ from current thinking in the field to advance the stem cell field. This program welcomes groundbreaking, high risk/high reward ideas with minimal preliminary data. Applicants for these grants may request up to $345,000 for a duration of up to 24 months.

Validation

The MSCRF Validation grant supports faculty with IP for promising human stem cell technologies that can be developed into products, services, or cures. This program enables faculty to meet critical milestones towards commercialization of innovative stem cell technologies through technology validation, market assessment, and the creation of university start-up companies in Maryland. Applicants for these grants may request up to $250,000 for a duration up to 24 months.

Launch

The MSCRF Launch grant program supports new, or new to the field, faculty to bring novel ideas and orthogonal expertise to the regenerative medicine field to develop innovative solutions to emerging challenges. Applicants for these grants may request up to $350,000 for a duration of up to 24 months.

Clinical

The MSCRF Clinical grant program supports human stem-cell based clinical trials to accelerate cures to patients in need. This program supports any US-Based organization with a clinical trial site in Maryland. Applicants for these grants may request up to $1,000,000 for a duration of up to 24 months.

Commercialization

The MSCRF Commercialization grant program supports start-up companies or established companies developing innovative human stem cell products. This program helps regenerative medicine companies in Maryland develop cutting-edge solutions to improve patient's lives and accelerate cures. Applicants for these grants may request up to $400,000 for a duration of up to 12 months.

Post Doctoral Fellowship

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

$11,232,734 Total 2022 Committed Funding to Awardees

36 AWARDS

RoosterBio

UNIVERSITY of MARYLAND BALTIMORE

RENOVATE

JOHNS HOPKINS UNIVERSITY

36 DISEASE INDICATIONS

Skull Bone Injuries
Barth Syndrome
thrombocytopenia
Ulcerative colitis
TET3 deficiency
Anemia
Glaucoma
leukopenia
Bone
Heart Disease
ALS
Diabetes, vascular disease
Cancer
Chronic Pain
Liver Insufficiency
Amputees with limb loss
Chronic Cutaneous Wounds
Ocular Graft versus Host Disea
Stroke
Liver Fibrosis
Huntington's Disease
Macular Degeneration
Acute Cutaneous Wounds
Alzheimer's Disea
Multiple Sclerosis
Beck-Fahrner Syndrom
Fragile X Syndrome
Demyelinating Diseases
Defects of Bone & Soft Tissue
Necrotizing Enterocolitis
Hematologic m
Corneal endothelial dystrophie
Long QT Syndrome Type 2

Vita Therapeutics, Inc. closed a $31 million Series B financing round, an investment which will be used to advance Vita's lead pre-clinical program VTA-100 for limb-girdle muscular dystrophy to the clinic. It will also fund the development of Vita's newest program, VTA-120 for the treatment of patients with facioscapulohumeral muscular dystrophy, and to further expand Vita's discovery pipeline. Since its inception, Vita has raised a total of $66 million.

RoosterBio, Inc. has entered into multiple collaborative partnerships in 2022. Among these is an exclusive distributor partnership with MBL Beijing Biotech, making RoosterBio's MSC and exosome therapy readily accessible to developers in China. Additionally, RoosterBio has entered into a partnership with two contract development and manufacturing organizations, FUJIFILM Diosynth Biotechnologies and AGC Biologics, for incorporating RoosterBio's products into their manufacturing capabilities and offerings.

Cartesian Therapeutics, Inc. dosed the first patient in its Phase 1/2a multicenter clinical study evaluating Descartes-25, the first off-the-shelf RNA cell therapy to enter clinical trials, in patients with multiple myeloma. Additionally, Cartesian presented its late-breaking interim data from its Phase 1/2a clinical trial of Descartes-08 in patients with generalized Myasthenia Gravis, demonstrating safety, tolerability, and significant clinical improvements in initial patients.

Longeveron, Inc. The FDA granted Fast Track Designation for Lomecel-B™, an investigational allogeneic, bone marrow-derived medicinal signaling cell product, for the treatment of Hypoplastic Left Heart Syndrome (HLHS) in infants, a condition affecting approximately 1,000 babies per year. Longeveron also completed enrollment of its Phase 2a trial of Lomecel-B for the treatment of Alzheimer's Disease.

Reprocell, Inc. completed their Phase II trial of Stemchymal, a regenerative medicine product derived from somatic stem cells for the treatment of spinocerebellar ataxia, in Japan. Additionally, Reprocell has entered into collaborative agreements with BioBridge Global to expand their induced pluripotent stem cell technology capabilities in the US.

Theradaptive, Inc. won an additional Breakthrough Designation, a process designed to expedite the development and review of medical devices which may demonstrate substantial improvement over available devices, for its OsteoAdapt SP Spinal Fusion implant indicated for posterolateral spinal fusion to treat degenerative disc disease, spondylolisthesis, or retrolisthesis. This is the second Breakthrough Designation for Theradaptive in spinal fusion.

BioCardia, Inc. was granted FDA approval of its Investigational New Drug application to initiate a first-in-human Phase I/II clinical trial of its Neurokinin-1 receptor positive (NK1R+) allogeneic human mesenchymal stem cell therapy for the treatment of patients with ischemic heart failure. This approval marks the second clinical trial approved by the FDA in 2022 for the Company's NK1R+ MSC platform.

Lifesprout, Inc. announced positive six-month interim results from its pivotal clinical trial of Lumina™, a revolutionary dermal filler. Lumina achieved its primary endpoint of correction of moderate-to-severe nasolabial folds with good safety. LifeSprout is a privately held regenerative medicine company founded with technology licensed from Johns Hopkins University.

MaxCyte, Inc. has entered into a Strategic Platform License agreement to partner with 3 additional companies; Vertex Pharmaceuticals Incorporated to advance their CRISPR/Cas9-based gene-editing program; LG Chem to advance their allogeneic CAR-T program; and Intima Bioscience to advance their Tumor Infiltrating Lymphocytes Program.

For more information on all our portfolio companies, visit https://www.mscrf.org/portfolio-companies.

ACCELERATING CURES Together!

MSCRF and Greenstone Biosciences, Inc. announce a collaboration to provide curated induced Pluripotent Stem Cell lines to stem cell investigators across the State of Maryland. The stem cell lines provided through this collaboration empower Maryland stem cell scientists to advance their research toward cures.

MSCRF Maryland Stem Cell Research Fund

Greenstone Biosciences, Inc. Announces Collaboration with the Maryland Stem Cell Research Fund to Provide Curated Induced Pluripotent Stem Cell Lines to Stem Cell Investigators Across the State of Maryland

The stem cell lines provided through this collaboration empower Maryland stem cell scientists to advance their research toward cures.

Palo Alto, CA, Aug 23, 2022 – Greenstone Biosciences, Inc. ("Greenstone Bio"), a commercial stage computational biology company announces today that the Company has entered into a collaboration with the Maryland Stem Cell Research Fund ("MSCRF") to provide, at no cost, human induced Pluripotent Stem Cell (iPSC) lines to investigators at academic universities and other research institutions who conduct human stem cell research in the State of Maryland.

Greenstone Bio (Stanford Research Park) focuses on clinical genomics, iPSC technology, AI/ML, and drug development. The company has established extensive knowledge and expertise in generating patient-derived human iPSCs and differentiation into numerous cell types for disease modeling and drug discovery.

Established in 2006 by the Governor and the Maryland General Assembly through the Maryland Stem Cell Research Act, MSCRF's goal is to accelerate stem cell-based research, commercialization, and cures by providing funding and resources to public and private entities in the State of Maryland.

Dr. Joseph Wu, Greenstone Bio co-founder said, "This is a great collaboration that translates Greenstone Bio's expertise in iPSC technology to accelerate therapies to improve patient care – fast-tracking bench to bedside." Dr. Wu is the Director of the Stanford Cardiovascular Institute and the Simon H. Stertzer, MD, Professor of Medicine & Radiology at Stanford University. Greenstone Board Chairman Lip-Bu Tan of Walden Catalyst Venture is a long-term strategic partner. Mr. Tan considers "this a wonderful and strategic translational medicine partnership with the Maryland Stem Cell Research Fund."

Dr. Amritha Jaishankar, MSCRF's Executive Director said, "We are delighted to collaborate with Dr. Joseph Wu and Greenstone Bio to make this valuable resource available to our faculty at research institutions across the State of Maryland. I'm confident that this open-source innovation and collaboration model will advance the stem cell field, and that our concerted efforts will accelerate research and therapies for diverse patients with unmet medical needs in the near term."

> This is a great collaboration that translates Greenstone Bio's expertise in iPSC technology to accelerating therapies to improve patient care - fast-tracking bench to bedside.

Joseph C. Wu, MD, PhD
Co-Founder
Greenstone Biosciences

> We are delighted to collaborate with Dr. Joseph Wu and Greenstone Bio to make this valuable resource available to our faculty at research institutions across the State of Maryland. I'm confident that this open-source innovation and collaboration model will advance the stem cell field, and that our concerted efforts will accelerate research and therapies for diverse patients with unmet medical needs in the near term.

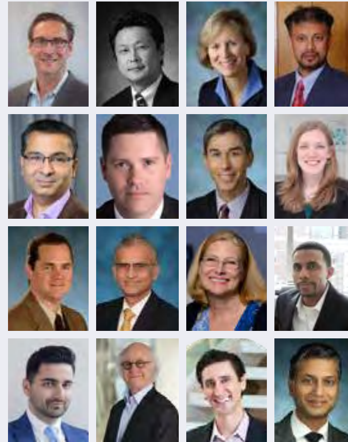
Amritha Jaishankar, PhD
Executive Director
MD Stem Cell Research Fund

MSCRF Wins Stem Cell & Regenerative Medicine Action Award for Leadership

Regenerative Medicine Foundation (RMF) awarded MSCRF the 2022 "Stem Cell and Regenerative Medicine Action Award." The Stem Cell and Regenerative Medicine Action Awards recognizes outstanding individuals and organizations for their devotion to improving health and developing cures through advocacy, innovation, leadership and education.

Scientific Thought Leadership

ISSCR 2022



The MSCRF Executive Director and the scientists we work with are globally recognized leaders and are regularly invited to speak at numerous stem cell conferences to share industry knowledge and insights so that we can advance the regenerative medicine field and address key hurdles.

Highlighted here are a focus session led by our Executive Director at the International Society for Stem Cell Research as well as sessions at the Advanced Therapies week, featuring talks by numerous members of our portfolio. MSCRF scientists were also featured at the World Stem Cell Summit.

In 2022, we also brought our scientists together virtually as well as in person, held several funding briefings in the State and participated in numerous local events to strengthen the life science ecosystem in Maryland.

Scientific Thought Leadership

Our Executive Director and the scientists & companies we support are invited speakers on podcasts and featured in articles. Below are a few highlights.

Ep. 218: "Commercializing Stem Cell-Based Technologies" Featuring Dr. Amritha Jaishankar

Accelerating cures: funding stem cell innovation & ideas in an evolving cell therapy space

Maryland Stem Cell Research Fund Launches Manufacturing Assistance Program

Top Stem Cell Companies Supported by the Maryland Stem Cell Research Fund

These Workforce Development Programs Help Ensure the Region Stays Competitive in Cell & Gene Therapy

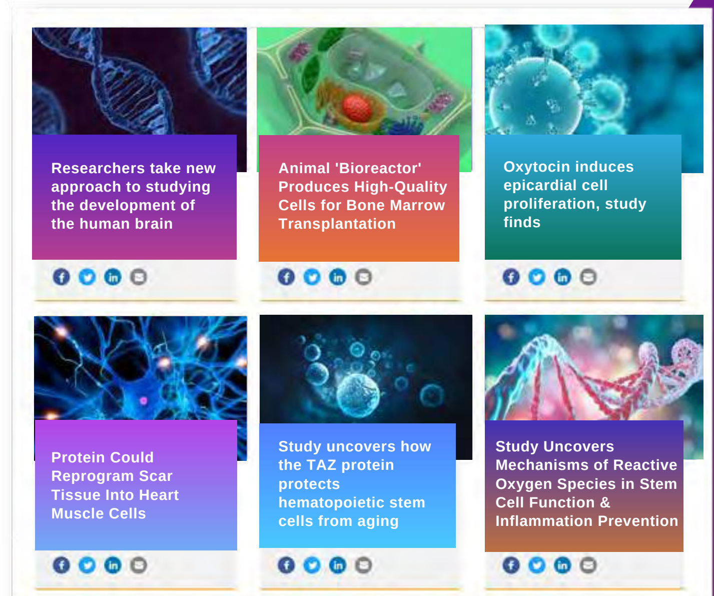
Maryland Stem Cell Research Fund launches stem cell therapy manufacturing grant program

Maryland Stem Cell Research Fund Supports Innovative Women Researchers, Part 1

Maryland Stem Cell Research Fund Supports Innovative Women Researchers, Part 2

Industry News

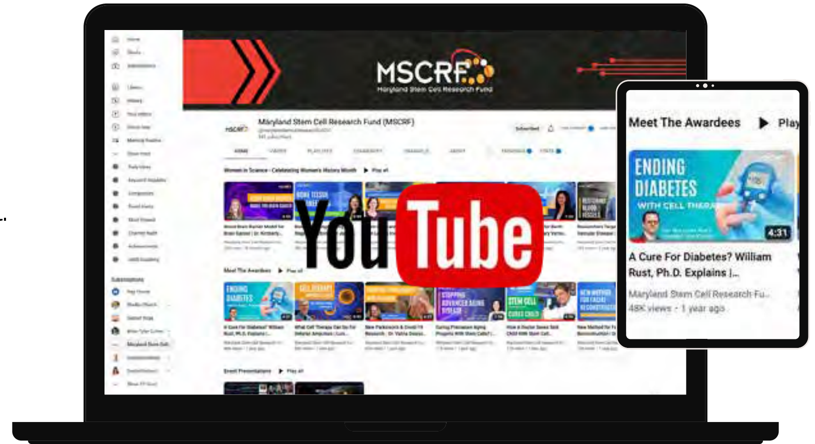
We keep our community connected to cutting-edge research and share the latest scientific advances in the stem cell field through our daily regenerative medicine industry news and updates on our website and app.

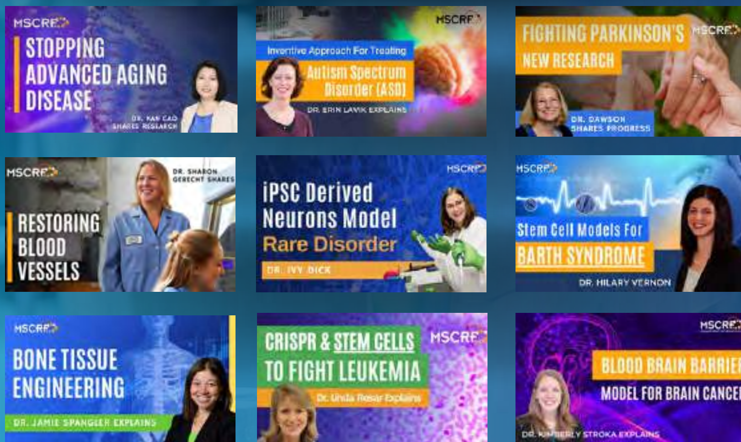


Public Outreach

YouTube Channel

Our scientists are international thought leaders, and their work can be accessed on our channel. With over 80,000 views and a growing community, we invite you to watch how our scientists are advancing treatments and cures.





Celebrating Women In Science

MSCRF Supports Innovative Women Researchers

We bring together and celebrate our scientists who are advancing stem cell research to cure diseases in Maryland that are positively impacting the world.

We highlight the work of several women in science who are accelerating the development of cures for Parkinson's, Autism, and a variety of Rare Diseases with our support.

Health Education

In collaboration with disease foundations and organizations dedicated to improving public health and accelerating cures, we provided health education and raised awareness about the need to address life-threatening diseases without treatments or cures.

COMMUNITY

Impact

IPSC DERIVED NEURONS

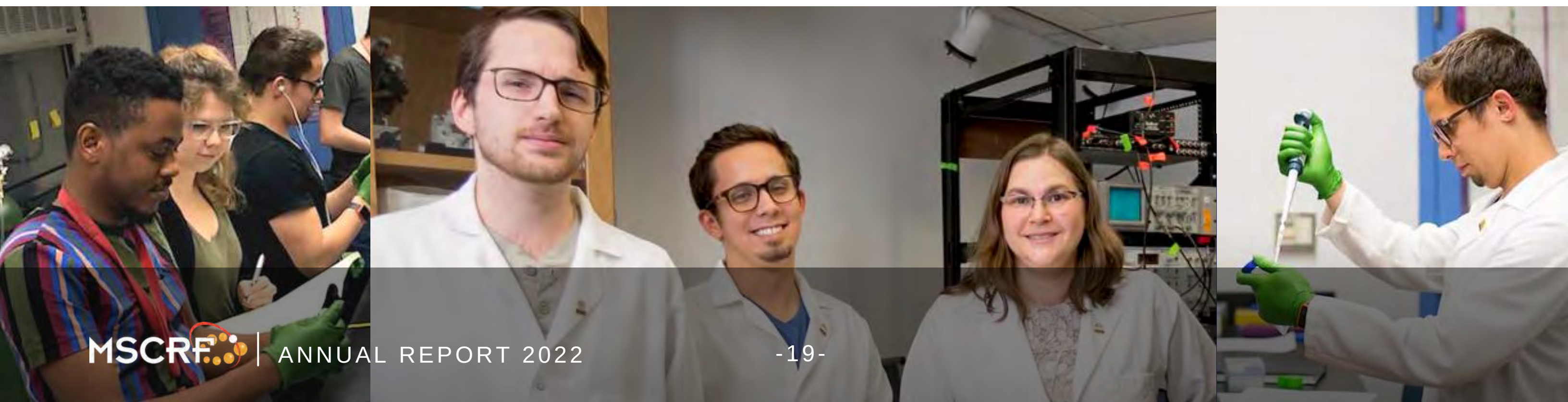
MODEL FOR TIMOTHY SYNDROME

DR. IVY E. DICK

Dr. Ivy E. Dick of the University of Maryland School of Medicine and her lab study the rare disorder **Timothy Syndrome (TS)**. TS is a **rare genetic disorder** that affects multiple organs, including the heart and brain. Sadly, the lifespan of someone with TS is only 2.5 years. It causes early childhood death in most cases.

Patients with TS have a common cardiac abnormality that predisposes them to cardiac arrhythmias that can be fatal. Dr. Dick studies this condition by implementing a genetically engineered **induced pluripotent stem cell (IPSC) system** that replicates the cardiac arrhythmias seen in TS patients. Using this system, Dr. Dick and her team have been developing and testing novel treatment strategies for this rare disorder.

Dr. Dick says the **Maryland Stem Cell Research Fund** has "enabled us to bring our mechanistic studies of Timothy Syndrome mutation into the context of a **human cell model**, allowing us to apply what we learned...to develop new treatment strategies."



BLOOD BRAIN BARRIER MODEL TO TREAT BRAIN CANCER

MSCRF

DR. KIMBERLY STROKA

Dr. Kimberly Stroka, Associate Professor in the Fischell Department of Bioengineering at the University of Maryland College Park, engineers cell model systems to study the blood brain barrier (BBB), a neurovascular system that regulates brain entry of ions, nutrients, cells, and drugs. The BBB can protect the brain from threats like infections, but it can also prevent treatments from getting to it when it's needed, making it very difficult to treat brain tumors.

Dr. Stroka explains that brain metastasis "remains a devastating prognosis due to limited treatment options," largely due to the lack of mechanistic understanding of how metastatic tumor cells invade across the highly selective brain barrier.

With support from the **Maryland Stem Cell Research Fund**, Dr. Stroka and her lab generated a "**blood brain barrier on a chip**," a model system that mimics the BBB's tissue microenvironment to further study brain metastasis. Their **research is unique** because it allows them to assess the movement of tumor cells and their interaction with other cell types in a model that is applicable to the human body.

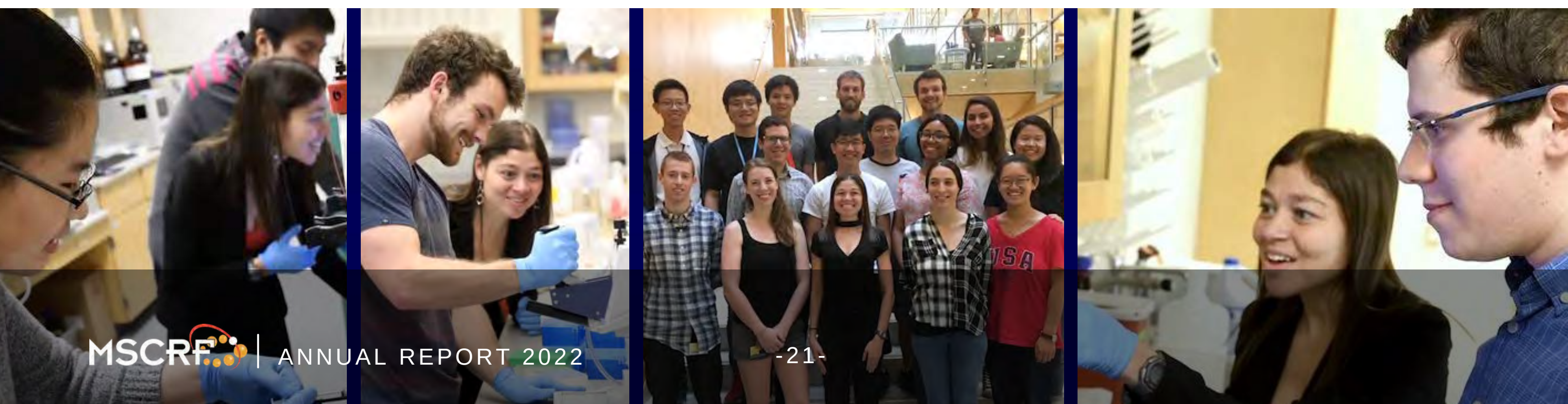
BIOMEDICAL ENGINEERING TO REGENERATE BONE

DR. JAMIE SPANGLER

In the United States, over 500,000 orthopedic procedures a year require bone grafts, a procedure in which bone is supplemented to a site of injury to provide strength and stability. Use of bone grafts suffers from pitfalls such as limited availability, risk of infection, and, in the case of allografts, may result in immune system attack.

In her lab at the Johns Hopkins School of Medicine, **Dr. Jamie Spangler and her team** are leveraging funding support from the **Maryland Stem Cell Research Fund (MSCRF)** to overcome key limitations of current tissue repair platforms by engineering new targeted growth factors that specifically and safely stimulate bone regeneration.

With the research from her lab, bone grafting procedures will be more accessible, more affordable, and safer for patients undergoing these therapies in the future. **Dr. Spangler credits MSCRF** for supporting the pursuit of this new area of research, allowing her lab to expand their bioengineering efforts into new frontiers in tissue regeneration.



Researchers Develop A New Method to Accelerate Cures

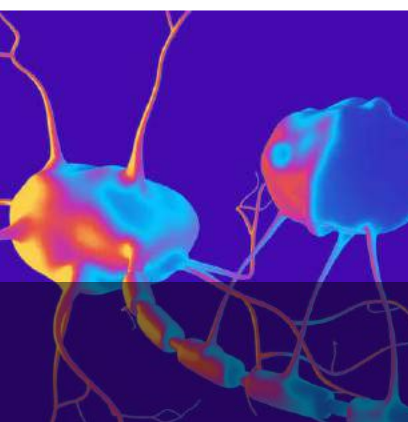
BRAIN REPAIR WITH STEM CELLS

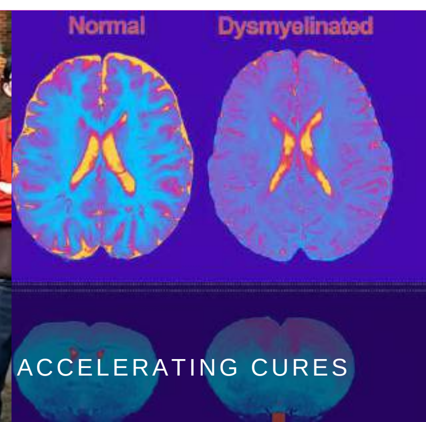
DR. PIOTR WALCZAK

At the University of Maryland, Baltimore, **Dr. Piotr Walczak** and his lab have dedicated themselves to examining how a variety of the 600 neurological disorders that pose a global burden on society can be treated in the best possible way. One method they have developed while studying these conditions is using **real-time image-guided neuro-interventions**, which are helpful to deliver stem cells in a targeted, effective, and safe way to the brain.

The Walczak lab demonstrated that transplantation of stem cells has a **positive therapeutic effect** in mouse models. His lab's novel technique of image-guided intra-arterial administration of stem cells is demonstrating clinical translation from mouse models to humans and it accelerates the development of therapies for people suffering from neurological diseases.

Dr. Walczak credits the Maryland Stem Cell Research Fund (MSCRF) for years of support and their aid in the removal of many obstacles "to the effective use of stem cells in patients suffering from devastating neurological disorders."





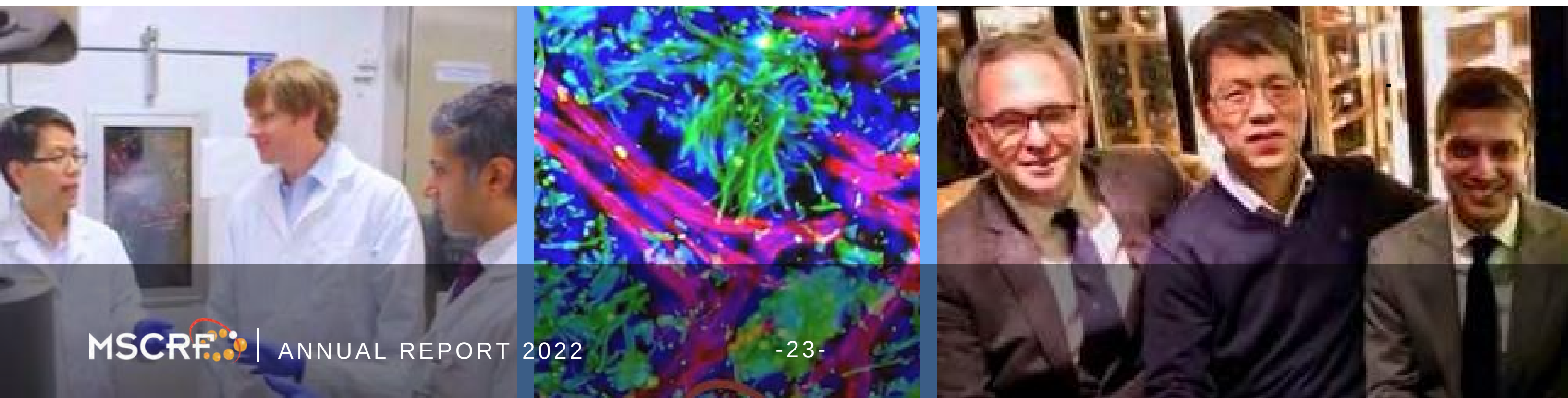
MSCRF

STEM CELL TECHNOLOGY
FOR SOFT TISSUE REGENERATION

Soft tissues are the body's first line of defense and play a role in everyday physical communication, including our facial expressions. Soft tissues can be damaged by trauma, excised as a part of treatments for skin disease, or deteriorate with aging. Current tissue regeneration technologies generate relatively "young" tissues and the homeostasis of these newly regenerated tissues is difficult to maintain.

To **improve tissue regeneration** and maturity, **Dr. Sashank Reddy's lab** at Johns Hopkins School of Medicine is studying the developmental progression of soft tissues and developing nanomaterials that behave like them. These biomaterials developed by the lab may be tailored to meet the needs of the soft tissue environment to help with cell differentiation and function, complementing the lab's developmental cell and homeostasis work.

Dr. Reddy credits the **Maryland Stem Cell Research Fund (MSCRF)** for not only supporting his research, but that of many other scientists he supports through his role as Senior Medical Director at Johns Hopkins Technology Ventures.



STEM CELL MODELS

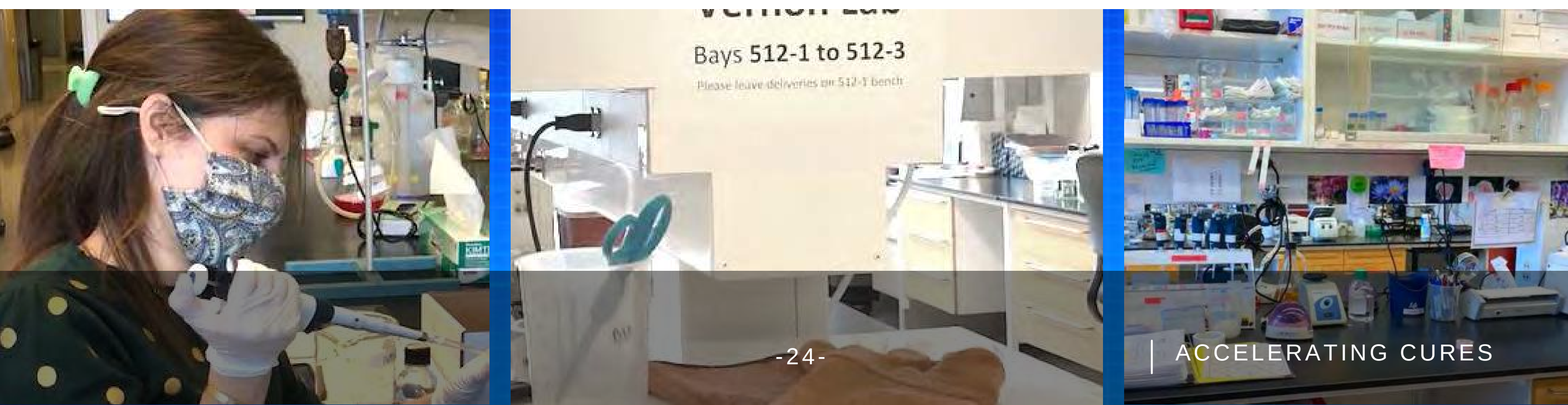
FOR BARTH SYNDROME

DR. HILARY VERNON

Barth Syndrome is a rare disease that presents as heart disease, weakened musculature, and neutropenia (a condition where patients are less able to fight off infection). While advancements have allowed those affected to see past infancy, a treatment that can rectify the impacted metabolic pathways would greatly improve the quality of life for individuals suffering from this disease.

Dr. Hilary Vernon and her lab at Johns Hopkins School of Medicine are using stem cell models to determine the mechanistic underpinnings of Barth Syndrome. To accomplish their goal, the Vernon lab is generating **induced pluripotent stem cells (iPSCs)** from Barth Syndrome patients. The Vernon lab hopes to identify a metabolite or pathway that can be used as a therapeutic target, potentially through dietary supplements or medications.

Dr. Vernon credits the **Maryland Stem Cell Research Fund (MSCRF)** for the support which allowed her lab to generate the foundational stem cell model that is pivotal to her research.



MSCRF

REGENERATING HEART TISSUE *With Stem Cells*

Heart disease is the number one cause of death in the United States and it is a major area of regenerative medicine research. However, a critical hurdle in developing stem cell-based technologies for cardiac indications is that newly differentiated cardiomyocytes (heart cells) are immature and are not a suitable replacement for mature heart tissues.

Dr. Chulan Kwon and his lab at Johns Hopkins School of Medicine have taken multidisciplinary approaches to identify factors crucial in promoting cell maturation. The Kwon lab's research advances efforts to produce adult-like heart cells from stem cells and offers the potential to be translated into better stem cell-based therapeutics and more effective drug development.

Dr. Kwon credits the **Maryland Stem Cell Research Fund (MSCRF)** for major support on these projects. Dr. Kwon explains that **MSCRF** contributions support the lab's research as well as the development and training of its students. MSCRF continues to support innovative cell therapy research and the current and future researchers who lead these efforts.

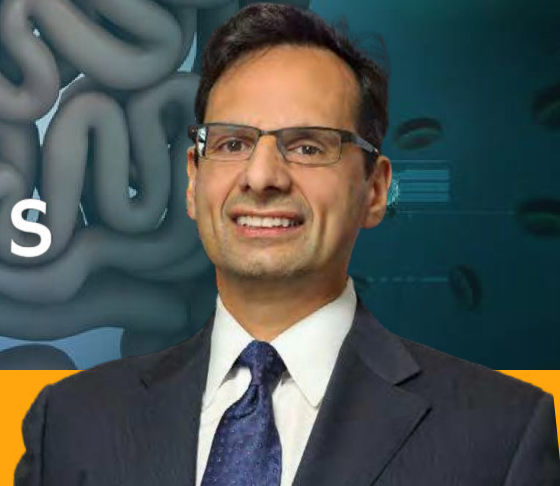
BIOENGINEERING REGENERATES TISSUE

Dr. John Fisher, Professor and Department Chair at the Fischell Department of Bioengineering at the University of Maryland College Park, uses a multitude of **bioengineering strategies to develop novel treatments to heal tissues and restore their function**.

Dr. Fisher's lab and his collaborators have worked with several tissues including bone, cartilage, cardiovascular, and placental. **Their goal is to build materials that can be easily implanted into a patient's body to help restore lost or damaged tissue**. Regenerative medicine often includes the use of **stem cells** because of their unique potential to give rise to cells of any tissue origin and ability to self-renew. As an example, Dr. Fisher notes his use of mesenchymal and hematopoietic stem cell co-cultures for engineering bone marrow. This particular **project combines 3D printing and a bioreactor system** to re-create the niche microenvironment needed for these stem cells to differentiate and expand. The innovative conjunction of these two techniques allows for efficient cell culture conditions and increase of cell yield for further downstream biological applications.

Dr. Fisher describes how support from the **Maryland Stem Cell Research Fund** "has not only impacted our lab and its growth, but it's also supported a number of trainees with graduate students and postdoctoral fellows". The novel and cutting-edge work of the Fisher Lab continuously advances the field of regenerative medicine and holds great impact for future clinical solutions.

Researchers use Stem Cell technology to FIGHT INTESTINAL DISEASES

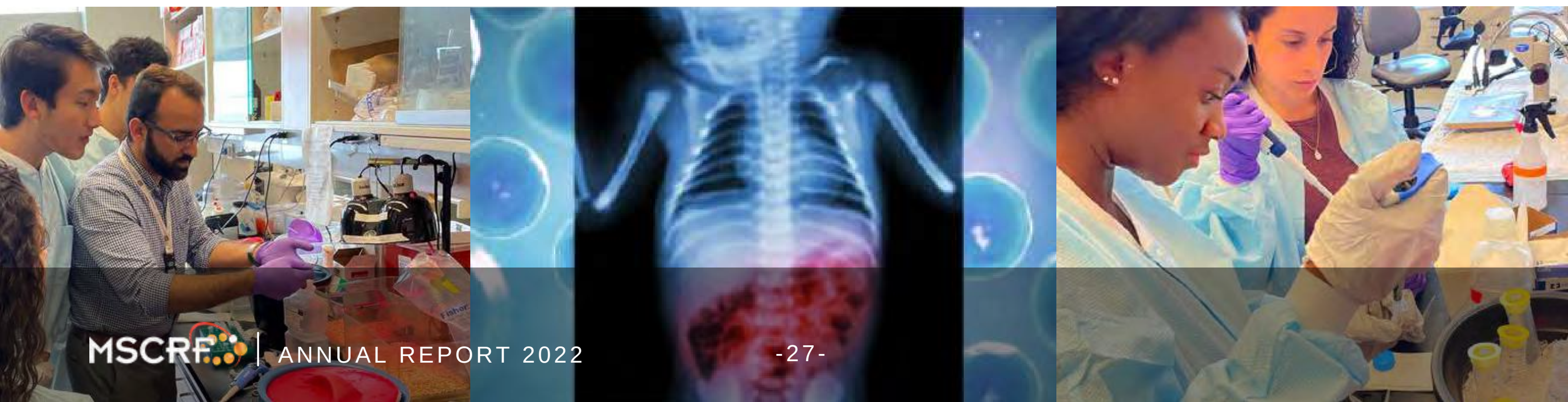


DR. DAVID HACKAM

Dr. David Hackam and his lab are utilizing human stem cell technologies to tackle the leading cause of death and disability in premature infants, necrotizing enterocolitis (NEC), as well as other chronic intestinal conditions that affect children and adults. As the Chief of Pediatric Surgery at Johns Hopkins University and Surgeon-in-Chief of Johns Hopkins Children's Center, Dr. Hackam knows firsthand the complicated neonatal surgery and the devastating long-term effects that result from NEC.

NEC, a disease that affects up to a third of premature infants, leads to sudden death of parts of the intestine and the only available countermeasure for severe cases is the surgical removal of the damaged intestine. If the infant survives surgery, they are left with lifelong short bowel syndrome and decreased quality of life.

Dr. Hackam's research focuses on using human stem cells to correct for the loss of intestine that results from NEC complications. His goal is not only to save lives but also to restore the quality of life of infants. He shares that the **Maryland Stem Cell Research Fund (MSCRF)** "**has been pivotal in helping advance this work.**" He credits MSCRF for investing in his vision, "We were funded through the mechanism that essentially takes an idea, invests in the idea, and allows the investigator to deliver on the promise of that idea." Dr. Hackam and his team have converted his idea into pivotal research and development that will benefit patients suffering from chronic and life-threatening conditions in Maryland and worldwide.



STEM CELLS HELP FIGHT HEART DISEASE

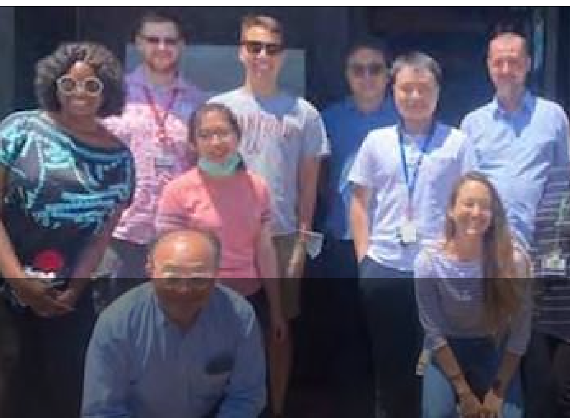
DR. CHARLES HONG

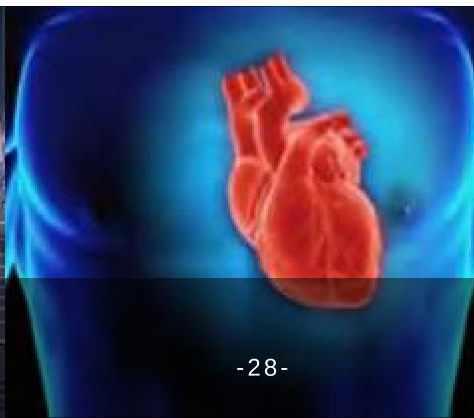
Cardiovascular disease is a leading cause of mortality worldwide, claiming the lives of an estimated **17.9 million** people each year. There is an urgent need to study this disease and discover new drugs.

Dr. Charles C. Hong and his lab at the University of Maryland School of Medicine are advancing cardiology research. The lab is utilizing cutting-edge approaches to develop new therapies for the 6 million Americans suffering from heart failure.

The Hong lab is implementing a model system using **human-derived induced pluripotent stem cells (iPSCs)**. Dr. Hong's goal is to use this system to develop a platform for drug discovery that will advance therapies for people suffering from heart failure.

Dr. Hong said the **Maryland Stem Cell Research Fund (MSCRF)** has "allowed us to continue our cutting-edge research in helping us to better use these stem cell-derived cardiomyocytes to faithfully recapitulate the human heart tissue."





MSCRF

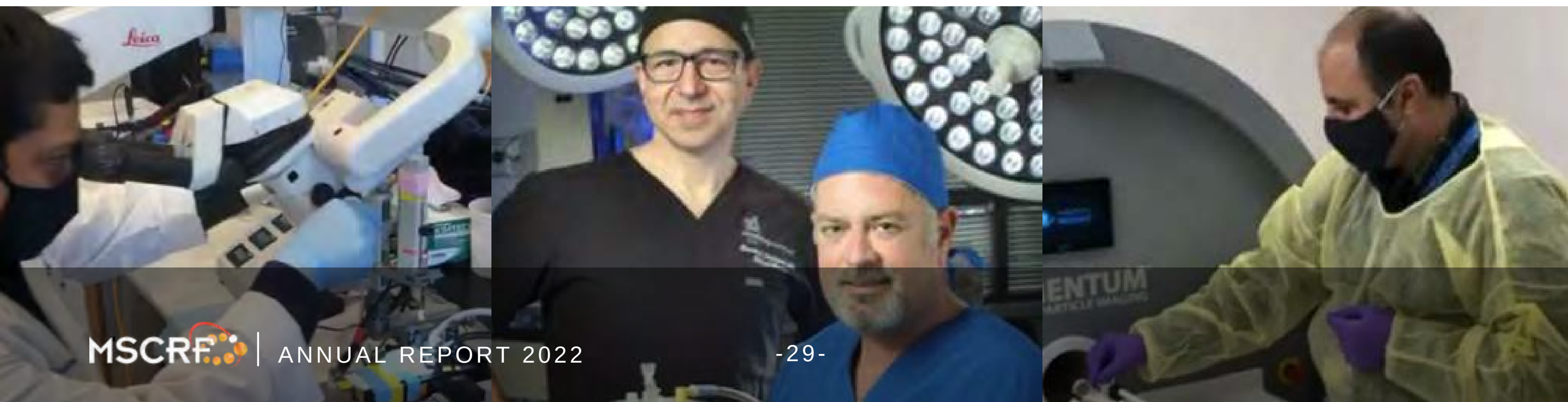
REAL-TIME STEM CELL TRACKING

Cell therapies and technologies involve injecting cells systemically or locally at sites of injury to replace defective or excised tissues. However, monitoring cells in real time after injection is impossible using conventional light-based imaging techniques. Real-time imaging allows physicians to visualize cell fate and determine treatment effectiveness **before disease progression** ensues, which may be too late for some patients.

Dr. Jeff Bulte and his lab, with long-term support from the **Maryland Stem Cell Research Fund (MSCRF)**, have developed Magnetic Resonance Imaging (MRI) and other imaging techniques for assessing stem-cell implantation in real-time.

The Bulte lab has applied advanced imaging techniques for the treatment development of many diseases and disorders, including **Multiple Sclerosis (MS), Amyotrophic Lateral Sclerosis (ALS or Lou Gehrig's disease), diabetes, stroke, and radiation damage.**

The lab credits **MSCRF** funding not only for supporting technological development of the Bulte lab, but also for professional development of its personnel. Several members of the Bulte lab have received post-doctoral support from **MSCRF** and have become research faculty and principal investigators of their own respective labs.



FIGHTING MUSCULAR DYSTROPHY *With Stem Cells*

MR. DOUGLAS FALK

Muscular Dystrophy is a group of 30+ diseases that is characterized by loss of muscle mass referred to as muscle wasting. A common form of muscular dystrophy is **Limb-Girdle Muscular Dystrophy (LGMD)**, which is a diverse subgroup of genetic disorders that usually manifests as weakness in the muscles around the hips and shoulders. In the US alone, there are approximately 4000 patients with up to 100 new patients identified each year.

Vita Therapeutics is a cell engineering company, founded at Johns Hopkins University in 2019 that seeks to develop life-transformative treatments. The company utilizes **induced Pluripotent Stem Cell (iPSC)** technology to develop regenerative therapies designed to support muscle regeneration and treat patients with muscle disorders.

Vita is getting ready to launch its first-in-human clinical trial to evaluate its leading product, VTA-100, for the treatment of LGMD 2A/R1. VTA-100 is designed as an autologous treatment that combines gene correction and iPSC technology to help repair and replace muscle cells. It is envisioned that transplanted Vita Therapeutics' muscle stem cells (satellite cells) will not only replace and repair damaged muscle tissue but also support long-lasting homeostasis, and repair future muscle damage. Douglas Falk, Vita's CEO, credits the **Maryland Stem Cell Research Fund (MSCRF)** with providing support early on to enable the development and preclinical testing of its muscle satellite cells, which led to the development of its lead product, VTA-100.

Technology to REGENERATE BONES

DR. LUIS ALVAREZ

Bone regeneration technologies can help prevent the need for amputation after traumatic injuries like those sustained by combat veterans. It can help alleviate debilitating pain in people with **degenerative spinal discs**, as well as treat a range of other debilitating conditions.

Theradaptive, a Frederick-based biotechnology company led by **CEO Luis Alvarez**, **Ph.D.**, is developing technologies that use **material-binding variant therapeutic proteins** to improve outcomes for future patients. By helping to deliver therapeutics directly to where they are needed, Theradaptive aims to reduce the number of patients having to make the life-altering decision to amputate. The company also seeks to use its technology to improve the spinal condition of patients who might otherwise suffer from significant loss of mobility. Among hundreds of other potential applications, Theradaptive's initial focus is on **spinal fusion, bone repair, and cartilage repair**.

The **Maryland Stem Cell Research Fund (MSCRF)** supported **Theradaptive** at an early stage in the company's development. Thanks to that early backing, Theradaptive was able to secure Federal funding, predominantly from the Department of Defense (DoD). **This unique blend of state and federal investment in Theradaptive highlights how MSCRF can jumpstart regenerative medicine efforts in biotechnology.**

Inventive Approach For Treating

AUTISM SPECTRUM DISORDER (ASD)

DR. ERIN LAVIK

Autism Spectrum Disorder (ASD) refers to a broad range of conditions related to brain development, estimated by the CDC to affect 1 in 54 children. **Dr. Erin Lavik**, Professor and Associate Dean at the University of Maryland Baltimore County, and her lab implement **innovative techniques** to study the brain and its associated neurological complications, such ASD. Dr. Lavik's research aims to build tissue models that mimic the environment of specific organs, such as the brain. She focuses on recreating the "neural stem cell niche" by combining neural stem cells and blood vessels. Neural stem cells give rise to neurons through an intricate developmental process involving multiplication, differentiation, and migration. Dr. Lavik combines these **stem cells and a screen-printing procedure to recreate a three-dimensional (3D) model of the brain** where she can better study the cells developmental process.

Dr. Lavik shares that "the **Maryland Stem Cell Research Fund** has been instrumental in our work" and hopes that this research can further elucidate the complications involved in the brains of people with ASD. She credits **MSCRF** as they "provided the support to allow us to develop the materials and approaches to build these structures". Dr. Lavik and her team's research provides the foundation to better understand intricate systems that may uncover novel therapeutic approaches.

Stem Cell Technology to GENERATE CORNEAL TISSUE

The eye is an incredibly complex organ that allows us to take in information from our surrounding world through sight. The **cornea** is the first layer of the eye, which helps us focus light and protect our eyes. When it is damaged, patients are in severe pain and, in some cases can become blind. As the outermost layer of the eye, the cornea is the most prone to damage. The current treatment for a damaged cornea is a cornea transplant; however, donor cornea is extremely limited. **Dr. Amer Riazuddin** and his lab at the Wilmer Eye Institute, Johns Hopkins University School of Medicine, are using **stem cell technology to generate corneal tissue that can be used in place of the cornea from a donor**.

In their work supported by the **Maryland Stem Cell Research Fund (MSCRF)**, the Riazuddin lab demonstrated that injection of human stem cell-derived corneal endothelial cells resulted in corneal endothelium restoration in both rabbits and primates during preclinical trials. **These therapies can benefit patients worldwide, as cryo-preserved human stem cells** can be transported to clinics and readily used. This has the potential to help many patients in countries with long waitlists awaiting donor tissue transplants.
Dr. Riazuddin's work, supported by the **MSCRF**, is moving into the clinical phase of this project and toward a solution for a corneal endothelium transplant.

CRISPR & STEM CELLS TO FIGHT LEUKEMIA

MSCRF

DR. LINDA RESAR

Leukemia is a debilitating blood cancer that can occur in childhood or throughout life and is particularly lethal in older populations. Leukemia is a complicated disease to target because blood cells are constantly turned over, which in turn requires frequent regeneration and means mutations can occur that could lead to leukemia. **The Resar lab** at Johns Hopkins University School of Medicine, led by **Dr. Linda Resar**, is working on a **molecular switch** that can control the proliferation of blood cells. They hope that by modulating levels of this protein, they can improve healthy cell growth while weakening leukemic cells.

In its work supported by the **Maryland Stem Cell Research Fund (MSCRF)**, the Resar lab is building upon its previous research by using **CRISPR**, a relatively new genetic engineering technology, to tune the expression of **HMGA1** to do just that. By turning down HMGA1 levels in cancerous cells, they will no longer be able to replicate and over-populate. Additionally, stem cells may be damaged during chemotherapy, leading to a decrease in HMGA1 levels. By **upregulating HMGA1 levels in stem cells** that have suffered from radiation or other injuries, the Resar lab hopes to **enhance stem cell performance** and, in turn, blood regeneration. The **Resar lab** is leveraging **MSCRF support** to identify new therapies **to prevent leukemia** and other forms of cancer, which will prevent the deaths of elderly patients and allow children suffering from childhood diseases to live healthier and fuller lives.

FUNDED

Abstracts

36 AWARDEES

Clinical:
Djordje Atanackovic, M.D. | University of Maryland, Baltimore
Luis Garza, M.D, Ph.D. | Johns Hopkins University
Sarah Sunshine, M.D. | University of Maryland, Baltimore

Commercial:
Alla Danilkovitch, Ph.D. | Britecyte, Inc.
Jonathan Rowley, Ph.D. | RoosterBio, Inc.
Bhanu Telugu, Ph.D. | RenOVAte Biosciences, Inc.

Validation:
Curt Civin, M.D. | University of Maryland, Baltimore
Xiaoming He, Ph.D. | University of Maryland, College Park
Tao Lowe, Ph.D. | University of Maryland, Baltimore
Sheikh Amer Riazuddin, Ph.D. | Johns Hopkins University
Elias Zambidis, M.D., Ph.D. | Johns Hopkins University

Discovery:
Seth Ament, Ph.D. | University of Maryland, Baltimore
Xinzhong Dong, Ph.D. | Johns Hopkins University
David Hackam, M.D., Ph.D. | Johns Hopkins University
Robert Johnston, Ph.D. | Johns Hopkins University
Hanseok Ko, Ph.D., M.S. | Johns Hopkins University
Sashank Reddy, M.D., Ph.D. | Johns Hopkins University
Srinivasa Rao Sripathi, Ph.D. | Johns Hopkins University
Hilary Vernon, M.D., Ph.D. | Johns Hopkins University
Piotr Walczak, M.D., Ph.D. | University of Maryland, Baltimore
Jiou Wang, M.D., Ph.D. | Johns Hopkins University

Launch:
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Chengpeng Chen, Ph.D. | University of Maryland, Baltimore Co.
Jill Fahrner, M.D., Ph.D. | Johns Hopkins University
Moonjung Jung, M.D., M.S. | Johns Hopkins University
Yajie Liang, M.D., Ph.D.| University of Maryland, Baltimore
Mollie Meffert, M.D., Ph.D., M.S.| Johns Hopkins University
Raphael Meier, M.D., Ph.D. | University of Maryland, Baltimore
Brian O'rourke, Ph.D. | Johns Hopkins University
Emmanouil Tampakakis, M.D. | Johns Hopkins University

Post-Doctoral Fellowship:
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Lukasz Kalkowski, Ph.D. | University of Maryland, Balitmore
Katherine Marshall, Ph.D. | Johns Hopkins University
Maryam Rahmati, Ph.D. | Johns Hopkins University
Mario Gomez Salazar, Ph.D. | Johns Hopkins University
Wonjin Yun, Ph.D. | Johns Hopkins University

CLINICAL

Program

2022

Djordje Atanackovic, M.D.
University of Maryland (UMB)
Awardee Amount: $996,846
Disease Target: Cancer

A Phase Ia Study of LT2950 Tri-Specific CAR T-Cells for Patients with Relapsed / Refractory B-Cell Lymphomas

Chimeric antigen receptor (CAR) T cell therapy has emerged as a powerful immunotherapeutic approach for various forms of cancer, especially hematologic malignancies. CAR T cells directed against the B cell-associated antigen CD19 have revolutionized the treatment of B cell lymphomas. Relapse after CAR T therapy remains an obstacle, with as many as 50% of all CAR19 T cell therapy treated patients relapsing within the first year after treatment and many of them manifesting CD19 antigen loss. Therefore, there is a critical and unmet need to enhance the efficacy of CAR T cells and improve the depth and durability of responses in relapsed/refractory B cell lymphoma. Multi-specific CAR-T cell targeting of tumor cells has been postulated to mitigate tumor antigen escape and improve treatment outcomes. We propose that multiantigen targeting of B cell lymphoma-associated antigens, especially the three B cell antigens CD19/CD20/CD22, will be a treatment strategy with superior efficacy. None of the multi-specific CART clinical studies performed to date have targeted more than two antigens. The CD19/CD20/CD22 tri-specific CAR T cell product was developed by Maryland-based biotech company on the hypothesis that B cell tumor antigen escape may be overcome by a CAR design that simultaneously targets three B cell leukemia/lymphoma antigens. Trispecific duoCAR-T cells were engineered with lentiviral vectors encoding two CAR open reading frames that target CD19, CD20, and CD22. In mice bearing CD19-, CD20-, and CD22-negative escape variants, only the trispecific duoCAR-T cells rapidly and efficiently rejected the tumors, indicating that multispecific duoCAR-T cells represent a unique and very promising strategy to prevent the downregulation of target antigen and subsequent antigen loss mediated relapse in patients with B cell malignancies. We will perform a first-in-human clinical trial investigating tri-specific CAR T cells in patients with relapsed/refractory B cell lymphomas at a cancer center. The tri-specific CAR T cells will be produced in-house. Twelve patients will be enrolled over the next two years. Primary endpoint will be safety and tolerability with efficacy, pharmacokinetics/pharmacodynamics, CAR immunogenicity, disease biomarkers, CAR T cell persistence/function, and markers of CAR T cell exhaustion as secondary endpoints. If successful, results from this trial will have the potential to change the standard of care for these patients and provide them with better outcomes, more durable responses, and maybe even cures. In the long term, this novel treatment could move to earlier lines of treatment potentially providing patients with newly diagnosed B cell lymphoma better options and improved outcomes. Strategically, as a first-in-human project which has been developed, this clinical study will help to: provide patients with B cell lymphoma with a unique treatment opportunity that is only available at our center in Maryland, promote cooperation with industry partners resident in Maryland.

Luis Garza, M.D., Ph.D.
Johns Hopkins University (JHU)
Awardee Amount: $650,000
Disease Target: Amputees with limb loss

Autologous Volar Fibroblast Injection into the Stump Site of Amputees

Stem cell therapy holds great promise in medicine, but research faces hurdles to define basic parameters such as ideal schedule, ideal dose, evidence of long-term engraftment, and evidence of long-term tissue alteration. The skin is an ideal model system to test these variables given the accessibility and low morbidity for intervention, and the results may prove generalizable to the field of regenerative medicine. To begin to address these questions in the pursuit of a defined clinical goal, we have been using a fibroblast cell therapy in an effort to help the more than 2 million amputees in the US. Many improvements have been made in prosthetic designs, but amputees still underuse prosthetics or abandon their use altogether because of the skin irritation and break down that they cause. To enhance prosthetic use, we propose to imbue normal palmo-plantar (VOLAR) skin features such as pressure adaptation to the non-volar stump site of amputees. Our solution hinges on the ability of mesenchyme (fibroblasts) to influence epithelial (keratinocyte) gene expression. Since 2013, we have been working under a CBER IND to test how ectopic volar fibroblasts can alter skin function in normal healthy volunteers. With MSCRF clinical program funding, we have shown that injecting volar fibroblasts into the non-volar skin of more than 30 normal human subjects produces promising and significant changes compared to vehicle. We first tested for histologic endpoints known to be greater in native volar skin to see if these were enhanced in injected non-volar skin. Markers of volar skin include higher KRT9 expression, greater epidermal thickness, larger keratinocyte cytoplasmic size, and longer collagen fibrils. All of these markers were ectopically increased in non-volar skin after volar fibroblast injection ($n=31$, $p<0.03$; $n=31$, $p<0.005$; $n=29$, $p<0.007$; $n=11$ $p<0.04$, respectively) even after 5 months. Notably, in scRNA seq data we find persistent cellular changes 17 months after cell injection. In physical tests with a durometer, we find increased physical firmness at the site of volar fibroblast injections. The long-term engraftment of these cells and tissue changes of our model create a robust platform to test concepts of stem cell therapy toward the development of a new therapeutic. This represents our FINAL grant solicitation for this project. The motivations for this final grant application include delays from COVID despite fixed costs, a new matching-requiring NIH RMIP grant, and novel testing for a mixed product containing cells + collagen extracellular matrix. Hypothesis: Our phase 1 trial show changes in genetic, histologic, and physical firmness measures in injected non-volar skin that made it more like volar skin. Our novel data also suggest that inclusion of collagen with our cell therapy enhances efficacy. We hypothesize that the effects will be even more dramatic in amputees given the local pressure at the stump site and the likely evolutionary adaptation of volar fibroblasts to respond to pressure. Trial Design for Phase 2 1. Biopsy amputees at a volar site; submit tissue to Hopkins Cellular Therapy Core to expand fibroblast stem cells as we have done previously. 2. Inject whole stump with vehicle or volar cells+collagen 3 times over 1 week as per our Phase 1 protocol. 3. Wait for 1-5 months. 4. Perform noninvasive testing for epidermal thickness, skin firmness, and physical activity. 5. Test for quality of life improvements by questionnaire. In parallel, under our previous MSCRF funding, we propose to continue analyzing previously obtained clinical trial biopsies from human subjects to define the molecular phenotype of modified skin.

Sarah Sunshine, M.D.

University of Maryland, Baltimore (UMB)

Awardee Amount: $269,400

Disease Target: Ocular Graft Versus Host Disease

Mesenchymal Stem Cells for the Treatment of Ocular Graft Versus Host Disease

Patients with ocular Graft versus Host Disease (oGVHD) are a critical patient population with a unique autoimmune ophthalmic disease. Many patients who have allogeneic hematopoietic stem cell transplantation (HSCT) as part of their cancer therapy achieve complete remission from their cancer and subsequently develop Graft versus Host Disease (GVHD), a chronic autoimmune disease that causes significant damage to many organs including their eyes. oGVHD affects approximately 50% of individuals who receive an allogeneic HSCT and is one of the most debilitating aspects of GVHD. oGVHD symptoms range from dry eye to chronic pain resulting in chronic corneal epithelial disease that is poorly treated with available therapies. Standard of care treatments for oGVHD include lubrication with tear supplementation, reduction in inflammation through immunosuppression via local or systemic routes and ultimately surgical treatment for corneal perforation, however, current treatments for oGVHD inadequately control the patients disease. No current treatments specifically target promotion or regeneration of healthy epithelial tissue in this patient population. This study will evaluate Mesenchymal Stem Cell (MSC) based therapy to promote growth and regeneration of corneal epithelial disease in patients with oGVHD. We are participating in a multi-center clinical trial evaluating the benefit of MSCs in the treatment of chronic epithelial corneal disease. This study is funded through the Department of Defense and will allow the University of Maryland to recruit 40 patients. We at the University of Maryland School of Medicine are interested in MSC therapy to promote growth and repair epithelial defects due to oGVHD.

The MSCRF grant will allow us to focus specifically on patients with oGVHD and develop a more robust patient cohort. Additionally we will be able to go beyond the primary end points of photo-documented epithelial staining by investigating the underlying mechanism of MSCs on epithelial cells and neurogenic cells in the cornea through advanced imaging modalities. The MSCs will be used under an Investigational New Drug (IND) application through the Food and Drug Administration (FDA). MSCs are under investigation in multiple clinical trials for promoting tissue repair and modulating immune responses. Early results from ongoing studies are showing compelling evidence for the therapeutic effects of MSCs for promoting corneal repair. Specifically, the PI on this multicenter study recently reported the results of the first three patients with persistent corneal epithelial defects who were enrolled in the study (results presented at ARVO 2022). All three patients healed their epithelial defects. The specific aim of the clinical trial is to determine if application of allogeneic MSCs will improve the epithelial health in patients with oGVHD. The MSRF grant will allow us to perform advanced imaging modalities including laser scanning confocal microscopy (LSCM) and Meibomian Gland Interferometry (MGI). The LCSM will evaluate the inflammatory cells and nerve density in the cornea and the MGI will evaluate the tear and meibomian gland changes. We hypothesize that application of allogeneic MSCs will significantly promote tissue repair and regeneration to improve ocular surface health compared to the standard of care, minimizing sequelae.

COMMERCIALIZATION

Program

2022

Alla Danilkovitch, Ph.D.
Britecyte, Inc.
Awardee Amount: $270,000
Disease Target: Liver Fibrosis

A Mesenchymal Stem Cell Containing Matrix for Nonalcoholic Steatohepatitis (NASH)

Nonalcoholic fatty liver disease (NAFLD) and its progressive stage non-alcoholic steatohepatitis (NASH) are metabolic diseases with white adipose tissue (WAT) dysfunction that represent a leading cause of liver-related morbidity and mortality in the US and worldwide. According to the American Liver Foundation, 16.4 million patients in the US are affected by NASH, which is predicted to become the #1 reason for liver transplants by 2030. The US spends $5 billion a year on NASH healthcare alone; this cost is expected to more than triple in ten years. Despite 40 years of development, there are no approved therapies for NAFLD/NASH. Given the high prevalence and rapid growth of NAFLD/NASH, the economic and health burden, and NASH-associated morbidity and mortality, there is an unmet medical need for therapies that can stop, slow, or reverse the progression of NAFLD/NASH. Britecyte is developing BRC001, an innovative regenerative medicine platform technology targeting diseases with WAT dysfunction including NAFLD/NASH. BRC001 is a first in class off-the-shelf allogeneic engineered adipose product composed of a combination of adipose-derived MSCs and adipose tissue. Our proprietary process eliminates BRC001 immunogenicity allowing allogeneic use of the functional adipose without immunosuppression. Our strategy is to target the disrupted metabolism in patients WAT, which is the root cause of NASH.Preliminary results demonstrated that rat BRC001 improved NASH in a relevant diet-induced obese Zucker rat model without toxic effects; however, the efficacy of human BRC001, which is the clinical product, has not been evaluated. The MSCRF project is focused on establishing the efficacy of human BRC001 in diet-induced NASH animal models representing different NASH stages. The project will provide robust in vivo BRC001 efficacy data, uncover mechanisms of action, and define a target NASH patient population for BRC001, allowing Britecyte to transition from the preclinical to clinical phase of development.

Jonathan Rowley, Ph.D.
RoosterBio, Inc.
Awardee Amount: $270,000
Disease Target: N/A

Simplified Solution for Enhanced Production of High Purity Extracellular Vesicles from Mesenchymal Stem Cells

Extracellular vesicles (EVs) are lipid bilayer membrane bound particles released from cells. EVs contain biological contents such as proteins and nucleic acids. Research shows that EVs derived from human mesenchymal stromal cells (hMSC-EVs) can be used for therapeutic applications. Specifically, hMSC-EVs have been studied in over 200 preclinical applications and dozens of clinical trials. A major bottleneck for hMSC-EV therapies is the manufacturing requirement for a large number of cells to produce a clinically relevant hMSC-EV dose. RoosterBio (RBI) is an industry leader in rapid hMSC expansion within 3D bioreactors, allowing us to move beyond this traditional bottleneck and solve new challenges in hMSC-EV manufacturing. Existing hMSC-EV collection processes allow for a cell growth phase (approximately 5 days) followed by an EV collection phase (approximately 3 days). Simplifying this process to include collection of hMSC-EVs during growth phase as well as during collection phase would enable a higher hMSC-EV yield. However, this will require use of a defined cell growth medium, as current growth media include undefined biological components that contain process impurities, complicating hMSC-EV isolation and purification. We propose to develop a simplified solution for hMSC-EV production that will eliminate media exchange steps and extend the collection window for hMSC-EVs, thus significantly increasing the number of hMSC-EVs collected per cell. Additionally, since the medium component will be defined and thus free of process impurities, the resulting hMSC-EV preparation will be of a higher purity than current preparations. In summary, this proposal will develop an instrumental solution for large-scale hMSC-EV production to improve hMSC- EV collection for researchers worldwide and provide the hMSC-EV material necessary for extensive clinical investigations. With development of this solution, RBI will continue to put Maryland on the map as a leader in stem cell technology through further generation of jobs and revenue for the state.

Bhanu Telugu, Ph.D.

RenOVAte Biosciences, Inc.
Awardee Amount: $269,500
Disease Target: Liver Insufficiency

Growing Exogenous Human Liver in Pigs

In the United States alone, more than 123,000 men, women and children currently need lifesaving organ transplants. Every 10 minutes another name is added to the national organ transplant waiting list. Sadly, an average of 22 people die each day due to lack of available life-saving organ, with the numbers expected to increase every year. The same is true for patients on liver transplantation waitlist. In the United States, there are an average of 12,000 patients waiting for a liver transplantation at any given time. The ability to generate exogenous organs in pig for transplantation into humans (xenotransplantation) is considered as one of the sources to bridge this shortfall. Pig is already being used for xenotransplanation studies as the size of the animal, organs and physiology are similar to humans. Several tissues from pigs (heart valves, bladder, cornea, etc) are already being used or in advanced stages of product development for transplantation into humans.

The main goal of our Company is to generate organs of endodermal origin, in this case liver from donor progenitor cells called extraembryonic endodermal cells or XEN cells established from patient- specific stem cells using pig as a bio-incubator. Following technical validation, this will provide a pathway for revenue generation by providing on-demand source of transplantation ready hepatic cells for cellular therapies and will plug-in into the associated technologies such as organ-on chips, 3D- printing, and pharmaceutical applications in the short-term. In the long-term, the goal is to generate immune-compatible transplantation ready solid organs for transplantation.

VALIDATION

Program

2022

Curt Civin, M.D.
University of Maryland, Baltimore (UMB)
Awardee Amount: $230,000
Disease Target: Hematologic Malignancies & Blood Disorders

Validation of the GPB Curate Cell Processing System for manufacture of therapeutic hematopoietic stem cells

Allogeneic hematopoietic stem cell (HSC) transplant has become a clinical standard treatment for several benign and malignant diseases, and gene therapy clinical trials utilizing genetically modified autologous HSCs are reporting early successes. HSCs comprise only <1% of immunophenotype-defined CD34+ hematopoietic stem-progenitor cells (HSPCs), but these rare HSCs are the only cells capable of in vivo hematopoietic engraftment and reconstitution. Therefore, high yield and good quality of HSCs are absolutely necessary for successful clinical HSC transplantation and HSC gene therapy. Our team recently developed Deterministic Lateral Displacement (DLD) microfluidic chips and the complete upscaled GPB CurateR system to optimally process full-size non-mobilized human blood leukaphereses for the purpose of harvesting WBCs in high yield and with T lymphocyte functionality, explicitly to enhance manufacturing of therapeutic CAR-T cells. The GPB DLD microfluidic cell separation techniques can process blood cells by automated flow through a microfluidic chip containing a specifically designed array of micropost-defined channels which is tilted a small angle from the fluid stream direction. This unique design of DLD microfluidic chip can allow to separate blood cells based on the cell size. The CurateR system is a disposable, closed fluid paths, and automated cell processing platform with a hand-on time of only 10 minutes. Therefore, we believe that the CurateR system will simplify cell processing workflows by reducing the number of cell washing steps and resources, as well as the hands-on time. In this Validation grant application, we propose to rigorously confirm (or deny) that this same CurateR system can process mobilized human peripheral blood leukaphereses in order to harvest hematopoietic progenitor cells (HPCs) and HSCs from either G-CSF- or Plerixafor-mobilized human blood leukaphereses in high yield and with HPC/HSC functionality for clinical HSC transplantation and gene therapies.

Xiaoming He, Ph.D.
University of Maryland, College Park (UMD)
Awardee Amount: $230,000
Disease Target: Heart Disease

ROCK Inhibitor-Free 3D Culture and Cardiac-Differentiation of Human iPSCs and Banking of iPSC-Derived Cardiac Organoids

Heart disease is the leading cause of death in the US. Deriving cardiomyocytes (particularly in the form of 3D beating cardiac organoids) from human induced pluripotent stem cells (iPSCs) has been proposed as a promising strategy/model for understanding, preventing, and treating heart disease. Recently, we discovered that ROCK inhibitor that has been commonly used in 3D cultures of human iPSCs for improving their viability/yield, compromises their quality. By reducing the RI concentration, the quality and efficiency of iPSC cardiac differentiation in 3D can be greatly improved, but at the cost of a reduced yield/viability of the human iPSCs in 3D culture. We further developed a novel method of cold-triggered detachment of the cells for culture in 3D with high viability and pluripotency/quality without using any RI. This enables highly efficient differentiation of the cells into 3D beating cardiac organoids with high quality and yield. We will further validate this RI-free approach for 3D culture and cardiac differentiation using another commonly used human iPSC line, as the first specific aim of this project. For the human iPSC-derived beating cardiac organoids to be a commercially available product, they must be banked via cryopreservation for wide distribution and ready-availability. We have successfully developed a novel natural sand-based technology to improve the quality and viability/yield of banking human iPSCs. We will further validate this technology for banking the human iPSC-derived 3D beating cardiac organoids with high post-cryopreservation functional survival, as the second specific aim of this project. In summary, this project will validate two innovative technologies for 1) high-yield production of high-quality human iPSC-derived cardiac organoids and 2) successful cryopreservation of the cardiac organoids for ready availability and wide distribution. Both technologies are urgently needed for understanding, preventing, and treating heart disease, the leading cause of death in the US.

Validation Grant Awards

Tao Lowe, Ph.D.

University of Maryland, Baltimore (UMB)

Awardee Amount: $230,000

Disease Target: Bone Defects

3D-Bioprinted Allograft-Polymer Hybrid Constructs for Bone Regeneration

Bone injuries and diseases continue to increase with an aging global population. Critical sized defects and degenerative diseases hinder endogenous bone regeneration. There is unmet clinical need to develop bone graft substitutes to treat skeletal and cranio maxillofacial fractures and defects, use for dental implant surgery, and replace and regenerate lost bone caused by osteonecrosis. Tissue engineering combining with stem cell therapy and 3D-bioprinting promises to replace damaged tissues via cell-laden scaffolds with good fabrication uniformity and reproducibility. However, currently there is not a scaffold system that can mimic the natural process to regenerate functional bone. The overall goal of this project is to develop scaffolds mimicking the natural bone architecture and signal pathways to differentiate stem cells into osteoblasts to construct patient-specific and defect-site-specific bone grafts. The objectives of this proposal are to 1) develop 3D-printed constructs containing bone allograft, biodegradable poly-caprolactone, biodegradable nanogels loaded with osteogenic growth factors bone morphogenetic protein-2 (BMP-2) and platelet derived growth factor-BB (PDGF-BB), and dental pulp or induced pluripotent stem cells (DPSCs or iPSCs), interconnected pores, and bone defect sizes; and 2) provide natural bone-mimicking chemical, physical, mechanical, and biological cues to maintain the stemness of DPSCs/iPSCs and preferentially differentiate the stem cells into osteoblasts to regenerate new bone tissues at the bone defect site after implantation in a rat model with critical bone defect size. The proposed 3D construct is highly significant and innovative with a solid premise and intellectual property protection. If successful, this project will present a new paradigm for both scientific understanding and clinical practice for bone regeneration. Experts in biomaterials, animal models for bone repair, bone and periodontal regeneration, and bone allografts and surgery will work together to translate the proposed revolutionary technology from bench to bedside to treat bone defects/injuries.

Sheikh Amer Riazuddin, Ph.D., M.S.

Johns Hopkins University (JHU)

Awardee Amount: $229,986

Disease Target: Corneal Endothelial Dystrophies

Validating the Utility of Pluripotent Stem Cell-Derived Corneal Endothelial Cell Sheets in Endothelial Keratoplasty

Cornea is the outermost, transparent tissue of the eye composed of five layers. Corneal endothelium (CE) is the innermost layer essential for maintaining corneal clarity by mediating hydration through barrier and pump functions. Corneal endothelial dystrophies are the leading cause of corneal transplantation performed in the United States (US) each year, and although keratoplasty, has been successful in treating corneal edema, the worldwide shortage of transplantable-grade donor CE remains an insurmountable obstacle in reducing corneal blindness. We previously generated corneal endothelial cells (CECs) from human embryonic stem cells (hESCs) and demonstrated that cryopreserved hESC-derived CECs can form a functional monolayer of CE on denuded Descemets membrane (DM) in rabbits and monkeys when injected into the anterior chamber (mimicking treatment for a mild phenotype). We recently further demonstrated that cryopreserved hESC-derived CECs can form a functional monolayer of CE on the denuded stroma in rabbits and monkeys (mimicking treatment for moderate phenotype where the pathology does not remain localized to CE but extends to DM, the second innermost layer of the cornea). In many corneal endothelial dystrophies, that are prevalent in the US, the pathology affects the DM, which is thickened exhibiting pathological microtopography, and therefore, the injection of CECs is not likely to form a monolayer necessary for the physiological functioning of the CE. Here, we propose to validate the hESC-derived CEC sheet (instead of dissociated hESC-derived CECs) as an alternative to donor tissue in corneal endothelial keratoplasty. In this approach, both CE and DM will be removed followed by transplantation of the hESC-derived CEC sheet in two preclinical models, rabbits, and monkeys. Validating the efficiency of the hESC-derived CEC sheet will broaden the clinical utility of hESC-derived CECs in keratoplasty to include corneal endothelial dystrophies with severe corneal edema.

Elias Zambidis, M.D., Ph.D.

Johns Hopkins University (JHU)

Awardee Amount: $230,000

Disease Target: Diabetes, Vascular Disease

Commercial Validation of Clinical-Grade Progenitors from TIRN hiPSC

We have filed IP claims and published methods for the derivation of a new class of human stem cells termed Tankyrase/PARP inhibitor-regulated nave human induced pluripotent stem cells (TIRN-hiPSC). Chemical reprograming of primed, conventional hPSC to TIRN-hPSC alleviates dysfunctional epigenetic and disease-associated donor cell memory and generates multi-lineage nave progenitors with reduced lineage-primed gene expression and dramatically improved in vivo engraftment and functionality. Although TIRN-hiPSC-derived nave progenitors have wide impact for regenerative medicine, their broad clinical application via autologous patient-specific approaches faces important practical limitations associated with hiPSC technology in general. For example, the cost involved in screening individual hiPSC lines for high-quality clones makes patient-specific therapies inaccessible in health care systems with limited resources. Moreover, the labor of validating genomic integrity and functionality of individual, autologous hiPSC is not sustainable in the long term.

Even if these challenges could be addressed, personalized hiPSC lines are not readily available for acute disorders requiring immediate therapeutic intervention (e.g., myocardial infarction, cerebro-vascular stroke). As an alternative to patient-specific cellular therapies, we have proposed a universal application of our tankyrase/PARP inhibitor nave (TIRN) methodology with universal donor banks; reprogrammed from inventories of clinical-grade, HLA-typed cord blood (CB) and peripheral blood (PB) samples. In this project we will validate the feasibility of the goal of creating HLA-defined UTIRN-hiPSC banks in collaboration with a commercial partner. Our Aim will functionally validate nave vascular progenitors (N-VP) differentiated from TIRN-hiPSC in defined xenofree (XF), feeder-free (FF) defined conditions with molecular and epigenetic testing for this future goal. This goal will be coordinated with a commercial partner to optimize our TIRN method into a commercial cell product in current good manufacturing practice (cGMP)-compliant conditions for future therapeutic Phase I/II clinical trials.

DISCOVERY

Program

2022

Seth Ament, Ph.D.

University of Maryland, Baltimore (UMB)
Awardee Amount: $343,477
Disease Target: Huntington's Disease

Developing A Stem Cell Model of Epigenetic Dysregulation in Huntington's Disease

Epigenetic dysregulation in neural cells is a hallmark of many neuro-degenerative diseases. Epigenetic therapies aim to restore the epigenome to a healthy state by targeting the activity of chromatin modifying enzymes. However, the development of epigenetic therapies has been hindered by incomplete knowledge about the specific epigenetic changes occurring in neurodegenerative diseases, as well as by the need for new therapies that more precisely modify the epigenome. Here, we will address these challenges, focusing on a model neurodegenerative disease, Huntington's disease (HD), due to its genetic and clinical homogeneity and well-established epigenetic dysregulation. HD is the most common monogenic cause of neurodegeneration, affecting ~1 in 10,000 individuals, all of whom share a causal trinucleotide repeat expansion in the HTT gene. Epigenetic and transcriptional dysregulation in affected brain regions is a pronounced feature of HD and in cellular and animal models expressing the mutant HTT (mHTT) protein. Our exciting preliminary data in HD mouse models suggest that epigenetic dysregulation is a direct effect of mHTT and implicate partial loss-of-function of the polycomb repressive complex (PRC2), including reduced levels of the H3K27me3 chromatin mark written by PRC2. However, the precise interactions between HTT and PRC2 on chromatin DNA and their impacts on gene regulation remain poorly understood, especially in disease-relevant human cell types. In our proposed work, we will characterize transcriptional and epigenetic dysregulation caused by HD mutations in human neural cells and establish proof-of-principle for an epigenetic therapy, using an established human pluripotent stem cell model (HD-hiPSCs). In Specific Aim 1, we will profile HD-hiPSC-derived neural cells at two disease-relevant time points: striatal spiny projection neuron (SPN)-like neurons, which model the neuronal subtype most vulnerable to neuro-degeneration, and neural rosettes, which model deficits in corticogenesis. We will apply single-cell multi-omics (RNA-seq + ATAC-seq co-assays in thousands of single-cells), as well as chromatin immunoprecipitation and sequencing (ChIP-seq) of the huntingtin protein, the PRC2 component EZH2, and the H3K27me3 chromatin mark. Integrated analyses of these data, we will provide insight into the interactions of HTT occupancy, PRC2 occupancy, chromatin accessibility, and their impacts on gene expression. In Specific Aim 2, we will test the hypothesis that restoring H3K27me3 levels rescues transcriptional and cellular effects of HD mutations. Our primary strategy is to silence the H3K27me3-specific histone lysine demethylases KDM6A and KDM6B. Previous studies have shown that silencing these enzymes can increase H3K27me3 levels in multiple cell types and is well tolerated in cells lacking PRC2. We will knockout KDM6A and KDM6B in HD-hiPSCs by CRISPR/Cas9 and test that silencing KDM6 rescues H3K27me3 levels, gene expression, vulnerability of SPN-like neurons to degeneration, and deficits in neural rosette formation. Results will be confirmed by silencing KDM6 using commercially available small molecule inhibitors.

Xinzhong Dong, Ph.D.

Johns Hopkins University (JHU)
Awardee Amount: $345,000
Disease Target: Chronic Pain

Generation and Characterization of Human Mrgprx1+ Sensory Neurons for Developing Novel Anti-Pain Therapies

Itch and pain sensation, a subtype of somatosensation, can be evoked by a wide range of compounds with a variety of chemical structures, including small molecule compounds, peptides, proteases, cytokines, and lipid components. These pain and itch-inducing substances activate the membrane receptors and/or ion channels that are expressed in sensory nerve endings in the skin, and the activation of these receptors triggers the electrical impulses that are delivered through a series of connected nerves to the brain, where the itch/pain sensation is generated. It is estimated that over one third of the world's population suffers from persistent or recurrent pain caused by neurological disorders, diabetes, car accident, war injuries, chemotherapy, etc. Most drugs on the market for pain and itch sensation have undesired side effects because their targets exist both inside and outside the pain pathways. Human Mas-related G protein-coupled receptor X1 (MRGPRX1) is a promising target of novel pain and itch inhibitors, mainly because of its restricted expression in primary nociceptive neurons. However, the functional properties of MRGPRX1 cannot be fully inferred from rodent analogs, owing to cross-species variations in MRGPR agonist activity and receptor function. To address this issue, we will generate human MRGPRX1+ neurons for understanding the cell specification processes and dissecting functional heterogeneity of MRGPRX1-expressing nociceptive, pruriceptive neurons in vitro and in vivo. Our proposal will not only gain new insight on stem cell fate determination processes toward a specific sensory neuronal subtype, but also build a foundation to develop novel drug candidates for managing persistent pain/itch conditions.

Discovery Grant Awards

David Hackam, M.D., Ph.D.

Johns Hopkins University (JHU)
Awardee Amount: $345,000
Disease Target: Necrotizing Enterocolitis

Amniotic Fluid Derived Stem Cells for the Treatment of Necrotizing Enterocolitis in Premature Infants

The current project advances the goals of the MSCRF and this RFA by seeking to develop an off-the-shelf treatment for necrotizing enterocolitis based upon the use of genetically optimized, amniotic fluid-derived human stem cells. To do so, we have assembled a diverse team that includes three Maryland-based clinician-scientists from Johns Hopkins University: pediatric surgeon-scientist and expert in stem cells in necrotizing enterocolitis, Dr. David Hackam (PI); obstetrician Dr. Irina Burd (co-investigator), and current MSCRF grantee and expert in stem cell biology, pediatric surgeon Dr. Shaun Kunisaki (co-investigator). The studies will be supported through a collaboration with Dr. Paolo de Coppi at University College who first described the amniotic fluid derived stem cells, and who is a leading authority on their biology. Necrotizing enterocolitis (NEC) is a leading cause of death and disability in premature infants. The typical child with NEC is an infant in the neonatal intensive care unit who acutely develops abdominal distention and bloody stools, and then after 24-48h is either dying or dead. There is no specific treatment for NEC, and approximately 1/3 of the 5000 infants in the USA who develop NEC will die. NEC is particularly devastating in premature infants from minority backgrounds, highlighting significant disparities in their neonatal outcomes. Given that NEC survival has not significantly improved in the past 30 years, and the disparate impact on the most vulnerable children, there is an urgent need for new NEC treatments. This proposal will seek to utilize human amniotic fluid stem cells as a novel treatment for infants with NEC. Amniotic fluid-derived stem cells (AFSCs) are present within the amniotic fluid from the first trimester, and can be generally classified as tyrosine kinase receptor c-kit positive or negative. This proposal will focus on human c-kit+ AFSCs that fulfill the properties consistent with the MSCRF RFA (divide indefinitely, give rise to many other types of specialized cells; give rise to new stem cells with identical potential mechanism). The proposal is innovative in utilizing human AFSCs that will be tested for their ability to treat NEC in mouse and piglet models, and validated in a human organoid based NEC-in-a-dish discovery platform after a single cell RNA-seq based optimization strategy. Hypothesis and specific aims (see figure):. We hypothesize that human c-kit+ amniotic fluid derived stem cells can be used to treat necrotizing enterocolitis in experimental models. We further hypothesize that the use of single cell RNA-seq will allow us to identify novel genes that drive the anti-NEC effects of human amniotic fluid-derived stem cells, and which can then be capitalized on to improve the ability of human AFSCs to treat NEC. We will test this hypothesis in two specific aims:. AIM 1. To evaluate the ability of amniotic fluid-derived stem cells to treat NEC in mice and piglets, and to determine the mechanisms involved. AIM 2. To identify and functionally characterize novel genes that regulate the anti-NEC properties of human amniotic fluid stem cells using single cell RNA-seq, and to optimize these stem cells for off-the-shelf NEC treatment.

Robert Johnston, Ph.D.

Johns Hopkins University (JHU)
Awardee Amount: $345,000
Disease Target: Glaucoma

A Human Retinal Organoid Model to Study Retinal Ganglion Cell Biology

Human retinal organoids hold promise to provide biologic agents for vision therapy. To realize this potential, we must develop methods to manipulate the generation of specific cell types. Retinal ganglion cells (RGCs) are projection neurons in the retina that transmit visual information from the eye to the brain. As RGCs die or become impaired during glaucoma, there is great interest in utilizing stem cell-based sources of RGCs for therapeutic applications. Though RGCs are produced in human retinal organoids, enthusiasm towards their translational use has been tempered by their reported loss/death. To address this challenge, we are developing human retinal organoids as a model to understand the biology of human RGCs. This project aims to develop methods to increase the production of RGCs, promote their survival, and understand the diversity of RGC subtypes in human retinal organoids. This project aligns with the goals of the MSCRF to develop new strategies for the treatment of human diseases through stem cell research. We established human retinal organoids as a model to study cone photoreceptor subtype specification. Our work advanced retinal organoids as a model for determining mechanisms of development with promising utility for therapeutics and vision repair (Eldred et al., 2018, Science; Hadyniak et al., 2021, bioRxiv, Science in revision)1,2. Based on this success, we next aimed to understand RGC biology in human retinal organoids. In our studies of signaling pathways, we found that high retinoic acid (RA) levels, as commonly used in many retinal organoid differentiation protocols, limited RGC production, whereas the absence of RA promoted RGC generation. To understand how RA influences RGC generation, we will alter the timing and concentrations of RA during organoid differentiation and quantify RGC yield. As RGC loss has been reported in human retinal organoids, we assessed the timeline of RGC generation, maintenance, and loss. A subpopulation of RGCs survive, whereas other RGCs are lost in a manner consistent with human/vertebrate retinal development, suggesting that preventing cell death will yield RGC-rich organoids. To prevent RGC death, we will genetically and pharmacologically inhibit the apoptotic pathway. As innervation is critical for RGC survival, we developed an RGC axon outgrowth assay, which we will implement to improve RGC maintenance. The diversity of human RGC subtypes remains unclear. As single cell RNA sequencing experiments have not provided a definitive classification of human RGC subtypes, we designed a mesoscale, multiplex RNA FISH approach to visualize gene expression in RGCs in sectioned tissue and characterize subtypes. We will integrate visual transcriptomic and scRNAseq methods to examine gene expression and RGC cell fates in normal and RGC-rich organoids. These studies will improve our understanding of RGC biology and generate a validated source of human RGCs for translational applications. Successful completion of these experiments will lay the groundwork to genetically and pharmacologically manipulate RGC subtypes in the future. This project will provide the basis to build a stem cell-derived assembloid model connecting human retinal and brain organoids in a physiologically meaningful way, changing how we study human visual system development, function, and disease.

Hanseok Ko, Ph.D., M.S.
Johns Hopkins University (JHU)
Awardee Amount: $345,000
Disease Target: Alzheimer's Disease

Validation of Ripk2 Kinase As A Novel Therapeutic Target for Alzheimer's Disease

Alzheimer's disease (AD) pathophysiology is a complex cascade of protein interactions and molecular pathway activation that is associated with pathologic amyloid beta folding and subsequent neuronal cell death. There is growing recognition that one of the critical steps in neurodegeneration in AD is neuroinflammation, characterized by activation of microglia and astrocytes. Recently, we described neurotoxic reactive astrocytes that we observed in various human neurodegenerative diseases including Parkinsons disease (PD) and AD. We found that activation of microglia leads to the conversion of resting astrocytes into neurotoxic reactive astrocytes via secretion of IL-1alpha, TNF-alpha, and C1q (reported as neurotoxic astrocytes inducers). Building on these findings in primary murine neuronal cultures, we discovered that amyloid beta oligomers, which mimic pathologic amyloid beta in AD, activate microglia to induce neurotoxic astrocyte formation and neuronal death in vitro. We observed evidence for microglial activation and neurotoxic astrocytes formation that correlates with neurobehavioral deficits in a mouse model of AD. However, the mechanisms underlying the activated microglia/astrocyte axis in the mediation and acceleration of the neurodegeneration in AD remains unclear. As such, an important role for the activated microglia/astrocyte axis in AD needs to be studied in great depth. Importantly, our preliminary study indicates that RIPK2 (receptor-interacting serine/threonine-protein kinase 2) is active in microglia in AD brains and genetic depletion and pharmacological inhibition of RIPK2 inhibit the microglia activation, neurotoxic astrocytes formation and neuronal death due to amyloid beta oligomers in murine cells suggesting RIPK2s role in the activated microglia/astrocyte axis in AD pathogenesis. Based on these findings, in Specific Aim 1, we will further determine the findings in human cells using microglia derived from healthy iPSCs (hiPSCs) and AD-iPSCs (APP-swe het and PS1-m146v het iPSCs) with or without homozygous RIPK2 depletion, and astrocytes and cortical neurons derived from hiPSCs that may afford us a more predictive model of human brain. Furthermore, we will identify and characterize the down-stream pathway associated with RIPK2 activation in neuroinflammation due to amyloid beta oligomers by employing proximity-dependent biotin identification (BioID) of RIPK2 from microglia derived from hiPSCs with or without RIPK2 depletion. In Specific Aim 2, we will validate the effect of a newly devolved RIPK2 inhibitor, CMPD0673 with highly potent and blood brain barrier (BBB) permeable on the microglia activation, neurotoxic astrocytes formation and neuronal death due to amyloid beta oligomers in human cells. To this end, microglia derived from hiPSCs and AD-iPSCs (APP-swe het and PS1-m146v het iPSCs) with pharmacological inhibition of RIPK2, and astrocytes and cortical neurons derived from hiPSCs will be utilized. This study enables us to uncover the role of RIPK2 activation on microglia activation, neurotoxic astrocytes formation and neuronal death due to amyloid beta oligomers in human cells. Also, this investigation will provide new insights into the pathogenesis in AD and leads to promising novel therapeutic agent for the treatment of AD.

Sashank Reddy, M.D., Ph.D.
Johns Hopkins University (JHU)
Awardee Amount: $344,432
Disease Target: Acute & Chronic Cutaneous Wounds

Mobilization of Skin Resident Stem Cells to Drive Skin Growth

Acute and chronic skin wounds are a major problem in clinical medicine. Chronic wounds alone afflict more than 8 million Americans and their management costs more than $25 billion per year. The burden of chronic wounds is growing with the aging of the population and increased incidence of co-morbidities such as obesity and diabetes mellitus. Similarly, acute skin deficits from trauma, burns, and surgical resection are extremely common, with more than half a million patients treated yearly for burns alone in the United States. Current strategies for replacing damaged or missing skin from these varied causes consist of autologous skin grafts and flaps and topical skin substitutes. Both approaches have significant drawbacks. Autologous skin reconstructions can cause donor site morbidities, transferred skin does not always survive in its new location, and it is often not as durable as the skin that was originally lost. Skin substitutes, on the other hand, have widely varying degrees of success and are prone to being rejected or infected. Given the problems with existing approaches, there is a major need for regenerative solutions that enhance the bodys ability to repair cutaneous defects with durable, full-thickness, color and texture matched skin. One approach to creating functional, autologous skin is known as tissue expansion. This entails the surgical placement of silicone balloons (tissue expanders) under the skin and subsequent filling with saline over months. The gradual volumetric expansion of the device leads to growth of the overlying skin much like a growing fetus expands abdominal skin in pregnant women. While this process can create full thickness autologous skin, it is hampered by serious limitations including the propensity for expanders to become exposed or infected, and the decreased tensile strength of the newly created skin. Hindering our ability to overcome these limitations is our poor mechanistic understanding of how skin growth is induced. Recently, we developed a system of tissue expansion in genetically tractable mice that revealed a critical role for Lgr6-expressing keratinocyte stem cells in creating new skin. In the studies proposed here, we aim to build on this promising work to define the cellular and molecular determinants of skin growth in human and mouse systems. By manipulating the signals and stem cells uncovered by these studies, we aim to enhance tissue expansion to create high quality, functional skin on demand. Our approach is readily translatable since it is grounded in two clinically proven techniques in reconstructive surgery tissue expansion and cutaneous stem cell therapy. The ex vivo expansion of keratinocyte stem cells represents the first successful example of a regenerative cell therapy, with cultured epithelial autografts from these cells first coming into clinical use in the 1980s. A recent successful effort to edit a patients keratinocyte stem cells prior to transplantation, and clinical transfer of stromal cells prior to tissue expansion, further validate the possibility of adoptive cell therapies for skin diseases. In the proposed studies we will discover the fate of key cutaneous stem cells and the signals driving their growth using cutting edge machine learning guided tissue reconstruction and genomics approaches. The insights gained from these studies will be used to augment skin resident cells and their pro-regenerative signals during expansion, creating a broadly applicable regenerative therapy for orphan and common causes of skin loss.

Discovery Grant Awards

Srinivasa Rao Sripathi, Ph.D.

Johns Hopkins University (JHU)
Awardee Amount: $345,000
Disease Target: Age-Related Macular Degeneration

High-Throughput Screen for Small Molecules That Inhibit RPE-EMT as an Approach for Treatment of AMD

Age-related macular degeneration (AMD) is a leading cause of irreversible vision loss and blindness in developed countries. Despite important advances in the treatment of the neovascular, or wet, form of the disease, there are currently no approved or validated treatments for the more common atrophic, or dry, form of the disease. Multiple environmental factors, including exposure to growth factors, cytokines, cigarette smoking, and oxidative stress, all of which accumulate with age, can promote injury of the retinal pigmented epithelium (RPE), the cells that support the health and function of photoreceptor cells, and increase the risk of developing AMD. Early indications of AMD-associated RPE damage include development of extracellular deposits of drusen between Bruchs membrane and RPE, appearance of clumps of pigment in the macula, and the formation of lipofuscin (subretinal deposits of oxidized proteins and lipids) within the RPE. Damage of RPE initiates stress response pathways that result in induction of the epithelial-mesenchymal-transition (EMT) which leads to RPE de-differentiation, dysfunction [1-4] and ultimately cell death. Indeed, the RPE-EMT response has been reported to be involved in both atrophic AMD as well as in the scarring associated with neovascular AMD[5-7]. To greater understand these early features of AMD, there is a critical need for better models to study both the pathways that lead to RPE damage, as well as the response pathways that result in RPE de-differentiation and death. The advent of efficient methods for differentiating human embryonic (hES) and induced pluripotent (hiPS) stem cells into RPE now makes it possible to study human dry AMD-associated RPE degeneration and dysfunction in an experimentally tractable fashion, which is a particularly significant development for the AMD field because of the relative lack of widely accepted and clinically relevant atrophic AMD animal models. The RPE generated from hES and hiPS cells [8, 9] have morphological, gene expression, and functional properties very similar to native human RPE cells[10, 11] so we can now use cultured hRPE as a model to identify pathways that modulate RPE response to specific AMD-associated stress, thus allowing the potential discovery of targets that can prevent and/or reduce RPE cell damage and death, and there by preserve vision. For this project, we are proposing to use hRPE as a model to better understand the key regulatory factors that initiate the common RPE-EMT. We will use hRPE to develop a platform to better understand the molecular mechanism and regulation of this stress response; a means to identify genes, using CRISPR-Cas9-based gene activation and interference (CRISPRa/i), that can be targeted to inhibit this early event that is associated with the progression of age-related macular degeneration (AMD) and other retinal diseases. Inhibition of the pathways that drive RPE-EMT can be a therapeutic intervention to maintain RPE integrity both for RPE transplantation applications as well as for potential treatment of RPE degeneration in disease states.

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

Johns Hopkins University (JHU)
Awardee Amount: $345,000
Disease Target: Barth Syndrome

Cardiolipin Modification In iPSCs: Developing A Treatment for Tissue-Specific Mitochondrial Defects in Barth Syndrome

Barth Syndrome (BTHS) is an X-linked genetic disease caused by defects in TAFAZZIN (TAZ), a gene encoding 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 and a reduction of mature CL, resulting in significant mitochondrial dysfunction in affected organs. Clinically, BTHS is characterized by intermittent neutropenia and early-onset cardiomyopathy, which carries a high risk of morbidity and mortality. Interestingly, and exceptional among most mitochondrial disorders, there are no central nervous system effects in BTHS. Two recent discoveries about the mitochondrial pathology in Barth syndrome have laid the groundwork for the research described in this grant proposal. (1) We recently identified two major areas of mitochondrial pathology in an HEK293 BTHS model with high relevance to cardiac function: defects in PARL and PGAM5, two key regulators of mitochondrial quality control (MQC), and decreased expression and function of respiratory complex I (CI). (2) Our collaborator established the molecular basis for the tissue-specific CL acyl chain profiles and found that affected and unaffected tissues in BTHS have very different acyl chain profiles. They further showed that these CL profiles are modifiable by altering the extracellular fatty acid environment. In this grant application, we propose to use a newly established CRISPR-edited TAFAZZIN-deficient iPSC model that will be differentiated into different cell types to establish the tissue-specific contribution of MQC and CI dysfunction in affected (cardiomyocyte) and unaffected (neuron) cell types in BTHS. We propose to then manipulate CL content by precisely controlled fatty acid supplementation in affected cells in order to remediate these mitochondrial pathologies. From a therapeutic development standpoint, dietary supplementation with lipids is an approach already in use in several inborn errors of metabolism, and significant shifts in cellular lipid content via changes in dietary intake and lipid supplementation is achievable within a short period of time. Therefore, we have a high expectation for the success of our approach. 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 at the Kennedy Krieger Institute in Baltimore (now in its 11th year), IRB approved longitudinal and cross-sectional natural history studies, an IRB approved biobank at Johns Hopkins University for biological materials from affected individuals (plasma, urine, whole blood), and establishment of model cell lines (patient derived lymphoblastoid and fibroblast cell lines, HEK-293 edited cell lines), and completion of the first clinical trial in Barth Syndrome. We have also established collaborations with multi-disciplinary academic teams, family organizations including the Barth Syndrome Foundation, and industry partners. Our established cellular models and research tools have been, and will continue to be, an invaluable tool available to the BTHS research community and to the wider community of scientists studying CL defects.

Piotr Walczak, M.D., Ph.D.
University of Maryland, Baltimore (UMB)
Awardee Amount: $345,000
Disease Target: Stroke

Intra-Arterial Administration of Human MSCs Secreting P2X7-Blocking Nanobody as Adjuvant Therapy for Acute Stroke

Stroke is the third leading cause of death and the leading cause of severe chronic disability in adults, affecting nearly 800,000 people in the U.S. each year. It burdens public health systems and is expected to increase hospitalization and rehabilitation costs due to the aging of Western societies. The efficacy of current treatments (mechanical thrombectomy, and pharmacological thrombolysis) is undisputed, but unfortunately, most patients still develop lifelong neurological deficits. As understanding of the pathomechanism of stroke increases, attractive therapeutic targets are identified. Hence, there is hope to develop adjuvant therapies that could be implemented immediately after thrombectomy. We argue that while thrombectomy creates an opportunity to save the brain, it is imperative to manage the acute neuroinflammatory insult, which causes more severe secondary brain damage. By managing this acute insult, we can buy time for stem cells to unfold their full biological potential. Among the many factors contributing to the acute neuroinflammatory cascades, a prominent one is the adenosine triphosphate (ATP), released by cells injured by ischemia and through its P2X7 receptor, that drives downstream inflammatory pathways. In this context, the selective blocking of these receptors has been shown to be an excellent therapeutic strategy for stroke. Nanobodies, recombinant single domain antibodies (sdAbs) derived from camelid heavy-chain antibodies, are a promising new technology platform. However, the challenge is that systemically delivered nanobodies fail to accumulate in the brain at the desired concentrations. Taking advantage of the unique properties of mesenchymal stem cells (MSCs), we propose to address this challenge by engineering them with the mRNA, encoding P2X7-blocking nanobody. MSCs are considered to be one of the safest and most versatile therapeutic agents, but it is now evident that transplanting nave MSCs yields only modest benefits. We have shown that intra-arterially (I.A.) delivered MSCs can cross the blood-brain barrier and thus serve as a carrier for the local production of neuroinflammation-blocking nanobodies in brain paranchyma. After the acute phase, when inflammation subsided, nanobody expression will naturally cease. MSCs will continue their developmental program, including pericyte formation, helping restore cerebral circulation and blood-brain barrier function. Our group has been developing over the last decade image-guided intra-arterial injection to the brain. Here we will explore utility of this technique for selective targeting of engineered MSCs. Notably, routine endovascular techniques of mechanical thrombectomy, used to treat growing number of patients, offer unprecedented opportunities for I.A. delivery of MSCs that block neuroinflammation as adjuvant therapy, immediately after reperfusion, using the same catheters as for thrombectomy. If our exploratory project demonstrates safety and efficacy of this approach, it could be rapidly implemented into clinical practice, potentially impacting the treatment of stroke.

Jiou Wang, M.D., Ph.D.
Johns Hopkins University (JHU)
Awardee Amount: $345,000
Disease Target: Alzheimer's Disease & Related Dementia

Investigating A Novel Signaling Pathway in iPSC-Derived Models of Tau-Associated Neurodegeneration

Neurodegeneration is an increasing public health challenge and remains an unsolved biomedical problem. Protein misfolding and aggregation are a central feature of neurodegenerative diseases. We have discovered a key stress-response signaling on translational control in cells, and this proposal is aimed at exploring the significant implication of this cellular program in basic and translational studies of a major form of neurodegeneration linked to tauopathy using human induced pluripotent stem cell derived model systems. The environment for life is in a constant state of flux, and living cells are faced with numerous challenges of various limitations and damaging insults from environmental agents. To maintain a state of fitness during the stress of environmental alterations or internal change, cells have evolved exquisite stress response programs that sense potentially harmful situations and make the necessary adaptations at the molecular and cellular levels. Defects in such stress response programs can have major consequences ranging from cell death to abnormal growth. Proteins are responsible for most cellular functions, and the maintenance of protein homeostasis (proteostasis) is required for the survival of cells, especially under stress conditions. Elucidating how cells respond and adapt to stimuli related to damaged or unfolded proteins is fundamental to the understanding of both physiological and pathological mechanisms of cellular homeostasis in health and disease. We have discovered a new pathway for unfolded protein and integrated stress responses [1], anchored in MARK2 as a novel eIF2 kinase, which is also known to promote tau hyperphosphorylation and its proteotoxicity. We have also delineated a signaling pathway that senses the proteotoxic stress and activates the newly identified eIF2 kinase. To maintain protein homeostasis, cells have evolved exquisite stress response programs that sense potentially harmful situations and make the necessary adaptations at the molecular and cellular levels. A key regulation of protein homeostasis occurs at the level of protein synthesis or translation. The specific aims are to determine the effects of tau-induced proteotoxic stress on the new signaling pathway and to examine the role of the signaling in controlling the tauopathy and proteotoxicity. These studies will have important implications for our understanding of the stress responses and the pathogenic process in the most common form of neurodegenerative diseases. The findings will not only provide novel entry points for understanding the tauopathy and proteotoxicity, but also reveal molecular targets for harnessing the cellular defense system to prevent and treat the relevant neurodegenerative diseases. We predict that the advances gained through our research efforts in human stem cell derived models will eventually lead to new therapeutic interventions to address these diseases in the worlds rapidly aging population.

LAUNCH

Program

2022

Patrick Cahan, Ph.D., M.S.
Johns Hopkins University (JHU)
Awardee Amount: $345,000
Disease Target: N/A

Distilling Predictive Models of Human Stem Cell Fate Control from Single Cell Omics Data

The primary goal of the proposed work is to develop a computational platform, OneStemCell (OneSC), that distills the flood of human single cell sequencing data down to specific, testable hypotheses for the more efficient and faithful derivation of desired cell populations from human pluripotent stem cells. We will prospectively determine the utility of this platform by testing its predictions to improve the fidelity of human pluripotent stem cell derived mesoderm. Furthermore, we will experimentally test whether this improves the functional maturation of one downstream lineage, the cardiomyocyte. Therefore, the work proposed here is a blend of computational tool development, analysis of existing single cell sequencing data, generation of new single cell sequencing data of human stem cells both at early stages (e.g. equivalent to gastrulation) and late stages (i.e. equivalent to a post-natal stage and later) of differentiation, and their in vitro functional assessment. The work proposed here falls squarely in line with the major goal of the MSCRF to fund stem cell research in Maryland that leads to the use of stem cells to improve human health. This is because our proposed research, if successful, will result in a system that can measure the fidelity of stem cell derived populations, which will facilitate the comparative assessment of new stem cell differentiation protocols; in a system that generates data-driven hypotheses to improve differentiation protocols; and in better protocols for the generation of human mesoderm progenitors, which may yield cardiomyocytes with increased mature phenotypes. Since launching his lab at Johns Hopkins, Dr. Cahan has focused his team's experimental efforts on mouse development and mouse embryonic stem cells. He has not performed any experimental work on human pluripotent stem cells or their derivatives. He wants to use the MSCRF Launch grant as a way to make his research more biomedically impactful and relevant to human disease. Therefore, the work proposed here falls squarely in line with the MSCRF's Launch program increase the number of Maryland researchers working on human stem cells.

Chengpeng Chen, Ph.D.
University of Maryland, Baltimore County (UMBC)
Awardee Amount: $350,000
Disease Target: Ulcerative Colitis

3D Model and in-line Assessment of Colon based on iPS cells for Ulcerative Colitis Treatment

Ulcerative colitis (UC) impacts 1 million people in the US, and that number is growing. UC is an inflammatory bowel disease leading to ulcers coupled with colon inflammation. While there are therapies available to treat UC, many are expensive and only work in a subset of patients. Currently, there is no way to screen therapies to determine their efficacy. Instead, patients try medications andlook at symptom progression, which can be an expensive and debilitating process. A the rapid formation of tissue models that mimic the critical features of the colon for high throughput and personalized therapies. The goal of this research is to apply the screen-printing process to build novel models of the colon for studying ulcerative colitis and high-content drug screening to treat the disease. The use of human inducible pluripotent stem cells (hiPSCs) presents a powerful research tool for modeling tissues, which has not been done to make colon models. Traditional 3D organoid models can exhibit a high level of heterogeneity of cell types and high culture-to-culture variability, along with little control over the growth of neural networks. In addition, pharmaceutical industry research suggests that most cerebral organoid models have not been commercially scalable. The combination of using the differentiation of specific subtypes hiPSC-derived epithelial cells and immune cells and the screen-printed hydrogel developed here in a highly controlled fashion will lead to a more homogenous and scalable system. We will test the scalability of such a system and its utility in a small library high-content drug screen. If successful, this approach will create a novel tissue model for the colon that will be used to identify early biomarkers, points of intervention, and potential targets for drug development. We have a focus on developing the assays in this proposal for automated, high-throughput methods geared towards drug discovery. Indeed, the assays proposed here (TEER and in-line media assessments) are used in the pharmaceutical industry in toxicology studies on potential therapeutic compounds before they go to clinical trials. Finally, Dr. Lavik has a relationship with Ken Malone at Early Charm Ventures in Baltimore and has begun to explore opportunities for licensing the patent based on the screen printing and technologies developed in this proposal. Because this protocol is designed to be high-content and scalable for lead generation and phenotype-driven drug discovery and is developed by UMBC, it can be rapidly commercialized.

Launch Grant Awards

Jill Fahrner, M.D., Ph.D.

Johns Hopkins University (JHU)
Awardee Amount: $345,000
Disease Target: Beck-Fahrner/TET3 Deficiency

Development of Patient iPSC-derived Organoid Models for Beck-Fahrner Syndrome: Probing DNA Methylation and Advancing Treatment

Beck-Fahrner syndrome (BEFAHRS) is the first described monogenic neurodevelopmental disorder (NDD) of DNA demethylation but lacks a human experimental model to study disease mechanisms and therapies. BEFAHRS results from germ-line mutations in the gene encoding TET3, which converts 5-methylcytosine (5mC) to 5-hydroxymethylcytosine (5hmC), the first step in demethylation. Our long-term goal is to understand how disrupted DNA methylation (DNAm) and other forms of epigenetic regulation cause Mendelian disorders of the epigenetic machinery (MDEMs)NDDs resulting from mutations in epigenetic machinery genes and to develop effective targeted treatments. The objective here is to use BEFAHRS to elucidate mechanisms by which reduced DNA demethylation leads to neurological dysfunction and to determine the therapeutic efficacy of treatment with TET3s cofactor ascorbic acid either alone or in combination with the epigenetic drug 5-azacytidine (5-aza). The central hypothesis is that in BEFAHRS, decreased conversion of 5mC to 5hmC alters gene expression in neural lineage cells, causing imbalanced cellular proliferation, differentiation and neuronal signaling and that treatment with ascorbic acid alone or with 5-aza, can restore TET activity and DNAm and ameliorate cellular disease manifestations. By understanding how impaired conversion of 5mC to 5hmC causes neuronal dysfunction, we will better understand BEFAHRS, allowing us to identify potential treatments, and importantly, gain an understanding of the fundamental role that DNA demethylation, 5mC, and 5hmC play in normal neurodevelopment and the pathogenesis of NDDs. The central hypothesis will be tested by pursuing three specific aims: 1. Identify cellular disease mechanisms for BEFAHRS in patient iPSC-derived organoids; 2. Determine DNAm and gene expression changes relevant to BEFAHRS pathogenesis; and 3. Evaluate efficacy of targeted therapies to restore DNAm and cellular phenotypes in BEFAHRS. The project aligns well with the goals of the Launch Program. It takes advantage of our recent discovery of a naturally-occurring and poorly-understood human disease to develop a novel human stem cell-derived experimental system. Cerebral organoids and neural lineage cells made from patient-derived iPSCs will be used to study the molecular underpinnings of the BEFAHRS phenotype, supporting the goal of developing new and innovative disease models; and cutting-edge bisulfite-based genome-wide sequencing techniques will be combined to analyze 5mC and 5hmC in this system, supporting the goal of developing technically innovative approaches. The proposed work aligns with the goal of the MSCRF to develop new medical treatments for diseases using human stem cell-based strategies. The approach is translationally innovative because it challenges the dogma that NDDs are irreversible and combines a nutritional agent ascorbic acid (vitamin C) with an epigenetic drug 5-azato treat a novel disorder. This type of target-specific combination therapy has not been used to treat MDEMs or other NDDs. This work has the potential to achieve new horizons in the treatment of BEFAHRS and related disorders with altered DNAm. These contributions will advance the field by establishing a causal role and mechanistic basis for altered DNAm in the pathogenesis of BEFAHRS and will inform our understanding of the role of disrupted DNAm in common NDDs, leading to mechanistic-based therapies.

Moonjung Jung, M.D., M.S.

Johns Hopkins University (JHU)
Awardee Amount: $350,000
Disease Target: Anemia, Thrombocytopenia, Leukopenia

Rejuvenate Hematopoietic Stem Cells by Counteracting the Alcohol-Induced Aging Process

The primary goal of this proposal is to develop technology to enhance blood stem cell function following DNA damage from alcohol exposure. Alcohol is a common source of DNA damage in humans. Indeed, its metabolic byproducts, acetaldehyde and reactive oxygen species (ROS), mutate hematopoietic stem cells (HSCs). Importantly, DNA damage is central to aging by contributing to stem cell exhaustion, cellular senescence, inflammation, and deregulated nutrient sensing. Together, these findings have led us to hypothesize that: 1) alcohol-induced DNA damage accelerates aging in HSCs, and, 2) innovative stem cell technology with CRISPR activators can be used to repair and protect aging HSCs. Alcohol suppresses bone marrow: patients with excessive drinking develop thrombocytopenia, anemia, leukopenia, or even pancytopenia. Dysregulated folate metabolism and iron utilization due to alcohol are contributing factors to bone marrow suppression. However, nutritional supplements do not reverse alcohol-induced hematopoietic suppression. Moreover, the effects of light-to-moderate alcohol consumption on overall health remain controversial. Thus, there is an unmet need to better understand the effects of alcohol consumption on HSC, particularly in the setting of our aging populations. While it is widely accepted that excessive alcohol use can cause DNA damage, we do not know exact mechanisms by which alcohol accelerates aging, nor do we have clinical interventions to prevent DNA damage from alcohol drinking. To fill this knowledge gap, we propose xenotransplantation experiments using human HSCs where we will model in vivo chronic moderate alcohol exposure followed by comparisons of self-renewal capacity by serial transplant, the gold standard method to assess HSC function. We also propose to determine genes that protect stem cells from DNA damage caused by alcohol, which will be validated in human HSCs by overexpression of each candidate gene. Identifying genetic pathways that protect HSCs will allow us to activate specific genes using innovative CRISPR/Cas9 transcriptional activator (CRISPRa) as therapy for HSCs exposed to alcohol and other DNA damaging agents in future studies. Our scientific premise that alcohol causes DNA damage and premature aging in HSCs is based on the literature and our preliminary data: 1) Alcohol suppresses the colony-forming capacity of human HSCs in vitro, 2) Chronic voluntary drinking in Rhesus Macaques leads to decreased in vitro colony-forming capacity and impaired mitochondrial function in HSCs, and BM niche remodeling, 3) Chronic, moderate-dose alcohol exposure in mice leads to increased mitochondrial DNA damage and upregulation in genes involved in base excision repair (BER) (Neil1, Ogg1) 4) Moderate-dose alcohol feeding for an extended duration (8-weeks) in a rat model leads to thrombocytopenia, decreased reticulocyte counts and HSC populations, and increased 8-hydroxy-2-deoxyguanosine (8-OHdG; a marker of oxidative DNA damage) in HSCs, 5) Low-to-moderate doses of alcohol induce gamma-H2AX (a marker of DNA damage) in the Jurkat cell line, 6) Aged HSCs show increased numbers of gamma-H2AX foci together with decreased DNA repair function compared to young HSCs, 7) Overexpression of Ogg1, a DNA repair protein in the BER pathway, mitigates oxidative DNA damage, 8) Overexpression of ALDH1A1 reduces alcohol-induced tissue damage. Based on these observations, we hypothesize that: 1) alcohol drinking causes DNA damage and accelerates aging in HSCs, and 2) activation of DNA repair genes or alcohol metabolic enzymes will prevent alcohol-induced DNA damage.

Yajie Liang, M.D., Ph.D.
University of Maryland, Baltimore (UMB)
Awardee Amount: $345,000
Disease Target: Ischemic Stroke

Intravital 2-Photon Imaging Human iPSC-Derived Progenitors Grafted into Ischemic Mouse Brain Assisted by Helper Cells

Interruption in the blood supply to the brain leads to stroke, which is the leading cause of severe chronic disability. Endovascular clot removal contributed to the breakthrough in clinical outcomes, though many patients still never return to premorbid status, especially for those who miss the clot removal treatment window. Restoration of damaged neuronal circuits by transplanted cells is highly desirable and would be an ultimate solution. However, to date only few studies demonstrated any integration of transplanted cells with host cytoarchitecture and functional benefit was modest if any. Progress in this effort is hindered because these studies rely on static outcome measures such as post-mortem assessment, lacking insight into the dynamic and functional features of cell integration with the host neuronal circuits. Therefore, we will employ intravital imaging with the goal of advancing our understanding about the graft-host interactions in the infarcted brain dynamically at the molecular level. Two-photon microscopy (2PM) has been increasingly used to study neuronal circuits in live animals, with the advantage of providing high spatial and temporal resolution images of single cells as well as insights into their function. Our previous work contributed to developing an optical cell positioning system (oCPS) using 2PM, to achieve long-term single-cell tracking of neural progenitors in the adult mouse brain. Here, we propose to upgrade oCPS for long-term functional single-cell tracking by combining state-of-the-art functional sensors and 2PM imaging system. The molecular and optical imaging tools will be tested on carefully selected source of highly potent cells. We will induce cortical progenitor specification of human induced pluripotent stem cells (iPSC) thus obtaining phenotypic identity of the cortical neurons we intend to replace. We hypothesize that the opening of a plasticity window in the subacute phase of stroke provides an opportunity for circuit re-organization and the integration of new cells. To address the survival issue of the transplanted cells, we will utilize our helper cell co-transplantation strategy in which iPSC-derived astrocytes will be used as helper cells. To avoid using immunosuppression or immunodeficient animals that compromise clinical relevance of stroke model, we recently developed an effective induction of tolerance to xenografts that allow human cells to be transplanted into the immunocompetent mouse brains without being rejected. Overall, our study will address the most burning and vital issue in regenerative medicine: integrating grafted cells into adult neural circuits in the injured brain. Unlike current therapeutic strategies based on clot removal within 24 hours after the onset of stroke, the focus on subacute phase of stroke substantially widens the treatment window for stroke. By developing innovative approaches for tracking migration, fate, and function of grafted cells and their interactions with host cells, this study will produce results that may reinvigorate the neural cell transplantation field for the treatment of neurological disorders, which is at the core of the MSCRF program.

Mollie Meffert, M.D., Ph.D.,
Johns Hopkins University (JHU)
Awardee Amount: $345,000
Disease Target: Fragile X Syndrome

Noncoding RNA Regulation in Fragile X-Syndrome Patient-Derived Stem Cells

We propose to apply a newly developed technology for sequencing of chimeric miRNA-mRNA reads to make direct comparative analyses of transcripts targeted in-vivo for repression by the RNA-induced silencing complex (RISC) in human healthy and Fragile X Syndrome (FXS) patient-derived iPSCs and excitatory neurons generated by programmed differentiation. Our approach will deliver genome-wide quantitative RISC-targeting information; we will also test candidate let-7 family miRNAs implicated by our preliminary data and manuscript in-revision as misregulated in a mouse model of FXS (Fmr1 KO). Datasets generated will be used to prioritize targets and pathways for functional validation. In addition, our approach will permit the discovery and quantitation of unanticipated small RNAmRNA target interactions that are distinct from miRNAs, such as the recently appreciated roles for tRNA fragments in RISC(3) control of translation. The goal of this proposal is to use stem cell technology to provide a rigorous examination and testing of the functional impact of altered RISC-mediated gene targeting in the setting of human FXS disease and, ultimately, to help advance the identification of new therapeutic targets and greater understanding of shared pathways in neurodevelopmental disorders. My laboratory is new to the field of stem cell research, without prior grants or publications in this area. We have a track record of contributions in neuronal gene expression and noncoding RNA, including more recently in neurological disease. Our innovative genome-wide approach to investigating noncoding RNA contributions to disease-mediated differences in gene expression is now primed for advancement to the setting of human stem cells and would greatly benefit from the support and scientific community of the MSCRF.

Raphael Meier, M.D., Ph.D.
University of Maryland, Baltimore (UMB)
Awardee Amount: $349,657
Disease Target: Liver Fibrosis

Enhancing Mesenchymal Stem Cells Intrinsic Expression of IL-10 and MMP-9 to Augment Capacities for the Treatment of Liver Diseases

The liver is a complex organ achieving multiple tasks including intestinal blood detoxification, processing metabolism, glucose metabolism, and coagulation factor production. An array of conditions can cause terminal or end-stage liver disease, namely, nonalcoholic steatohepatitis (NASH), biliary disease (in children and adults), viral hepatitis (B and C), and excessive alcohol intake (on the uprise since the COVID-19 pandemic). In patients with end-stage liver disease, an irreversible scarring and inflammation of the liver take place, and the only therapeutic options are supportive/palliative care or liver transplantation. Due to organ shortage in the United States and globally, more than half of the patients never get transplanted and three patient per day die while waiting for an organ in the US (1,155 lives were lost last year). Medication which blocks or reverse the inflammation/scarring in the liver does not yet exists. In this context, we propose to use Mesenchymal Stem Cells (MSCs), which are adult progenitor cells with anti-inflammatory and fibrinolytic effects, modify them genetically to increase their anti-inflammatory/ fibrinolytic secretion potential, and use them as a tool to block/reverse the scarring process occurring in chronic liver diseases. Based on our previous research, we plan to focus on IL-10 and MMP (matrix metalloproteinase)-9 production by MSCs to achieve the desired effect. Objective of proposal / central hypothesis Enhanced MSCs secrete high levels of IL-10 and MMP-9 and can reverse inflammation and liver fibrosis in vitro and in vivo. Aims/Approach 1)Modify human MSCs in vitro using the new technology CRISPR/Cas9 in order to boost the production of IL-10 and MMP-9 and microencapsulate the cells in microbeads of 0.5 mm, constituting a cytokine/enzyme production unit. 2) Test the modified and encapsulated MSCs ability in reducing inflammation in vitro, using the hepatic stellate cell model, and in vivo, using different models of liver fibrosis in mice (high fat diet, surgical bile duct ligation (BDL), and CCl-4 injection). Anticipated outcome and impact The estimated impact is immense since liver disease accounts for approximately two million deaths per year worldwide. Generating an alternative treatment, that is less invasive, based on stem cell, CRISPR/Cas9 technology, and microencapsulation would represent a new therapeutic hope, applicable for liver disease treatment and beyond.

Brian O'rourke, Ph.D.
Johns Hopkins University (JHU)
Awardee Amount: $349,436
Disease Target: Heart Disease

Effects of Innate Immune Signaling on Ischemia-Reperfusion Tolerance of Human Stem Cell-Derived Cardiomyocytes

Sudden Cardiac Arrest from ventricular arrhythmias, occurring after myocardial infarction (MI) or during chronic heart failure, is a leading cause of death in the United States (>300k/yr). Regeneration of heart tissue after MI, using autologous or allogenic stem cells, is a promising strategy to restore contractile function, but efforts have been hampered by the susceptibility of cardiomyocytes derived from human embryonic- (hESC-CM) or induced pluripotent- (iCM) stem cells to cell death and arrhythmias. These limitations are related to the immature nature of the donor myocytes, favoring electrophysiological instability, to poor electrical coupling with the host myocardium, and to the inability of the donor cells to survive the hostile environment created by the innate and adaptive immune systems of host during the inflammatory, proliferative, and healing phases of infarct resolution. In this dynamic milieu, the complex interactions between stem cell-derived myocytes and non-myocytes, including fibroblasts and resident and recruited immune cells, are poorly understood. We recently obtained compelling preliminary data showing that activation of an innate immune type I interferon response (using the dsRNA mimetic Poly I:C) in iCM monolayers (~95% myocyte purity) results in the robust production of cytokine mediators, including a >70-fold increase in CXCL10 (an immune cell chemoattractant and marker of heart failure), and a 2-10 fold increase in more than 30 other cytokines. The increase in secreted factors was associated with impaired excitation-contraction coupling and suppression of mitochondrial function; however, it is unknown if these autocrine/paracrine mediators underlie the effects on cardiomyocyte function or on myocyte/non-myocyte interactions. Moreover, it is unknown if the sensitivity of iCMs to ischemia-reperfusion (I/R) injury is modified by innate immune pathway activation. The latter question is particularly interesting since post-ischemic inflammation or interferon pathway activation plays a role in myocardial injury, yet, interferon pathway activation has also been reported to be a preconditioning method to enhance I/R tolerance. Here, we test the hypothesis that the activation of the iCM type I interferon response and concomitant cytokine secretion contribute to electrophysiological, contractile, and mitochondrial dysfunction, and increases iCM vulnerability to I/R injury. The project leverages 2D iCM monolayers and 3D Engineered Heart Tissues (EHTs), with or without co-cultured non-myocytes (macrophages, fibroblasts) to assess how innate immune activation alters cardiomyocyte function and affects I/R sensitivity in an in vitro I/R model that recapitulates mitochondrial membrane potential (m) instability, and arrhythmias upon reperfusion. With this cardiac microphysiological system, for the first time, we can address fundamental questions about the behavior of stem-cell derived tissues in a hostile ischemic/immune active environment with conditions encountered in the post-infarct myocardium.

Emmanouil Tampakakis, M.D.

John Hopkins University (JHU)

Awardee Amount: $350,000

Disease Target: Heart Disease

Leveraging Innervated Human Cardioids to Study Cardiac Disease

Human pluripotent stem cell-derived cardiomyocytes (hPSC-CMs) have provided unprecedented opportunities for modeling heart development and disease, drug discovery and regenerative medicine. However, the fact that they remain immature, and they lack interactions with non-myocytes in a chamber-like formation that simulates cardiac morphology and function, limits their applicability. To address these limitations, we, and others generated cardiac organoids, or in vitro 3D miniaturized hearts from PSCs. However, organoids lack most non-myocyte cell types. This likely reflects the inability of current organoids to integrate extracardiac cells that contribute to heart development. Therefore, recent advances in cardiac organoids have stalled as they fail to recapitulate the complexity of native heart. Sympathetic neurons (SNs) control cardiac function and disruption of SNs often leads to cardiac disease in adults. Maturational adjustments in cardiomyocytes coincide with hearts sympathetic innervation. To determine the effect of SNs on cardiac maturation, we co-cultured hPSC-SNs and hPSC-CMs. This resulted in hPSC-CMs with adult-like structure and function, and consistent transcriptomic changes. In addition, using mouse models, we demonstrated that SNs suppress CM proliferation and regulate cardiac metabolism, and structural and calcium handling genes, supporting novel neuromodulation therapies for heart regeneration and disease. However, currently there are no in vitro human heart models or tissue samples to assess neuromodulatory effects or treatments for cardiac disease.
Based on a recent study, we generated cardiac organoids that form a chamber-like structure with an inner endocardial layer surrounded by CMs. We then co-cultured organoids with hPSC-derived epicardial cells, which covered the outer layer to form a tri-lineage cardiac organoid described as cardioid. Next, we successfully innervated human cardioids by co-culturing them with hPSC-SNs. Because SNs affect CM maturation and proliferation, we hypothesize that innervated cardioids recapitulate cardiac physiology with more mature CMs and can serve as a system to study the neuromodulation of heart regeneration and disease. We plan to address this hypothesis with two specific aims.

Aim 1: Determine the innervation patterns and maturation of hPSC-CMs in cardioids with hPSC-SNs. Cardiac SNs demonstrate significant spatial diversity and they can innervate noncardiomyocytes. Additionally, SNs and non-myocytes would likely promote hPSC-CM maturation in cardioids. Therefore, we will characterize the innervation patterns of cardioids with hPSC-SNs. We will also examine hPSC-CM maturation by analyzing their morphology and function. In addition, we will utilize single cell transcriptomics to determine maturation-related gene expression patterns. Aim 2: Determine the effect of hPSC-SNs on human cardioids after injury. To overcome the lack of human heart regeneration models, we will perform cryoinjury in human innervated cardioids and analyze the effect of hPSC-SNs on hPSC-CMs proliferation, and apoptosis, and cardioid fibrosis. Activation of SNs after myocardial injury results in increased arrhythmogenesis, thus we will use optical mapping to study arrhythmias in innervated cardioids after injury. This work aims to utilize novel innervated miniaturized human hearts to study the neuroregulatory effects of SNs on CM maturation and disease. Ultimately, it paves the way for the generation of future more mature and complex human heart models for drug development, disease modeling and regenerative therapies.

POST-DOCTORAL FELLOWSHIP

Program

2022

Ileana Hernández Araiza, Ph.D.

University of Maryland, Baltimore (UMB)
Mentor: Matthew Trudeau, Ph.D.
Awardee Amount: $130,000
Disease Target: Long QT Syndrome Type 2

Restoring the Function of hERG LQT2 N-terminal Mutants with Non-Canonical Amino Acids in hiPSC-CMs

Long QT (LQT) syndrome is a severe cardiac disorder characterized by arrhythmia that can result in sudden cardiac arrest. Hereditary LQT syndrome type 2 is related to mutations in the human EAG related (hERG) potassium channel, some of those mutations introduce an amber stop codon (TAG). hERG channels produce the cardiac IKr current, essential in terminating the action potential in cardiomyocytes, so understanding the effect of these mutations in the properties of IKr currents and in consequence, the electrical properties of cardiomyocytes is vital for the understanding and proper treatment of this disorder. This proposal aims to study the putative loss of function produced by these mutations in the expression and biophysiological properties of hERG channels. We also propose a possible therapeutic approach incorporating non-canonical amino acids (ncAA) using TAG codon suppression. We will use electrophysiological techniques in HEK293 cells as a heterologous expression system that allows for a controlled study of the hERG TAG LQT2 mutants currents and in human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CM) to assess the effect in a physiological setting. Currently, hiPSC-CMs are the most advantageous study model for several cardiac disorders, especially channelopathies, as they provide a physiological context including the expression of cardiac-specific currents like IKr. Also, hiPSC-CMs derived from a LQT2 patient showed reduced IKr, increased action potential duration (APD), and early afterdepolarizations (EAD) characteristic of LQT2 syndrome. Gene-edited hiPSC-CMs will allow the study of the hERG TAG LQT2 mutants effect on the electrical activity on individual cardiomyocytes and the potential rescue of a wild-type phenotype with the incorporation on ncAAs. Our current lack of understanding of the molecular basis of hereditary LQT2 syndrome limits the development of effective treatment. The present proposal is directly translational as it centers in understanding the effect of different LQT2-linked hERG TAG mutants on the electrical properties of human induced pluripotent stem cell derived cardiomyocytes (hiPSC-CMs). We also propose the incorporation of a ncAA at the TAG codon using amber codon suppression technology as a method to restore the WT phenotype of hERG channels in hiPSC-CMs. This approach has previously worked in a heterologous expression system and the results of this work will provide information on its therapeutical potential. Although animal models have provided some information on the etiology of LQT2 syndrome, those findings are not easily translated to LQT2 patients due to differences in the ventricular action potential, ion channel expression and heart physiology. The use of hiPSC-CMs reduces drastically this differences and gene-editing technology allow to model patient-specific mutations. Overall, the results of this proposal will help elucidate the etiology of familial LQT2 syndrome and can lead to personalized treatment for patients with different mutations.

Lukasz Kalkowski, Ph.D.

University of Maryland, Balitmore (UMB)
Mentor: Piotr Walczak, M.D., Ph.D..
Awardee Amount: $130,000
Disease Target: Multiple Sclerosis (MS)

Homing of Intravascularly Injected Glial Progenitors to Demyelinating Brain Lesions Enhanced by Focused Ultrasound Treatment

Multiple Sclerosis is one of the most frequent neurological diseases without cure. Transplantation of stem cells have been attempted to boost myelin repair but so far with no effective cures. Dr Walczaks group have demonstrated high therapeutic potential of glial-restricted progenitors (GRPs), global replacement of oligodendrocytes and robust therapeutic effect in neonatal dysmyelinated mice. Similar therapeutic approaches in older recipients proved challenging. Therapeutic effects were modest at best, because of poor cell engraftment and protocol optimization was difficult because of high variability of lesion distribution and disease severity at baseline. To address these issues we worked on developing animal model of MS with full control of the lesion size and location. We established local autoimmune encephalomyelitis model in rats characterized by excellent clinical relevance and high reproducibility. We also worked on minimally invasive intravascular administration of cells to the brain. In this project we propose to implement focused ultrasound (FUS) technology with two unique modalities: MR-guided FUS-mediated blood-brain barrier opening to induce focal lesion and then at the chronic stage, FUS-mediated parenchymal stimulation to induce expression of adhesion molecules thus improving capture and diapedesis of intraarterially injected human GRPs (Aim 1). Second aim is genetic engineering of hGRPs to further enhance their therapeutic properties. We will use mRNA transfection to minimize the risk of long-term disruption of cell functionality. The cells will be transfected to direct their differentiation towards oligodendrocytes. Overall, we propose implementation of advanced technologies to: (i) improve utility of animal models; (ii) improve homing of glial progenitors using image-guided, minimally invasive technique; and (iii) improve differentiation of progenitor cells towards oligodendrocytes through mRNA-based expression of transcription factor. All these techniques can be readily translated to the clinical setting and as such bring opportunity to improve prospects of patients suffering from multiple sclerosis and other myelin diseases.

Post-Doctoral Fellowship Grant Awards

Katherine Marshall, Ph.D.

Johns Hopkins University (JHU)
Mentor: Nicholas Maragakis, M.D.
Awardee Amount: $130,000
Disease Target: ALS

Modeling Corticospinal Tract Pathophysiology with ALS iPSC-Derived Corticospinal Motor Neurons

ALS is a devastating neurodegenerative disease in which progressive degeneration of the motor system leads to muscle weakness, paralysis, and death, typically fewer than 5 years following diagnosis. ALS is characterized by death of both upper (corticospinal motor neurons, CSMNs) and lower (spinal motor neurons, spMNs) motor neurons. Though there is substantial heterogeneity in the clinical course and involvement of upper or lower motor neuron symptoms, CSMN dysfunction and hyperexcitability are typical pathological and clinical features in ALS patients. CSMNs synapse on spMNs, thus communicating signals from the brain to the spinal cord. Considering their involvement in ALS pathogenesis, CSMNs are relatively understudied compared to spMNs. However, it has been increasingly recognized that cortical dysfunction is an early symptom in ALS, including in pre-symptomatic familial ALS patients with SOD1 mutations. Post-mortem samples from ALS patients reveal various morphological changes and death of CSMNs. Interestingly, pools of spMNs that receive more CSMN input, such as those that innervate distal and laryngeal muscles, degenerate more quickly compared to those that receive less, such as the extraocular muscles. The relative sparing of spMN pools that receive little CSMN input suggests that CSMN dysfunction contributes to vulnerability of spMNs. We hypothesize that ALS CSMN hyperexcitability will contribute to a diminished ability to form and maintain connections with spMNs. Modeling CSMN pathobiology in ALS is particularly challenging. Transgenic mouse models are important for understanding pathogenic mechanisms of ALS, and multiple models have demonstrated sophisticated ways to document early degeneration of CSMNs and explore avenues for improving CSMN health. However, mouse CSMNs have different connectivity to spMNs than human CSMNs, which may mask the observable pathology stemming from CSMNs in ALS models.

Maryam Rahmati, Ph.D.

Johns Hopkins University (JHU)
Mentor: Warren Grayson, Ph.D.
Awardee Amount: $130,000
Disease Target: Skull Bone Injuries

Mitigating the Impact of Trauma-Induced Senescent Cells on Stem Cell-Mediated Bone Regeneration

Adipose-derived stromal/stem cell (ASC) transplantation has emerged as a promising clinical strategy to promote craniofacial bone regeneration. ASCs have great regenerative capacities over bone marrow stem cells and/or autogenous bone grafts because of their higher cell-to-volume proportion, less sensitivity to aging, and their ease of harvest. However, understanding the fundamental cell-cell communication and signaling crosstalk between the transplanted ASCs and the cranial defect microenvironment remains elusive. Senescent cells (SnCs) are key players in directing tissue healing mechanisms in several tissues by stimulating chronic inflammation and DNA damage. If the number of SnCs are increased after trauma, their presence in the defect microenvironment could severely reduce the potency of ASCs. There are no published studies on how SnCs affect craniofacial bone maintenance and regeneration after stem cell transplantation. There is a need to evaluate the identity, spatial location, and physiologic impacts of SnCs that accumulate in craniofacial bone with trauma and their impact on ASCs fate after transplantation. Using our developed quantitative light-sheet microscopy (QLSM) imaging platform, we generated compelling preliminary data to show the presence of putative SnCs in the cranial bone of young mice. To further explore the cellular and molecular crosstalk between transplanted ASCs and SnCs in the cranial tissue microenvironment, we propose to identify the cellular phenotypes of SnCs and to assess their impact on stem cell guided bone regeneration using scRNA-seq combined with histology and immunohistochemistry. To deeply explore the effects of SnCs on stem cell-guided bone regeneration, we will create 4-mm critical size skull defects in 3-month-old mice skull and study the crosstalk between ASCs and SnCs after transplantation. We will do this study to test the hypotheses that SnCs accumulate in the young murine skull after trauma injuries and that their presence would hamper ASC-guided craniofacial bone regeneration.

Mario Gomez Salazar, Ph.D.

Johns Hopkins University (JHU)
Mentor: Aaron James, M.D., Ph.D.
Awardee Amount: $130,000
Disease Target: Defects of Bone & Soft Tissue

Exploration of Human Perivascular Stem Cell Diversity

Mesenchymal progenitors reside in the perivascular niche and hold promise for treatment of different pathologies including bone defects. However, recent clinical trials using adipose-derived cell therapies for bone tissue healing have demonstrated suboptimal results. Indeed, contrasting results using stromal cells have made it difficult to get approval from the FDA, being approved only in rare occasions. Our team has a strong record track in the field of perivascular stem/progenitor cells from adipose tissue for skeletal engineering and regeneration, having been first described by our group. The tunica adventitia of arteries and veins contain most progenitor cells (adventicytes / adventitial cells) displaying high degree of heterogeneity of unknown potential. Recent single cell molecular analysis by our group confirms this heterogeneity and suggests a hierarchy of stem cells based on spatial organization in the adventitia niche. This postdoctoral fellowship grant will allow the training and professional development of the applicant. On the other hand, this study will help gain insight into the relationship between spatial location and stem cell features in adventicytes, we proposed to fully study the tunica adventitia niche using spatial transcriptomics in combination with scRNA sequencing. Our main aim is to identify novel functional subsets and define the skeletogenic potential of these subsets. Our advances will not only help understand this stem cell niche, but also improve and standardize the use of adipose-derived cell therapies, making its approval more feasible in comparison with other cell preparations.

Wonjin Yun, Ph.D.

Johns Hopkins University (JHU)
Mentor: Gabsang Lee, Ph.D.
Awardee Amount: $130,000
Disease Target: Demyelinating Diseases

Functional Schwann Cells Direct Induced from Hypo-Immunogenic Human Fibroblasts

Schwann cells are primarily responsible for the myelin wrapping of peripheral nerve axons and play pivotal roles in regeneration of the peripheral nervous system (PNS). Transplantation of Schwann cells have been considered highly desirable for neural diseases, but to date the production of myelination-competent functional Schwann cells are extremely limited. In addition, there are immunological barriers to the clinical usage of allogeneic transplant, and autologous human induced pluripotent stem cells (hiPSCs)-based therapy requires much resources and tedious laborious procedures for preparing patient-specific clinical-grade cells. To this end, we propose a novel approach to establish universal induced Schwann cells that are directed converted from hypoimmunogenic fibroblasts with a set of core transcription factors (TFs) licensing Schwann cell myelination. Our approach will not only be beneficial to identify core TFs for human Schwann cells myelination and augmenting human Schwann cells functionality, but also enable us to develop a novel cell therapy paradigm for demyelinating disorders. The translational plan for this work is a unique opportunity to identify a set of genetic core factors governing the myelination and generate functional myelination competent Schwann cells through direct conversion for future cell therapy from our proposal. Furthermore, we will also generate hypo-immunogenic hPSCs and their derivative fibroblasts to establish universal induced Schwann cells. As a combined above proposes, we will pursue developing new methods for enhancing Schwann cell myelination and for further pre-clinical phase with such as Validation and/or Commercialization program for the MSCRF. In addition, the development of TFs-induced direct conversion strategies can be a great platform not only to investigate new candidate genes and functions expected to be less important but also recapitulate human disease or myelin development in a rapid and feasible manner, facilitating drug discovery and cell replacement therapy.

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