MSCRF
Maryland Stem Cell Research Fund

MARYLAND

STEM CELL RESEARCH FUND

ANNUAL REPORT

-2024-

MSCRF
Maryland Stem Cell Research Fund

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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 close to 600 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**.

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.

Maryland Stem Cell Resarch Commission

Rachel Brewster, Ph.D.
MSCRC Chair
Appointed by the University System of Maryland

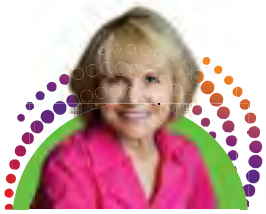
Scott Bailey, Ph.D.
MSCRC Vice Chair
Appointed by Johns Hopkins University

Ira Schwartz, Esq.
Attorney General's Designee

Mary Armanios, M.D.
Appointed by Johns Hopkins University

Margaret Conn Himelfarb
Appointed by the Governor

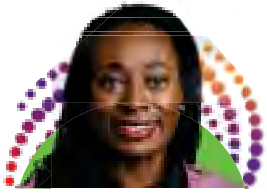
Mamta Gautam-Basak, Ph.D.
Appointed by the Governor



Diane Hoffmann, M.S., J.D.
Appointed by the University System of Maryland

Debra Mathews, Ph.D, M.A.
Appointed by Johns Hopkins University

David Mosser, Ph.D.
Appointed by the University System of Maryland



Barbara Nsiah, Ph.D.
Appointed by the President of the Senate

Linda Powers, J.D.
Appointed by the President of the Senate

Rabbi Avram Reisner, Ph.D.
Appointed by the Governor

Curt Van Tassell, Ph.D.
Appointed by the Speaker of the House of Delegates

MSCRF

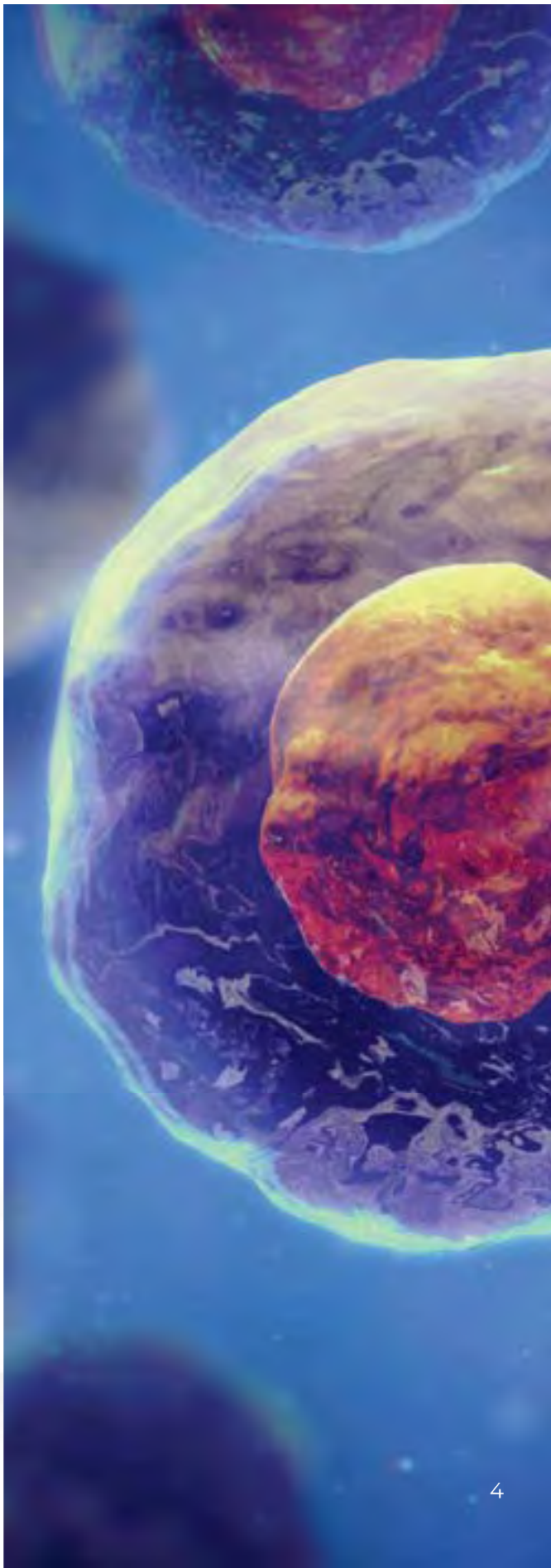
EXECUTIVE *Overview*

Investing in a Brighter Future

Executive Overview

The year 2024 marked a year of tremendous growth, success and synergy for Maryland Stem Cell Research Fund (MSCRF) as well as for the regenerative medicine sector. It has been an exciting first, full-year as Executive Director of MSCRF. There has been an incredible amount of progress within the field of stem cell research being led by research teams from Maryland academic and commercial entities, and MSCRF has been a cornerstone of Maryland's thriving bio-economy, demonstrating the transformative power of public funding to drive private sector innovation. **Through strategic investments, MSCRF has fueled economic growth, created jobs, and advanced groundbreaking scientific and healthcare innovations that directly improve lives.**

Regenerative medicine sector has made significant advancement, with clinical successes and regulatory approvals. This year saw a breakthrough in treating solid tumors with a cell therapy. The FDA approved Amtagvi, which harnesses the power of tumor-infiltrating lymphocytes to treat advanced melanoma. The therapy uses enhanced T cells from the patient to target and fight the cancer. FDA approved stem cell therapies such as Lantidra, a novel cell therapy for type 1 diabetes and Omisirge, a stem cell therapy patients with blood cancers approved by FDA reflect the FDA's efforts to support innovative cellular therapies that address complex diseases, especially in cases with limited treatment options.



Executive Overview

These advancement over the past year demonstrate the promise of regenerative medicines, including those being developed in Maryland. Funding from MSCRF supports ongoing research and development by academic researchers and private companies, driving transformative research that is powerfully impacting the lives of patients and their loved ones.

We would like to extend our heartfelt thanks to the Maryland legislature for maintaining the funding level of $20.5 million for Fiscal Year 2025 (FY2025), consistent with the previous years. This continued support is vital for providing unwavering assistance to Maryland companies and innovators to drive advancements in healthcare solutions and uphold Maryland's economic competitiveness.

In addition to the recent approvals, there have been significant clinical advancements as well. In Japan, researchers achieved a breakthrough in treating limbal stem cell deficiency, which causes severe vision loss. The scientists were able to restore vision in three of four patients by transplanting corneal epithelium derived from induced pluripotent stem cells. Findings from the study were published in The Lancet in November.

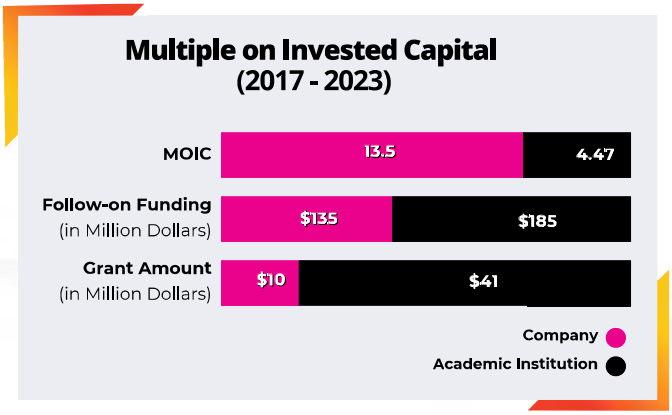
Another clinical advancement include progress in a stem cell study at Cedars-Sinai Hospital to treat amyotrophic lateral sclerosis (ALS). Stem cells injected into the spinal cord showed signs of slowing disease progression. However, this approach is still in the early stages.

Strengthening the Ecosystem

The demand for MSCRF funding continues to grow. Since its inception in 2006, MSCRF programs have bolstered the foundation of Maryland's life sciences ecosystem by supporting entrepreneurial ventures, fostering job creation and advancing transformative treatments for multiple disease indications. The funds also serve to cultivate a collaborative ecosystem throughout academic institutions, research organizations, and businesses to ensure Maryland remains a leader in regenerative medicine.

654 Projects Supported by MSCRF

To date, we have allocated $213 million to 654 innovative technologies aimed at addressing a broad spectrum of diseases affecting human health. **In the past six years, an investment of $51 million in companies and scientists has yielded an impressive return of $320 million in capital for the state from private and federal funding sources.** From 2017-2023, MSCRF allocated $10 million in grant funds to companies that have gone on to raise $135 million in outside capital, more than 13 times the state's investment. In that same time frame, $41 million directed toward scientists resulted in $185 million in follow-on funding, achieving more than four times the return.



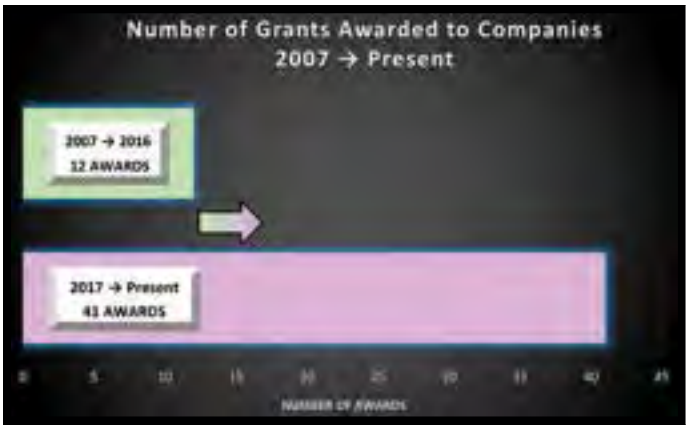
13-Fold Return on MSCRF's Investment in Companies

Notably, these outcomes far surpass typical venture capital return expectations, which are generally two to three times the initial investment.

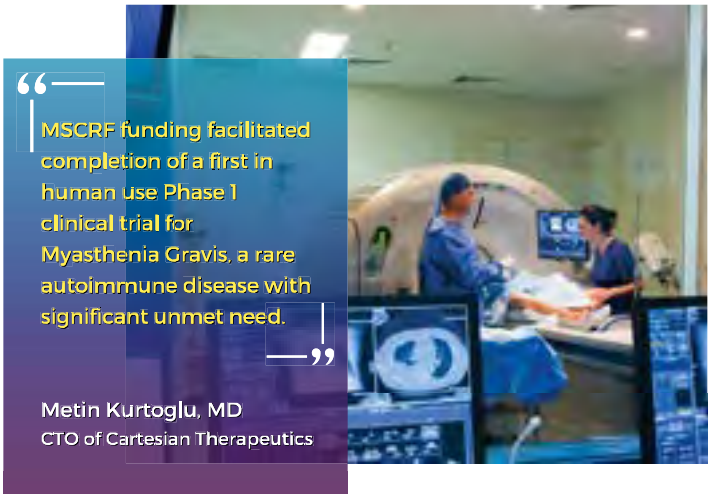
Further, MSCRF has significantly enhanced the state's cell manufacturing capabilities through the Manufacturing Assistance Funding Program unveiled in 2023. **An investment of $3.2 million over the past two years is already yielding promising results, directly supporting companies' manufacturing optimization and facility establishment**. See pages 39-41.

Executive Overview

This support allows MSCRF-funded companies to maintain their R&D and manufacturing capabilities in Maryland and attract additional capital investments to the state which bolsters the state's economy and provides a strong foundation for high-paying employment.



Over the past seven years, MSCRF funding to companies has tripled. More than 75% of companies backed by MSCRF remain in business, with many of them going on to raise additional funding from other sources. For example, **Germantown-based Seraxis, which received $700,000 in grants from MSCRF, raised an additional $50 million from other investors**. Likewise, Cartesian Therapeutics was initially backed by MSCRF and is now a publicly-traded company advancing their clinical pipeline closer to patients.

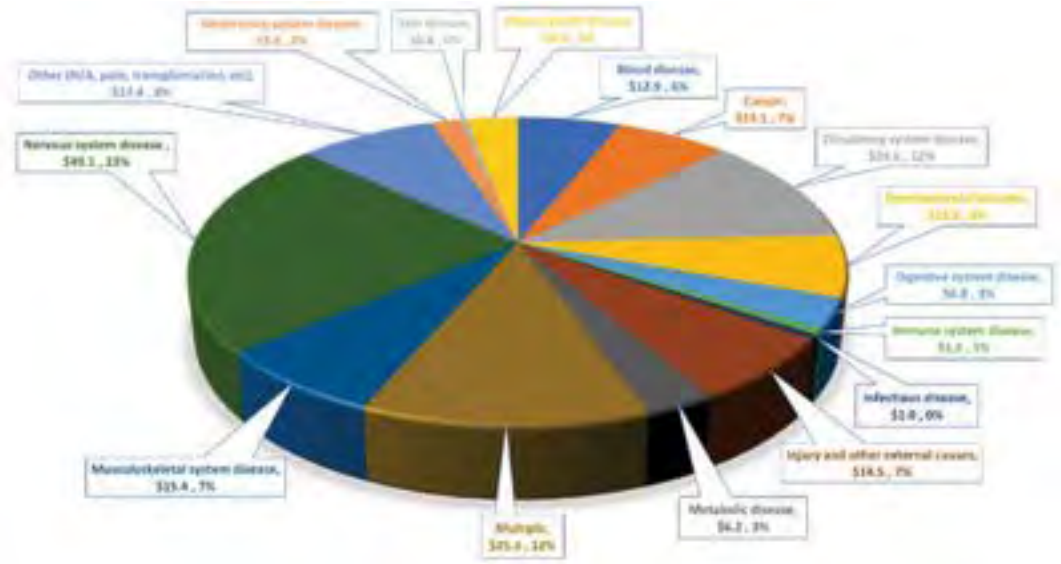


More than 30 companies have benefitted from MSCRF grants, including 15 startup ventures. **In 2024, MSCRF backed 5 startups that are focused on transitioning stem cell discoveries into successful products.** Successful companies create high-paying employment opportunities. **Fiscal data from 2007 to 2024 shows that MSCRF funding supported 2000 jobs** (Sage Policy Group MSCRF's Economic Impact Report, December 2024). **Theradaptive, Inc.** in Frederick, MD **expanded their employee base from 3 to 33 people** and plan to double the workforce in the next two years. **RoosterBio, Inc.,** another Maryland-based company **expanded their employee base from 4 to 43 and generated revenues of more than $50 million over the past few years.**

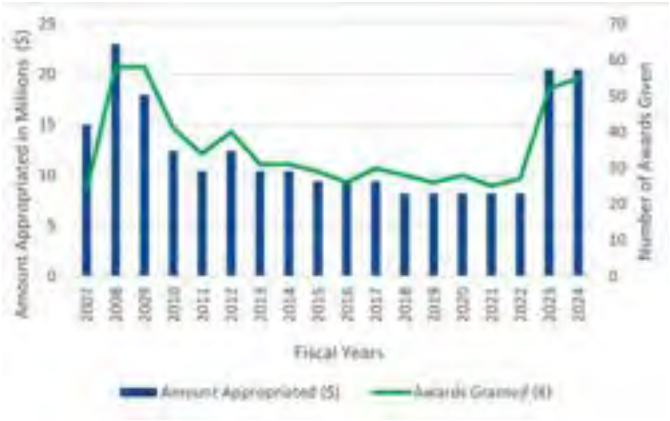
MSCRF funds help create high-salary jobs that average more than $100,000. **From 2007-2024, every $1 million in MSCRF investment supported 10 statewide jobs and supported an estimated $525 million in economic activity** (Sage Policy Group MSCRF's Economic Impact Report, December 2024). MSCRF's investments foster a vibrant entrepreneurial ecosystem that seeds the state with the best and the brightest talent and attracts a growing number of entrepreneurs.

MSCRF Remains Dedicated to **Advancing Medical Innovations** in Maryland

MSCRF's dedication to advancing cutting-edge research has positioned it as a catalyst for groundbreaking discoveries. With support for 650 distinct research projects since its inception, MSCRF funding targets critical health challenges and diseases affecting diverse populations. The grants encompass a wide range of stem cell research across multiple disease indications, funding 190 different diseases and human conditions since the program's launch. Below is a detailed breakdown of the disease categories supported by MSCRF from 2007 to 2024:

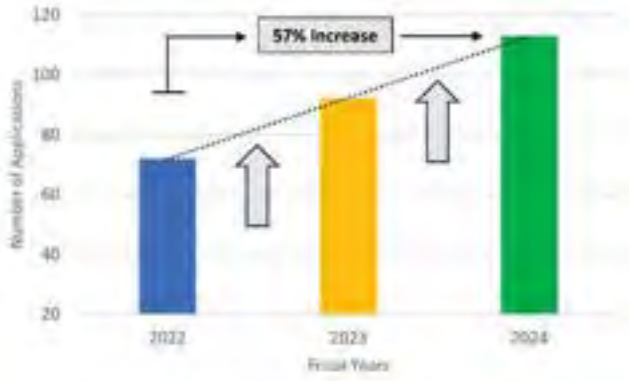


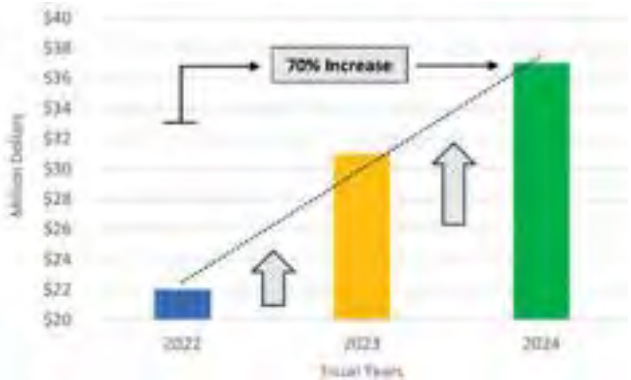
Supporting a Growing Need
Strengthening the Ecosystem



Regenerative medicine and stem cell ecosystem is maturing and expanding in Maryland. With the support from Maryland Governor and General Assembly, we attempted to address the needs of the growing ecosystem. Over the past two years MSCRF grant awards returned to a level similar to what was allocated by the legislature in 2008 but the need for more funding continues.

Since 2022, MSCRF has seen a 57% increase in the number of grant applications, translating to a 70% increase in requested funding, reaching $37 million- nearly double the current appropriation for MSCRF.





Additionally, **MSCRF has seen a 200% increase in applications for Launch Awards, reflecting on the expansion of regenerative medicine ecosystem in Maryland.** These grants are designed to encourage new and new-to-the-field faculty to bring innovative research and technology to the regenerative medicine field. These awards also support established researchers who seek to gain experience in the stem cell field. The funds are a pivotal tool as they advance their research and the field.

New MSCRF Initiatives
Strengthening the Ecosystem

In our commitment to innovation and enhancing the ecosystem, MSCRF has launched new initiatives to elevate our grant funding strategy and broaden our community impact.

For the first time, commercialization and validation awardees have benefited from a unique opportunity called **Second-Tiered Funding, receiving an additional $100,000 for collaborative projects between companies and academic institutions.** This initiative has been positively received by both companies and academic scientists.

Increased funding
for public-private partnership

In 2024, MSCRF introduced another transformative initiative, expanding funding eligibility to companies outside Maryland, provided they conduct their research funded by MSCRF within the state.

These strategic efforts not only accelerate the translation of breakthrough innovations to Maryland but also deliver substantial economic benefits for the state.

MSCRF

AWARDEES to Date

University of Maryland
Johns Hopkins University
The Geneva Foundation
MedStar Health Research Institute
LIBD Lieber Institute for Brain Development
Hussman Institute for Autism
UMBC
CaringCross
Johns Hopkins School of Medicine
University of Maryland Baltimore
Kennedy Krieger Institute
University of Maryland School of Medicine
RENOVATE
HOHCells
Phycin
Orthobiologics Research Initiative
RoosterBio
Theradaptive
REPROCELL
Secretome Therapeutics
TheraCord Solving Collection + Saving Lives
3Dnamics
seraxis
VITA Therapeutics
Britecyte
KOLON TISSUEGENE
elixirgen scientific
LIFESPROUT
MaxCyte
propagenix
Orgenesis
SR Synaptic Research
MTI-GlobalStem Acquired by Thermo Fisher Scientific
Cartesian Therapeutics
GEMSTONE
DOMICELL The Implantable Bioreactor Company
LONGEVERON CELL-BASED THERAPIES
BIOCARDIA
sentien biotechnologies

190
Different Disease Indications Supported

$19.2 Million
*Tax Revenues for Maryland and local governments

2,000
*Jobs Supported

41
Different Entities Supported

$213 Million
In MSCRF Awarded Grants

$3.2 Million
Funds Supporting Biomanufacturing in Maryland

654
Funded Research Projects

$525 Million
*In Economic Activity

$200 Million
*In Labor Income

* MSCRF's Economic Impact Report by Sage Policy Group, December 2024.

Every dollar invested by the state in MSCRF is a commitment to a brighter, more prosperous future!

Impact Beyond Economic Benefits

The impact of MSCRF has extended far beyond fiscal benefits; it continues to play a crucial role in advancing new medical strategies for treating diseases. Stem cell transplants conducted at Johns Hopkins are providing patients with new lives. Dr. Luis Garza's MSCRF-funded research at Johns Hopkins is helping heal damaged skin for amputees, including veterans. **A half-match bone marrow transplant, supported by MSCRF, functionally cured 16-year-old Nicholas Brown of Chronic Granulomatous Disease**. **LaShanta "Shauna" Whisenton underwent a similar transplant procedure and is now free from sickle cell disease.** See pages 35-38.

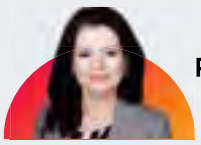
Additionally, **Germantown-based Seraxis, Inc., supported by MSCRF, is set to begin human testing on a curative treatment for Type I diabetes using stem cells.** Renovate Inc. is developing technologies to provide solutions for organ shortages through genetically modified animal organs. That research is backed by the MSCRF grants.

The value of these innovative cures and treatments, which offer substantial medical benefits, extends beyond monetary values, making their economic impact challenging to quantify.

MSCRF has cultivated a dynamic regenerative medicine ecosystem in Maryland, advancing innovative scientific concepts through capital, mentorship, and vital connections. We've forged meaningful partnerships and investment opportunities while training the future workforce in this crucial field. We are deeply grateful to lead this community and foster a culture of excellence, collaboration, and innovation that drives groundbreaking research and improves human health.

With continued funding, we can sustain our momentum, fostering innovation and job creation right here in Maryland. This ongoing support will enable transformative, life-saving advancements for people in Maryland and across the globe. As we continue to elevate this organization—backed by the commitment of the Maryland Governor and legislators, we look forward to accelerating further cures together and making a meaningful impact on the scientists and communities we serve.

With optimism and appreciation,



Ruchika Nijhara

Ruchika Nijhara, PhD, MBA
Executive Director, Maryland Stem Cell Research Fund

Rachel Brewster

Rachel Brewster, Ph.D.
Chair, Maryland Stem Cell Research Commission

MSCRF

2024
At-A-Glance

Investing in a Brighter Future

MSCRF Funding Opportunities

Clinical

Funding for clinical development of stem cell-based therapies.

Commercialization

Helping companies transition their work into marketable products.

Manufacturing Assistance

Funding to support manufacturing infrastructure/processes for stem cell therapies.

Discovery

Support for early-stage stem cell research projects.

Launch

Enabling new faculty members to pursue innovative stem cell research.

Validation

Funding for projects ready for translational development.

Post-Doctoral Fellowship

Support for post-doctoral researchers specializing in stem cell biology.

MSCRF offers funding opportunities through seven unique programs, designed to advance groundbreaking stem cell research and regenerative medicine.

2024

By the NUMBERS

111
Applications Submitted

$19.7 Million
Total Amount Funded

57
MSCRF Grant Awards

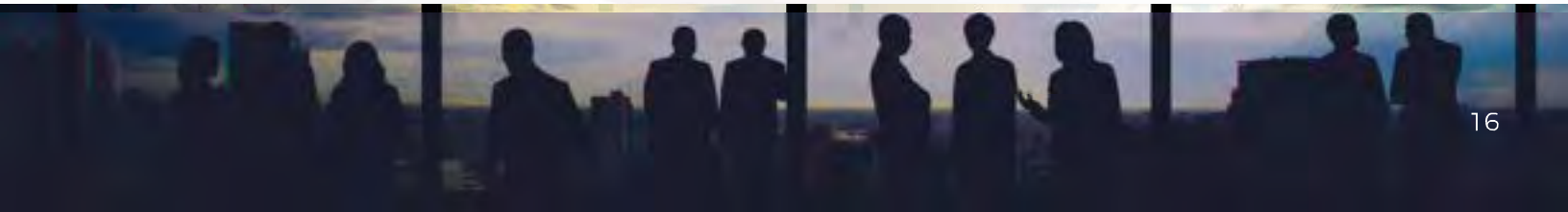
40+
Disease Indications

$4.5 Million
Awarded to Companies

56%
New MSCRF Awardees

12
Maryland Entities Funded

$1.3 Million
Awarded for Manufacturing



2024 At-a-Glance

A year of Momentum

2024 marks MSCRF's 18th anniversary as Maryland's premier granting agency dedicated to advancing research and development in regenerative medicine. **We have seen a substantial increase in demand for state funding, with requests soaring to $37 million—almost double the FY24 appropriation for MSCRF.**

We have experienced significant growth and success across multiple fronts, including the introduction of our new initiatives, advancements by scientists and innovators bringing breakthroughs closer to patients, the expansion and fortification of Maryland's biotech ecosystem, and increased economic benefits for the state.

A pillar of our success is our unwavering commitment to research, specifically in supporting innovative projects within academic institutions and companies. **This year, we proudly awarded over $19 million across 57 new research initiatives, driving forward basic, translational, and clinical research that holds tremendous promise for slowing disease progression, providing treatments, and ultimately curing human ailments that enhance overall health**. The foundation of our mission is rooted in leveraging stem cells to propel disease research. Our scientists have achieved numerous breakthroughs that are paving the way for a brighter future for patients everywhere.

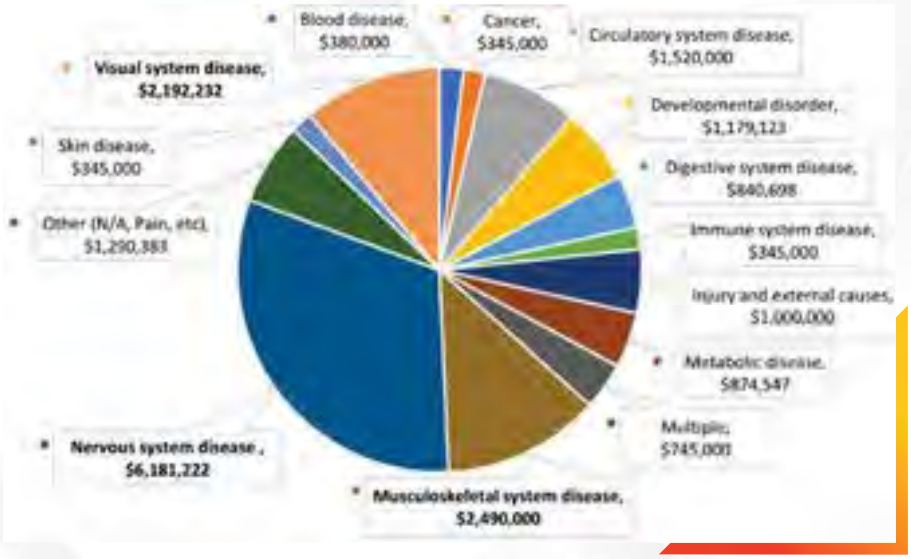
OVER $19M invested in 2024

Just alone in 2024, MSCRF has made a significant difference by supporting research across more than 40 different diseases, ranging from rare genetic disorders to common health issues. This diversity underscores the broad impact of our investments in driving innovation that transforms lives. Notably, the most ambitious research initiatives funded through our discovery grants comprised 36% of the total funding. These groundbreaking experimental projects and creative ideas are igniting revolutionary approaches that pave the way for life-saving treatments and a brighter future.

36% Funding Directed towards Innovative Research Projects

Remarkably, 56% of the grant recipients were new awardees. This corroborates the expansion of Maryland ecosystem and the growing recognition of regenerative medicine's transformative potential. This also reflects MSCRF's dedication to empower a wide range of brilliant minds to lead advancements in stem cell research. Additionally, this year saw a record annual increase of nearly 20% in applications, as the Maryland life sciences community increasingly recognizes MSCRF as an essential foundation for their innovative research.

- Nervous system: 33%
- Other: 9%
- Visual system: 9%
- Musculoskeletal system: 8%
- Circulatory system: 8%



2024 funding was allocated to 12 different public and private entities. The Orthobiologics Research Institute, a non-profit organization along with **six companies were grant recipients—three of which were startups, including Caleo Biotechnologies, Inc., HOH Cells, LLC, and Agnos Therapeutics along with thre young but successful enterprises like Reprocell USA, Seraxis, Inc., and Theradaptive Inc**. Among the other grant recipients were five esteemed academic institutions such as Johns Hopkins University, the University of Maryland at Baltimore, the University of Maryland, College Park, the Lieber Institute for Brain Development, and the Hugo W. Moser Research Institute at Kennedy Krieger. This diverse group exemplifies Maryland's commitment to advancing scientific research and breakthroughs in regenerative medicine.

> "At MSCRF, our commitment to driving positive change in Maryland and advancing regenerative medicine fuels our daily efforts. These core principles motivate our team to meet the needs of Maryland's life sciences ecosystem, pursuing what once seemed impossible. We are profoundly thankful to the Maryland legislature for their visionary support. Together, we are shaping a brighter future for every Marylander"
>
> **Ruchika Nijhara, PhD, MBA**
> Executive Director, Maryland Stem Cell Research Fund

Maryland Stem Cell Research Commission Highlights

New Leadership at the Maryland Stem Cell Research Commission (MSCRC)

Rachel Brewster, PhD, a distinguished professor in the biology department at the University of Maryland, Baltimore County (UMBC), has been unanimously appointed as the new chairperson of the MSCRF's Commission (board), beginning her term on July 1, 2024, for the next two years. Having initially joined the board in 2013 through an appointment by the University System of Maryland, Brewster was named co-Vice Chair in 2022, alongside Dr. Scott Bailey, who currently holds the Vice Chair position.

For two decades, the Brewster Laboratory has focused on the development of the central nervous system (CNS), which comprises the brain and spinal cord. Recently, their research has expanded to investigate how brain cells and cells in other organs respond to low oxygen levels (hypoxia). These vital studies aim to address significant public health challenges, from preventing CNS birth defects to developing better therapies for cellular damage associated with hypoxia, a condition linked to many major diseases.

> MSCRF grants support groundbreaking research and collaborations on stem cells across Maryland. We are proud to foster a future of hope and healing for patients.
>
> **Rachel Brewster, Ph.D**
> New Chair,
> MD Stem Cell Research Commission (MSCRC)

As she steps into her new role, Brewster is poised to bring a fresh perspective dedicated to advancing stem cell research and driving economic growth in Maryland.

She succeeds **Diane Hoffmann**, **M.S., J.D.,** Director of the Law & Health Care Program at the University of Maryland School of Law, who continues to contribute to the Board of Commissioners.

During Hoffmann's term, MSCRF's annual budget doubled to $20.5 million, significantly boosting our ability to support transformative research and create a lasting impact in Maryland and beyond. Under her leadership, MSCRF also launched the Manufacturing Assistance Grant Program in 2023, expanding funding support for companies to develop their manufacturing infrastructure and processes. The entire MSCRF team and the Commission extends their heartfelt gratitude for her unwavering dedication and visionary leadership.

New Maryland Stem Cell Research Commissioner

In 2024, MSCRF welcomed drug development and regulatory affairs expert **Mamta Gautam-Basak, PhD** to its Board of Commissioners, which reviews and accepts or rejects applications

Gautam-Basak was appointed to the MSCRF Board of Directors in January by Gov. Wes Moore's office. The appointment is for two years. She currently serves as Senior Director, Regulatory Affairs CMC at BridgeBio, a company focused on the development of transformative therapies for genetic diseases.
Prior to BridgeBio, Gautam-Basak spent two years as Senior Director, Regulatory Affairs CMC at Celldex Therapeutics. And before that, was a Senior Technical ASdvisor at US Pharmacopeia. For 26 years, Gautam-Basak served as a Senior CMC Policy Advisor at the Food and Drug Administration. In that role, she served as a primary CMC contact for drug manufacturers. She provided insight regarding CMC queries and decisions. Gautam-Basak also offered industry guidance from preIND, NDA approval and life cycle management of all CMC changes such as CMOs, drug shortages, recalls and inspectional issues.

"It was truly a privilege to serve as Chair of the Commission. Among the many highlights of my term was the hiring of Ruchika Nijhara as Executive Director. Under her leadership and dedication, she has led the Commission to new heights and achievements. It was also an honor to work with such a committed group of Commissioners. During my term we were able to expand the type and number of grants we have awarded and improve the quality of our review process and oversight of awardees. We have also progressed the field of stem cell research in the state and moved closer to our goal of seeing the development of new stem cell cures and therapies."

Diane Hoffmann, M.S., J.D.
Immediate Past Chair
MSCRC

Ira Schwarz, Esq.
The Daily Record's 2024 Leaders in Law Recognition

Ira Schwarz, a 2024 Leader in Law

MSCRF Commissioner **Ira Schwarz, Esq.** was named one of The Daily Record's 2024 Leaders in Law. Schwarz serves as legal counsel for both MSCRF and TEDCO. The prestigious honor is awarded to those who are not only dedicated to the legal profession, but also to the community at large. Schwartz was recognized for his commitment to supporting Maryland's innovation ecosystem. He was one of five selected to be part of the "In-House Counsel" category. Schwarz has served TEDCO for the past 25 years and is with MSCRF since its inception. The Daily Record's Leaders in Law awards are presented in collaboration with the Maryland State Bar Association.

Building a Manufacturing Infrastructure

The young companies established in recent years are now ready to expand their operations and scale their groundbreaking technologies for human testing and commercialization. **To create a thriving and sustainable ecosystem for company growth, MSCRF aimed to establish "sticky" initiatives that encourage companies to expand their R&D efforts within the state. This vision was the impetus behind the launch of the Manufacturing Assistance Grant Program in 2023**.

This program is designed to cultivate an environment that promotes the circulation and retention of talent, both entrepreneurial and scientific. The grant extends support to companies navigating the complex manufacturing needs inherent in the regenerative medicine sector as they advance their technologies toward clinical development. **With robust manufacturing capabilities comes the potential for talent development and economic growth. The first awards from this program were issued in 2023, and the positive outcomes are already becoming evident**. See pages 39-41.

In 2024, the Manufacturing Assistance Grant Program emerged as a focal point for numerous companies, leading to a competitive selection process. Among the numerous applications received, two companies- **Reprocell Inc. and Theradaptive, Inc. were awarded grants to support their manufacturing efforts.**

REPROCELL: Transforming Stem Cell Research with State-of-the-Art Manufacturing

Reprocell's large-scale GMP Grade iPSC Master Cell Bank facility with semi-automated filling capabilities

In May 2024, **REPROCELL** celebrated the inauguration of its state-of-the-art GMP (Good Manufacturing Practices) manufacturing facility in Beltsville, MD (see the picture), with the invaluable support of an MSCRF manufacturing assistance grant. This advanced facility is at the forefront of innovation, enabling the production of off-the-shelf GMP-grade Human Induced Pluripotent Cells (hiPSCs) and Human Mesenchymal Stem Cells (hMSCs) using cutting-edge, footprint-free RNA reprogramming technology.

The company also acquired a closed GMP cell culturing system, designed specifically for manufacturing clinically relevant hiPSCs and hMSCs. This advanced Cytocentric Xvivo Model 2 system, sourced from BioSpherix in New York, excels in producing highly sophisticated laboratory equipment that maintains optimal in-vitro (cell culture) and in-vivo (animal) environments.

With these improved manufacturing capabilities, REPROCELL is now capable of producing off-the-shelf GMP-grade hiPSCs and hMSCs for its internal development programs, as well as for client companies. **As the demand for GMP-grade stem cell products continues to rise, this state-of-the-art manufacturing facility positions REPROCELL to support stem cell researchers and companies, both in Maryland and beyond**. Their advances are already accelerating the translation of groundbreaking discoveries into clinical applications, paving the way for transformative therapies that could change countless lives.

Theradaptive, Inc.: Expansion of the Facility for Manufacturing its Product

The new manufacturing site in Frederick represents Theradaptive's continued growth to becoming a commercial company with MSCRF's support.

Frederick-based Theradaptive has made significant strides with the support of MSCRF's Manufacturing Assistance Program, which facilitated the establishment of their new 28,000 square Facility dedicated to their OsteoAdapt regenerative therapeutic product for spine and trauma repair. The new facility, which more than quadrupled, opened in September and is already being used to support Theradaptive's ongoing Phase I/II human clinical trial for OsteoAdapt. The expansion enables Theradaptive to manufacture their product in house bringing all their research and development in Maryland. **"Bringing manufacturing in-house improves product quality and control, accelerating our timeline for clinical testing and commercial release.**

This strategy allows for on-demand production, free from the limitations of Contract Manufacturing Organizations (CMO) availability and staffing, leveraging Maryland's rich biopharma talent, which is often scarce at many CMO locations", stated Dr. Luis Alvarez, CEO and founder of Theradaptive.

Theradaptive employs 33 people but anticipates adding approximately 50 more jobs in the coming years.

MSCRF New Initiatives- Expanding funding for Companies

MSCRF's new strategic initiatives are attracting outside companies to Maryland, nurturing public-private partnerships that drive the development of cures and expedite returns on investment.

In 2024, MSCRF opened its commercialization grant program to companies outside of Maryland, provided their research activities are conducted within the state of Maryland. **This initiative aims to foster research and development, enhance collaboration between entities, drive economic benefits for the state, and establish Maryland as a leader in the field of regenerative medicine.**

Recognizing that breakthroughs in healthcare are rooted in collaboration, MSCRF's initiative of second-tiered funding is connecting the two- often siloed but complementary worlds of academic and industry. In 2024, **three awardees- secured additional funding of $100,000 (second-tiered funding) each, through this initiative, by proposing collaborative efforts between industry and academic laboratory to advance their technologies**. "Second-tier funding" is a supplemental funding over the maximum grant amount, available to Validation and Commercialization grant awardees for public-private collaborative projects.

> "We're proud to establish Maryland as a nexus for stem cell innovation. These grants empower scientists and companies to accelerate scientific and commercial breakthroughs that can bring positive outcome to the patients."
>
> **Rachel Brewster, PhD**
> Chair, MSCRC

Company awardees, Agnos Therapeutics and Caleo Biotechnologies, Inc., each received second-tiered funding) on top of over $400,000 in Commercialization grants funding amount for collaborating with academic scientists at John Hopkins University. Additionally, **Renovate Biosciences, obtained $100,000 through second-tiered funding** alongside a $250,000 validation grant awarded to Dr. Elias Zambidis at Johns Hopkins University to advance his technology. These awards highlight MSCRF's unwavering commitment to accelerating the development of innovative stem cell-based therapies and technologies in Maryland, ultimately creating a positive impact on human lives.

Turning **Innovative Research** into **Real-World Companies**

Increase in the number of Maryland-Based **Start-up Companies**

The legacy of MSCRF funding has cultivated a vibrant community of exceptional scientists. The advancements made by these academic stem cell researchers, supported by MSCRF, are driving the creation of new companies. **MSCRF has facilitated the launch of 15 startups, five of which emerged in FY2024 alone.** MSCRF not only supports research in academic institutions that leads to the formation of these innovative companies but also plays a crucial role in nurturing the surge of new stem cell-focused startups throughout Maryland. In 2024, MSCRF supported three promising start ups through commercialization grants, further fueling innovation in Maryland as they represent the future of healthcare and life sciences.

Agnos Therapeutics

Agnos Therapeutics, a newly formed company from John Hopkins University, secured a Commercialization grant from MSCRF. The company is focused on the development of cellular component transfer (CCT) as a treatment to restore vision in retinitis pigmentosa (RP), a rare genetic eye disease that causes progressive vision loss. CCT is a cell therapy that allows donor cells to merge with the patient's own cells to replenish missing or defective proteins. Agnos Therapeutics was founded by Dr. Mandeep Singh, at Wilmer Eye Institute, Johns Hopkins University. **MSCRF grant will enable the company to lay the foundation for manufacturing and human clinical testing of CCT, which seems to be a safe, durable, and highly effective treatment for Retinitis Pigmentosa.** Retinitis Pigmentosa is among the most common causes of incurable blindness, and its treatment is an unmet need.

Caleo Biotechnologies

The recipient of a MSCRF Commercialization Award, **Caleo Biotechnologies,** a Maryland-based company, was recently founded by **Dr. Samaneh Kamali.**

The company developed the first comprehensive patient-derived Organ-Dish preclinical model for IBD. **Kamali and her team will pursue the IBD Organ-Dish Models to test the response of clinical drugs in a patient-specific manner.** Inflammatory bowel disease (IBD) is fast becoming a growing problem with patients often cycle through multiple chronic therapies. The response rates to existing therapies remain suboptimal, and most clinical trials fail. This may be traced back to the dearth of reliable and relevant preclinical models.

Caleo Biotechnologies aims to fill this gap using their novel and comprehensive Organ-Dish Models for IBD. The company secured Second-tiered MSCRF funding of nearly $100,000 on top of $400,000, to advance their technology via collaboration with Dr. David Hackam at Johns Hopkins University.

HOH Cells, LLC

HOH Cells, LLC, a spin-off company from University of Maryland, College Park, received a Commercialization grant to support the development of devices for 3D Culture and banking of human stem cells and their derivatives with high viability and function. Dr. Xiaoming (Shawn) He founded HOH LLC in 2023 to develop and manufacture ingenious products and devices for cell culture, banking, analysis, and therapy, and for drug screening. **Shawn received grant funds from MSCRF in 2021 and 2023, which enabled him to validate HOHCells technology and start his company.**

These ingenious technologies/devices will enable not only large-scale production of homogeneous high-quality human stem cells in 3D, but also convenient distribution and ready availability of human stem cells and their derivatives to end users, which are indispensable for the eventual success of the burgeoning stem cell-based medicine.

MSCRF

CLINICAL *Impact*

Investing in a Brighter Future

Demonstrating MSCRF's **Clinical Impact**

MSCRF has consistently focused on accelerating the development of new treatments and cures for the major diseases facing our society today. **Over the past seven years, MSCRF supported over 15 clinical trials**. This remarkable progress showcases how stem cell research is evolving from concept to reality.

In 2024, MSCRF awarded multiple grants to facilitate research advancing into clinical studies. Simultaneously, **ongoing MSCRF-backed clinical trials have already demonstrated successful patient outcomes**, underscoring the effectiveness of stem cells in creating innovative treatments.

These accomplishments highlight the transformative power of stem cells and reaffirm our commitment to fostering advances in regenerative medicine. We eagerly anticipate seeing even more groundbreaking therapies make their way into clinical practice in the near future. Here are some examples of clinical trials supported by MSCRF.

Regenerative Therapeutic Product for Spine & Trauma Repair

In 2024, MSCRF proudly funded the Phase I/II OASIS Clinical Trial, which assesses the safety and efficacy of Theradaptive's lead asset, OsteoAdapt SP, in spinal fusion procedures. **Three patients have already been dosed with OsteoAdapt, showing promising outcomes.**

The primary aim of the OASIS trial is to evaluate the dosing, safety, and efficacy of OsteoAdapt SP as an alternative to autologous bone grafts, the current standard of care in spinal fusion surgery. OsteoAdapt SP is a cutting-edge regenerative therapeutic product specifically designed for spine and trauma repair. It features a next-generation protein, AMP2, a proprietary variant of BMP2 (a bone-inducing protein), which is bound to scaffold implant materials to ensure sustained local delivery at critical defect sites.

OsteoAdapt SP

This innovative regenerative implant enhances the body's own stem cells to precisely regenerate bone tissue without the adverse off-target effects commonly associated with traditional methods. Beyond spinal injuries, OsteoAdapt is being developed for applications in dentistry, orthopedics, sports medicine, and veterinary medicine.

In November, preclinical data demonstrating the safety and efficacy of OsteoAdapt SP in a clinically relevant large animal model were published in Spine. In the article titled "In Vivo Assessment of AMP2, a Novel Ceramic-Binding BMP-2, in Ovine Lumbar Interbody Fusion," the findings indicated faster and more robust bone formation within the interbody cage. This preclinical study compared OsteoAdapt SP with iliac crest bone graft (ICBG) in a lumbar interbody fusion model, revealing that Theradaptive's therapy performed as well as or better than the gold standard in all assessed metrics.

As the demand for spinal fusion surgery rises, particularly among the aging population—where 40% of adults over 40 and 80% of those over 80 experience at least one degenerated disc—the need for effective alternatives is critical. While autologous bone grafting, which requires harvesting bone from the patient's body, remains the treatment of choice, it presents challenges such as limited availability and donor-site complications. Furthermore, alternative options like artificial implants and donor bones come with notable failure rates, sometimes as high as 35%. **OsteoAdapt SP addresses these challenges by offering a potent, precise solution that overcomes the limitations of existing treatments.** Additionally, it has been granted three breakthrough designations for spinal applications, paving a faster path to market.

Non-surgical **Treatment** using Adipose Derived **Stem Cells, MFAT, for Rotator Cuff Tears**

In September 2024, MSCRF awarded OrthoBilogics Research Initiative, Inc., to support a clinical trial that is a stem cell therapy (Micro-Fragmented Adipose Transfer, MFAT) for Partial Thickness Rotator Cuff Tears.

Patients with partial thickness rotator cuff tears typically have three treatment avenues: cortisone injections, physical therapy, or surgery. Unfortunately, these methods do not always deliver satisfactory results. **The implementation of MFAT offers a potential long-term regenerative solution, improving pain management and enhancing functionality for patients with these injuries.**

Dr. John Ferrell, III, founder of ORI and the principal investigator on the clinical trial will evaluate the effectiveness of MFAT grafts in treating partial thickness rotator cuff tears, focusing on patients who currently face less-than-ideal standard care options.

Slated to commence in early 2025 in partnership with Regenerative Orthopedics and Sports Medicine (ROSM), the trial will include up to 60 patients. Dr. Ferrell acknowledges the growth potential in regenerative medicine over the next decade and highlights that much work remains to make stem cell-based therapies widely accessible.

> "We are at a pivotal moment where regenerative medicine has the potential to revolutionize orthopedic care, shifting the focus from reactive treatments to preventative solutions. Research shows that a significant percentage of individuals over the age of 60 have rotator cuff tears. By treating these conditions earlier with non-invasive regenerative approaches, we can prevent countless unnecessary surgeries and improve patient outcomes."
>
> Dr. John Ferrell,
> Founder of OrthoBiologics Research Initiatives, Inc.

"Currently, this procedure is not covered by insurance, making it cost-prohibitive." he explains. **"We believe this landmark study could reshape the landscape of MFAT for partial thickness rotator cuff tears, with the hope that such procedures will eventually gain insurance coverage for this and other indications."**

Stem cells to Cure Type-1 Diabetes

Germantown-based Seraxis is one step closer to bringing a potential functional cure for insulin-requiring diabetes. In October, the FDA greenlit a Phase I/II human clinical trial evaluating Seraxis' lead stem cell therapy for diabetes.

Seraxis' SR-02 is a cluster of pancreatic cells that closely resemble the native pancreatic "islets" that are damaged in diabetes patients. The islets secrete hormones, such as insulin and glucagon, which regulate the amount of glucose in blood. SR-02 is the first reprogrammed stem cell-derived pancreatic product candidate authorized by the FDA for clinical assessment as a potential functional cure for insulin-requiring diabetes. This approach is expected to improve patient outcomes and be more broadly accessible to a broader population of diabetes patients.

Over the past several years there have been significant advancements in treating diabetes, including the use of automated insulin delivery devices. However, much still need to be done. Poorly regulated levels of blood glucose lead to kidney failure, cardiac and neurologic disease, blindness, limb amputation and shortened lifespan.

The Seraxis therapy is intended to restore normal blood glucose regulation to patients of diabetes.

> "MSCRF investment accelerates our timelines to IND clearance for our next generation therapy, having an oversized impact on our goals of benefitting the largest patient population possible."
>
> Dr. William Rust,
> CEO of Seraxis

The Phase I/II clinical trial will assess safety and efficacy of SR-02 after it is implanted in the abdomen of patients with insulin-requiring diabetes. SR-02 will be evaluated in a small number of patients, and will monitor pancreatic endocrine function in patients of poorly controlled diabetes.

Over the years, MSCRF has supported Seraxis' efforts to bring a potential functional cure for diabetes to the market. **As Seraxis continues to advance its assets through the clinic, company officials are confident that all patients of diabetes can look forward to a day free of insulin injections**. Within the next five years, Seraxis believes it will be near to completing late-stage clinical studies of SR-02 and expanding manufacturing to commercial scale.

Novel **Cell Therapy** for Amputees

MSCRF awarded Dr. Luis Garza clinical grants to support testing stem cells to regenerate skin at the stump site by converting stump skin to palmo-plantar skin in amputees. This work will benefit individuals, including military personnel who rely on prostheses after an amputation. Promising results have emerged from over two dozen patients who do not use prosthetics; this group was chosen to establish best practices and gather baseline data, and they have shown positive outcomes with this method. **The ultimate goal of the cell therapy is for amputees to develop a callous at their stump site as opposed to an ulcer; a change that would likely allow them to wear their prosthetics comfortably.**

Dr. Garza, an associate professor of dermatology at Johns Hopkins University School of Medicine, explains that the stem cell therapy aims to enhance the health of skin cells at the amputation site, facilitating a more comfortable prosthetic experience. "After the stem cell injection, the skin at the stump site feels firmer," he notes, adding that a microscopic examination reveals that the top layer of skin cells at the injection site appears thicker, with collagen that is longer and stronger. "These are powerful indicators that the skin is changing.

These findings from the first MSCRF -funded clinical trial were recently published in the reputable Science Journal, highlighting the efficacy of this cellular therapy shows efficacy in human subjects.

Looking ahead, the 2024 MSCRF-funded trial aims to investigate whether pre-conditioning the skin can enhance stem cell engraftment and improve clinical outcomes.

Through these innovative efforts, Dr. Garza and the MSCRF are committed to transforming the lives of amputees, paving the way for better prosthetic integration and overall quality of life.

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

Dr. Sarah Sunshine's clinical trial supported by MSCRF clinical grant uses mesenchymal stem cells for the treatment of ocular graft versus host disease (GvHD).Ocular GvHD is a severe inflammatory dry eye disease that affects patients who have undergone a bone marrow transplant. It results from the donor immune system attacking the host ocular surface or eyes. Despite knowing that the donor cells are the cause, there are no targeted therapies for this issue.

It's the first time this approach is being assessed in a clinical trial. If successful, it will expand the role of stem cell therapy to treat eye diseases. Currently four patients have been enrolled in the MSCRF-supported clinical trial and the study will continue to recruit patients through 2025.

Sunshine, an assistant professor at the University of Maryland, specializes in treating corneal and ocular surface diseases focusing on complications of oncologic therapy in the eye, most specifically on ocular Graft vs Host Disease.

"Despite the disease being caused by the bone marrow transplant, our current therapies are limited, with only lubrication, environmental changes, and steroids as treatment options, which result in complications including cataracts and glaucoma. Therefore, it is critical to find a safer and more targeted therapy.

> "I am honored to be a part of the MSCRF community and am so fortunate to see the potential benefit that the stem cell therapy has had on my patients who have enrolled in the study. To be a part of moving the field of stem cell therapy forward is exciting and inspiring to continue working and studying stem cell therapies in our most challenging patients."
>
> Dr. Sarah B. Sunshine,
> University of Maryland, Baltimore

Mesenchymal stem cells (MSC) are a potentially ideal treatment option as they have immune-modulatory and other protective effects as well as the potential to differentiate into local tissue types. We believe that the main mediators of the effect are actually factors that are secreted by the MSCs."

MSCRF support has been essential for Sunshine's research. **In addition to the financial assistance, Sunshine says MSCRF has created a community of scientists focused on stem cell biology and regenerative medicine that has boosted opportunities for collaboration that will expand the capabilities of this rapidly-evolving field.**

MSCRF

Celebrating TRANSFORMATIVE SUCCESSES

Investing in a Brighter Future

Celebrating Patient Cures & the Success of MSCRF Awardees

Every advancement that positively impact the Maryland community deserves recognition and celebration. This section highlight the inspiring stories of patients who have been cured by stem cell therapies and our awardee companies that have achieved significant milestones . These successes also illustrate the significant benefits derived from the state's investment in MSCRF, enhancing both the Maryland economy and its communities.

Celebrating Cures

We are proud to honor the lives transformed by stem cell therapies, celebrating the significant cures supported by MSCRF. Our programs have empowered researchers and clinicians to break away from traditional models that merely address symptoms, steering their focus toward restoring health and function. This shift not only directly enhances patient outcomes but also reinstates hope and enriches the quality of life for individuals and their families.

Curing Sickle Cell Disease

For the past 14 years, LaShanta "Shauna" Whisenton has been living her best life free from the pain caused by sickle cell disease.

Extreme pain is a constant companion for people living with sickle cell disease. That pain can be so severe that patients such as Whisenton seek medical attention if over-the-counter remedies do not work. Throughout her life, Whisenton sought medical attention to alleviate the pain caused by Hemoglobin S-C disease, a rare type of sickle cell.

In the early 2000s, her hematologist informed her of a sickle cell clinical research program at Johns Hopkins that offered a potential functional cure of her disease. To Whisenton, that kind of potential was a complete shock. She believed sickle cell was a burden she would bear her whole life. After discussing the opportunity with her family, Whisenton decided to pursue the bone marrow transplant treatment. The first step was finding a suitable marrow donor. The type of procedure Hopkins was conducting was a haploidentical transplant.

Also known as a half-match bone marrow stem cell transplant, a haploidentical procedure uses cells from a related donor who has a partial match to the patient's human leukocyte antigens. The best match was Whisenton's eldest son, who was eight years old at the time. The boy who had witnessed his mother's many trips to the hospital to address her sickle cell-related pain was eager to help. **The procedure was a resounding success**. Within six months, routine testing conducted by the Hopkins team revealed she was 96% sickle cell free.

Whisenton hopes others can experience the same kind of freedom that she's come to know. **She says it's "absolutely imperative that there is state and government funds, as well as private money, to help people shape this kind of research."**

"I was so excited to see these results. Being free from sickle cell opened up so many opportunities for me. The pain from sickle cell is so severe. I haven't experienced that pain in more than 10 years, but I'll never forget how it feels-"

LaShanta Whisenton

Now that she's functionally free from the chains of Sickle Cell, Whisenton, who was a fierce advocate for herself, is now lending her voice in support of others who are navigating their way through life with the burden of the disease. She has written a series of children's books that could help other families who are experiencing many of the same issues she and her family did. The series, "Niam's Journey," is about a middle-school aged boy with sickle cell disease.

The stories focus on the ways sickle cell interrupts people lives, how he navigates through each day with the support of his family and friends. Her goal is to have the illustrated book be available to patients through hospitals, doctor's offices and libraries, anywhere a sickle cell patient or family member can find it.

Stem Cells Offer Cure for Chronic Granulomatous Disease (CGD)

The first few years of Nicholas Brown's life were challenging. Weeks after his birth, Nicholas faced a myriad of mysterious medical challenges, including inflammation of the gut, infections and high fevers.

Caroline Laguerre-Brown, Nicholas' mother, says no one understood the root of Nicholas' problems. Nicholas underwent a battery of tests at Johns Hopkins University conducted by every major service" before a diagnosis appeared. Nicholas was diagnosed with chronic granulomatous disease (CGD), a rare, inherited genetic disorder that causes the body's immune system to malfunction due to improper function of the phagocyte, a type of white blood cell that fights infections.

Initially, Nicholas was required to take numerous medications, including interferon gamma shots. It was a tough life for the youngster. Because of how easily he caught infections, Nicholas was unable to associate with other children his age. In 2011, when Nicholas was about four years old, he became seriously ill with recurrent pneumonia. He spent significant time in the care of his family and healthcare providers. His mother says the challenges that winter were the most difficult her son faced. But, it's always darkest before dawn. Later that year, Nicholas came under the care of Dr. Heather Symons, a pediatric oncologist and Clinical Director, Pediatric Blood and Marrow Transplant at Johns Hopkins University.

Symons offered a glimmer of hope for Nicholas with a bone marrow transplant. Although the prospect sounded "terrifying" to Caroline and her husband, Symons was able to explain the process and bring the Browns to a "place of comfort." While none of the Browns were a good match for Nicholas, Symons explained to the family about the half-match procedures that Johns Hopkins was seeing success with. The procedure, called a haploidentical transplant, uses stem cells from a donor who is a partial genetic match to the patient.

Nicholas' father met this criterion and was able to donate his bone marrow. The procedure was a resounding success. Tests following the procedure showed that Nicholas' bone marrow was 100 percent his father's. This meant it was 100 percent free from GCD.

"He has no need to take any medicines for GCD. It's absolutely crazy when you think about how much medicine this kid was taking," Caroline says. "It's hard to put into words how consequential this was for Nicholas, for us. What this team has done to arrive at a procedure is give every kid with a live parent a chance. The research was life transforming. It's profoundly impactful for families who are going through terrible medical crises. It's literally the difference between life and death. A life where your child can live to their full potential."

That was 10 years ago. Today, Nicholas is 16 years old. He is in a science and tech program at his high school, plays lacrosse and serves as a mentor to middle school students.

"We celebrated Nicholas' 10-year transplant birthday in 2023. **That groundbreaking research brought our family out of a dark place. It was transformative. I can't think of anything more impactful than the ability to transform lives." Caroline adds.**

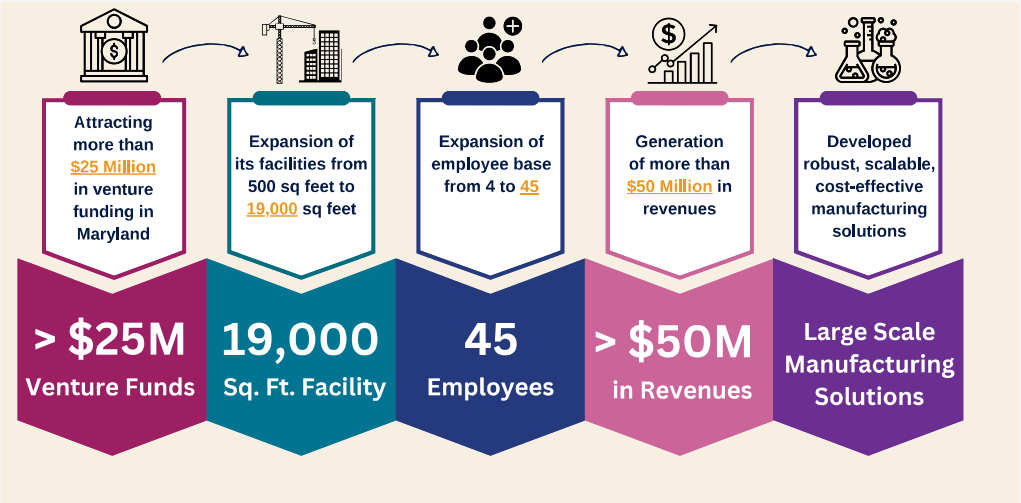
Symons says continued funding through the Maryland Stem Cell Research Fund is crucial for stem cell research. Without the kind of funding MSCRF provides, innovation can be stymied.

"We can't learn as much if we don't have adequate funding. **This funding is saving lives. The value of that... you can't put a monetary value on that. The commitment that the state of Maryland has made to stem cell research means that we can provide cutting edge, innovative treatments right here in Maryland. It gives us the opportunity to move the bar for treating patients who otherwise have no cure,"** adds Symons.

Celebrating RoosterBio, Inc.

Founded in 2013 by bioentrepreneur Jon Rowley, PhD, RoosterBio began its journey within the Frederick Innovative Technology Center, Inc. (FITCI), Maryland's local technology incubator. Today, RoosterBio stands at the forefront of advancing biomanufacturing processes that are crucial for the commercialization of Cell and Gene Therapy. **Over the past decade, MSCRF has invested a total of $1.89 million in RoosterBio through 4 grants**, including the grant for enhancing the efficiency of their biomanufacturing processes.

This investment has yielded nearly a 100X return to the state while bolstering the local economy. To date, RoosterBio has attracted $25 million in venture capital to Maryland and has generated over $50 million in revenue in just a short span of time, experiencing nearly 50X revenue growth since its first year on the market. This success has been instrumental in creating high-paying jobs within the state.



RoosterBio has expanded from 500 square-feet to a state-of-the-art 19,000-square-feet facility in Fredrick. The company has launched four innovative stem-cell products. Their stem cell products have been utilized in multiple clinical trials, successfully treating hundreds of patients. RoosterBio has also gained international recognition, extending their services to the Asia Pacific, Europe, and South America in addition to North America.

MSCRF's manufacturing assistance grant enabled RoosterBio to strategically position itself as a leader in exosome bioprocessing, reinforcing its role in advancing biomanufacturing. In 2024, the company formed several strategic partnerships, including collaborations with Wisconsin-based Waisman Biomanufacturing, a leading contract development and manufacturing organization, and BioSolution Designs, creating an end-to-end solution for the engineering and development of cell- and exosome-based therapeutics.

Celebrating Theradaptive, Inc.

2024 has been a landmark year for Theradaptive Inc., a Maryland based company at the forefront of targeted regenerative therapeutics. In September 2024, the company initiated clinical Phase I/II testing for its lead product, OsteoAdapt, for spine and trauma repair. See page 29 for clinical trial details. This year, **Theradaptive opened a new facility in Frederick, expanding its space from 6,000 to 28,000 square feet. The company has increased its workforce from 3 to 33 employees, with plans to double the team as manufacturing operations ramp up.**

This facility was partially funded by a MSCRF manufacturing grant awarded in 2023, allowing Theradaptive to consolidate all its manufacturing efforts in Maryland. **With a total investment of $3.5 million from MSCRF over the years, Theradaptive has attracted $60 million in venture capital, representing nearly a 20X return on the state's investment.**

Dr. Luis Alvarez, CEO of Theradaptive was motivated to start a company focused on bone regeneration products due to the injuries he witnessed among soldiers during his time as a Lieutenant Colonel in the US Army.

The company has received the Gold Level Award for Best Technology in Spine 2024 from Orthopedics This Week, a recognition from a panel of spine and neurosurgeons that highlights the advancements Theradaptive is making in neurosurgery. Alvarez anticipates having insights from the ongoing OsteoAdapt SP Clinical trial in 2025 to support a planned Series B financing round later next year.

"MSCRF has enabled us to establish our operations in Maryland, supporting the local economy. Within a year or two, the benefits of our manufacturing efforts will manifest in economic stimulation. **MSCRF grant funds are critical to expanding cell therapy and biopharma across Maryland. The funds will support long-term activity in Maryland." said Alvarez**.

Alvarez envisions Theradaptive as a "commercial stage biopharma firm based in Frederick with an expanding clinical pipeline, manufacturing operations, and R&D all happening in the state of Maryland." He emphasizes, **"If you want to build Maryland's bio-economy, MSCRF is one of the best ways to do it. Their support can make the difference between a company operating in Maryland or elsewhere**."

Celebrating **Reprocell, USA, Inc.**

In May 2024, REPROCELL proudly inaugurated a new GMP biomanufacturing laboratory facility in Maryland, partially funded by MSCRF through its Manufacturing Assistance Grant Program. As Rama Modali, Chief Executive Officer of REPROCELL USA, Ltd., explains, this advanced biomanufacturing facility addresses the growing needs of companies and scientists, propelling the clinical development of innovative stem cell therapies and treatments. See page 22.

Reprocell Inc, USA (formerly BioServe) expanded its focus in regenerative medicine after being acquired by Reprocell Japan in 2014. At the time of acquisition, the company employed just 10 people.

Over the past decade, MSCRF's investment of $1.4 million has yielded tens of millions in sales revenue, with annual revenues approaching $10 million each year. Notably, 80% of Reprocell's annual revenue comes from clients outside Maryland, contributing to Maryland economy.

Reprocell has expanded its workforce from 10 to 25 employees, filling high-paying salaried positions.

"MSCRF support is enormous, particularly when we talk about bringing some unique therapeutic agents to market. It's helped us position ourselves as one of the leading companies developing preclinical assets for neurodegenerative diseases. MSCRF is a great vehicle for us." Modali says.

Looking ahead, Modali anticipates further growth, with Reprocell planning to enhance its capabilities to produce four cell lines simul-taneously in 2025, effectively doubling its current capacity. This growth trajectory is expected to continue, with both the company's operational footprint and employee base in Maryland to set to grow by 50% in the next five years.

Celebrating 25 Years of MaxCyte: Innovating Cell Engineering Therapies

For 25 years, Rockville-based MaxCyte, Inc. has made a profound impact on the field of cell therapy through its proprietary ExPERT cell engineering platform, which has become an industry standard. Since its inception in 1999, MaxCyte and its Flow Electroporation platform have established themselves as cornerstones of regenerative medicine both in Maryland and around the globe.

MSCRF has invested nearly $0.8 million in MaxCyte through grants in 2015 and 2017, which have yielded impressive returns as the company experienced significant revenue growth and expanded its presence in Maryland. Last year, MaxCyte reported total revenues exceeding $40 million, contributing positively to the Maryland economy.

"We've made tremendous progress over the last 25 years," said Maher Masoud, President and CEO of MaxCyte. "As advanced cell and gene therapy modalities transition from concept to clinic, new cell engineering approaches will continue to evolve. With every milestone, we leverage our best-in-class electroporation technology and industry expertise to support our customers in advancing bio-based medicines and improving patients' lives." The ExPERT platform was specifically designed to engineer blood cells for disease treatment and served as the foundational technology for the first FDA-approved CRISPR-based gene therapy for severe sickle cell disease.

In December 2023, the FDA approved Casgevy (exa-cel), a groundbreaking treatment developed by CRISPR Therapeutics & Vertex Pharmaceuticals.

In 2022, the company unveiled a new 67,000 square-foot facility in Rockville, enhancing its process development capabilities and providing additional office, laboratory, and manufacturing space. This expansion allowed MaxCyte to strengthen its operations and innovate further in cell engineering.

MaxCyte's technology has not only made a significant impact in regenerative medicine but has also fostered substantial growth within Maryland, emphasizing the company's role as a leader in the industry over the years.

Celebrating Dr. Xiaoming He's Journey: Translating Research into HOHCells, LLC

Dr. Xiaoming (Shawn) He's remarkable journey with MSCRF illustrates the powerful transition from groundbreaking research to the formation of a successful commercial venture, **HOHCells LLC**. Initially propelled by MSCRF Discovery Grant in 2021, his path was further strengthened by a MSCRF's Validation Grant in 2023 and MSCRF's Commercialization Grant for HOHCells in 2024.

Shawn is driven by a vision to revolutionize the culture of Induced Pluripotent Stem Cells (iPSCs), addressing the traditional reliance on ROCK inhibitors, which can lead to spontaneous cell differentiation and compromise cell quality. He is a professor at Fischell Department of Bioengineering at University of Maryland, College Park.

In July 2023, Shawn founded HOH LLC, humorously noting that "HOH" represents his "head over heels" enthusiasm for cells while also alluding to the chemical formula for water. Reflecting on the transformative impact of MSCRF grants, he emphasizes that these resources facilitated the crucial first step toward establishing his company. "**Without this financial support from MSCRF," Dr. He states, "the prospect of starting a company would likely have remained just an idea**."

The 2021 Discovery Grant supported his research in 3D Culture and Differentiation of Human iPSCs for Cardiac Tissue Engineering and Regeneration. The subsequent Validation Grant in 2023 reinforced his commitment to developing innovative solutions for prevalent diseases in Maryland, including cancer, diabetes, cardiovascular diseases, and neurological disorders. The 2024 Commercialization Grant focuses on developing devices for 3D culture and banking of human iPSCs and their derivatives, ensuring high viability and functionality.

Dr. He acknowledges the critical role MSCRF grants have played in advancing his technology toward commercialization. The banked iPSC-Derived Cardiac Organoids, made possible by MSCRF funding, present versatile applications in research laboratories, fueling advancements in various cell therapy research that will ultimately contribute to clinical testing. **He highlights the "tremendous advantage" that MSCRF's financial support provides to Maryland's stem cell research community, equipping local researchers with a competitive edge.**

MSCRF not only empowers groundbreaking research but also enables researchers like Dr. He to take transformative steps, cultivating a robust and dynamic entrepreneurial ecosystem in Maryland.

MSCRF

REFLECTIONS
from the MSCRF Ecosystem

Investing in a Brighter Future

The impact of MSCRF extends far beyond funding; it resonates deeply within the community it serves. Feedback from stakeholders in our ecosystem—innovators, entrepreneurs, companies, postdoctoral fellows, and other stakeholders—reveals a strong appreciation for the support and growth opportunities provided by MSCRF. Many express gratitude for the collaborative environment fostered by our initiatives, which has led to innovative partnerships and transformative research.

Participants in the MSCRF ecosystem consistently highlight the value of our funding in bridging the gap between groundbreaking ideas and clinical applications. They commend the proactive efforts of MSCRF to engage with the community, ensuring that every voice is heard and valued. Whether through grants that bolster research or initiatives that encourage collaboration among local institutions and companies, the overarching sentiment is one of optimism and inspiration.

The collective feedback underscores the belief that with continued support and investment, the Maryland ecosystem can drive significant advancements in regenerative medicine, ultimately improving patient outcomes and enhancing quality of life for individuals affected by various health challenges. Together, we are building a stronger ecosystem and a brighter future in Maryland.

Christy Wyskiel, Senior Advisor to the President for Innovation and Entrepreneurship, Johns Hopkins University

MSCRF plays a pivotal role in the Johns Hopkins innovation ecosystem, empowering our scientists and entrepreneurs to transform early-stage innovations into investable assets and thriving startups. **This funding has strengthened our entrepreneurial ecosystem and attracted significant company investments**. As I review our pipeline of life sciences technologies poised to become new cures, I am struck by how many benefited from this great non-dilutive funding source.

Alla Danilkovitch, Founder and Chief Scientific Officer, Britecyte, Inc.

MSCRF funding helped advance Britecyte's adipose technology for liver diseases and supported the commercial launch of the Liposana product for adipose defects. *Britecyte is very thankful for MSCRF's support and looks forward to continuing to work with the MSCRF team.*

Ha Nam Nguyen, Founder and CEO, 3Dnamics, Inc

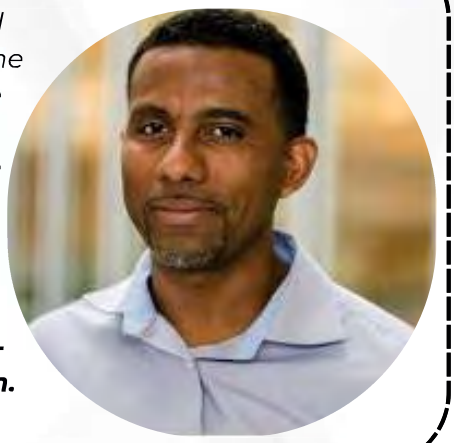
MSCRF was instrumental in the establishment of our company. Without it, we would not have started the company.

Rachana Mishra, Founder and Director, Secretome Therapeutics, Inc.

MSCRF has been great to work with. They understand the environment of a start-up and the ability to be agile along the way with us as we try to accomplish our goals. **I was able to grow as a faculty member at the University of Maryland and publish because of MSCRF funding before I transitioned to my spin-off company, Secretome, Inc., also later supported by MSCRF.**

Warren Grayson, Professor, Johns Hopkins University

This funding was instrumental in facilitating the continued progress of the bone regeneration studies in my lab. It was the first major funding for my lab in the area of skeletal muscle regeneration and has been transformative for the work we have been able to do. We are now recognized as one of the leading labs nationally in the field of skeletal muscle regeneration.



The continuity of funding for the bone regeneration projects has been pivotal in moving our team from early-stage, basic science research into the realm of translation. We are currently transitioning into a company.

Jennifer Erwin, Assistant Professor, Lieber Institute for Brain Development

The MSCRF launch funding came at a crucial time in our scientific research and helped bridge the gap before obtaining significant NIH funding.

Nancy Cowger, Executive Director, Licensing and Alliances, University of Maryland, Baltimore

*Our team at UM Ventures, Baltimore, is very encouraged by the outreach and support of the MSCRF stage and the enhancements to MSCRF funding programs under Dr. Nijhara's leadership," said Dr. Nancy Cowger, Executive Director, Licensing & Alliances. "***We've noted that certain MSCRF funding programs have been revised to spur more public-private collaborations, and we expect to see a positive impact on UMB's technology commercialization goals as a result of our faculty innovators and startups participating in these programs.**

Ricardo A. Feldman, Professor, University of Maryland Baltimore

In 2009, I received a 5-year, $1,500,000 grant from the MSCRF, which allowed me to establish an internationally recognized research program on Gaucher disease and GBA1-associated Parkinson's disease. Without this and subsequent MSCRF funding, we would not have been able to uncover new disease mechanisms, identify druggable therapeutic targets, or validate new drugs from biotech companies (such as Gain Therapeutics). Our laboratory is now one of the go-to places for testing new biotech drugs. We are now on the cusp of developing a new test to identify individuals at the highest risk of developing Parkinson's disease years ahead of clinical symptoms.

The visionary leadership of the MSCRF has made Maryland one of the two premier promoters of stem cell-based treatments for diseases for which there is no cure. **Considering that the other state-funded engine for stem cell research (California's CIRM) has 100-fold more funding than Maryland, the MSCRF's impact is tremendous. More funding would greatly hasten the timeline to find cures for intractable diseases, which significantly impact quality of life and weigh heavily on healthcare budgets.**

Arthur S. Feltrin, Postdoctoral research Fellow, Lieber Institute for Brain Development

*MS*CRF funding has been pivotal in advancing my research. It has enabled us to apply advanced machine learning techniques to analyze complex neurogenetic data from SETD1A mediated regulation in human-derived stem cells, providing critical insights into the mechanisms underlying both schizophrenia and human neurodevelopment. **The MSCRF Postdoctoral Fellowship is an excellent program that significantly supports the advancement of stem cell research.** It offers a unique and valuable opportunity for non-American citizens and residents to secure funding for their research, promoting diversity and fostering innovative ideas from a global pool of scientists working in the USA

Daniel Lobo, Associate Professor, University of Maryland, Baltimore County

This MSCRF funding has been essential to expand my research to human stem cells and establish a new collaboration with University of Maryland, Baltimore.

Valina Dawson, Professor, Johns Hopkins University

Funding has allowed research in areas not supported by federal funds, producing new knowledge and advancing the field.

Mingyao, Ying, Associate Professor, Kennedy Krieger Institute

The MSCRF funding is critical for my career development in the field of human stem cell research**. I have established a research network involving industrial and academic scientists** to apply our technology in human iPS cell differentiation for disease modeling and cell replacement therapy.

Elias T. Zambidis, Johns Hopkins University

I am fortunate to have been awarded several grants from the MSCRF, which have permitted the development of an academic program aimed at treating children with life-threatening diseases that can only be cured with stem cell transplantation.

Additionally, MSCRF funding allowed me to perform research with human embryonic stem cells that otherwise cannot be funded by the NIH, due to current federal restrictions on the use of certain stem cells and fetal tissues.

Finally, the insights gained from aligning with the goals of the MSCRF further inspired me to engage in national leadership in public education of regenerative medicine for other stem cell scientists.
I have advocated for several policy efforts for the promotion and funding of human stem cell research at the federal and state levels. For example, I recently joined a consortium of international scientists organized by the International Society of Stem Cell Research (ISSCR) to testify and advise lawmakers at the U.S. Congress on matters that exactly align with the mission of the MSCRF: the liberalization of federal funding of human fetal and human embryonic stem cell research.

MSCRF

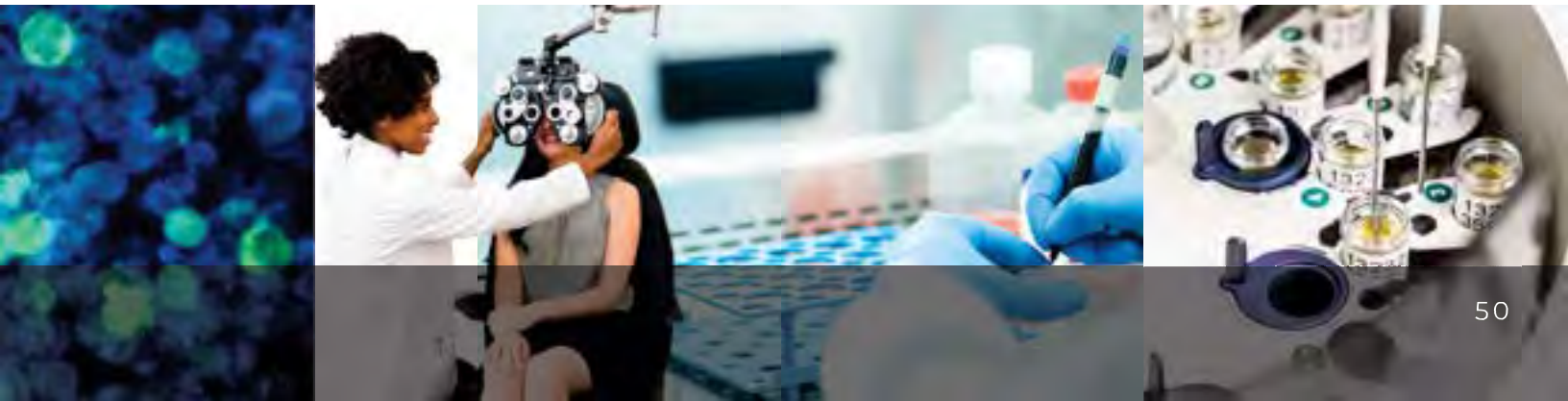
COMMUNITY *Impact*

Investing in a Brighter Future

Optic neuropathies, including glaucoma, are the world's most prevalent cause of irreversible blindness. These diseases cause permanent vision loss by killing retinal ganglion cells (RGCs), the nerve cells that send visual information from the retina of the eye to the brain though fibers (called axons) within the optic nerve. Though some types of optic neuropathies can be treated to slow or stop disease worsening, vision restoration would require replacement of RGCs and their connections within the eye and brain.

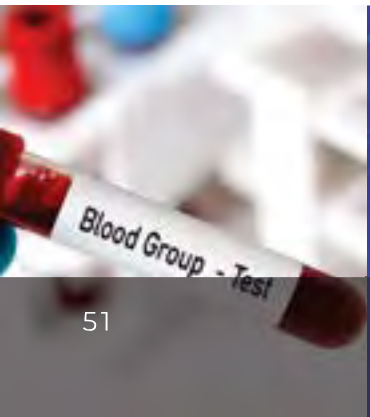
Dr. Thomas Johnson from Johns Hopkins University has recently developed human stem cell derived RGCs to treat optic neuropathy. However, after implantation, cell (neuron) survival, extension of cell fibers into the retina and brain, and formation of "communication stations" (functional synapses) within the visual pathway remain challenging. To overcome this, Dr. Johnson is developing a specialized biomaterial (scaffold) into which donor RGCs can be added. The scaffold mimics critical properties of the retina and can be loaded with bioactive molecules to provide donor neurons with important signals that improve their survival and direct their functional engraftment into the eye.

In this project. Dr. Johnson will evaluate several design characteristics of the scaffold to achieve optimal regenerative potential. In addition, Dr. Johnson will develop a surgical technique to implant the scaffold through a small incision and evaluate the survival and engraftment of donor neurons, which will set the stage for human clinical trials.



There is an urgent need for new prevention and treatment strategies for Alzheimer's disease, with anti-inflammatory approaches as a promising yet under-explored avenue. Microglial activation has been implicated in neuronal damage, which suggests that modifying this activation could be a viable treatment target. **Dr. Machairaki**, a scientist from John Hopkins University, focuses her research on discovering anti-inflammatory drugs using a personalized medicine approach with induced Pluripotent Stem Cells (iPSCs) from Alzheimer's patients and healthy individuals. By capturing unique genetic information, the study will assess individual responses to inhibitors on iPSC-derived microglia.

Previous attempts at using anti-inflammatory strategies for Alzheimer's have often failed due to variability in inflammatory responses between individuals and the lack of relevant models for validating treatments. This initiative seeks to address these challenges, driving the development of effective therapies for Alzheimer's disease.



Stem Cell Therapy to Treat CHRONIC PAIN

DR. XINZHONG DONG

Dr. Xinzhong Dong, Over one-third of the world's population suffers from devastating pain caused by neurological disorders, diseases, car accidents, war injuries, chemotherapy, etc. Most drugs on the market for pain treatment have undesired side adversely affects the quality of life.

Dr. Xinzhong Dong from Johns Hopkins University have recently developed novel (pain-sensing) neurons from human pluripotent stem cells (hPSCs) that can survive for prolonged periods of time in the body after implantation. In doing so, they appear to establish functional connections with the body's own neurons to potentially sensitize or directly activate them to alleviate pain.

In this validation project Dr. Dong will test if these novel hPSC-derived sensory neurons can mitigate the pain (responses) in vivo, potentially leading to the development of a novel stem cell-based therapy for treating chronic pain.

Neurodevelopmental disorders (NDDs), such as schizophrenia, are the outcome of trajectories that start early in life. Although it is obvious that prevention should start early, there are no specific interventions to prevent risk. This is because there is a gap in the knowledge of the mechanisms through which genomic and environmental risk factors act in early life and affect brain development. Dr. Gianluca Ursini from the Lieber Institute for Brain Development (LIBD) has previously identified schizophrenia-risk genes in placenta. In this project, Dr. Ursini will use a stem-cell derived model to uncover the exact placental mechanisms contributing to risk for NDDs, like schizophrenia.

The knowledge derived from this research will help Dr. Ursini develop novel strategies for prevention and treatment of NDDs in vulnerable populations, like expecting mothers, who are in need of more predictable diagnostic interventions. Further, it will define individuals at high risk for schizophrenia, who will potentially benefit from postnatal strategies of prevention.

MSCRF

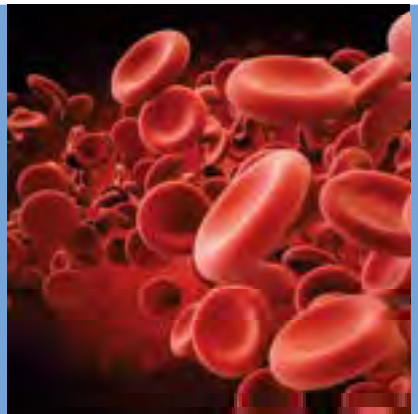
Novel Stem Cell Derived Therapy to Treat ALSAMYOTROPHIC LATERAL SCLEROSIS (ALS)

DR. JEFFREY ROTHSTEIN

Defects in an essential cellular process that maintains communication between cell components have recently emerged as a critical mechanism underlying Amyotrophic lateral sclerosis (ALS), a fatal neurological disorder that causes the progressive loss of motor neurons in the brain and spinal cord.

In collaboration with an industry partner, Ionis Pharmaceuticals, and their expert team, Dr. Jeffrey Rothstein from Johns Hopkins University has been developing a human therapy, termed antisense oligonucleotides (ASOs), which is derived from the patient's induced pluripotent stem cell (iPSC) to treat ALS.

The impact for this drug development program has enormous potential for a range of debilitating neurological diseases in which cell communication is dysfunctional, such as ALS, frontotemporal dementia and Alzheimer's disease. Should this ASO therapy prove potent in delaying the disease and allow functional recovery, the health impact would be substantial.



Novel Approach to Treat

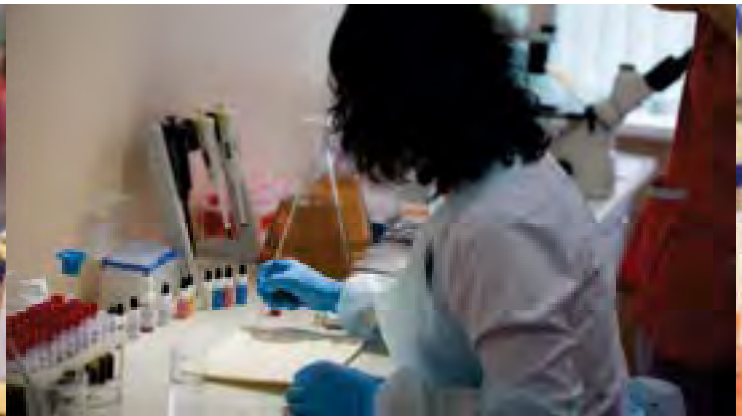
DUCHENNE MUSCULAR DYSTROPHY (DMD)

DR. RENYUAN BAI

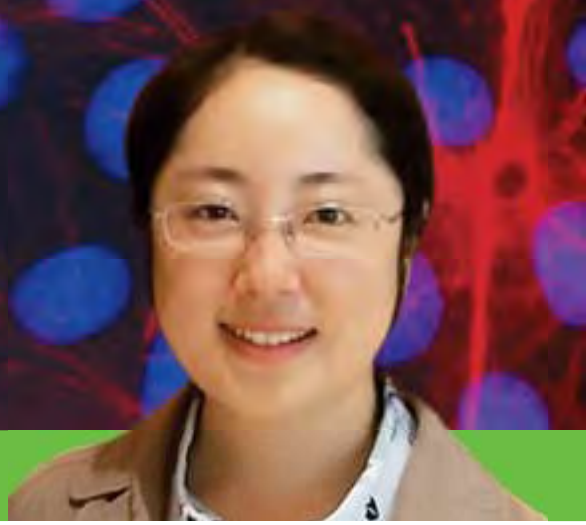
Duchenne muscular dystrophy (DMD) is a common genetic muscle disorder and is caused by mutations in DYSTROPHIN. Recent studies show the important functions of DYSTROPHIN protein in skeletal muscle stem cells, however, there is no gene delivery system specifically targeting the skeletal muscle stem cells. Recently, several Adeno-associated virus (AAV)-based gene therapy strategies have been developed for DMD, delivering DYSTROPHIN to skeletal muscle tissues, but these methods have been mostly unsuccessful due to the nonspecificity of the AAV.

Dr. Renyuan Bai from the Hugo W. Moser Research Institute at Kennedy Krieger has established a new (MYOrganoid) system, derived from human induced pluripotent stem cells (hiPSCs), and skeletal muscle stem cells. Using MYOrganoids and AAV expertise, his team aims to identify a muscle stem cell protein that could retarget AAV to specific cell types, such as skeletal muscle stem cells to treat DMD.

In this project, Dr. Bai will use his innovative MYOrganoid system to not only develop a new gene therapy strategy, but also to validate the therapeutic effect of novel AAV vectors, which will lay the foundation for meaningful and long-lasting gene therapies for DMD as well as other types of muscular dystrophies.



Investigating Brain Stiffness in NEUROLOGICAL DISEASES



DR. LI YAN

The human neural vascular unit governs the dynamic interaction among brain cells and the blood-brain barrier (BBB). The BBB can sense and adapt its behavior in response to the brain's stiffness. Dysfunction of the BBB is considered an early marker of several neurological diseases. While prior studies confirm the impact of matrix stiffness on BBB function, the effect of stiffness in the brain microenvironments on various cells remains largely unclear.

In this study, Dr. Li Yan from the University of Maryland at College Park will investigate these effects by adjusting the stiffness of the vascular bed in a model that mimics the BBB microenvironment.

Through this comprehensive approach, Dr. Yan aims to advance our understanding of the implications of vascular stiffness in the brain for various neurovascular diseases, such as Alzheimer's disease, as well as breast cancer metastasis to the brain. By unraveling the mechanisms involved, Dr. Yan strives to contribute to the development of innovative therapeutic approaches that will improve outcomes in patients suffering from these debilitating conditions.

Novel Strategy for Enhancing Recovery from

BRAIN INJURY POST-CARDIAC ARREST

DR. XIAOFENG JIA

Cardiac arrest (CA) is a leading cause of death worldwide. Despite advances in cardiopulmonary resuscitation methods, only about 10 % of adult victims survive hospital discharge. Among CA survivors, brain injury is the biggest impediment to functional recovery as current strategies cannot reverse neural injury resulting from CA.

Stem cell transplantation holds great potential in treating neuronal injury. Dr. Xiaofeng Jia from the University of Maryland in Baltimore recently reported that human neural stem cell (NSC) markedly improved neurologic outcomes after CA. Despite significant progress, stem cell survival after implantation is still extremely problematic. To overcome this, Dr. Jia recently developed a novel strategy which significantly improved the survival and boosted the therapeutic efficacy of transplanted human NSCs as well as their secreted factors, termed extracellular vesicles (EVs).

In this project, Dr. Jia will employ an innovative approach, termed metabolic glycoengineering, to investigate the effect of stem cell-derived EV therapy on functional outcomes from brain injury with the overall goal of improving the patient's quality of life after CA.

Innovating Genome Editing:

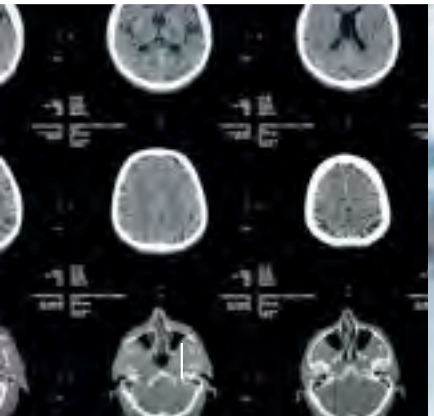
Harnessing Exosomes for Targeted BRAIN THERAPY

DR. MIROSLAW JANOWSKI

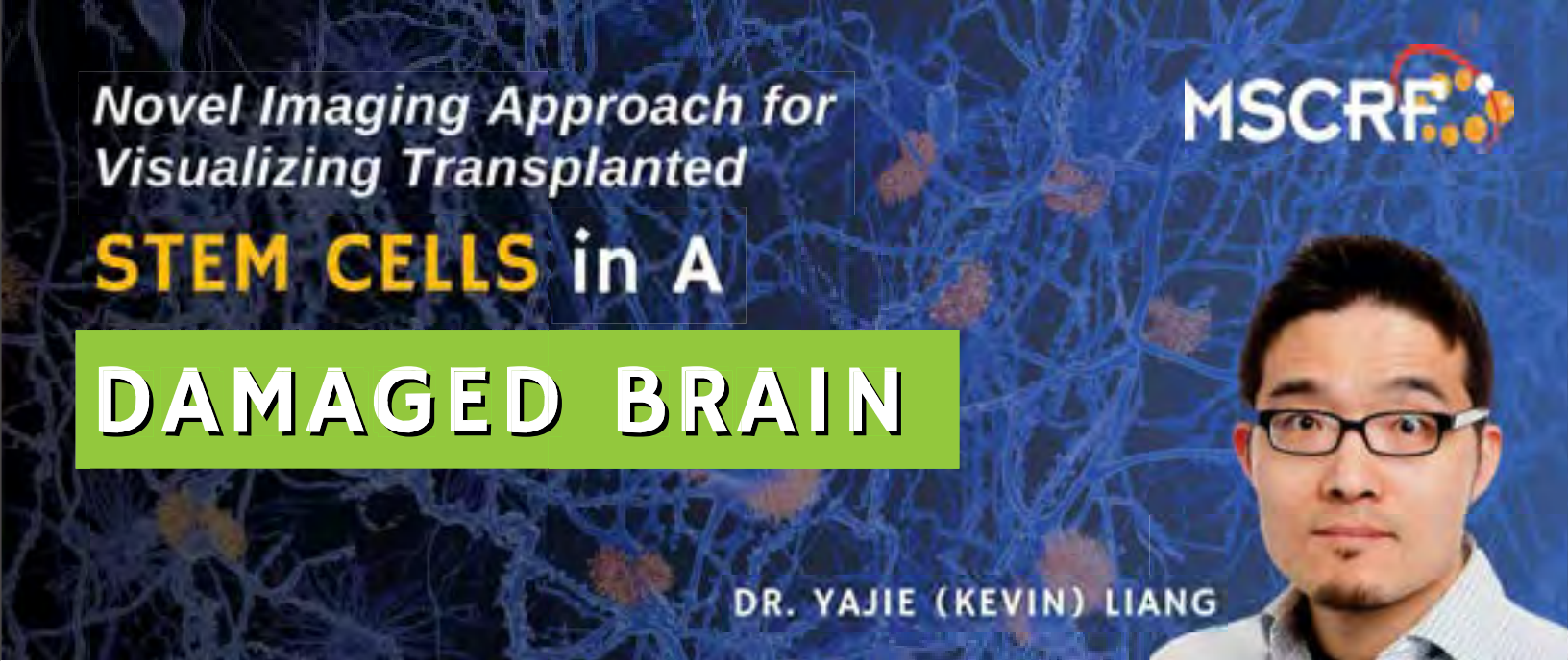
Genetic disorders are caused by abnormalities in a person's genetic material or DNA. They can affect many body systems and are often incurable and involve diseases, such as down syndrome, autism, sickle cell disease, and many others.

Current methods of genome editing in the brain are ineffective. In fact, the brain is a difficult target for any therapeutic agent. However, thanks to MSCRF's previously funded research, Dr. Miroslaw Janowski from the University of Maryland in Baltimore has established innovative protocols for delivery and broad distribution of stem cells in the brain.

Dr. Janowski will design a novel genome editing strategy for delivery of therapeutic cargo into the brain using very small particles secreted from stem cells, termed exosomes. This novel strategy will be specifically used for genome editing mutation in the brain causing familial amyotrophic lateral sclerosis (ALS), a lethal rare neurodegenerative disorder. If successful, this therapeutic strategy may be applicable to any genetic disorder.



Novel Imaging Approach for Visualizing Transplanted STEM CELLS in A DAMAGED BRAIN



Stroke is a leading cause of mortality and disability worldwide, including more than 795,000 cases in the U.S. per year, with 15–30% of survivors being permanently disabled. Restoration of damaged neuronal circuits by transplanted cells is highly desirable and could present as the ultimate solution. However, the inability to monitor dynamic cell behaviors after transplantation represents a huge knowledge gap and impedes the advancement of cures.

Dr. Yajie (Kevin) Liang from the University of Maryland – Baltimore has developed an imaging approach using a specialized (multiphoton) microscope.

This unique approach enables the real-time visualization of transplanted induced pluripotent stem cells (iPSC)-derived neural cells to study their behavior and uncover strategies to enhance their survival and integration in the damaged brain.

Dr. Liang's goal is to enhance the efficacy of neural stem cell therapies by advancing our understanding of the cellular interactions in the injured brain. By developing innovative approaches for tracking and enhancing the integration of implanted neural stem cells in the body's own tissues, this project has the potential to reinvigorate the cell transplantation field for the treatment of neurological disorders, such as stroke.

MSCRF

Neural Stem Cells to treat

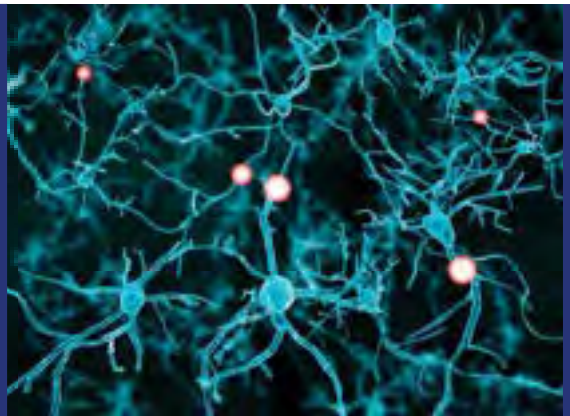
DEGENERATIVE DISORDERS

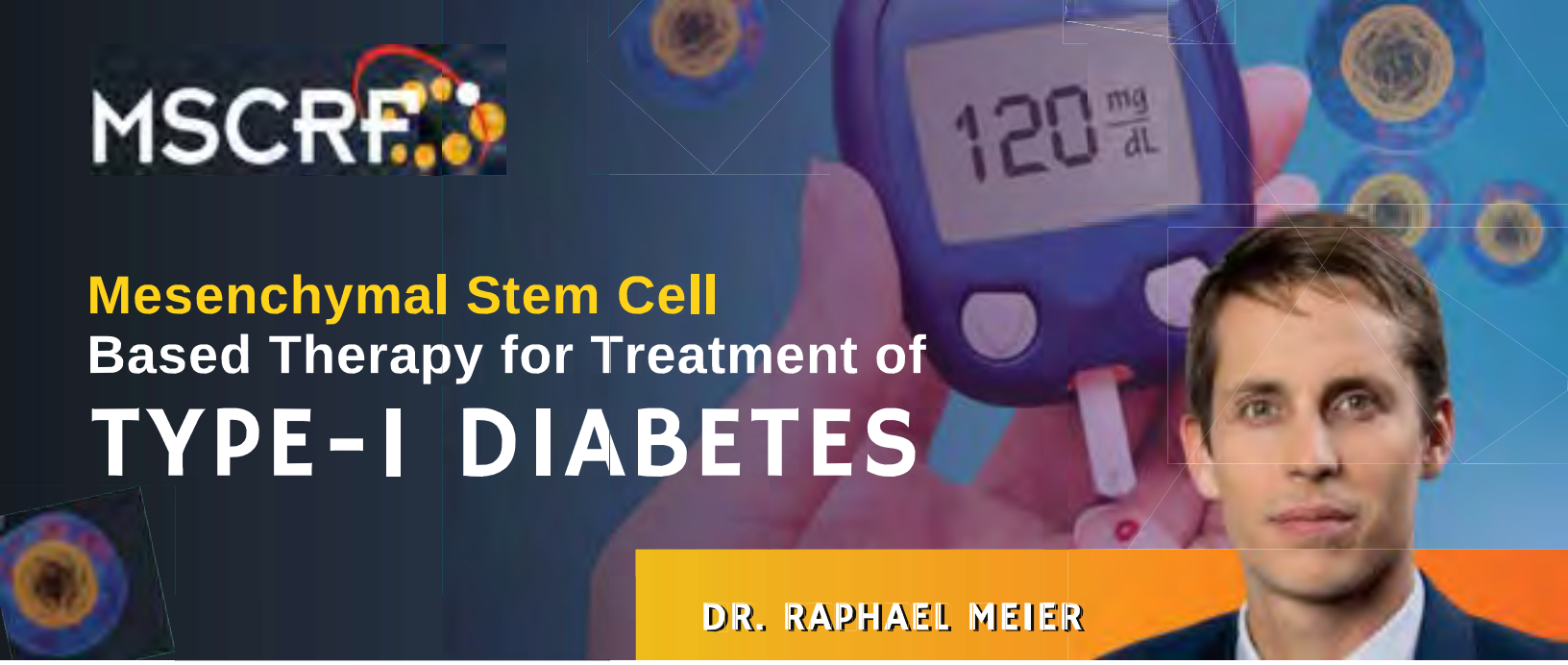
DR. GRAEME WOODWORTH

Mechanical interactions between neural stem cells and the brain microenvironment profoundly affect their behavior and function. The brain microenvironment is among the most confined and tortuous of all tissues in the human body with potential dramatic consequences to brain stem cells, including DNA damage and transformation to cancer cells.

Dr. Graeme Woodworth from the University of Maryland, Baltimore, in collaboration with three different labs in Maryland, will for the first time, employ a novel device that enables the collection and real-time observation of 10,000+ neural stem cells in a brain-like microenvironment over long timeframes.

This research project aims to uncover the relationship between stem cell mechanics and confined movement to establish the impact on neural stem cell behavior and function. The proposed work will provide an understanding of the potential consequences of human neural stem cells biomechanics in a confined and extremely specialized microenvironment. The impact and results of this work will inform new research directions, therapeutic strategies, and new opportunities to diagnose and treat neurological diseases, such as brain cancer.





Mesenchymal Stem Cell Based Therapy for Treatment of TYPE-I DIABETES

DR. RAPHAEL MEIER

Type 1 diabetes (T1D) is a chronic condition resulting from the destruction of insulin-producing pancreatic beta cells, forcing patients to rely on exogenous insulin for blood sugar management. While insulin therapy is crucial, it carries challenges including hypoglycemic episodes, meal management, dosing accuracy, and the risk of severe long-term complications. On June 28, 2023, the FDA approved the first allogeneic pancreatic islet cell therapy for T1D. However, the limited viability and poor engraftment of pancreatic islets after infusion hinder the potential for insulin independence.

Dr. Meier's previous research has shown that Mesenchymal Stem Cells (MSCs) can enhance the survival and function of encapsulated pancreatic islets through cell-to-cell contact. By genetically modifying MSCs to improve the secretion of key moleculeS vital for beta cell function and islet vascularization, Dr. Meier is developing a novel therapy that co-encapsulates these modified MSCs with pancreatic islets to treat T1D. Given that T1D is projected to affect 629 million people worldwide by 2045, developing effective therapeutic strategies is essential to combat this public health concern.

MSCRF

PUBLIC *Engagement*

Investing in a Brighter Future

Where was MSCRF in 2024?

Fostering Connections and Building Communities

Throughout 2024, MSCRF leadership hosted and attended multiple conferences, programs and stem cell-related events both within Maryland and outside. From April to June 2024, the Maryland Stem Cell Research Fund (MSCRF) was active in hosting and attending events that strengthened the state's stem cell community and fostered vital connections.

The purpose of hosting and attending these events was to support ongoing research and advancement within the field, particularly the scientific work undertaken by MSCRF-backed researchers and companies and to engage with the ecosystem to understand and address their needs.

Below are some highlights of those engagements:

Legislative Showcase

On March 18, MSCRF, alongside its portfolio company Rooster Bio, participated in the TEDCO (Technology Development Corporation) Legislative Technology Showcase, where Maryland lawmakers had the opportunity to witness firsthand the innovative technologies being developed by companies and entrepreneurs in the state.

Attendees were engaged by presentations and demonstrations from various companies and startups, with Rooster Bio proudly among the 12 companies showcasing their research. Based in Frederick, Maryland, Rooster Bio specializes in developing and manufacturing human mesenchymal stem/stromal cell (hMSC) and exosome/extracellular vesicle (EV) products, driving the rapid implementation of scalable advanced therapies.

Additionally, Rooster Bio was one of the inaugural awardees of MSCRF's new Manufacturing Assistance Program, which focuses on advancing GMP production of cell therapy products in Maryland.

Through this engagement, MSCRF highlighted the positive impacts of its funding on portfolio companies and the broader Maryland innovation landscape.

Maryland Stem Cell and Regenerative Medicine Technology Showcase

On April 25, 2024, MSCRF, in partnership with the Maryland Department of Commerce, proudly hosted the Maryland Stem Cell and Regenerative Medicine Technology Showcase. This premier event provided a platform for MSCRF-funded companies and academic researchers to showcase their innovative stem cell-based approaches. Highlights included presentations from MSCRF portfolio companies such as Secretome Therapeutics, Renovate Biosciences, Phycin, Seraxis, Theradaptive, and SereNeuro Therapeutics, alongside cutting-edge research from esteemed entrepreneurial scientists like Drs. Luis Garza, Sheikh Amer Riazuddin, Curt Civin, Elias Zambidis, Xiaoming (Shawn) He, and Tao Lowe.

The showcase attracted a distinguished panel of investors, including Sally Allain from JLabs, Matt Tremblay from Blackbird Laboratories, Bradford Young from B.A.Y. Biotech Consulting, Rosemarie Truman from the Center for Advancing Innovation, and Brock Reeve from Eos Bioinnovation.

The event was further enhanced by insights from Dr. Peter Marks of the FDA and Maryland Comptroller Brooke Lierman, who emphasized the significant advancements and promising future of Maryland's stem cell research sector.

By fostering such connections between scientists and investors, MSCRF aims to accelerate the development of transformative therapies, ensuring that innovative solutions reach patients in need while bolstering the growth of Maryland's life sciences ecosystem.

Maryland Stem Cell and Regenerative Medicine Technology Showcase

Spring Stem Cell Symposium and Workshop

On May 17, 2024 MSCRF, in collaboration with University of Maryland School of Medicine and John Hopkins Institute of Cell Engineering, co-hosted the first-annual Spring Stem Cell Symposium and Workshop.

The one-day program provided a forum for stem cell and regenerative medicine researchers from across Maryland to collaborate and advance patient care. It served as a vital platform for trainees and junior faculty in stem cell and regenerative medicine to showcase their research and build collaborative networks across Maryland's institutions.

The symposium was highlighted by inspiring remarks from USM Vice Chancellor Dr. Jay A. Perman, followed by a keynote address from Dr. Curt Civin, a renowned leader in hematopoietic stem cells.

The day featured five engaging oral sessions covering a diverse range of stem cell research topics, culminating in awards for the best presentations presented by Maryland State Delegate Catherine M. Forbes, who also emphasized the importance of continued investment in stem cell research.

AURP BIO and BIO

In June 2024, I traveled to San Diego to serve on a panel at AURP (Association of University Research Parks) BIO Health Caucus 2024.

The event took place before the annual BIO International convention, also held in San Diego. At AURP BIO, I served on the "Finding Funding for BIO Research/ Startups: Federal, State and Private Sources." Other panelists included Liz Powell, founder and president of G2G Consulting and Caroline Arzoo, director of partnerships and business development at OmniSync. The discussion focused on ways companies and organizations can find and apply nondilutive funds from government agencies.

Felicitation Ceremony

Also in June, MSCRF grant awardees were honored at the second annual Felicitation Ceremony,

The event, which was held at the Hotel at Anne Arundel Preserve, provided the awardees with an opportunity to highlight their innovative stem cell research.

The ceremony also afforded awardees the chance to explore potential collaborations with their peers during a networking event. The Felicitation Ceremony wasn't all business, though. Attendees participated in a science trivia challenge.

MSCRF also had the opportunity to participate in other events in addition to the ones previously mentioned.

MSCRF strives to connect with a larger ecosystem and bolster a growing ecosystem of stem cell-focused researchers throughout Maryland.

MSCRF

GRANT AWARDS
Funded

Investing in a Brighter Future

CLINICAL:
Luis Alvarez, PhD | Theradaptive, Inc.
John Ferrell, MD | Orthobiologics Research Initiative (ORI)
Luis Garza, MD, PhD | Johns Hopkins University

COMMERCIALIZATION:
Samaneh Kamali, PhD | Caleo Biotechnologies, Inc.
Xiaoming (Shawn) He, PhD | HOHCells, Inc.
Jean-Philippe Richard, PhD | Reprocell, Inc.
William Rust, PhD | Seraxis, Inc.
Mandeep Singh, MD, PhD | Agnos Therapeutics, Inc.

MANUFACTURING ASSISTANCE:
Luis Alvarez, PhD | Theradaptive, Inc.
Evelyn Chukwurah, PhD | Reprocell, Inc.

VALIDATION:
Curt Civin, MD | University of Maryland, Baltimore
Xinzhong Dong, PhD | Johns Hopkins University
Jeffrey D Rothstein, MD, PhD | Johns Hopkins University
Elias Zambidis, MD, PhD | Johns Hopkins University

LAUNCH:
Marios Arvanitis, MD | Johns Hopkins University
Renyuan Bai, PhD | Hugo W. Moser Research Institute at Kennedy Krieger
Man-Kyo Chung, PhD | University of Maryland, Baltimore
Allen Eghrari, MD | Johns Hopkins University
Konstantinos Konstantopoulos, PhD | Johns Hopkins University
Payam Mohassel, MD | Johns Hopkins University
Chan-Hyun Na, PhD | Johns Hopkins University
Alexandros Poulopoulos, PhD | University of Maryland, Baltimore
Robin Roychaudhuri, PhD | University of Maryland, Baltimore
Tomoyo Sawada, PhD | Lieber Institute for Brain Development
Debasish Sinha, MS, PhD | Johns Hopkins University
Dudley Strickland, PhD | University of Maryland, Baltimore
Gianluca Ursini, MD, PhD | Lieber Institute for Brain Development
Li Yan, PhD | University of Maryland, College Park

DISCOVERY:
Chengyan Chu, PhD | University of Maryland, Baltimore
Ivy E. Dick, PhD | University of Maryland, Baltimore
Miroslaw Janowski, MD, PhD | University of Maryland, Baltimore
Xiaofeng Jia, PhD | University of Maryland, Baltimore
Thomas Johnson, MD, PhD | Johns Hopkins University
Annie Kathuria, PhD | Johns Hopkins University
Chulan Kwon, PhD | Johns Hopkins University
Gabsang Lee, PhD | Johns Hopkins University
Yajie (Kevin) Liang, PhD | University of Maryland, Baltimore
Iris Lindberg, PhD | University of Maryland, Baltimore
Vasiliki Machairaki, PhD | Johns Hopkins University
Xiaobo Mao, PhD | Johns Hopkins University
Raphael Meier, MD, PhD | Johns Hopkins University
Byoung Chol Oh, PhD | Johns Hopkins University
Sashank Reddy, MD, PhD | Johns Hopkins University
Sheikh Amer Riazuddin, PhD | Johns Hopkins University
Hiromi Sesaki, PhD | Johns Hopkins University
Charlotte Sumner, MD | Johns Hopkins University
Emmanouil Tampakakis, MD | Johns Hopkins University
Zack Wang, PhD | Johns Hopkins University
Graeme Woodworth, MD | University of Maryland, Baltimore

POST-DOCTORAL FELLOWSHIP:
Mohit Kwatra, PhD | Johns Hopkins University
Shalini Sharma , PhD | University of Maryland, Baltimore
Neelima Thottappillil, PhD | Johns Hopkins University
Jinghui Wang, PhD | University of Maryland, Baltimore
Wenshen Wang, PhD | Hugo W. Moser Research Institute at Kennedy Krieger
Feiyu Yang, PhD | Johns Hopkins University
Ridzky Yuda, PhD | Johns Hopkins University
Heng Zhao, PhD | Johns Hopkins University

MSCRF

Clinical
Grant Awards

2024

Luis Alvarez, PhD

Theradaptive, Inc.
Awardee Amount: $1,000,000
Disease Target: Bone Regeneration Spinal Fusion

John Ferrell, MD

Orthobiologics Research Initiative (ORI)
Awardee Amount: $360,000
Disease Target: Rotator Cuff Tears; Musculoskeletal System Disease
(2025 1st Funding Cycle)

OASIS: Clinical Investigation of OsteoAdapt SP in Single-level Transforaminal Lumbar Interbody Fusion (TLIF)

Theradaptive, Inc. is a regenerative medicine company spun out of Massachusetts Institute of Technology (MIT) in 2017. It has developed a platform for targeted delivery of regenerative therapeutics that promotes tissue regeneration in a controlled manner. This platform converts recombinantly-expressed therapeutic proteins to bind scaffold implant materials extremely tightly, thus achieving persistent local delivery at critical defect sites. This eliminates diffusion of biologics from the implant site, and significantly reduces off target effects and serious adverse events (SAEs). This technology enables a new class of regenerative implants that potentiate in situ stem cells to regenerate tissue in an anatomically precise manner. For spinal fusion applications, Theradaptive developed OsteoAdapt SP™, a combination product consisting of a proprietary modified variant of human Bone Morphogenetic Protein 2 (BMP2) called AMP2, together with a carrier implant. BMP2 is commonly found in the body and stimulates stem cells to promote bone regeneration. The AMP2 protein binds extremely tightly to resorbable implants, permitting precise delivery of osteoinductive bioactivity, and eliminating the risk of protein diffusion and off-target effects. Stimulation of new bone formation is synchronized with AMP2 and implant resorption, thus effecting robust bone formation. Importantly, the ability to activate a patient's own stem cells to potentiate the desired therapeutic effect eliminates the need to harvest autologous stem cells, significantly improving patient outcomes. While there are other regenerative orthopedic products available on the market, none has demonstrated safety and effectiveness in posterior spinal fusion. This prospective, open label, randomized trial will determine the safety and performance of two doses of OsteoAdapt SP for the treatment of degenerative diseases of the lumbosacral spine in patients having transforaminal lumbar interbody fusion. If successful, the use of OsteoAdapt SP could contribute to reduced chronic pain, disability, and neurological deficits and improved quality of life for these patients. In late 2019, the FDA Office of Combination Products (OCP) formally designated OsteoAdapt SP a Class III Medical Device (Combination Product) and assigned the Center for Devices and Radiological Health (CDRH) as having jurisdiction with support from the Center for Drug Evaluation and Research (CDER). FDA CDRH rules will apply to all regulatory filings for this product and Indication for Use. Manufacturing processes will comply with both drug and device cGMP regulations. OsteoAdapt SP will follow a Premarket Approval (PMA) regulatory path. Theradaptive is the regulatory Sponsor and clinical trial Sponsor. FDA has established the Breakthrough Medical Devices (BMD) Program to fast-track novel technologies that are likely to offer a significant advance in patient outcomes. Devices designated with breakthrough status are given priority throughout the application and approval status. Meeting requests are accommodated in less than half the typical time and all clinical IDE and PMA reviews are expedited. OsteoAdapt SP has been granted three breakthrough designations for spinal applications, allowing for a faster path to market. Theradaptive is the only company in the orthopedics market with three Breakthrough Medical Device designations granted, including designations approved for OsteoAdapt SP indications in Transforaminal Lumbar Interbody Fusion (TLIF), Anterior Lumbar Interbody Fusion (ALIF), Posterolateral Fusion (PLF).

Ultrasound Guided Micro-Fragmented Adipose Transfer Graft for Partial Thickness Rotator Cuff Tears, Single-Blind Randomized Controlled Trial

Partial thickness rotator cuff tears (PTRCT) have a variety of etiologies. It is one of the most common musculoskeletal pathologies and is frequently treated with surgical reconstruction of the rotator cuff (RCR). In the absence of surgery and usually following failed conservative care, traditional orthopedic medicine utilizes corticosteroid injections into the bursa over the pathologic tendon in patients with rotator cuff tears to reduce pain, secondary to reduction in inflammation [1]. While corticosteroids may provide interval relief of pain, this intervention typically fails to provide a long-term solution to this disease process. Micro-Fragmented Adipose Transfer (MFAT) or Adipose Derived Autologous Stem Cells (ADASC's) are a widely used investigational orthobiologic method for the treatment of joint osteoarthritis and tendinopathy within clinical, non-surgical medicine. For patients with high-grade tears of the supraspinatus/infraspinatus distal tendons of the rotator cuff, MFAT is used to mimic a biological matrix that adds stability to the damaged tissue chemically and physically. There is growing, published evidence suggesting efficacy of MFAT for the indication of partial thickness rotator cuff tears. While MFAT injection will be the tested interventional treatment, corticosteroid injection (CSI) will serve as the control intervention for this study. We hypothesize that MFAT injection will provide superior improvement in pain and functional patient reported outcome scores (PROMs) which will be measured by VAS pain and QuickDASH relative to CSI, and that MFAT treatment will stimulate a robust remodeling of the affected tendon as measured by MRI imaging.

Luis Garza, MD, PhD

Johns Hopkins University
Awardee Amount: $650,000
Disease Target: Amputations

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

Cell and gene therapy are considered an emerging novel third arm of medicine after traditional small molecule pharmacology and surgery with already ground-breaking FDA approved therapies such as CAR T cells for cancer. In this vein, the skin dermis might be a convenient fixed reservoir for cellular depots like an ectopic pancreas given the proven success of skin-based glucose sensing and insulin injection. However, before its routine practical use, there are important practical questions that must be addressed. We have recently completed a phase 1 clinical trial on cellular therapy in human subjects that raise such critical questions. This grant aims to enhance issues related to poor levels of cellular engraftment in solid tissue. Our completed phase 1 trial began to assess the utility of autologous volar (palmo-plantar) fibroblasts to imbue volar features to the non-volar skin at the stump site of an amputee to make the skin more pressure resistant and less likely to develop pressure ulcers. In this grant and in a manuscript with favorable 1st round reviews invited for resubmission at Science magazine we show data that this cellular therapy shows efficacy in human subjects, but with low levels of engraftment a year after injection such that ~1% of fibroblasts consist of ectopic injected fibroblasts. This begs the important question of defining barriers and solutions to ectopic cell survival. In this grant we propose a new clinical trial to test a novel way of preparing solid tissue for cellular therapy. The initial success of hematopoetic stem cells by E. Donnall Thomas that led to the Nobel Prize in 1990 hinged on the use of non-lethal irradiation to clear the bone marrow stem cell niche and allow for full stem cell engraftment. In an analogy for solid organ, we hypothesize that the adjunctive use ultraviolet (UV) light will induce fibroblast apoptosis and open cellular niches for improved ectopic fibroblast stem cell engraftment. In the present grant we show promising animal and human data that ultraviolet light leads to decreased fibroblast numbers in the skin. We also have completed a pilot human subject treated with ultraviolet light prior to cellular therapy. In a comparison to previously treated patients without ultraviolet with MSCRF support, we show significantly improved endpoints and here propose to test more subjects

Hypothesis: Pre-treatment with 3x Minimal Erythema Dose (MED) of Narrow Band (nb) UV light will induce fibroblast apoptosis to vacate occupied cellular niches and allow improved ectopic fibroblast stem cell engraftment with improved clinical endpoints.

Trial design:

1. Biopsy normal healthy subjects at a volar site; submit tissue to Hopkins Cellular Therapy Core to expand fibroblast stem cells as we have done previously.
2. Pre treat human subject with 3x MED nbUVB X 1 on day 1
3. On days 2 and 4 inject 5 million volar fibroblasts.
4. Perform noninvasive testing for epidermal thickness and skin firmness.
5. At 2 months after injection, remove skin containing injected cells for dual single cell ATAC seq and RNA-seq.
6. Compare data to historical MSCRF-funded treated subjects without UV light.

The results of this grant will inform an ongoing phase 2 trial in amputees to test if outcomes of physical activity and prosthetic use are better for those injected with volar fibroblasts after UV light pre-treatment. This grant will use matching funds from a peer-reviewed and awarded NIH RMIP grant that will synergize with the present effort.

MSCRF

Commercialization
Grant Awards

2024

Samaneh Kamali, PhD

Caleo Biotechnologies, Inc.
Awardee Amount: $490,700
Disease Target: Inflammatory Bowel Disease
(2025 1st Funding Cycle)

Xiaoming (Shawn) He, PhD

HOHCells, Inc.
Awardee Amount: $400,000
Disease Target: Devices-Focused Project for All Diseases

Accelerating the Next Generation Therapies for Inflammatory Bowel Disease

Inflammatory bowel disease (IBD) is fast becoming a growing problem with patients often cycle through multiple chronic therapies. The response rates to existing therapies remain suboptimal, and despite the significant progress in IBD treatment most clinical trials fail. Therefore, several challenges, including the cost of clinical trials and the failure in drug development, remain the primary risk of investment dollars and translating discoveries to the clinic. This may be traced back to the dearth of reliable and relevant preclinical models. Current preclinical models of IBD are inadequate for maintaining the genetic diversity of the diseases and demonstrating the capacity for regeneration. The continued existence of this gap represents an important issue because the regenerative diversity of human IBD will not be represented, and thus further approaches to these enigmatic diseases will remain challenging. To fill this gap, at Caleo Biotechnologies, we combined our expertise in disease modeling with the most advanced discoveries in stem cell biology and regenerative medicine and successfully developed the first comprehensive patient-derived Organ-Dish preclinical model for IBD (DODs). In brief, we generate induced pluripotent stem cells (iPSCs) originated from the patient suffering from IBD, direct- differentiate them into definitive endoderm (DE), and further to DODs. Notably, self-organizing DODs comprise epithelial, mesenxhymal, and immune compartments. The presence of these cell types working in concert leads to faithful recapitulation of in vivo physiology and diseased organ structure. With these comprehensive patient-first tools in hand, our long-term objective is to develop more effective treatment and preventive strategies for IBD.

Medical Devices for RI-free 3D Culture and Improved Banking of Human iPSCs and Derivatives

Human induced pluripotent stem cells (iPSCs) show great promise for personalized medicine, as they can be reprogrammed from somatic cells in easy-to-access organs (e.g., skin) and differentiate into cells of all three germ layers without any ethical concern. However, large-scale production of human iPSCs in 3D is indispensable for cell-based medicine. We discovered that ROCK inhibitor (RI, 10 μM in concentration) that has been commonly used for 3D culture of human iPSCs to improve their viability and yield, significantly compromises their quality. To address this issue, we developed a novel method of cold-triggered detachment of human iPSCs for culture in 3D with high viability (~100%), yield, and quality without using RI. However, the device for the novel method needs to be made fresh and has a short shelf life at room temperature, which is not ideal as a commercially available product. Therefore, we will further develop the device to have a long shelf life for RI-free 3D culture of human iPSCs, as the first specific aim of this project. In the same line, for the human iPSCs and their derivatives as commercially available products, they must be banked via cryopreservation for convenient distribution and ready-availability. We have successfully developed a novel natural sandbased technology to improve the banking of human iPSCs and mouse ovarian follicles. We will further develop this technology for banking the 3D human iPSC-derived mesenchymal stem cells (iMSCs) with high postcryopreservation functional survival, as the second specific aim of this project. In summary, this project will develop two ingenious technologies for 1) RI-free production of 3D human iPSCs with high viability, yield, and quality and 2) cryopreservation of 3D human iMSCs with high viability, yield, and quality. Both technologies/devices are of urgent need for the understanding, drug-screening, and treatment of various diseases, particularly in a personalized manner.

Jean-Philippe Richard, PhD

Reprocell, USA
Awardee Amount: $394,005
Disease Target: Cell Therapy

William Rust, PhD

Seraxis, Inc.
Awardee Amount: $399,560
Disease Target: Type 1 Diabetes

Generation of human iPSC and MSC MCBs and Derivative Products in an Enclosed GMP System

REPROCELL is a manufacturer and vendor of cells and products for stem cell research that specializes in providing biologically relevant human tissue models for drug discovery and regenerative medicine. REPROCELL offers a range of products and services encompassing biobanking and induced pluripotent stem cell (iPSC) and mesenchymal stem cell (MSC) line generation, as well as development and services to differentiate cells into multiple lineages. We propose to expand REPROCELL's current capabilities to provide GMP manufactured clinically relevant Master Cell Banks (MCB) of iPSCs and MSCs toresearch, therapeutic and biotech companies. We have an active partnership with BioSpherix, and we have established a Xvivo model 2 Cytocentric Closed GMP System in our facilities. In the timeframe of the grant, this system will be used to generate one GMP MCB of iPSCs from one of our current qualified seed stock clones, one GMP MCB of iPSC derived MSCs, and several derivative products such as exosomes and feeder cell lines. REPROCELL will have complete authority over the process, which permits the flexibility to adapt to custom requests and related partnerships. These products will be a proof of concept for our service capabilities as GMP iPSC MCB manufacturer, as we can adapt our processes to clients' request and own lines. These products will be available to local stem cell companies and various Maryland research institutions. Public health impact: We believe the Maryland stem cell community will benefit greatly from these new offers, indirectly from the MCB of hiPSC and more directly from the MCB of MSCs, feeder cell lines and MSC-derived exosome products. These cell lines would be used for the development of new cell-based therapeutics by our clients.

Clinical Development of a Universal Cure for Insulin-Requiring Diabetes

Pancreatic islet cell cluster implants containing insulin-secreting beta cells are proven to reverse diabetes in humans. Pancreatic islet cell clusters can be harvested from donor human cadaveric pancreases or manufactured from human stem cells. After transplant, the patient requires immune suppressing drugs which have serious side effects to prevent rejection of the islet cell clusters. This requirement limits the applicability of the cure to only a small fraction of diabetes patients whose disease is complicated by recurrent severe (life threatening) hypoglycemia (low blood sugar). Seraxis manufactures best-in-class islet cell clusters from its proprietary stem cell line at its GMP facility in Germantown, Maryland to support clinical studies in patients of diabetes with severe recurrent hypoglycemia. To overcome the requirement for life-long immune suppression post-transplant, Seraxis has completed development of a next generation genetically modified version of its islet cell clusters that are invisible to host immune defenses. These "invisible" islets do not stimulate a human antigraft immune response in vitro and are capable of surviving without rejection in chimeric mice with human immune function, creating a therapy for the broad insulin requiring diabetes market. Funding of this Research Project will advance the "invisible" islet cell clusters towards IND submission and clinical testing by completing 1) Testing the existing next generation genetically modified master cell bank: Characterization of SR1423-HI (MCB) to demonstrate stability, purity and lack of adventitious agents 2) Manufacturing and testing the working cell bank (WCB) 3) Efficacy study: a six-month study in 20 mice. Long-standing type 1 diabetes and advanced type 2 diabetes (patients who require insulin) is associated with many co-morbidities including heart disease, eye disease (leading to blindness), kidney disease (leading to kidney failure and dialysis), neurologic disease (painful diabetic neuropathy and progression to foot ulcers and amputation), and peripheral artery disease (foot ulcers). About 9 million American require ongoing glucose monitoring and use of exogenous insulin. The cost insulin therapy to the US health system is enormous – for example, today the annual market for just continuous glucose monitors (CGMs) alone is over $7 billion. Seraxis aspires to create a practical cure for those with diabetes requiring insulin, eliminating the requirement for exogenous insulin and drastically improving health outcomes including preserved organ function and longevity.

Mandeep Singh, MD, PhD

Agnos Therapeutics, Inc
Awardee Amount: $498,863.47
Disease Target: Retinitis Pigmentosa

Development of Cellular Component Transfer (CCT) as a Treatment to Restore Vision in Retinitis Pigmentosa

Agnos, a spinout of Johns Hopkins University, is developing cellular component transfer (CCT) therapy as a variant-independent protein augmentation treatment for retinitis pigmentosa (RP). This grant will take us to the pre-IND stage by de-risking Good Laboratory Practices (GLP) manufacturing concerns and performing large animal studies incorporating regulatory guidance on study design. The success of this project proposal will lay the foundation for manufacturing and first-in-human clinical testing of CCT, which we have designed as a safe, durable, and highly effective treatment for RP. With CCT treatment, affected people will be able to preserve or restore their sight. The scientific premise of CCT is that donor precursor cells can transfer multiple wild-type proteins directly into diseased recipient photoreceptor cells in the retina. The transferred proteins are in amounts sufficient to complement single-gene deficiencies and produce variant-independent functional repair. Thus, CCT aims to replenish wild-type proteins into pre-existing, endogenous, albeit functionally deficient photoreceptors to restore their functional capacity, thus providing years of improved vision and quality of life for RP patients.

The development of CCT technology will advance the biotechnology sector in Maryland. CCT treatment will fill a global need and will establish the reputation of Maryland Biotech as being at the frontier of novel therapies. We will also create new biotechnology jobs at our company AGNOS in Maryland. Specifically, the know-how of the retinal stem cell culture protocol used for CCT is housed in Maryland and our scientists will be located close to Johns Hopkins for quality control over the process of f this potential product. To Society – Patients affected by the rare disease, retinitis pigmentosa (RP), currently have no available treatment options. For them, the onset of mild vision disturbances in adolescence culminates in legal blindness and often total vision loss by middle age. Rare diseases affecting adult populations often severely impact individuals' ability to complete education and participate or remain in the workforce. The impact to the young and working age group, and thereby to society at large, will be immeasurable.

MSCRF

Manufacturing Assistance Grant Awards

2024

Luis Alvarez, PhD

Theradaptive, Inc.
Awardee Amount: $1,000,000
Disease Target: Intervertebral Disc Degeneration
(2025 1st Funding Cycle)

Evelyn Chukwurah, PhD

Reprocell, Inc.
Awardee Amount: $344,402
Disease Target: Cell Therapy

Theradaptive GMP OsteoAdapt Manufacturing and QC Facility Build Out and Validation in Frederick Maryland

Theradaptive is a leading biotechnology firm that develops bioactive stem cell implants that stimulate tissue regeneration. Our lead therapeutic is AMP2 which is an implant-binding engineered variant of bone morphogenetic protein 2 (BMP2) that promotes bone growth by stimulating osteogenic stem cells to regenerate native bone. Using this technology, we have created resorbable implants that are surface coated with AMP2 that retain the biologic at the implant site over the extended period of time required to achieve stem cell activation and regeneration. This permits precise delivery of bone-healing bioactivity that eliminates the risk of off target effects. The release of AMP2 from the implant is synchronized with implant resorption, thus producing robust bone formation. We used this technology to develop a product called OsteoAdapt SP, the first biological device of its kind. We have validated OsteoAdapt in models of long-bone repair in rodents, goats, and sheep and demonstrated superiority over the current best treatments available. We are entering clinical trials in Q3 2024 for our lead indication in spinal repair and will use outsourced GMP manufacturing for our Phase I/II study. In preparation for Phase III and large-scale commercial production we have expanded our facility and are building a GMP manufacturing suite at our Frederick headquarters. Additionally, we are conducting scale up process development for GMP manufacturing and are implementing QC labs to support release testing on our final drug substance and final product. This will enable us to tap into the talent rich I-270 biotech corridor while de-risking our current CMO based production. Degenerative disc disease affects most people during their lifetime and is prevalent in aging populations. Consequences are chronic pain, reduced mobility, and impacted work, rest and fitness. Current treatments are limited in the ability to rapidly stabilize spinal vertebral structures. Theradaptive's advanced bone void filler OsteoAdapt is a promising new tool with the potential to significantly improve efficacy and safety of bone tissue repair in spine, orthopedics, dental, and other areas.

Enhancement of Capabilities of Existing Cytocentric Xvivo System Model 2 from BioSpherix

Induced pluripotent stem cells (iPSC) are used in cell therapy, drug discovery and drug development. REPROCELL USA (REPROCELL) supports the entire workflow of stem cell research and pre-clinical drug development. Our unique portfolio includes proprietary RNA reprogramming technology, commercial biorepository of ethically sourced human tissues and iPSC line generation and development. Our additional services include multilineage differentiation of iPSCs, stem cell culture media and reagents. With our deep knowledge of stem cell biology, REPROCELL is a recognized leader in cutting-edge tools and services to accelerate regenerative medicine and drug development. To further speed up clinical and translational research we have recently installed the Cytocentic, Xvivo system model X2 Closed GMP System (Xvivo System) manufactured by BioSpherix Medical, Ltd. (Parish, NY). We are currently using this system to manufacture GMP grade iPSC and mesenchymal stem cells (MSC) master cell banks (MCB). This system provides an ISO 5 quality GMP working environment approved by FDA. The current system at REPROCELL has the capacity to produce one GMP quality MCB at a time. In this proposal we request funding to enhance the capacity of the existing system by adding more modules. These additional modules will allow REPROCELL to work with up to 4 iPSC lines or other differentiated cell products at the same time. This additional capacity will let us meet the current market demand for the GMP-grade iPSCs master cell banks and related GMP-grade off-the shelf products for internal use. It will also allow us to satisfy the demands of therapeutic, pharmaceutical and biotechnology companies all at a competitive price. REPROCELL is well positioned with more than 15 years of expertise, qualified stem cell scientists, the Xvivo system in place and technical support, to successfully generate GMP MCBs and other differentiated cell products. We believe the Maryland stem cell community will benefit from having a local GMP facility that can provide cell lines that are ready for making therapeutic products. We will also be supplying these MCBs to grantees of California Institute for Regenerative Medicine (CIRM) for the development of therapeutic products.

MSCRF

Validation
Grant Awards

Curt Civin, MD

University of Maryland, Baltimore
Awardee Amount: $250,000
Disease Target: Sickle Cell Anemia

Xinzhong Dong, PhD

Johns Hopkins University
Awardee Amount: $250,000
Disease Target: Chronic Pain

Curate Processing of Hematopoietic Stem-Progenitor Cells for Gene Therapies

In 2023, the FDA approved genetic modification of hematopoietic stem-progenitor cells (HSPCs) for treatment of sickle cell anemia, setting the stage for HSPC gene therapy to become established similarly for the many rare monogenic inherited diseases involving the blood-immune system, e.g. hemoglobinopathies, immunodeficiencies. Our GPB-Princeton-UMB team developed Deterministic Cell SeparationTM (DCS™) microfluidic chips and the GPB Curate® Cell Processing System to efficiently harvest viable white blood cells (WBCs), containing functional T lymphocytes, while depleting red blood cells (RBCs) and platelets (PLTs), from full-size non-mobilized blood leukapheresis, specifically to enhance therapeutic chimeric antigen receptor (CAR)-T lymphocyte manufacture. The patented GPB DCS™ chip gently separates blood cells based solely on cell size, and our developed Curate® System includes a closed, automated cell processing platform with cell suspensions and buffers contained in disposable sterile cassettes, bags and tubing. Reproducible Curate® processing of non-mobilized leukapheresis requires <1h and is to enter clinical trials at several institutions including University of Maryland and NIH. We believe that results of long-term HSPC transplantation experiments from our ongoing 2023-MSCRFV-5986 project (Validation of the GPB Curate Cell Processing System for manufacture of therapeutic hematopoietic stem cells) will confirm that Curate® processing to deplete RBCs and PLTs from mobilized blood leukapheresis efficiently recovers HSPCs within the WBC ("DCS™ Product") fraction. This anticipated result will indicate that the Curate® System, essentially as is, can serve a 2nd use: HSPC manufacture from mobilized blood. We now propose a new MSCRF Validation project designed to further increase the value of UMB co-owned intellectual property (IP). Herein, we plan to optimize and evaluate genetic modification of CD34+ HSPCs harvested from mobilized blood via the Curate® System, using both technologies recently approved for gene therapies, i.e. 1) lentiviral (LV) transduction for overexpression, and 2) electroporation (nucleofection) of CRISPR/Cas9 reagents for gene editing. Two technologies for genetic modification of HSPCs are now FDA-approved for treatment of sickle cell anemia, a disease that plagues African-Americans; and HSPC gene therapies for many rare but severe monogenic inherited diseases involving the lympho-hematopoietic system are in development. Accomplishing the milestones of this project will demonstrate that Curate® processing can enhance and contribute to automation and standardization of genetically modified CD34+ HSPC manufacture for gene therapies. Moreover, we will identify any needed modifications of current Curate® processing, which will guide further research and development. These outcomes will substantially increase the value of UMB co-owned IP.

Validation of in Vivo Pain-Relieving Efficacies of Human Pain-Sensing Neurons

Over one-third of the world's population suffers from devastating pain caused by neurological disorders, diseases, car accidents, war injuries, chemotherapy, etc. Most drugs on the market for pain treatment have undesired side effects because their targets exist both inside and outside the pain pathways. Recent studies have revealed that nociceptive (pain-sensing) neurons encompass a remarkably heterogeneous population that entails various transductions of noxious stimuli through numerous ion channels and receptors, hampering detailed understanding of human nociception as well as analgesic drug development. Human pluripotent stem cells (hPSCs) have emerged as a new cellular source for developing novel cell therapies, because they produce large quantities of otherwise extremely rare cell populations. For example, we and others have shown that nociceptive neuron population is readily generated from hPSCs; however, the resulting nociceptive neuron populations were highly heterogeneous, and subtypes of nociceptive neurons were not sufficiently defined, purified, or characterized. To harness the promise of hPSCs and address such issues, we generated multiple genetic reporter hPSCs by knocking in GFP-tagged constructs into the 3' UTR of SCN9A, TRPV1, and MRGPRX1 loci. Pure subtypes of SCN9A::GFP+, MRGPRX1::GFP+, and TRPV1::GFP+ nociceptive neurons can be readily generated, isolated from hPSCs, and cultured for weeks showing preferential responses to painful and irritating. Moreover, we found that these human nociceptive neurons can rapidly generate extended axons and survive for prolonged periods after implanted into immunodeficient rats. Interestingly, the survived hPSC-derived donor nociceptive neurons could establish functional connections with host neurons to sensitize or direct activate endogenous neurons. In this validation proposal, we will optimize the sensory neuron purification approach and test if the hPSC-derived sensory neurons can mitigate the pain responses in an immunodeficient rat chronic pain model. The results from this project will potentially lead to novel cell therapy for treating chronic pain. There is no effective treatment for many chronic pain patients and currently available therapeutic options brought significant addiction problems with huge socioeconomic burdens. With ample support from the Maryland State and Johns Hopkins University, this proposed study can lead to a new patent with potential therapeutics to treat chronic pain. This achievement could yield patent royalties and pave the way for a new startup company in Maryland.

Jeffrey Rothstein, MD, PhD

Johns Hopkins University
Awardee Amount: $249,073
Disease Target: Motor Neuron Diseases; Dementia
(2025 1st Funding Cycle)

Elias Zambidis, MD, PhD

Johns Hopkins University
Awardee Amount: $250,000
Disease Target: Blindness; Eye disease; Visual System Disease
(2025 1st Funding Cycle)

CHMP7 Antisense Oligonucleotide: Candidate Therapy for ALS/FTD Dementia and TDP-43 proteinopathies

Defects in an essential cellular process that maintains communication between nuclear and cytoplasmic compartments of the cell (nucleo-cytoplasmic transport, NCT) have recently emerged as a prominent pathogenic mechanism underlying sporadic and familial ALS when studied in large numbers of patientderived induced pluripotent stem cell-derived neuron lines and human brain tissues. Setting the stage for the development of a CHMP7 ASO human therapy, CHMP7 targeting antisense oligonucleotides (ASOs) alleviates nuclear pore complex injury and mitigates TDP-43 dysfunction which is significantly trigger in ALS as a result of NPC injury. The current program will advance the CHMP7 ASO through the necessary steps to eventually make it ready for human ALS trial in sporadic and C9orf72 ALS, and other conditions associated with TDP-43 dysfunction by our pharma company collaborator, Ionis Pharmaceuticals, and their expert team. The impact for this drug development program has enormous potential for a range of debilitating neurological diseases. Basic research has identified nuclear pore and associated TDP-43 dysfunction as a fundamental biological defect in Amyotrophic Lateral Sclerosis, frontotemporal dementia and Alzheimer's disease. Should this ASO prove potent in delaying disease and allowing some functional recovery in the more common sporadic forms of the disease—the health impact would be quite impressive.

Validation of MoroPLUR Induced Totipotent Stem Cells for Generating Whole Human Eye Organs

We recently developed a new class of human induced totipotent stem cells termed 'MoroPLUR' Tankyrase/PARP (poly (ADP-ribose) polymerase) Inhibitor-Regulated Naïve Stem Cells (TIRN-SC) that possess high differentiation performance and a capacity to contribute differentiated human neural tissues into the central nervous systems of developing animal embryos. In this project, we will expand our academiccommercial collaborations to exploit TIRN-SC for generating interspecies chimeric mice and pigs with physiologically mature, laminated, and functional human retinal tissues. We propose to commercially validate our patent-pending TIRN-SC technology for this goal by first generating mice with fully humanized retinal tissues in novel interspecies neural-retinal blastocyst complementation (IBC) approaches using mutant mice that are incapable of generating endogenous neural-retinal tissues. We will also validate the patient safety of IBC-generated neural-retinal tissue from chemically reprogrammed TIRN-SC by testing their genomic integrity before and after retinal differentiation; using retinal organoid (RO) tissues as pre-clinical surrogates. These pilot pre-commercial validation experiments will be important for determining the future feasibility of TIRN-SC for generating neural-retinal tissues in adult pigs; prior to investing in more complicated and expensive models of human retinal tissue development within gene-edited eyeless pig IBC models. If successful, MoroPLUR-derived mice and pigs with whole human eye organs will be a disruptive technology that will enable reliable pre-clinical research testing for ocular regenerative therapies, as well as serving as a source of mature human retinal sheets for clinical transplantation. Mice and pigs harboring experimental, gene-engineered human retinal tissues will enable the research community to model eye diseases and investigate high-risk ophthalmologic therapies that are not possible or ethical to perform in patients. Moreover, a large animal porcine model than can generate developmentally mature, patientspecific neuro-retinal tissues will have valuable, high impact as a future source of full retinal sheets for future clinical transplantation approaches to cure blindness.

MSCRF

Launch
Grant Awards

2024

Marios Arvanitis, MD

Johns Hopkins University
Awardee Amount: $350,000
Disease Target: Vascular & Circulatory System Diseases
(2025 1st Funding Cycle)

Renyuan Bai, PhD

Hugo W. Moser Research Institute at Kennedy Krieger
Awardee Amount: $350,000
Disease Target: Duchenne Muscular Dystrophy

The Role of Fate-Determining Transcription Factor REST in Endothelial Differentiation

Endothelial differentiation and specialization are essential for the development of new blood vessels from pre-existing blood vessels in our body, in a process termed angiogenesis. Angiogenesis is beneficial during wound healing but when dysregulated, it can lead to vascular disease and contribute to pathologic cancer growth. However, our current knowledge on the mechanisms of endothelial differentiation and angiogenesis are incomplete which limits our ability to target those mechanisms to treat human disease. In prior work in our lab, we discovered a new gene, REST, that affects endothelial function and leads to atherosclerosis. We found that REST affects processes related to endothelial differentiation and angiogenesis and affects neo-angiogenic growth within atherosclerotic plaques. In this proposal, we aim to study the effects of REST in the different steps of endothelial differentiation and specialization, along with the underlying mechanisms involved using a human embryonic stem cell to endothelial cell differentiation model. This study will allow us to identify novel therapeutic targets to regulate endothelial differentiation and angiogenesis for the treatment of vascular disease. This project has a high impact on public health for several reasons. First, improving our ability to develop specialized endothelial cells from stem cells is important to vascular biology research and will serve as a platform for future studies in vascular disease. Second, understanding the mechanisms and pathways via which REST regulates endothelial differentiation and angiogenesis will allow for the development of new therapeutic strategies to target angiogenesis to treat human disease.

Develop A Satellite Cells-Specific AAV Vector for Duchenne Muscular Dystrophy Using A Human PSC-Based Platform

Duchenne muscular dystrophy (DMD) is a common genetic muscle disorder and is caused by mutations in DYSTROPHIN. Recently, several Adeno-associated virus (AAV)-based gene therapy strategies have been developed for DMD, delivering truncated forms of DYSTROPHIN to skeletal muscle tissues. Many muscle tissues in pathological conditions undergo a high rate of turnover, meaning the damaged muscle tissues constantly degenerate and new muscle tissue regenerate, although healthy muscle is quite stable. Therefore, if the genetic payload is delivered to injured muscle tissue in patients via nonselective AAV, it will be difficult to expect long-term therapeutic effects. Recent studies show the important functions of DYSTROPHIN protein in skeletal muscle stem cells, however, there is no gene delivery system specifically targeting the skeletal muscle stem cells, partly due to the lack of a humanized in vitro skeletal muscle model resembling the complex in vivo environment that contains satellite cells. Our group has established a new MYOrganoid system derived from human induced pluripotent stem cells (hiPSCs), with PAX7+ putative skeletal muscle stem cells. With MYOrganoids and AAV expertise in our team, we aim to identify a muscle stem cell-specific AAV capsid protein by screening capsid libraries derived from the AAV8 or AAV-rh74, using the human PAX7+ cells in the MYOrganoids. AAV8 and AAV-rh74 have shown safety, efficacies and favorable biodistribution in human patients and novel variants with heptapeptide insertion in AAV capsid's VR-VIII region could retarget AAV to new cell types such as skeletal muscle stem cells without altering the overall in vivo behavior of the parental AAV capsids. We anticipate the selected AAV capsid protein will have a high infection rate in the PAX7+ cells in MYOrganoids derived from DMD hiPSC lines. Our proposed study will lay the foundation for meaningful and long-lasting gene therapies for DMD as well as other muscular dystrophies. Our MYOrganoid system is not only useful for developing a new gene therapy strategy, but also for validating the infectivity and therapeutic effect of novel AAV vectors in a humanized DMD model. Vita Therapeutics shows interest to potentially license out this technology. Once successful in generating novel AAV vectors, the translational plan will follow the similar path that led to the FDA-approval of AAV-rh74 with micro-dystrophin as payload via systemic administration (IV), namely test of efficacies in rodent disease models and safety in non-human primates, and clinical trials in DMD patients.

Man-Kyo Chung, PhD

University of Maryland, Baltimore
Awardee Amount: $349,997.80
Disease Target: Dental Caries & Pulpitis

Development of hiPSC-Derived Odontoblast Scaffold for Future Cell Therapy In Dentin Regeneration

Previously, while mesenchymal stem cells and dental pulp stem cells have been considered as new cellular sources to promote dental tissue regeneration, these cells are not ideal due to the irreversible proliferation-arrested state, declined differentiation capability after long-term culture, and 'batch-tobatch' variations of the biological properties. To address these issues, we propose to utilize human induced pluripotent stem cells (hiPSCs) to generate odontoblasts. In this proposal, we will develop a new hiPSC-based direct odontoblast differentiation and prospective isolation methodology using our established expertise. In addition, we will develop novel human odontoblast scaffolds for odontoblast transplantation to determine the efficacy of dentin regeneration in damaged teeth. Dental caries and following pulpitis is a common condition that greatly affects human health with huge socioeconomic burden. Current treatment approaches in dentistry is mostly symptomatic and cell replacement therapy for regeneration will bring more long-lasting solutions for patients. Successful outcomes will develop a defined direct hiPSC differentiation protocol to generate functional odontoblasts that can be potentially useful for cell therapy for dentin regeneration as well as to develop novel scaffold with human odontoblasts for the effective regeneration of dentin. Therefore, our study will be highly translational for regenerative dentistry.

Allen Eghrari, MD

Johns Hopkins University
Awardee Amount: $348,432
Disease Target: Corneal Dystrophies

Building a Gene-Corrected, Personalized Approach to Corneal Transplantation

Corneal endothelial dystrophies affect approximately 1 in 40 Americans and are the leading indication for corneal transplantation in the United States. Recent advances in regenerative medicine that draw on cultured corneal endothelial cells (CECs) from a donor or from pluripotent human embryonic stem cells are limited by a risk of rejection due to sourcing from a separate donor, and risk of glaucoma or infection from the need for ocular immuno-suppression. Here, we propose the use of corneal endothelial cells derived from gene-corrected, induced pluripotent stem cells that can be acquired from the patient's own blood sample. This means not only producing CECs but also correcting the genetic mutation within stem cells so that the new CECs do not carry the mutation causing the disease. This would open the door for a personalized approach that uses cells bearing genetic material familiar to the patient's immune system and that is expected to result in a decreased inflammatory response relative to typical transplants. In this study, we plan two aims. The first is to validate gene-corrected CECs from a blood sample provided by an individual with the L450W mutation in COL8A2. To achieve this aim, we will produce induced pluripotent stem cells from a blood sample, correct the mutation at the stem cell stage, and then produce CECs. We will then test these cells to ensure they are demonstrating the same physical properties and appearance as healthy CECs and that the expression of genes, which is unique for each cell type, is similar to typical CECs and improved from affected cells. In the second aim of this study, we will utilize CECs to heal corneal swelling in an animal model. We hypothesize that the edema-reducing effect of gene-corrected CECs will be similar to CECs derived from a healthy donor. If successful, this approach would allow for access to personalized corneal endothelial cell transplants in areas where cultural, ethical or economic reasons have prevented systems of organ and tissue donation. It also allows for resolution of rare genetic mutations that otherwise would not receive attention for potential pharmacological treatment.

Konstantinos Konstantopoulos, PhD

Johns Hopkins University
Awardee Amount: $350,000
Disease Target: Organ Transplantation
(2025 1st Funding Cycle)

Payam Mohassel, MD

Johns Hopkins University
Awardee Amount: $350,000
Disease Target: Duchenne Muscular Dystrophy

Extracellular Fluid Viscosity is a Novel Physical Cue that Regulates Human Mesenchymal Stem Cell Function

Human mesenchymal stem cells (hMSCs) are multipotent, stroma cells present in many tissues. hMSCs have been extensively studied because of their promising clinical applications in tissue regeneration, treatment of bone and cartilage defects etc. hMSCs respond to different physiologically relevant mechanical stimuli, such as stiffness, viscoelasticity, and confinement. All previous studies were performed in hMSC media with a viscosity close to water (0.7 cP). However, the viscosity of interstitial fluids is elevated, and can exceed 3 cP, whereas blood viscosity can reach 8 cP during pathological abnormalities. Extracellular fluid viscosity is thus a physiologically relevant physical cue, which has been overlooked. We were the first to establish that elevated, yet physiological, levels of viscosity (8 cP) regulate tumor cell function in vitro and in vivo. However, it is currently unknown how fluid viscosity affects hMSCs differentiation on substrates of prescribed stiffness, viscoelasticity, or confinement. Intriguing preliminary data reveal that although hMSCs on soft substrates and baseline (0.7 cP) viscosity undergo adipogenic differentiation, elevated viscosity (8 cP) is sufficient to induce osteogenic differentiation in these soft substrata. In light of our preliminary data, we propose to establish extracellular fluid viscosity as a novel physical cue that regulates hMSCs differentiation, and elucidate the underlying signaling mechanisms by which viscosity biases hMSCs towards osteogenic differentiation (Aim 1). In Aim 2, we will decouple the effects of stiffness from confinement using a novel microfluidic device, and delineate their interplay (as well as that of viscoelasticity) with fluid viscosity in hMSC differentiation. Lastly, we will decipher how fluid viscosity imprints mechanomemory to hMSCs, and alters their immunosuppressive potential. In sum, our proposed research will establish fluid viscosity as a novel physical cue that regulates the differentiation and immunosuppressive potential of hMSCs. This discovery will open new avenues for stem cell therapies relevant to tissue regeneration. Our research will establish fluid viscosity as a novel physical cue that regulates the differentiation and immunosuppressive potential of hMSCs. This discovery will open new avenues for stem cell therapeutic applications, including the design of precisely temporal tissue regenerative or immunosuppressive therapies, which could be used in various diseased conditions, such as injury and tissue transplants.

hPSC-Derived Skeletal Muscle Organoid Transplantation as a Therapy for Duchenne Muscular Dystrophy

Muscle atrophy and muscle wasting can be caused by different disease processes including genetic disease, systemic diseases including auto-immune disease or cancer, or as part of age-related muscle loss (sarcopenia). Muscle weakness is correlated with morbidity and mortality and currently, except for exercise, there are no effective treatments to reverse muscle weakness. Muscle tissue naturally contains adult muscle stem cells that enable its regenerative capacity. Thus, cell-based therapies hold great promise to help harness and accentuate this natural process as a treatment for a wide variety of muscle diseases. In this application, we propose to use human pluripotent stem cells as a therapeutic strategy, focusing on treating a mouse model of Duchenne muscular dystrophy. Building on prior work, we propose a novel human pluripotent stem cell derived organoid model that will improve some of the prior limitations of cell transplantation. This proposal will advance our understanding of cell-based therapies for treatment of skeletal muscle disease. In particular, it will aim to test the preclinical efficacy of cell-based therapies for Duchenne muscular dystrophy, a rare and life limiting disease that manifests in children.

Chan-Hyun Na, PhD

Johns Hopkins University
Awardee Amount: $350,000
Disease Target: Alzheimer's Disease & Frontotemporal Dementia

Cell-Type-Specific Proteome Analysis of Human Brain Organoid

Understanding proteomic changes of human iPSC-derived brain organoids, which is crucial for studying human brain diseases, is essential to studying the pathogenesis mechanisms of human brain diseases. Mass spectrometry has become central in proteomics, but conventional methods analyze whole organoids, not accounting for distinct cell types' roles and signaling. This general approach hinders understanding the interactions between different cell types. Techniques like BONCAT, TurboID, or APEX provide cell-type-specific analysis but require genetic manipulation, limiting their use. We propose a new method, iCAB, combining immunohistochemistry and biotin-tyramide, targeting specific cell types or organelles with antibodies and biotinylating nearby proteins. Biotinylated proteins are then enriched and identified via mass spectrometry, enabling precise proteome profiling. This project has two aims: (1) Develop antibodymediated biotinylation for cell-type-specific proteome analysis in iPSC-derived brain organoids. (2) Develop a differential tagging-based multiple-cell type analysis from the same organoids, overcoming the limitation of isolating proteins from only one cell type per tissue section. This method will be userfriendly, straightforward, and free from genetic modification, making cell-type-specific proteome analysis more accessible and broadening its application in brain organoid research.
By completing this project, we will establish an effective and straightforward celltype-specific proteomic analysis method and gain a deeper understanding of the dysregulated intercellular signals for each cell type of human iPSC-derived brain organoids from patients with neurodegenerative diseases. Ultimately, this new technology will serve as a crucial foundation for identifying mechanisms underlying neurodegenerative diseases, screening drugs, establishing personalized medicine, and finding effective treatments.

Alexandros Poulopoulos, PhD

University of Maryland, Baltimore
Awardee Amount: $349,995
Disease Target: Circulatory System Disease
(2025 1st Funding Cycle)

iPSC Platforms to Develop Genome Restoration Strategies Using Cas9-RC

This project aims to leverage the Cas9-RC genome editor developed by our team to create innovative genome restoration therapies for genetic diseases with heterogeneous rare variants. Cas9-RC enhances Homology-Directed Recombination (HDR), allowing for precise replacement of large genomic segments. This capability enables targeting multiple pathogenic variants within a gene using a single agent, simplifying translation and regulatory approval. Previously, our research utilized mice and cell lines. We now seek to transition to human cells, using induced pluripotent stem cells (iPSCs) to develop genome-restoring therapeutics. Specifically, we will use iPSCs with patient variants causing Timothy Syndrome (TS) as a model to demonstrate the efficacy of Cas9-RC for genome restoration. TS's diverse pathogenic variants make it an ideal candidate for this approach. We will also establish an innovative in vivo model using stem cell xenotransplantation to test these therapeutics in a live setting, optimizing delivery and dosing. This model involves transplanting human iPSC-derived neurons into the rodent brain, to accelerate determining clinically-relevant parameters. By shifting to human iPSC research, we aim to contribute valuable resources to the stem cell community, including the development of knock-in iPSC lines. These efforts will enhance collaborative research and advance the application of genome-restoring therapeutics. This project aims to develop Cas9-RC genome-restoring therapeutics, targeting rare genetic diseases like Timothy Syndrome. By leveraging human iPSC models, we will create precise treatments, reducing chronic treatment needs and healthcare costs. This research has the potential to create systematic gene therapies, particularly for those without current treatment options

Robin Roychaudhuri, PhD

University of Maryland, Baltimore
Awardee Amount: $345,896.34
Disease Target: Alzheimer's Disease; Nervous System Disease
(2025 1st Funding Cycle)

Role of Lipid Metabolic Signatures in Induced Pluripotent Stem Cell Derived Alzheimer's Disease Neurons

Alzheimer's disease (AD) is a complex and multifactorial disease that features extracellular accumulations of amyloid - protein (A) and intracellular tangles of tau and neuroinflammatory responses involving activation of microglia and astrocytes. Progression of AD leads to synaptic dysfunction, altered neural excitability and neuronal death and is a disease of synaptic failure. Synaptic junctions are the building blocks of chemical synapse and compelling evidence points to essential function of lipids in synaptic neurotransmission. Lipids including phosphatidyl inositol phosphates (PIP) and phosphatidylserine (PS) are crucial for synaptic vesicle cycle and fusion. Lipids also play major roles in other synaptic processes including formation and shaping of membranes, lipid mediated signal transmission and endocytosis. Multiple AD risk factor genes are involved in cholesterol metabolism in addition to ApoE4. Lipid accumulation containing cholesterol are common in the brains of AD patients as are abnormal endosomes and lysosomes suggestion dysfunction of lipid metabolism in neurons in AD. How lipid metabolism contributes to neuronal impairments in AD is unknown. Since AD is a disease of synaptic failure, determining the effect of lipid metabolism on synaptic function in AD neurons may open therapeutic avenues in the treatment of cognitive decline that manifest in later stages of AD. The project proposed is in strong alignment with the goals of MSCRF in accelerating novel stem cell technologies aimed at developing novel cures. The proposed project has strong translational value as novel therapies based on patient derived iPSCs may be a possibility. Dietary supplementation of lipids is a strong outcome that may benefit cognitive outcomes in larger Alzheimer's disease patient studies.

Tomoyo Sawada, PhD

Lieber Institute for Brain Development
Awardee Amount: $349,780.18
Disease Target: Down Syndrome

Exploring the Contribution of The Placenta-Brain Axis to Neurodevelopmental Abnormalities In Down Syndrome

The proposed research project aims to explore the intricate relationship between placental function and brain development in individuals with Down syndrome (trisomy 21, T21), the most common genetic condition. Despite established abnormalities in both the placenta and developing brain, the impact of placental changes on fetal brain development in T21 remains inadequately understood. With a focus on the placenta's essential endocrine role in delivering neurotrophic and neuroprotective compounds to the fetal brain, our goal is to determine the consequences of impaired placental signaling on neurodevelopmental processes in T21. This study will utilize hiPSC-based models of placental cells and the developing brain to demonstrate the consequences of compromised placenta-brain axis in T21 brain development. Two primary aims drive this project: First, establishing a novel platform to investigate the placenta brain axis in T21, an area receiving limited attention in T21 research and hiPSC-based neurodevelopmental models. Second, assessing the consequences of compromised placental endocrine function and increased oxidative stress on neurodevelopment in T21, focusing on the role of human chorionic gonadotropin (hCG) secreted by syncytiotrophoblasts (STBs). The hypothesis proposes that improving hCG signals by reducing placental oxidative stress and enhancing STB formation may attenuate neurodevelopmental abnormalities in T21. This project intends to explore the role of placentally-produced compounds and their protective effects on human neurodevelopment. In addition to our primary goals, we aim to explore T21 STBs' response to oxidative stress by assessing hCG secretion, potentially an indicator of the severity of placental impairment that could be used in prenatal assessments. We expect our findings to identify potential prenatal treatment targets and enable real-time T21 assessment. Additionally, our innovative hiPSC-derived placenta-brain axis model will serve as a foundational platform to comprehend impact of high-risk pregnancies, substance misuse, and environmental chemicals on fetal brain development, which holds promise for significant contributions to maternal-fetal medicine. Considering the rising number of babies born with Down syndrome and the increasing births among women aged 35 to 44 in Maryland and the U.S., elevating the risk of T21, our proposed research becomes especially relevant as it will contribute to identifying potential therapeutic targets for prenatal treatment and enable real-time assessment of T21. Furthermore, the innovative hiPSC based placenta-brain axis will be instrumental in comprehending how high-risk pregnancies, substance misuse, and environmental chemical exposure impact fetal brain develop-ment. Taking into account Maryland's historically higher infant mortality rate compared to the national average, our project holds promise for substantial advancements in maternal-fetal medicine.

Debasish Sinha, MS, PhD

Johns Hopkins University
Awardee Amount: $349,984
Disease Target: Age-Related Macular Degeneration
(2025 1st Funding Cycle)

Dudley Strickland, PhD

University of Maryland, Baltimore
Awardee Amount: $350,000
Disease Target: Vascular & Circulatory System Diseases
(2025 1st Funding Cycle)

Preclinical Investigation of Dysregulated Autophagy-Induced EMT Drives Secretoryautophagy & Ferroptosis in Human iPSC-Derived RPE Cells with the CFH402 Risk Allele

Age-related macular degeneration (AMD) is a leading cause of vision loss in the elderly, with no effective treatments currently available for dry AMD. This research proposal aims to evaluate a novel therapeutic approach for dry AMD using a monoclonal antibody against Lipocalin-2 (LCN-2). LCN-2 is upregulated in aged RPE cells from AMD mouse models and human AMD donor samples. Impaired autophagy in RPE cells is a significant factor in early AMD development. Previous attempts to target autophagy through mechanistic target of rapamycin, complex1 (mTORC1) inhibition have been unsuccessful due to side effects. We have discovered that LCN-2 regulates autophagy. The proposed study will use induced pluripotent stem cell (iPSC)-derived RPE cells with specific genetic risk polymorphisms to model dry AMD in vitro. This model system will be used to determine if increased LCN-2 levels initiate epithelial-mesenchymal transition (EMT) due to abnormal autophagy via Epidermal Growth Factor Receptor (EGFR) signaling, explore the link between macro-autophagy, EMT, secretory autophagy, and ferroptosis in AMD pathogenesis using the CFH Y402H variant, and attempt to rescue the observed effects using the LCN-2 antibody. This novel strategy could have significant clinical value and may stimulate new entrepreneurial ventures in Maryland, driving economic growth and job creation in the healthcare sector and broader economy. By targeting the autophagy pathway through LCN-2 inhibition, this research offers a promising alternative to previous unsuccessful attempts at modulating autophagy in AMD. If successful, this approach could address a significant unmet medical need and improve the quality of life for millions of elderly individuals affected by dry AMD. The RPE is centrally involved in the pathogenesis of dry AMD. We aim to test a novel monoclonal antibody in genetically modified iPSC-derived RPE cells, an optimal in vitro model for dry AMD, which could lead to non-human primate studies and ultimately a first-in-human clinical trial. This work has the potential to address a major unmet medical need, potentially improving life quality for millions of elderly individuals affected by dry AMD, a condition currently lacking effective treatments.

Use of iPSC-Derived Smooth Muscle Cells to Identify Functional Defects in LRP1

Aortic aneurysms and aortic dissections account for 1% to 2% of all deaths in Western countries and are usually asymptomatic until they rupture. Thoracic aortic aneurysms occur in all age groups and are more highly associated with single gene mutations such as those found in Marfan syndrome, a connective tissue disorder resulting from mutations in FBN1, the gene encoding fibrillin-1. On the other hand, abdominal aortic aneurysms are most common and are typically associated with advanced age and atherosclerosis, and until now, no single gene has been identified that is causative. Our recent studies reveal that mutations in the LDL receptor-related protein 1 (LRP1) may be causative for abdominal aortic aneurysms. The objective of these studies are to use iPSC harboring the LRP1 mutation to test the hypothesis that specific mutations in LRP1 impact smooth muscle cell biology that results in aneurysm formation.Over 150,000 people die annually from abdominal aortic aneurysms. Until now, no single causative gene has been identified. We have identified mutations in LRP1 that may be causative for abdominal aortic aneurysms. Identifying genetic risk factors that predispose for the development of this disease, will allow for genetic screening of at-risk patients and are likely to uncover common pathways that contribute to this disease to allow for a targeted intervention and drug development.

Gianluca Ursini, MD, PhD

Lieber Institute for Brain Development
Awardee Amount: $349,343.28
Disease Target: Schizophrenia

Li Yan, PhD

University of Maryland, College Park
Awardee Amount: $350,000
Disease Target: Neurological Diseases

Detecting The Effect of Genomic Risk for Schizophrenia on Placenta Development & Function, Using iPSC-Derived Trophoblast Cultures

Neurodevelopmental disorders (NDDs) such as schizophrenia are the outcome of trajectories that start in early life, are sensitive to early life complications (ELCs) and affected by genetic susceptibility. Although it is obvious that prevention should start early, there are no specific interventions that can be implemented in early life to prevent or rescue trajectories of risk. This is because there is a gap in the knowledge of the mechanisms through which genomic and environmental risk factors act in early life. We hypothesize that changes in the development and function of the placenta play a role in mediating the effect of genetic and environmental risk factors on such trajectories of risk. We have previously found that genomic factors converge with ELCs in affecting risk for schizophrenia, and we have identified schizophrenia-risk genes in placenta, that imply the nutrient sensing capabilities and invasiveness of placenta. By using a stem-cell derived model, our objective is now to detect the exact placental mechanisms contributing to risk for NDDs, and the placental biomarkers that mediate and/or reveal such mechanisms. We will differentiate induced pluripotent stem cells of controls and patients with schizophrenia (N=20) in trophoblast stem cells, syncytiotrophoblast, and extravillous trophoblasts. Then, we will assess whether genomic risk for schizophrenia and placental genes associated with risk factors for NDDs affect placenta development and functions like nutrient sensing, hormone secretion, and trophoblast invasion; and whether they affect the secretion of factors that influence human brain development. Further, we will identify the placental molecules that mediate possible effects on placenta development and functions. We will perform analyses stratified by sex. The knowledge derived by this research will help defining novel strategies for prevention and treatment of NDDs that would not be triggered by the study of the brain alone. This research will identify the specific placental mechanisms contributing to trajectories of risk for neurodevelopmental disorders like schizophrenia, and the placental and genomic biomarkers. This will help to identify potential strategies of prenatal prevention of neurodevelopmental alterations, targeted to pregnant mothers and their offspring, which represent a vulnerable population in need of more effective therapeutic interventions. Further, it will define individuals at high-risk for schizophrenia, who will benefit from postnatal strategies of prevention, based on a more careful monitoring of their developmental trajectory, as well as subsets of patients who may have a different clinical course and response to treatment, tailored to the etiopathogenetic mechanisms of their condition.

The Role of Vascular Bed Stiffness in Regulating BBB Phenotypes in iPSC-Derived Brain Endothelial Cells

The human neural vascular unit (NVU) governs the dynamic interaction among brain cells and the blood-brain barrier (BBB), comprising brain endothelial cells, pericytes, astrocytes, neurons, microglia, and oligodendrocytes. These components intricately embed in the extracellular matrix (ECM), forming a complex NVU cell network. The composition and organization of the ECM and NVU cells determine the stiffness of the brain and vascular bed. The BBB, a vital NVU component, can sense and adapt its behavior in response to the cerebral vascular bed's stiffness. Dysfunction of the BBB is considered an early marker of several neurological diseases. Brain microvascular endothelial cells (BMECs), together with pericytes and astrocytes, form the vascular bed by embedding the basement membrane. While prior studies confirm the impact of matrix stiffness on the barrier function of iPSC-derived BMECs at maturation stages, it remains unclear whether vascular bed stiffness and its microenvironments influence BMEC differentiation towards endothelial phenotypes, particularly during induction and specification stages. The downstream effects of these influences on endothelial maturation are not well understood. Additionally, the impact of vascular bed stiffness on BMEC phenotypes and barrier function within the NVU environment remains largely unknown. To address these gaps, this study proposes two specific aims: Aim 1 aims to investigate the effects of matrix stiffness applied to iPSC-derived BMECs on inducing BBB phenotypes. Aim 2 seeks to evaluate BBB phenotype induction mediated by vascular bed stiffness within the NVU. The proposed research will assess how vascular bed stiffness influences phenotypes and barriers at various stages of differentiation within NVU microenvironments. This comprehensive approach aims to shed light on the intricate interplay of substrate and vascular bed stiffness in BMEC differentiation within the NVU, providing valuable insights for understanding and engineering a more physiological BBB. Vascular bed stiffness is vital for the proper function of the blood-brain barrier (BBB) in a healthy brain, and alterations in brain stiffness and BBB function are associated with various neurological diseases. While current in vitro models of the neurovascular unit (NVU) derived from induced pluripotent stem cells can partially replicate key BBB features, they lack the necessary interactions involving mechanical and cellular cues to fully represent BBB phenotypes. In this study, we will finely adjust the stiffness of the vascular bed within an NVU model that incorporates extracellular matrix, BBB cells, and brain organoids to study the BMEC differentiation, which will facilitate the basic study of BBB function and the development of innovative therapeutics.

MSCRF

Discovery
Grant Awards

2024

Chengyan Chu, PhD

University of Maryland, Baltimore
Awardee Amount: $345,000
Disease Target: Cerebral Small Vessel Disease

Ivy E. Dick, PhD

University of Maryland, Baltimore
Awardee Amount: $345,000
Disease Target: CACNA1A Related Disorder

Human GRPs As Drug Factories for Local Modulation Of Neuroinflammation In Cerebral Small Vessel Disease

Cerebral small vessel disease (CSVD) is a disorder that affects perforating cerebral arterioles, capillaries, and venules. The main clinical manifestations of CSVD are diverse, including motor deficits, cognitive impairment, mood disturbances, and abnormal gait. CSVD is now recognized as a major risk factor for stroke, contributing to about 25% of ischemic strokes and most hemorrhagic strokes. Notably, CSVD is responsible for most cognitive impairments and contributes to 50% of dementia cases, posing a massive burden to societies and health-care systems, particularly compounded by increased life expectancy and an aging population. Unfortunately, there is no effective preventative or therapeutic approach for CSVD. Neuroinflammation and white matter degeneration are increasingly recognized as key drivers of CSVD progression, which could be attractive therapeutic targets for CSVD. Thus, this project will use human glial-restricted progenitors (GRPs) with mRNA encoding P2X7-blocking nanobody to repair the white matter in CSVD via remyelination; and block P2X7-mediated inflammatory pathways through the nanobodies secreted from engineered GRPs to inhibit neuroinflammation. The strategy not only assures long-term survival and differentiation of transplanted GRPs, but also targets multiple critical pathophysiological aspects including neuroinflammation, endothelial dyfunction and BBB breakdown with the goal to prevent and repair or even reverse the brain damage in CSVD. The exploratory project aims to demonstrate the safety and efficacy of engineered GRPs in treating CSVD. It will open up a new therapeutic strategy to improve the quality of life of patients suffering from CSVD and other debilitating diseases.

Induced Pluripotent Stem Cell Derived Models for the Study and Treatment of CACNA1A Related Disorders

CACNA1A encodes the voltage-gated calcium channel CaV2.1, which plays a critical role in synaptic transmission throughout the brain. Mutations within CACNA1A can produce diverse and profound symptoms in patients, including ataxia, migraine, and seizures. As the number of known mutations has grown, the phenotypes presented by individual patients have become increasingly complex, with many patients exhibiting a unique combination of symptoms which cannot be explained entirely by studying the effects of the mutation in heterologous expression systems. These complex clinical presentations drive the need to evaluate CaV2.1 mutations within relevant neuronal contexts replete with neuronal proteins and signaling molecules. We will therefore generate two types of induced pluripotent stem cell (iPSC) derived models harboring CACNA1A mutations associated with distinct patient phenotypes. First, iPSC derived neurons, which display features similar to excitatory cortical neurons, will provide a robust model system within which to evaluate the impact of CACNA1A mutations on neuronal function. Second, we will generate cerebellar organoids containing Purkinje cells, known to be critical in the coordination and accuracy of movement. As CaV2.1 is particularly enriched within these specialized cells, they will provide a unique opportunity to evaluate the pathogenesis of mutations associated with ataxia and balance disorders. By studying the impact of these mutations within these two distinct neuron types, we will enable evaluation of context specific effects of each mutation, fostering new progress in understanding the underlying pathogenesis of CACNA1A related disorders and facilitating the development of new treatment strategies. CACNA1A related disorder is a rare disease which is often associated with a diverse array of phenotypes. Treatment options remain limited, leaving many patients without relief of debilitating symptoms. The generation of two relevant human neuronal models harboring CACNA1A mutations will be invaluable to evaluate the efficacy of current drugs on specific channel mutations and test new therapeutic options for these patients.

Miroslaw Janowski, MD, PhD

University of Maryland, Balitmore
Awardee Amount: $350,000
Disease Target: Amyotrophic Lateral Sclerosis

Xiaofeng Jia, PhD

University of Maryland, Balitmore
Awardee Amount: $345,000
Disease Target: Ischemic Brain Injury

Computational Approach to In Vivo Genome Editing in the Brain

The overarching goal of the project is to design a novel brain genome editing strategy based on exosomes as delivery vehicles. Current methods of genome editing in the brain are ineffective, largely due to suboptimal delivery of editing tools. In fact, the brain is a difficult target for any therapeutic agent; however, thanks to our previous research, we have established robust protocols for delivery and broad distribution of stem cells in the brain, exploiting their high migratory capacity. Here, we will tap into these properties, and more specifically, we will design stem cells that are capable of releasing exosomes as vehicles to implement genome editing machinery throughout the brain. Exosomes are naturally produced and released from the cells, making them a good choice for delivering a genome editing cargo in the neighborhood of recipient cells. Indeed, the concept of using exogenous exosomes (but not transplantation of exosome producing cells as in our project) has already been investigated, including the ingenious concept of routing molecular cargo for packaging within exosomes by fusing an exosome-bearing CD63 molecule with a target sequence, and fusing single domain antibody (sdAb) or aptamer against this target sequence with Cas9 protein. This facilitates the intracellular binding of the Cas9/sdAb/aptamer fusion protein to CD63 fused with a target sequence, which routes CRISPR/Cas9 to exosomes. While such a system has demonstrated feasibility of exosome-based genome editing, the need of fusing the endogenous CD63 molecule adds unnecessary complexity, chiefly, the logistical challenges related to in vivo production of exosomes by transplanted stem cells, which is a roadblock to clinical translation. Therefore, our project is focused on overcoming it. Then, our strategy will be specifically used for genome editing of point mutation causing familial amyotrophic lateral sclerosis (ALS) – unequivocally lethal rare neurodegenerative disorder, but it is applicable to any genetic disorder. The sanctuary site of the central nervous system shields it from benefits of correcting mutations by CRISPR-based genome editing. Here, we will use artificial intelligence for in silico design of critical molecules, which are expected to overcome this limitation. While we propose to test our novel approach in unequivocally lethal amyotrophic lateral sclerosis, we expect that our approach will be able to correct any mutation in the brain.

Developing and Improving Human Stem Cell-Derived Extracellular Vesicle Therapy for Brain Recovery After Cardiac Arrest

Cardiac arrest (CA) is a leading cause of death worldwide. Despite advances in cardiopulmonary resuscitation methods, only about 10 % of adult out-of-hospital CA victims survive hospital discharge. Among CA survivors, brain injury is the biggest impediment to functional recovery. Currently, neither pharmacological intervention nor therapeutic hypothermia can reverse CA-caused neural injury. Stem cell transplantation holds great potential in alleviating ischemic neuronal injury. We recently reported that intracerebral ventricular (ICV) administrated human neural stem cell (NSC) markedly improved neurologic outcomes after CA. Despite significant progress, tumourigenicity and immunogenicity have long been a challenge for stem cell therapy and hinder clinical applications. Increasing evidence suggests that extracellular vehicle (EV) therapy represents a novel cell-free treatment with compelling advantages over whole cell therapy by their parent cells. Our preliminary data showed human neural progenitor/stem cells (NPSCs) derived EVs improve brain recovery after CA. We recently developed a novel strategy which significantly improved the survival and boosted therapeutic efficacy of transplanted human NSCs by modification of these cells' glycans using recent advances in metabolic glycoengineering (MGE). With the long-term goal to develop and advance EV-based therapeutic intervention to ultimately improve the patient's quality of life, we will test the hypotheses: NSC-derived EV will improve neurological recovery, possibly through decreasing neuronal ischemic injuries and/or lessening immune reactions; optimized EVs will be more neuroprotective; and MGE-hNSC derived EV can further improve functional outcomes after CA. This proposal will investigate the effect of stem cell-derived EV therapy in a rat CA model via electrophysiological, functional outcomes, and histopathological measurements. For the first time, we will not only develop EV therapy but also further enhance the therapeutic effect by metabolic glycoengineering (MGE) to improve functional outcomes after CA. Our project focuses on developing cell-free interventions to improve functional outcomes from brain injury after CA in a clinically motivated, translational experimental design with systematic in vivo measurements. Our technology and protocols are highly translatable to the clinic and success in this project will have direct translational implications for patients resuscitated from CA.

Thomas Johnson, MD, PhD

Johns Hopkins University
Awardee Amount: $299,998
Disease Target: Optic Neuropathies (Glaucoma & Others)

Annie Kathuria, PhD

Johns Hopkins University
Awardee Amount: $345,000
Disease Target: Schizophrenia

A Multifunctional Biocompatible Scaffold for Cell Replacement Therapy to Restore Vision in Optic Neuropathy

Optic neuropathies, including glaucoma, are the world's most prevalent cause of irreversible blindness. These diseases cause permanent vision loss by killing retinal ganglion cells (RGCs), the nerve cells that send visual information from the retina of the eye to the brain though fibers (called axons) within the optic nerve. Though some types of optic neuropathies can be treated to slow or stop disease worsening, vision restoration would require replacement of RGCs and their connections within the eye and brain. We have attained qualified success in transplanting human stem cell derived RGCs into small and large animal models of optic neuropathy, but translating this approach to human clinical trials will require improvements in donor neuron survival, extension of fibers into the retina and brain, and formation of "communication stations" (functional synapses) within the visual pathway. To achieve these goals, we are developing a specialized biocompatible scaffold into which donor RGCs can be seeded. The scaffold is rationally engineered with three separate functional layers that mimic critical developmental properties of the retina. The scaffold can be loaded with bioactive molecules released in a sustained fashion to provide donor neurons with important signals that improve their survival and direct their maturation and functional engraftment into the eye, as a complement to the regenerative effects and guidance cues of the nanoarchitecture of the scaffold itself. This project aims to evaluate several design characteristics of the scaffold to achieve optimal neuroprotection, polarization, and regenerative potential of the graft pay load, as benchmarked against rigorous outcomes that assay cell survival and dendrite & axonal outgrowth in a directed fashion. In addition, we will develop a clinically translatable surgical technique to implant the scaffold through a small incision and evaluate the survival and engraftment of donor neurons in a large animal model. This work will set the stage for human clinical translation. This work aims to develop the tools and methodology that will be necessary to functionally replace RGCs for the treatment of optic neuropathies, and if successful will provide the world's first therapy capable of reversing vision loss for these patients. The approach is based on considerable prior data, experience in ophthalmic regenerative medicine, and expertise in medical devices and biomaterials which we will leverage to propel RGC transplantation towards clinical translation. The development of a novel biomaterial scaffold will produce intellectual property that will serve as the basis for a commercial venture capable of bringing this technology to clinical trial.

Elucidating Excitation-Inhibition Imbalance in Schizophrenia Using Patient-Derived Cerebral Organoids

Schizophrenia is a severe psychiatric illness that afflicts over 21 million people globally. Currently available antipsychotic medications fail to adequately address the widespread cognitive deficits that serve as the primary source of disability and predictor of poor functional outcomes in this disorder. There is strong evidence that imbalance between excitatory and inhibitory neurotransmission plays a key role in mediating these cognitive impairments. However, a comprehensive understanding of the specific cellular and molecular substrates underlying excitation-inhibition dysfunction remains lacking. This project will leverage cutting-edge stem cell models and genomic approaches to elucidate mechanisms contributing to dysfun-ctional excitation-inhibition homeostasis in schizophrenia patient neurons. The research specifically focuses on profiling transcriptional, proteomic, and functional abnormalities in patient-derived cerebral organ-oids and cortical neuronal cultures. Single-cell RNA sequencing experiments will definitively identify specific differences in gene expression and signaling pathways across distinct neuronal subtypes in patient organoids compared to healthy controls. Multielectrode array analysis will further characterize network-level deficits in neural oscillations and synchrony. Comparative analysis of cellular features in organoids generated from clozapine responder and non-responder patients holds promise for developing predictive biomarkers. Elucidating cell type-specific pathways mediating excitation-inhibition imbalance will fundamentally advance understanding of schizophrenia disease biology. By establishing links between molecular abnormalities, electrophysiological deficits, and medication response phenotypes, this work can unravel new therapeutic targets and pave the wave for precision treatment approaches tailored to rectify circuit-level disturbances underlying disabling cognitive deficits in individual patients. Findings from this study have the exciting capacity to reshape clinical care and improve real-world outcomes for millions afflicted by this enigmatic disorder worldwide. By elucidating cellular and molecular underpinnings of excitation-inhibition imbalance, this project holds remarkable potential to transform treatment approaches and dramatically improve functional outcomes for millions afflicted by schizophrenia worldwide.

Chulan Kwon, PhD

Johns Hopkins University
Awardee Amount: $344,999
Disease Target: Muscular Dystrophy

Correcting Muscular Dystrophy Using Microexons

Dystrophin-related genetic abnormalities cause Duchenne Muscular Dystrophy (DMD), a progressive illness marked by severe muscle weakening and cardiac problems in humans. However, there is no cure for the illness. While genome editing technologies like CRISPR-Cas9 have emerged as promising therapeutic tools for repairing DMD mutations, their utilities are constrained by the limited number of target exons for guide RNA design and the reliance on the non-homologous end joining mechanism. Here we propose to develop a novel genome engineering method based on "spliceable microexons (SME)," which allows for the removal of mutated sequences via a powerful splicing capacity in the genome. To apply this method for various DMD mutations, we will identify optimal genomic locations for the SME insertion and test if the insertion can repair DMD mutations in human iPSC models. A successful demonstration will validate the feasibility of utilizing SMEs for genome engineering, providing a novel avenue for the treatment of human genetic problems. This proposed research aims to develop a novel genome engineering technology that improves the efficiency, applicability, and stability of gene correction. A successful demonstration will lead to a major advance in the treatment of human genetic illnesses, such as Duchenne muscular dystrophy.

Gabsang Lee, PhD

Johns Hopkins University
Awardee Amount: $345,000
Disease Target: Neurodegenerative

Modeling Neurodegenerative Diseases with a New Optical TMEM106B Aggregation in hiPSC-Derived Cortical Neurons

Very recently, several studies uncovered the widespread pathology of TMEM106B aggregates in the post- mortem brain tissues of diverse neurodegenerative diseases, however, there is no sophisticated system to induce pathological TMEM106B aggregation in a human neuronal model. Using our established optogenetic protein aggregation system, we will develop a new optical TMEM106B aggregation platform in human induced pluripotent stem cell-derived cortical neurons to study its potential neuropathologic effects and perform a compound screening to reverse any neurodegenerative phenotypes. Our TMEM106B optical aggregation platform can lead us to define the pathogenic mechanism and discover new drug candidates. In last 2 years, the widespread pathology of TMEM106B aggregates were discovered in the postmortem brain tissues of diverse neurodegenerative diseases, including frontotemporal lobar degeneration (FTLD), limbic-predominant age-related TAR DNA binding protein 43 (TDP-43) encephalopathy (LATE), Alzheimer's disease (AD), Parkinson's disease (PD), and amyotrophic lateral sclerosis (ALS). However there is no model system to understand the pathophysiology and disease mechanism. Our proposed studies will bring a new TMEM106B aggregation platform for disease modeling and drug discovery.

Yajie (Kevin) Liang, PhD

University of Maryland, Baltimore
Awardee Amount: $345,000
Disease Target: Stroke

Iris Lindberg, PhD

University of Maryland, Baltimore
Awardee Amount: $342,233.88
Disease Target: Parkinson's Disease

Smart Intravital Multiphoton Imaging iPSC-Derived Cells Grafted into Ischemic Brain Assisted by Empowered Helper Cells

Stroke is a leading cause of mortality and disability worldwide, including more than 795,000 cases in the U.S. per year, with 15–30% of survivors being permanently disabled. Restoration of damaged neuronal circuits by transplanted cells is highly desirable and would be an ultimate solution especially for subacute or chronic stroke. However, progress in this effort is hindered by the lack of proper tools for monitoring the dynamic cell behaviors after transplantation, such as migration, fate, or the integration of grafted cells with the host neuronal circuits, which represents a huge gap of knowledge. Multiphoton microscopy has been increasingly used for monitoring cell behaviors in live animals, which could be used to address this knowledge gap for stem cell therapy of stroke. This proposal is a continuation of our Launch project funded by MRCSF in which we employed intravital imaging to take a close look at how transplanted human iPSC-derived neural progenitors' behavior in stroke animals and exploring measures to enhance their survival and integration in the damaged neural circuits. Our long-term goal is to advance the understanding about the graft-host interactions in the infarcted brain dynamically at the cellular and molecular level for enhancing the efficacy of neural stem cell therapy of stroke. 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 and enhancing the integration of grafted cells with host cells, this study will produce results that may reinvigorate the neural cell transplantation field for the treatment of neurological disorders.

Neuroprotection by An Endogenous Chaperone in a Stem Cell Model of Parkinson's Disease

The aim of this project is to study the role of a neuroprotective chaperone in a genetically controlled, differentiated human stem cell environment. This work will elaborate the biochemical mechanisms that an endogenous neural chaperone uses to reduce the toxicity of synuclein oligomers using new human stem cell models relevant to Parkinson's disease. We will genetically engineer iPSC cells to overexpress or lack the proSAAS chaperone. We will then differentiate these lines to dopaminergic neurons and test the protective effects of the proSAAS chaperone on dopaminergic neuronal survival following challenge with excess alpha synuclein. In accordance with the MSCRF goal of developing new strategies for the prevention and treatment of human disease through human stem cell research, this work is expected to provide proof-of-concept for proSAAS as a protective factor in dopaminergic stem cell survival; this is highly relevant to Parkinson's disease therapy. The work in this project will elaborate the control mechanisms neurons use to handle neurotoxic protein aggregation in Parkinson's disease. Parkinson's disease represents the second most common neurodegenerative disease; a recent epidemiological metaanalysis (1) indicates that the incidence of Parkinson's disease in people 65 and older ranges from 108 to 212 per 100,000 persons. The long time course of the disease exacts a huge burden on the health care system as well as on personal caregiving. However, to date the protective strategies that nigrostriatal dopaminergic neurons employ to block synuclein neurotoxicity have not been investigated. By exploiting endogenous synaptic chaperone pathways which act to save neurons from proteostatic distress, this work has the potential to greatly impact public health. The technology described here can ultimately be exploited to create further novel Parkinson's stem cell models (bearing risk-inducing mutations) and potentially lead to novel therapeutics.

Vasiliki Machairaki, PhD

Johns Hopkins University
Awardee Amount: $345,000
Disease Target: Alzheimer's Disease

Xiaobo Mao, PhD

Johns Hopkins University
Awardee Amount: $345,000
Disease Target: Alzheimer's Disease & Related Tauopathies

Development of hiPSC-Derived Microglia Drug Screening Platforms for Alzheimer's Disease

The problem of how to study the Alzheimer's Disease (AD) in the lab for the purposes of treatment development was initially tackled by "transgenic" mouse models. While the models advanced the study of AD, they have failed to lead to effective treatments because of species specific differences in brain development. Human models of the AD disease are needed to advance treatment development. The discovery of human induced pluripotent stem cells (iPSCs) derived from a person's blood now provides unprecedented opportunities for AD treatment development. iPSCs from individuals can be differentiated in the lab into all brain functional cell types and can capture unique genetic information, thus serving as excellent personalized models to understand molecular pathogenesis and develop treatment targets. Many previous attempts to treat/prevent AD with anti-inflammatory strategies have failed, very possibly for two reasons: 1) the role of inflammation in AD biological mechaanisms may vary between individuals (heterogeneity), and 2) inability to validate target engagement in physiologically valid models. This proposal addresses both of these issues by 1) testing drugs on microglia derived from 40 individuals, allowing us to assess heterogeneity in vivo; 2) using such microglia for high through put screening, which is likely to be a much more physiologically valid model system than 'old' methods using cell lines. In view of several recent negative trials for AD, the field is in dire need of novel, relatively simple, accessible screening platforms in which large numbers of neuroprotective compounds can be rapidly tested. For the experiments proposed in this project, we will be using a number of hiPSC lines from Johns Hopkins Alzheimer's Disease Research Center (ADRC) participants. These cell lines will be differentiated to each person's microglia, allowing them to be used as platforms for anti-inflammatory drug screening at the single person level. There is a crying need for new strategies for Alzheimer's disease prevention and treatment, and anti-inflammatory strategies are a promising and relatively unexplored target for AD drug discovery. Work proposed here will lay the groundwork to accelerate the development of a personalized treatment and define the patients for who it will be most effective.

Tauopathy Therapy using FGL1 Delivery via Engineering iPS Cell-Derived Exosomes

Neurofibrillary tangles (NFTs), prominent in Alzheimer's disease (AD), feature aberrant tau aggregates within neurons, contributing to widespread neurodegeneration. Recent breakthroughs revealed neuronto-neuron tau propagation as a self-perpetuating mechanism in AD pathogenesis. Our pioneering work identified lymphocyte-activation gene 3 (Lag3) as a pivotal receptor mediating prion-like α-synuclein fibril transmission and facilitating pathogenic tau spread. Building on this, we discovered a potential Lag3 inhibitory ligand with promising tau pathology-blocking capabilities. This ligand, hypothesized as a potent inhibitor of pathogenic tau spread, presents a novel avenue for therapeutic intervention. Our overarching goal is to translate this groundbreaking technology into effective therapies for AD and related tauopathies. Our strategy involves evaluating the Lag3 inhibitory ligand's efficacy in halting pathological tau spread within human iPS cell-derived neurons and organoids. Furthermore, we aim to explore iPS cell-derived exosomes as a potential vehicle for delivering the Lag3 inhibitory ligand to the brain, providing a targeted approach for inhibiting pathological tau spread. This translational research holds immense promise for developing innovative treatments against AD and related tauopathies. By elucidating the Lag3 inhibitory ligand's impact on pathological tau trans-mission and assessing the efficacy of exosome-mediated delivery, we aim to pave the way for transformative therapies that address the root causes of AD neurodegeneration. The successful outcome of this project holds the potential to revolutionize our understanding of Alzheimer's disease and related disorders. By providing innovative human iPS cell-based models and identifying a promising therapeutic target, this research lays the foundation for breakthroughs in drug development and clinical translation. The method-ologies developed here are poised to contribute significantly to biotechnology in Maryland, with a specific focus on advancing drug development and delivery for various brain diseases. This project not only addresses critical gaps in our knowledge of neurodegenerative conditions but also has far-reaching implications for the broader field of biomedicine, positioning Maryland at the forefront of cutting-edge research and innovation in the fight against brain diseases.

Raphael Meier, MD, PhD

Johns Hopkins University
Awardee Amount: $344,987.28
Disease Target: Type 1 Diabetes

Byoung Chol Oh, PhD

Johns Hopkins University
Awardee Amount: $301,976
Disease Target: Transplant

Co-transplantation of MMP-9 Enhanced Mesenchymal Stem Cells and Pancreatic Islets for Treatment of Type-1 Diabetes

Type 1 diabetes (T1D) is a chronic condition characterized by the destruction of insulinproducing pancreatic beta cells. T1D patients need exogenous insulin to maintain their blood sugar. While insulin therapy is essential for individuals with T1D, it comes with its own set of challenges such as episodes of hypoglycemia, mealtime management, dosing accuracy, and severe long-term complications. On June 28, 2023, the FDA approved the first allogeneic pancreatic islet cell therapy for the treatment of T1D. Currently, limited viability and poor engraftment of pancreatic islets after intraportal infusion drastically reduce the chances of insulin independence and limit its application. Our previous work demonstrated that Mesenchymal Stem Cells (MSCs) can improve survival/engraftment and function of encapsulated pancreatic islets by cell-to-cell contact. We genetically modify MSC for the enhanced secretion of certain molecules which are essential for pancreatic beta cell function and islet vascularization. Here, we propose to develop a novel cell therapy based on the co-encapsulation of genetically modified MSCs and pancreatic islets to treat diabetic patients. Type-1 Diabetes (T1D) is a global public health concern, expected to impact 629 million people by 2045. The need for therapy to cure T1D is a must. We aim to develop a novel cell therapy involving co-encapsulation of MMP9-enhanced MSC and pancreatic islets to treat T1D patients. Diabetes is one of the most prevalent diseases worldwide, expected to impact 629 million patients by 2045[1, 2]. In both children and adults, the hallmark of Type 1 diabetes (T1D) is the loss of insulin-secreting pancreatic β-cells. About 700,000 new cases are diagnosed each year[3, 4]. T1D patients require lifelong insulin injections with the risk of life-threatening hypoglycemia events. The disease is hard on many organs, the odds ratio of developing kidney failure is particularly high. Pancreatic islet transplantation has the potential to restore physiological insulin secretion and to achieve better glycemic control than exogenous insulin alone. It is far less invasive than a whole pancreas transplant and virtually eliminates hypoglycemia unawareness episodes. Currently, poor engraftment of pancreatic islets and their long-term survival are the major challenges for the successful T1D treatment[5]. Previously, we demonstrated that Mesenchymal Stem Cells (MSCs) can improve survival, engraftment and function of encapsulated pancreatic islets by cell-to-cell contact. Matrix Metalloproteinase-9 (MMP-9), one of the key molecules secreted by MSCs, is essential for beta cell function and islet vascularization[7]. The positive effect of MSCs on encapsulated islets has been well documented, including independent confirmation by another member of our group [8]. However, given the modest magnitude of the effect, we hypothesize that function and vascularization can be further improved by specifically increasing MMP-9 levels. Therefore, we modified MSCs to enhance the secretion of MMP-9 over several fold. Here, we propose to develop a novel cell therapy based on the transplantation of co-encapsulated MMP-9-secreting MSCs and pancreatic islets for the successful treatment of T1D.

A Novel Stem Cell-Based Therapy Utilizing hiPSC-PNSorganoid-Derived Schwann Cells to Enhance Nerve Regeneration in VCA

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

Sashank Reddy, MD, PhD

Johns Hopkins University
Awardee Amount: $345,000
Disease Target: Cancer, Radiation Dermatitis

Sheikh Amer Riazuddin, PhD

Johns Hopkins University
Awardee Amount: $344,953
Disease Target: Glaucoma

Regenerative Cell Therapies for Radiation Injury

Radiation dermatitis (RD) is a devastating and common complication of radiation treatment for cancer, occurring in almost 95% of patients undergoing radiation therapy. The burns, acute and chronic wounds, fibrosis, joint contracture, pain, and impaired speech and swallow from RD severely affect quality of life and can lead to treatment discontinuation. Despite the severity of the problem, there are no FDA-approved therapies for RD. In clinical studies, lipotransfer of autologous adipose derived stem cells (ADSCs) and adipocytes has been shown to be effective for RD, but it is hampered by the need for invasive surgical harvest and limited duration of cell survival. We have developed a biomimetic and biodegradable nanofiber-hydrogel complex (NHC) that encourages vascularization, modulates inflammation, and provides a secure substrate for cell attachment and survival. Here we propose a regenerative cell therapy approach to RD that combines allogeneic ADSCs and ADSC-derived EVs with NHC as a first in class, off the shelf therapy, eliminating the need for surgical harvest of stem cells. Importantly, both NHC and allogeneic ADSCs have proven safe in clinical trials, easing the path for translation for the synergistic approach. In the proposed studies, we will define the optimal NHC/ADSC/EV combinations needed to promote stem cell retention and heal skin damage in RD. We will engineer NHC variants capable of supporting durable delivery of allogeneic ADSCs and EVs. We will quantify mechanisms of skin damage and repair using machine learning guided 3D tissue reconstruction. Finally, we will define the cellular and molecular basis for therapeutic effect using single cell sequencing, immunoprofiling, and lineage tracing approaches. These studies will provide the foundational understanding for planned clinical trials. Our ultimate goal is to develop the first truly effective stem cell-based therapy for the millions of cancer patients suffering from RD. Half of all cancer patients undergo radiation therapy during their course of care, with nearly all of them developing radiation dermatitis – a severe complication mimicking acute and chronic skin burns. We aim to unite two synergistic and clinically validated technologies, allogeneic adipose derived stem cells and regenerative nanomaterials to deliver the first effective, off the shelf therapy for the millions of patients suffering with radiation dermatitis.

Pluripotent Stem Cell-Derived Trabecular Meshwork Cells as A Potential Treatment of Glaucoma

The front of the eye consists of the cornea, iris, ciliary body, and lens. Aqueous humor (AH) produced by the ciliary body provides nutrition to the eye and helps in retaining the shape of the eye by maintaining intraocular pressure (IOP). The physiological IOP is maintained by a balance between the production and outflow of AH, which exits the eye through trabecular meshwork (TM). Obstruction in drainage of the AH results in elevated IOP, a risk factor for glaucoma. Glaucoma refers to a group of eye diseases that can cause vision loss due to damage to the optic nerve. It is the leading cause of irreversible loss of vision worldwide. Multiple studies have reported decreased cellularity of TM cells in glaucomatous eyes, which has been presumed to be the basis of increased resistance to the outflow of AH and elevated IOP. Our long-term goal is to develop a minimally invasive, stem cell-based therapeutic approach for restoring normal IOP in patients with a diminished outflow of AH. We envision a procedure where the malfunctioned TM as a result of glaucoma will be removed surgically followed by the injection of pluripotent stem cell-derived TM cells incorporated with magnetic nanoparticles (mNPs), which will help with steering the injected cells to the iridocorneal angle of the eye to regenerate TM. To this end, we have developed a xeno-free and feeder-free protocol to differentiate human embryonic stem cells (hESCs) into TM cells. We propose three aims in this application. First, incorporate mNPs into the hESC-derived TM cells, second, optimize the delivery of mNPincorporated, hESC-derived TM cells to iridocorneal angle to regenerate TM in rabbits, and third, examine the efficacy of mNP-incorporated, hESC-derived TM cells to restore the drainage of AH in glaucomatous eyes of a canine model of glaucoma. Glaucoma is the leading cause of irreversible blindness worldwide. While topical medications are prescribed to control elevated IOP in glaucomatous eyes, there is currently no treatment for permanently rectifying the hindrance in drainage of the AH that is presumed to be the basis of elevated IOP. The successful outcome of the proposed research will serve as the basis for the development of a new potential treatment for TM dysfunction and glaucoma.

Hiromi Sesaki, PhD

Johns Hopkins University
Awardee Amount: $345,000
Disease Target: Inclusion Body Myopathy

Charlotte Sumner, MD

Johns Hopkins University
Awardee Amount: $344,999
Disease Target: Distal Spinal Muscular Atrophy

Mitigating Myopathy by Promoting Mitochondrial Fusion and Mitophagy

This research program focuses on hereditary inclusion body myopathy (hIBM), a severe disease caused by mutations in valosin-containing protein (VCP). VCP functions in numerous cellular processes, including protein degradation, transcription, and organelle fusion, by acting in different cellular locations such as mitochondria, endoplasmic reticulum, Golgi complex, endosome-lysosome system, and nuclei. Mutations in VCP lead to severe diseases, hIBM with Paget's disease of the bone and frontotemporal dementia (also called multisystem proteinopathy 1) and amyotrophic lateral sclerosis. These diseases accumulate damaged proteins and form inclusion bodies in muscle cells and neurons. hIBM is the most prominent manifestation, affecting 90% of patients with VCP mutations. A hallmark of hIBM is mitochondrial dysfunction. Muscle cells, needing high energy, are sensitive to changes in mitochondrial function. Studies have suggested that hIBM involves reduced mitochondrial fusion and impaired mitophagy. Mitochondrial fusion distributes electron transport chain components, crucial for cellular energy, through the mitochondrial network. Mitophagy, which removes damaged mitochondria, is also hindered in hIBM, leading to the accumulation of dysfunctional mitochondria. We will test whether enhancing mitochondrial health by promoting mitochondrial fusion and mitophagy mitigate hIBM. Our research program addresses the critical gap in mitochondrial-focused treatments for hIBM and utilizes our advanced protocol for myoblast differentiation from patient-derived hiPSCs, as well as innovative biosensors for mitochondrial fusion and mitophagy. Central to our approach is the synergistic integration of two unique and complementary areas of expertise through interdisciplinary collaboration by Dr. Sesaki (mitochondrial dynamics, function, and pathology) and Dr. Lee (stem cell biology, skeletal muscle, & disease modeling). The outcomes are expected to significantly inform the development of mitochondria-targeted therapies for hIBM, which currently lacks effective treatments. Furthermore, the insight gained from our study potentially open new therapeutic avenues for many other diseases associated with mitochondrial dysfunctions, such as Alzheimer's, Parkinson's, and amyotrophic lateral sclerosis. The outcomes of this research program are expected to significantly inform the development of mitochondria-focused therapies for hIBM, a rare disease currently lacking effective treatments. Additionally, the insights gained from this study are likely to have a broad and positive impact on the treatment of many other mitochondrial-associated diseases, including major conditions such as Alzheimer's, Parkinson's, and ALS.

Repairing Human iPSC-Derived Blood Neural Barriers with TRPV4 Antagonists

Dominant gain-of-function missense mutations of the cell surfaceexpressed cation channel TRPV4 (transient receptor potential vanilloid 4) cause distal spinal muscular atrophy and Charcot-Marie-Tooth disease 2C. Recently, we demonstrated in mutant TRPV4 knock-in mice that motor neuron disease is caused by TRPV4 expression in neural vascular endothelial cells (NVECs) and disruption of blood-neural barriers (BNBs). These phenotypes were reversed by genetic ablation of TRPV4 specifically from endothelial cells, or by treating mice at an early disease stage with a small molecule TRPV4 antagonist. In this proposal, we aim to further advance the development of small molecule TRPV4 antagonists for patients with TRPV4 channelopathies by characterizing the endogenous, human mutant TRPV4 channel in NVECs derived from isogenic human induced pluripotent stem cell (iPSC) lines in which human disease-causing mutations have been introduced utilizing CRISPR/Cas9 gene editing. Given the complexity of the CNS vasculature in vivo, experimental assessment of the regulatory effects of individual signaling molecules and pathways on BNB integrity requires sophisticated in vitro models. Recent advances in tissue engineering, pioneered by the Searson laboratory, have led to innovative, physiologically benchmarked in vitro models that recapitulate key aspects of the human BNB, including barrier properties in the physiological range. Taking advantage of an ongoing collaboration between the Sumner and Searson laboratories, we will use monolayers (2D models) or microvessels (3D models) composed of NVECs derived from isogenic iPSC lines to characterize how activation of the endogenous wild type and mutant human TRPV4 channel disrupts human BNBs, as well as the ability of TRPV4 small molecule antagonists to repair these disrupted barriers. Impact statement: This research project will inform the development of small molecule TRPV4 antagonists for the treatment of patients with rare neuromuscular diseases caused by dominant mutations of the TRPV4 channel. Confirmation of the ability of TRPV4 antagonists to inhibit the endogenous mutant channel in human NVECs and repair disrupted BNBs will provide critical insights regarding the potential of this therapeutic approach in patients with TRPV4 channelopathies.

Emmanouil Tampakakis, MD

Johns Hopkins University
Awardee Amount: $345,000
Disease Target: Cardiac Diseases

Zack Wang, PhD

Johns Hopkins University
Awardee Amount: $345,000
Disease Target: Leukemia

Developing a CRISPR-Based Platform to Interrogate Enhancers & Super Enhancers

Enhancers activate gene expression. Recently, new enhancers in clusters have been described as super enhancers (SEs). These SEs tightly control gene transcription to regulate cell identity and are linked to cardiac defects. However, the effects of SEs during cardiac development remain largely unknown. Human pluripotent stem cell-derived CMs (hPSC-CMs), are lacking essential maturation elements, thus they remain embryonic-like impeding their applications. To examine the role of enhancers during cardiac maturation, we developed a novel hPSC-based enhancer CRISPR activation (enCRISPRa) system. We also created a list of SEs that regulate CM maturation genes. Thus, we hypothesize that by applying our enCRISPRa platform we could interrogate the effects of maturation-related SEs. The role of SEs in cardiac diseases is unknown. To examine the applicability of enCRISPRa in investigating enhancer-related defects, we developed a hPSC-CM model where deletion of a cardiac enhancer (part of known SE) disrupted CM maturation. Therefore, we hypothesize that leveraging this specific SE for gene activation at physiologic levels will promote CM maturation and rescue the disease phenotype. We will test our hypotheses with two specific aims:

Aim 1: Determine the effects of SEs on CM maturation by developing a high throughput screen. Using a lentiviral library of sgRNAs targeting SEs and enCRISPRa to optimize gene expression, we will develop a high-throughput single cell RNA-seq screen.

Aim 2: Determine whether targeted cardiac SE activation promotes CM maturation. To provide proof-of-concept evidence regarding the applicability of enCRISPRa to study enhancer-related diseases, we will target enhancers within a cardiac SE and analyze CM maturation.

This work aims to develop novel CRISPR-activation technologies to interrogate SEs and provide a high throughput screening platform to investigate SE effects. Ultimately, our protocols could be applied to any hPSC-derived cell and can be an invaluable tool to study the roles of enhancers in human diseases. Our research aims to provide novel research tools to investigate the role of the understudied and overlooked transcrip-tional enhancers. As genetic variants within enhancers have been linked with diseases and enhancers appear to regulate the development and maturation of various tissues, having more sophisticated protocols to interrogate and leverage the specific effects of enhancers could have tremendous benefits for public health.

Efficient Generation of T-Cells from iPSCs for Immunotherapy

Chimeric antigen receptor (CAR) T cell therapies have transformed cancer treatment, yet a persistent challenge in the field is the creation of "off-the-shelf" CAR-T cells to streamline production and reduce costs. Human induced pluripotent stem cells (iPSCs) offer a promising solution due to their unlimited self-renewal capacity and ability to generate scalable differentiating cells. iPSC-derived T cells, when combined with gene-editing technology, present an avenue for developing "universal" T cells, mitigating graft-versus-host disease (GvHD), and addressing host immune rejection arising from HLA mismatches. Despite this potential, the efficiency of generating functional T cells from iPSCs lags in vivo processes, emphasizing the need for an appropriate microenvironment to guide stage-specific T-cell development. Our recent research has unveiled the significance of a hypoxic condition during iPSC differentiation, promoting an arterial program that enhances the endothelial-to-hematopoietic transition (EHT) of hemogenic endothelial (HE) cells. This process leads to the generation of hematopoietic stem/progenitor cells (HSPCs) with T-cell potential, mirroring the embryonic development stage where most functional T cells originate. In this project, our goal is to establish a highly efficient system for differentiating iPSCs into definitive HSCs with CAR-T cell potential, with a specific focus on the arterial endothelial niche. Specifically, (1) we aim to manipulate cellular niches by identifying the microenvironmental cues to promote the efficiency of generating HSPCs with T-cell potential, and (2) we will use our established CAR-CD19 to demonstrate the capability of iPSC-derived CAR-CD19 T-cells in inhibiting CD19+ B cell leukemia both in vitro and in vivo. We expect to optimize our iPSC differentiation system with cellular niche signals that enable us to leverage extrinsic signals to instruct definitive T cell development, ultimately leading to the efficient generation of CAR-T cells from iPSCs for future immunotherapy. The anticipated outcome of efficiently generating CAR-T cells from iPSCs holds promise for broader applicability in future immunotherapy by significantly reducing costs and production time and making advanced cancer treatments more accessible. By focusing on the arterial endothelial niche and manipulating cellular niches, the research aims to address challenges in understanding the microenvironment of T cell development, potentially advancing cancer immunotherapies and positively impacting public health by offering more effective tools in the treatment of cancer.

Graeme Woodworth, MD

University of Maryland, Baltimore

Awardee Amount: $345,000

Disease Target: Degenerative/Aging, Neoplastic

Mechano-Biological Analyses of Human Neural Stem Cells in Confined Microenvironments

Mechanical interactions between neural stem cells (NSC) and the brain microenvironment profoundly affect stemness, migration/invasion, and tumorigenicity. Indeed, cell migration in confined microenvironments is central to many biological processes (e.g., morphogenesis, immune and stem cell trafficking, metastasis) and is of great relevance for the brain as its extracellular space (ECS) is among the most confined and tortuous of all tissues in the human body. Confined migration presents an intrinsic mechanical challenge as cells must squeeze through constrictions smaller than the nuclear size with dramatic consequences for DNA repair/regulation and genomic variations5. However, our understanding of the complex and dynamic interaction between cell mechanics and biological function in confined migration is scarce mostly because the instruments used for mechanical measurements, such as atomic force microscopy (AFM) and micropipette aspiration, are based on physical contact and thus need direct access to the cell. This has prevented mechanical measurements of cells in complex microenvironments and over several time points, two essential requirements for understanding the biology and potential consequences of confined migration. To address this need, this proposal features the collaboration of three labs to provide A) advanced microfluidic devices that enable analyses of single to thousands of NSCs under confined micro-environment conditions, including tightly controlled brain-mimicking physiologic conditions and B) an all-optical approach to mechanical measurements using Brillouin microscopy which uses the material longitudinal elastic modulus as label-free contrast mechanism for imaging. Brillouin microscopy has been validated/established for cell and tissue applications and is ideal for studying neural stem cell mechanics and behavior in brain-mimicking confined environments by enabling the dynamic characterization of neural stem cell mechanics during confined migration.
This research program aims to elucidate the interplay between stem cell mechanics and confined migration to establish the impact on stemness /differentiation /fate and migration/invasiveness.

Our central hypothesis is that the dynamic regulation of cell modulus (and specifically nuclear modulus) is a critical determinant of cell fate and tolerance to migration through small constrictions. Our hypothesis is based on robust preliminary data showing that cells adapt their modulus under varying environmental conditions and that low nuclear modulus correlates with efficient confined migration. Our proposed work will transform our biomechanical understanding of migration in the brain and ultimately lead to identifying novel mechano-biological mechanisms related to confined migration, stem cell fate, and tumorigenicity.

Specific Aim 1: Quantify the mechanical modulus of stem cells in response to changing intra and extracellular factors. We will experimentally quantify the modulation of cell/nuclear modulus of NSCs in response to intracellular / extracellular physicochemical cues found in brain-specific physiological and pathological settings such as dimensionality/stiffness, adhesion conditions, and other cues (e.g., hypoxia). We will also identify the intracellular mediators of the nuclear mechanical response to ECM cues by characterizing the effects of individual cytoskeletal factors.

Specific Aim 2: Correlate the fate of stem cells as a function of their modulus during migration in confined microenvironments. We will determine how the specific cell/nuclear modulus and confined migration conditions affect the differentiation and migration of human NSCs. Cell and nuclear modulus will be measured directly during migration in specific conditions mimicking brain and tumor microenvironments. The cells from each condition will be collected and analyzed for stemness, differentiation, chromosomal stability, and alterations, including extrachromosomal DNA.

Expected outcomes and impact: This proposal will provide a mechanistic understanding of the biomechanics of confined migration, answering critical questions regarding the role of cell stiffness in confined cell migration, its relationship to cell stemness, and potential transformation.

MSCRF

Post-Doctoral Fellowship Grant Awards

2024

Mohit Kwatra, PhD

Johns Hopkins University
Mentor: Hanseok Ko, PhD
Awardee Amount: $130,000
Disease Target: Parkinson's Disease

Shalini Sharma, PhD

University of Maryland, Baltimore
Mentor: Miroslaw Janowski, MD, PhD
Awardee Amount: $130,000
Disease Target: Stroke

Role of OSM OSMR Signaling in Human Microglia Astrocyte Axis Mediated Neurodegeneration in Parkinson Disease

Neuroinflammation is a prominent process in Parkinson's disease (PD), with α-synuclein (α-syn) aggregates recognized as a key trigger for brain microglial activation. In primary murine cultures, we observed that α-synuclein preformed fibrils (PFF), mirroring PD-related misfolded α-synuclein, activate microglia, generate neurotoxic reactive astrocytes, and induce neurodegeneration. However, the precise cellular mechanisms behind these neuroinflammatory events, particularly reactive astrocyte formation triggered by α-syn PFF, remain unclear. To comprehensively understand α-syn PFF-induced microglial activation and the ensuing formation of neurotoxic reactive astrocytes leading to neurotoxicity, we conducted mass spectrometry (MS) analysis combined with SILAC (Stable Isotope Labeling by Amino acids in Cell culture) using neurotoxic astrocytes induced by conditional medium (MCM) collected from α-synuclein preformed fibrils (α-syn PFF)-activated microglia. Intriguingly, Oncostatin M receptor (OSMR) was identified from the MS analysis. Preliminary findings show notable OSM release from PFF-treated murine microglia, elevated OSMR levels in PFF-MCM-treated murine and human astrocytes, and PD brain samples. OSM plays a role in reactive astrocyte formation, and an OSM specific antibody effectively rescues neurotoxic astrocytes-induced neuronal death. This suggests OSM/OSMR signaling involvement in PFF-induced microglial activation, astrocyte formation, and neurodegeneration. Based on these observations, our investigation aims to explore the pivotal role in the microglia/reactive astrocyte axis and neurodegeneration in PD, utilizing genetic depletion and pharmacological intervention strategies in human microglia, astrocytes, and dopamine (DA) neurons. This investigation provides novel insights into PD pathogenesis and potential therapeutic treatment strategies. This proposal is based on accomplishment of two foremost aims:

Aim 1: To investigate the preventive effect of the OSM-specific antibody on neurotoxic astrocyte-induced neuronal death: OSM-specific antibody mediated pharmacological Inhibition of PFF-stimulated released OSM (from reactive human Microglia) on reactive astrocyte formation mediated toxicity in DA neurons.

Aim 2: Determine the impact of depletion of OSMR in astrocytes in PFF microgliainduced reactive astrocyte formation and neurotoxicity: Effect of PFF-intoxicated microglia released OSM on genetically depleted OSMR in astrocyte mediated toxicity in DA neurons.

Magnetic and Radioactive Labeling of Human Mesenchymal Stem Cells Secreting P2X7-Blocking Nanobody for their Precise Intra-Arterial Delivery in Stroke

Stroke is the third leading cause of death of women in the United States and the second leading cause of death globally. Currently available treatments for acute ischemic stroke are limited to mechanical thrombectomy and pharmacological thrombolysis but unfortunately, most patients nevertheless develop lifelong neurological deficits. These clinical consequences may result from secondary brain damage upon initiation of the neuroinflammatory processes at the time of insult and subsequent pericyte dysfunction. However, importantly, thrombectomy elicits reperfusion and access of therapeutic agents to the infarcted area. Therefore, human mesenchymal stem cells (hMSCs) secreting P2x7 nanobody are a very attractive two-prong approach to address key pathological features of acute stroke and recently has been funded by MSCRF Discovery Grant. It has previously been shown that intra-arterial delivery of hMSCs is more effective than intravenous administration, as it benefits from the effect of a first pass through cerebral circulation and avoids pulmonary entrapment. However, clinical translation of this approach may be hindered by a variability of outcomes due to imprecision of cell delivery, both spatial (delivery of cells to the desired brain area), as well as quantitative (delivery of the desired number of cells via the first pass). We have successfully labeled hMSCs and tracked them in vivo by MRI, but the signal decayed within a few days. Radioactivity is generally more sensitive and quantitative, but it is logistically more demanding. Therefore, the overarching goal of this project is to compare two most frequently applied clinically applicable cell labeling strategies, magnetic and radioactive, to report on the location and dose of intra-arterially infused hMSCs in terms of signal-to-noise ratio and potential negative impact on cell quality. We expect that radiolabeling will be superior to magnetic labeling for tracking of intra-arterially delivered hMSCs, and the benefits will offset additional logistical efforts. Our deliverable will be an optimal protocol for labeling and in vivo imaging of hMSCs secreting P2x7 nanobody, ready for turning into standard operating protocol (SOP) under current Good Manufacturing Practice cGMP conditions whenever needed.

Neelima Thottappillil, PhD

Johns Hopkins University
Mentor: Aaron James, MD, PhD
Awardee Amount: $130,000
Disease Target: Bone Defects & Other Orthopaedic Conditions

Jinghui Wang, PhD

University of Maryland, Baltimore
Mentor: Yajie Liang, MB, PhD
Awardee Amount: $130,000
Disease Target: Stroke

Endothelial Protein C Receptor Expression Identifies Perivascular Osteoprogenitors Modulated by WNT Signaling for Bone Regeneration

Bone defects either due to trauma or diseases are common and devastating conditions which cause a substantial socioeconomic burden. Therapeutic strategies for bone healing should target improved bone regeneration with ease of translation. The application of autologous stem/progenitor cells to promote bone healing is promising and is under active research. The stromal vascular fraction of adipose tissue is known to house multipotent progenitor cells6, including skeletogenic cells. However, to date, no adipose-derived stem cell therapies have met the clinical capability to heal bone defects8. Based on these past results, our group has held that translatable stem cell-based therapies for bone regeneration hinge on the identification of a more well-defined osteoprogenitor subpopulation. Our team has a long-standing interest in perivascular stem/progenitor cells from adipose tissue for bone engineering and regeneration (reviewed in9), having been first described by our group. Vascular structures are composed of three concentric layers, of which the outermost is termed the tunica adventitia. A major pool of unipotent, bipotent or multipotent progenitors within adipose stromal vascular fraction is believed to originate from the tunica adventitia (termed adventitial cells or adventicytes). Recent single-cell molecular analysis by our group identified several immunophenotypes of adventitial cells with distinct multilineage differentiation potential, which has also been confirmed by others using alternative approaches. In a series of new observations, we recently employed several high throughput approaches, including spatial transcriptomics and single-cell RNA sequencing to further define the continuum of CD34+ human adventicytes that exist within the perivascular stem cell niche. Our preliminary findings suggest that 1) novel cell surface markers such as CD201(Endothelial Protein C Receptor/PROCR) highlight histologically restricted and functionally relevant adventitial subpopulations with distinct osteogenic potential, and 2) CD201 may play important regulatory functions of adventitial progenitor cell behaviour via the novel canonical Wnt regulatory protein DACT2 (DAPPER2). In aggregate, our findings have uncovered an unexpected and functionally relevant stem cell subtype based on the expression of CD201 within the outermost layer of blood vessels in human white subcutaneous adipose tissue. This marker procures progenitor cells in sufficient numbers for bone tissue regeneration. This MSCRF proposal capitalises on this series of new observations. In an exploratory approach, we seek to define the cellular properties of perivascular stem cells characterised by the cell surface marker CD201 and leverage this knowledge for improved skeletal tissue regeneration (Aim 1). We further seek to determine the role of CD201 and its downstream intermediary DACT2 in the regulation of stemness/differentiation potential via canonical WNT signalling (Aim 2).

Multiphoton Imaging iPSC Derived NPCS Co-Transplanted with Helper Cells Expressing VEGF for Stroke Treatment

Stroke is an abrupt interruption of constant blood flow to the brain that causes loss of neurological function. The interruption of blood flow can be caused by a blockage, leading to the more common ischemic stroke, or by bleeding in the brain, leading to the more deadly hemorrhagic stroke. The advancement in clinical outcomes owes much to endovascular clot removal. However, a considerable number of patients fail to regain their pre-injury status, particularly when the clot removal treatment window is missed. One promising solution, out of many, lies in the restoration of damaged neuronal circuits through the transplantation of neural stem or progenitor cells. Unfortunately, progress in this endeavor faces challenges as current studies heavily depend on static outcome measures, such as post-mortem assessments. These approaches lack the ability to provide insights into the dynamic and functional aspects of cell integration with the host neuronal circuits. Dr. Liang's group developed the functional longitudinal tracking of individual cells (fLoTIC) under 2-photon microscopy and developed the cell labeling strategy LIASN (Location, Identify, Activity of Self and Neighbors) for fLoTIC toward observation of the migration, fate and function of transplanted cells as well as their communication with the host brain. From the therapeutic perspective, Dr. Liang's group invented the Helper-cell strategy and successfully showed its efficacy for improving survival of grafted neural stem cells co-transplanted with helper cells expressing bFGF. In this postdoc fellowship application, the applicant (Dr. Jinghui Wang) will leverage her prior experience in culturing and manipulating human iPSC-derived neural progenitor cells (NPCs) and push forward the endeavor in Dr. Liang's lab from both the imaging and therapeutic perspectives. In Aim 1, she will work on the imaging branch by optimizing our current multicolor cell labeling kit for functional single-cell tracking of human iPSC-NPCs in live animals. This effort may develop an enabling technology for the study of the dynamics of stem cell behaviors in live animals after transplantation. In Aim 2, she will delve into the Helper-cell strategy by testing another growth factor, VEGF, hypothesizing that the survival and integration of human iPSC-NPC will be significantly improved by co-transplanting helper cells expressing VEGF after stroke through enhanced vascularization of the graft. This is one step further into the Helper-cell strategy that Liang lab has developed. Overall, our study will address the most burning and vital issue in regenerative medicine: survival and integration of grafted cells into adult neural circuits in the injured brain. By developing innovative approaches for tracking and enhancing the integration of grafted cells with host cells, this study will produce results that may reinvigorate the neural cell transplantation field for the treatment of neurological disorders, which perfectly fits the mission of the MSCRF program.

Wenshen Wang, PhD

Hugo W. Moser Research Institute at Kennedy Krieger
Mentor: Guanshu Liu, PhD
Awardee Amount: $130,000
Disease Target: Myocardial Infarction

Feiyu Yang, PhD

Johns Hopkins University
Mentor: Deok-Ho Kim , PhD
Awardee Amount: $130,000
Disease Target: Neuropsychiatric Disorders

ModRNA-Delivering, Engineered Human iPSC-Derived Extracellular Vesicles for Image-Guided Targeted Treatment of Myocardial Infarction

According to 2023 statistics update from the American Heart Association (AHA), approximately every 40 seconds, someone in the United States will have a myocardial infarction (MI). Due to the low intrinsic regenerative capacity in adult mammalian hearts, cardiac function was permanently impaired after MI due to cardiomyocyte death and heart failure (HF). Effective regenerative treatments for myocardial infarction remain an unmet need. Human stem cells (hSCs) derived extracellular vesicles (EVs) have entered clinical trials, showing great potential as a cell-free regenerative therapy for protecting and repairing myocardial damage following MI. However, there are a few formidable challenges in the path towards the clinical application of hSC-EVs. First, native EVs exhibit limited targeting ability upon systemic administration. While cell engineering strategies have been successfully employed in engineering EVs for highly specific targeting of diseased organs or tumors, along with the benefits of high-quality and scalable production, the use of cell engineering for developing heart-homing EVs to better treat cardiovascular diseases is still largely unexplored. This study aims to leverage the advanced genome editing technology to endow hSC-EVs with an ischemic myocardium-targeting peptide (IMTP) to achieve substantially enhanced targeting and subsequent higher therapeutic efficacy. Secondly, while hSC-EVs exhibit strong regenerative potential, they may not be able to address all the treatment requirements satisfactorily when used alone. To overcome this challenge, a combination treatment that integrates EVs with other advanced therapies is expected to be more effective in rescuing damaged cardiomyocytes. In this context, we propose utilizing hSC-EVs as vectors for delivering modified mRNA (modRNA). We anticipate that incorporating vascular endothelial growth factor (VEGF) modRNA will significantly improve treatment outcomes due to its established effects in inducing angiogenesis, a crucial process in cardiac tissue regeneration. Third, the large-scale production of hSC-EVs presents technical challenges that could limit their clinical utility. To tackle this, our approach will use induced pluripotent stem cells (iPSCs) to generate MItargeted iPSC-EVs (iEVs) instead of the commonly used mesenchymal stem cells (MSCs). iEVs offer 16-fold higher yields and can be autologously produced directly from the patients themselves. Finally, extensive preclinical optimization and rigorous clinical trials are needed before these EV products can be made clinically available. In this stage, non-invasive, quantitative imaging methods are crucial for accurately assessing the delivery efficiency of therapeutic EVs to MI-induced damage sites and for predicting therapeutic outcomes on an individual basis. In our design, we will employ magnetic particle imaging (MPI), an emerging imaging technology providing hotspot imaging (without background signal) and linear signal from agents like superparamagnetic iron oxide nanoparticles (SPIONs). Based on the optimized electroporation protocol established in our preliminary studies, we will load EVs with both modRNA and Resovist, a commercially available SPION, simultaneously

Dorsal-Ventral and Rostral-Caudal Patterning of Brain Organoids using a Localized Passive Diffusion-Based Morphogen Gradient Generator

The goal of the proposed research is to Rationally design a novel bioengineering device and protocols to reliably establish human pluripotent stem cells (hPSCs)-derived brain organoids with dorsal-ventral (D-V) and rostral-caudal (R-C) patterning. Map the whole spatial atlas and analyze the gene developmental trajectories of patterned brain organoids using spatial transcriptomic technology. The positional identity patterning during human brain development is orchestrated in a highly coordinated manner by a handful of inductive signals locally produced by surrounding tissues. The signal dynamics, such as the duration, timings, and concentrations, are precisely controlled by genetic programs, which play vital roles in the proper completion of embryogenesis. While previous studies have used animal models to reveal general signaling pathways during the early developmental process, the signal dynamics underlying the proper development of the human brain remain obscure. Recent studies highlight the unique advantage of brain organoids in modeling neurological development and disease. Aim 1, we propose to develop a diffusion-based morphogen gradient generator to establish precise extrinsic morphogen gradients in brain organoids. To investigate the role of signaling dynamic for organoid differentiation, we plan to make the device flexible for tuning the morphogen source during culturing. We will then develop reproducible protocols by combining small molecules and growth factors for D-V and R-C brain organoid patterning using the device. The proposed method will reveal how spatial-temporal morphogen dynamics regulate the cell fate specification and axis formation of hPSCs-derived brain organoids. As patterned brain organoids mature, a highly diverse group of neurons and glial cells are expected to be spatially distributed in multiple regions. Aim 2 proposes to map the spatial gene atlas at single-cell resolution and profile the gene trajectories of patterned brain organoids with spatial context using the Visium spatial transcriptomic technology. We plan to collect patterned organoids at various ages to analysis neurodevelopment trajectories. Moreover, we will model the interneuron saltatory and tangential migrations using D-V patterned organoids, which is difficult to study using animal models. This study will build a comprehensive, spatial transcriptomic atlas and decipher key developmental trajectories using patterned brain organoid models. Human and animal brains significantly differ in functionality, complexity, and structure. Neurulation-stage human embryos are ethnically restricted in access9. Moreover, neuropsychiatric disorders are difficult to model using animal models. In contrast, hPSCs-derived brain organoids provide a unique opportunity to study the early developmental process of the human brain and the mechanisms underlying neuropsychiatric disorders. This study will unveil the fundamental mechanisms that govern the propagation of signaling activation for patterning brain organoids. Furthermore, the proposed approach can be broadly applied to study not only the basic developmental process of the human brain but also the genetic trajectories and pathological mechanisms of neuropsychiatric disorders. In the future, the proposed approach and patterned brain organoids can be used to study diseases such as Timothy syndrome and Rett syndrome, which involve the dysfunctions of cross-regional interactions of the cortex and ganglionic eminence in vivo.

Ridzky Yuda, PhD

Johns Hopkins University
Mentor: MoonJung Jung, PhD
Awardee Amount: $130,000
Disease Target: Bone Marrow Failure & Inflammation in Alcohol

Heng Zhao, PhD

Johns Hopkins University
Mentor: Dian Arifin, PhD
Awardee Amount: $130,000
Disease Target: Diabetes

Epigenetic Regulation Underlying Chronic Alcohol-Induced Double-Stranded RNA Accumulation and Inflammation in Hematopoietic Stem Progenitor Cells

Pitt-Hopkins Syndrome (PTHS) is a rare form of autism spectrum disorder (ASD) characterized by developmental deThe primary goals of this project are to: 1) elucidate the effects of chronic alcohol exposure on the hematopoietic stem and progenitor cell (HSPC) epigenome, and, 2) develop cell-based therapy to reverse the effects of alcohol on HSPCs. Chronic alcohol drinking negatively affects hematopoietic stem cells, leading to impairment of blood cell production, such as anemia, thrombocytopenia, leukopenia, and pancytopenia. The negative effects of alcohol stem from its metabolites, such as reactive oxygen species and acetaldehyde, both of which cause DNA damage, culminating in senescence, stem cell exhaustion, and inflammation during aging. DNA damage also reactivates retro transposable elements (RTE). Mechanistically, DNA damage redistributes chromatin-modifying enzymes, such as Sirtuins (SIRT1 and SIRT6), preventing them from repressing RTE-enriched genomic. Notably, the absence of SIRT6 reactivates RTE in ging and cellular stresses, fueling inflammation. Moreover, RTE-derived RNA forms double-stranded RNA (dsRNA) that can also elicit inflammation via the activation of nucleic acid sensors. Prior studies established the importance of RTE in promoting inflammation: Inhibition of the transposition activity by nucleoside reverse transcriptase inhibitor (NRTI) reduced inflammation in human fibroblasts, neurons, and retinal pigmented epithelium. Finally, owing to their capacity to inflict DNA damage and inflammation, we hypothesize that alcohol disrupts the epigenetic silencing of RTE, leading to dsRNA accumulation. However, whether alcohol causes the epigenetic reactivation of RTE or targeting RTE epigenetic regulation ameliorates alcohol-induced HSPC inflammation is unknown. Our scientific premise that alcohol causes epigenetic dysregulation in HSPCs leading to dsRNA accumulation is based on our compelling preliminary data in human CD34+ (huCD34+) HSPCs: 1) Alcohol promotes myeloid-biased differentiation and Type I interferon (IFN-1) signature in xenotransplanted huCD34+ HSPCs. 2) In vitro alcohol exposed huCD34+ HSPCs downregulate DNA methylation and histone modification signatures. 3) Alcohol-exposed huCD34+ HSPCs upregulate RTE. 4) dsRNA accumulates in alcohol-exposed huCD34+ HSPC. To determine if patient-specific mutations in TCF4 results in dysregulation of the OL lineage we first differentiated iPSC using a two-dimensional OL differentiation protocol. Preliminary results show increased proliferation of OLIG2+ OPCs at early time points, and a reduction in the expression of mature OL genes, consistent with phenotypes observed in PTHS mouse models. In addition, using a previously described three-dimensional differentiation protocol that generates human oligodendrocyte spheroids (hOLS), we observed a significant variation in the diameter of hOLS derived from PTHS lines that appears specific to patients harboring mutations causing TCF4 haploinsufficiency. Together, these results suggest TCF4 to be a critical regulator of OL development and therefore predict myelination deficits as a potential pathophysiological mechanism underlying neurodevelopmental abnormalities in PTHS. The goal of this proposal is to identify cellular and molecular mechanisms underlying OL deficits in a PTHS patient-derived iPSC model and to test the efficacy of promyelinating therapeutic interventions for the treatment of PTHS.

Theranostic Mesenchymal Stem Cell-Derived Extracellular Vesicle-Liposome Hybrids as Adjuvant Therapy for Pancreatic Islet Transplantation

Type 1 diabetes mellitus (T1DM) or juvenile-onset DM is a chronic disorder characterized by immune-mediated destruction of pancreatic insulin-producing β cells. T1DM accounts for an estimated 5–10% of diabetic Americans. Islet transplantation has shown promising potential to provide long-term and precise glucose control for T1DM patients. However, in addition to a shortage of donor islets, other challenges, such as islet recipients' immune rejection and excessive oxidative stress, lead to a low islet graft survival or function. Extracellular vesicles (EVs) are lipid membrane-enclosed vesicles which play key roles in a diverse range of biological processes. EVs, mainly from stem cells, have recently gained considerable attention as an adjuvant therapy to improve the therapeutic efficacy of islet transplantation. Human mesenchymal stem cells (MSCs) derived-EVs possess regenerative and immunomodulatory functions similar to MSCs but with lower immunogenicity, and, more importantly, without tumorigenicity risks. MSC-EV therapy also benefits from ease of storage and transport of EVs. However, developing theranostic (i.e. a combination of therapy and diagnosis in one platform) EV therapy is constrained by low tunability, poor reproducibility, single modal imaging, and even unsatisfactory therapeutic outputs. We propose to develop a novel multifunctional, liposome-EV hybrids (LPEV) for multimodal in vivo tracking, enhanced inflammation modulation, and to support islet transplantation for T1DM treatment. The hybrids will be created by fusing MSC-EVs with liposomes. Herein, liposomes will be loaded with two promising nanoparticles (NPs): biosafe, renal clearable supersmall anti-oxidative nanozyme CeO2 NPs and superparamagnetic iron oxide (SPIO) NPs. In addition to their anti-oxidative and anti-inflammatory property, CeO2 NPs are good tracers for computed tomography (CT). SPIO NPs act as strong magnetic particle imaging (MPI) tracer as well as T2-w magnetic resonance imaging (MRI) contrast agent. Liposome membranes will be decorated with triphenylphosphonium (TPP) for targeting mitochondria. The TPP strategy has been shown to enhance the anti-inflammatory property of CeO2 NPs. LPEV will therefore retain the therapeutic property of MSC-EVs with an enhanced anti-inflammatory function and CT/MRI/MPI imaging capability. EVs will be isolated from MSCs using differential centrifugation and qEV purification, followed by fusion with magnetic anti-oxidative liposomes in the assistance of polyethylene glycol-8000 (PEG8000). EV biomarkers (CD63, CD9, CD81), nanozyme content, and integrity of LPEV will be examined by Oni super-resolution microscopy, quantitative real-time polymerase chain reaction (qRT-PCR), inductively coupled plasma atomic emission spectroscopy (ICP-MS), cryogenic transmission electron microscopy (Cryo-TEM) and dynamic laser scattering (DLS). Their in vitro imaging sensitivity, anti-oxidative, anti-inflammatory and islet protective properties will also be examined by CT, MRI and MPI, reactive oxygen species, inflammatory molecules secretion and islet cell viability assays, respectively. As a proof-of-concept, LPEV will be co-transplanted with human islets into immunodeficient mice. We will follow the graft survival and function, and to test if LPEV can be detected in vivo using pre-clinical CT, MPI and T2-w MRI. This research may aid the development of MSC-EVs therapy for clinical applications and potentially contribute to the goals of MSCRF.

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