MSCRF

MARYLAND
STEM CELL RESEARCH FUND

2023 ANNUAL REPORT

SUPPORTING THE NEXT FRONTIERS OF MEDICINE

MSCRF
Maryland Stem Cell Research Fund

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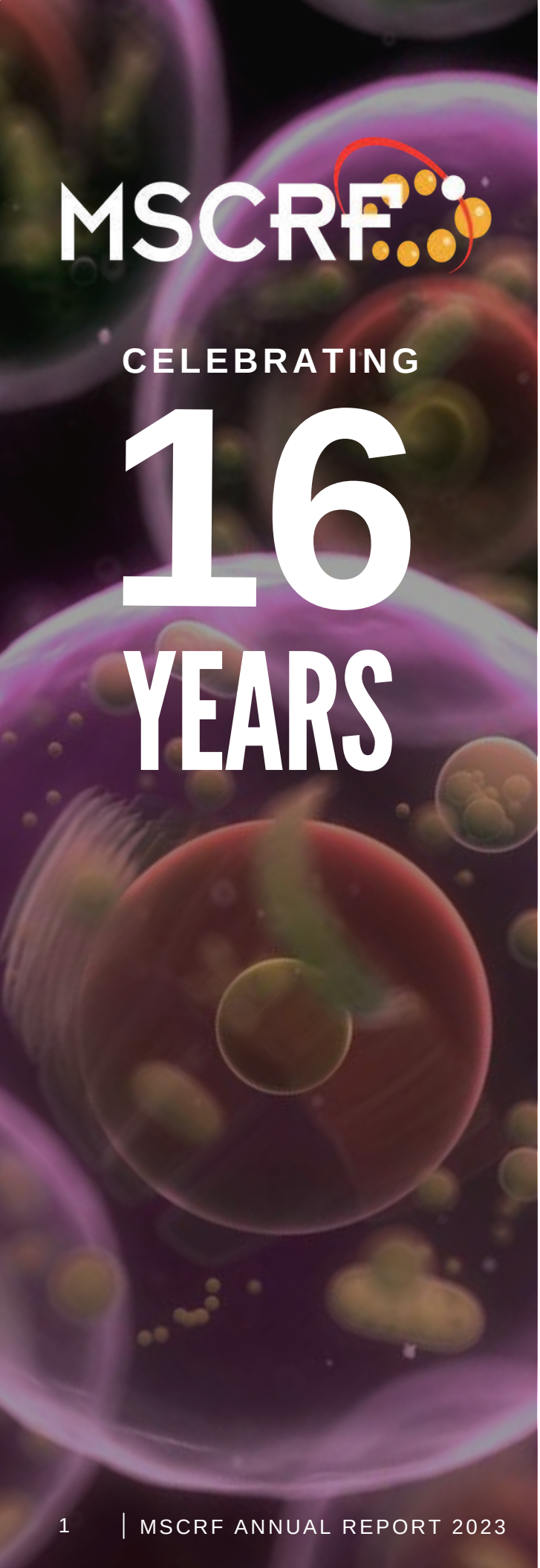


About Us

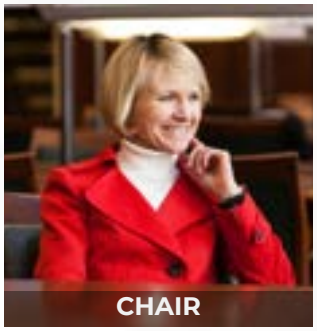
The Maryland Stem Cell Research Fund (MSCRF) is focused on identifying and fostering cutting-edge research and innovation in the field of regenerative medicine in Maryland. Our Accelerating Cures initiative comprises programs that help transition human stem cell-based technologies from the bench to the 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.

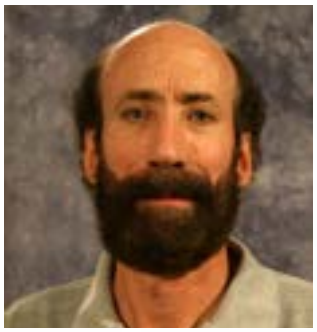
Maryland Stem Cell Research Commission



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

Scott Bailey, Ph.D.
Appointed by Johns Hopkins University

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



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

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

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Appointed by the Speaker of the House of Delegates

MSCRF

EXECUTIVE Overview

Supporting the Next Frontiers of Medicine

Executive Overview

Welcome to the 2023 MSCRF Annual Report!

MSCRF experienced a transformative year in 2023, characterized not only by a new strategic emphasis on fostering biomanufacturing in Maryland. This pivotal year also witnessed a notable increase in both the number and amount of grant awards, along with significant achievements made by our awardees, propelling their research and commercialization efforts to unprecedented levels.

The Maryland Stem Cell Research Fund (MSCRF) was established by the Governor and the Maryland General Assembly through the Maryland Stem Cell Research Act of 2006 during the 2006 General Assembly Session. **Maryland was one of the few states that made a forward-thinking investment in stem cell-based regenerative medicine approaches believed to have curative potential and to date remains committed to supporting the field.**

Executive Overview

Stem cell-based regenerative medicine holds immense promise in revolutionizing healthcare. These versatile cells have the unique ability to develop into various cell types, offering the potential to repair, replace, or regenerate damaged tissues and organs. Current research explores applications in treating a range of conditions, from degenerative diseases and injuries to genetic disorders. Clinical trials and breakthroughs indicate progress in harnessing the therapeutic potential of stem cells, with ongoing efforts to address challenges like immune rejection and ethical considerations.

The field's future envisions personalized treatments, tissue engineering, and disease interventions, positioning stem cell-based regenerative medicine at the forefront of transformative medical advancements.

MSCRF provides support to this field of study by dedicating funding through grants and awards to fuel the research, development, and commercialization of cell therapy and regenerative medicine. 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.

In this report, we maintain our tradition of commemorating a fruitful year by showcasing key statistics, metrics, and new programs that underscore the program's achievements. We present our recent triumphs and share the advancements our awardees have achieved in translating discoveries into tangible solutions that enhance human lives. **Their successes unfold the narrative of MSCRF's influence in strengthening the landscape of stem cell research, providing hope to countless patients and their families in Maryland and beyond**. We are grateful to the community for another transformational year at MSCRF.

MSCRF Remains Strong and Laser-focused on its Mission

MSCRF remains dedicated and resolute in its mission to spearhead innovative stem cell research and catalyze groundbreaking discoveries in healthcare. **What commenced with three MSCRF grant programs in 2007 has burgeoned into seven grant programs in 2023, with the newest being the manufacturing assistance program**. These seven grant programs cover various facets of stem cell research and its development and commercialization.

With a new executive director dedicated to the advancement of regenerative medicine, MSCRF's commitment to growing the stem cell research field shines through, as it continues its 16-year journey dedicated to discovering improved treatments and cures. **The fund supports a diverse portfolio of programs that span the entire research lifecycle—from initial discovery to clinical trials, commercialization, and manufacturing.**

The grants have played a pivotal role in advancing projects through different developmental stages, from basic and translational research to clinical trials presenting the possibility of soon launching FDA-approved products. MSCRF has enabled groundbreaking discoveries in human trials, demonstrating that regenerative therapies have the potential to become the third pillar of medicine.

State lawmakers continue to honor the vision of supporting scientific research initially established in the 2006 funding of the MSCRF. **Demonstrating a steadfast commitment, the state legislature approved a substantial allocation of $20.5 million to MSCRF for both fiscal years 2023 and 2024.**

The evident appreciation for MSCRF's endeavors by Governor Moore and Lieutenant Governor Miller, expressed during our meetings this year, is truly valued.

Since its inception, MSCRF has **allocated nearly $200 million, supporting approximately 600 projects centered around stem cell research across nearly 40 public and private entities in Maryland.** Stem cell research in Maryland has gained substantial traction, demonstrating promising avenues for treating a spectrum of diseases.

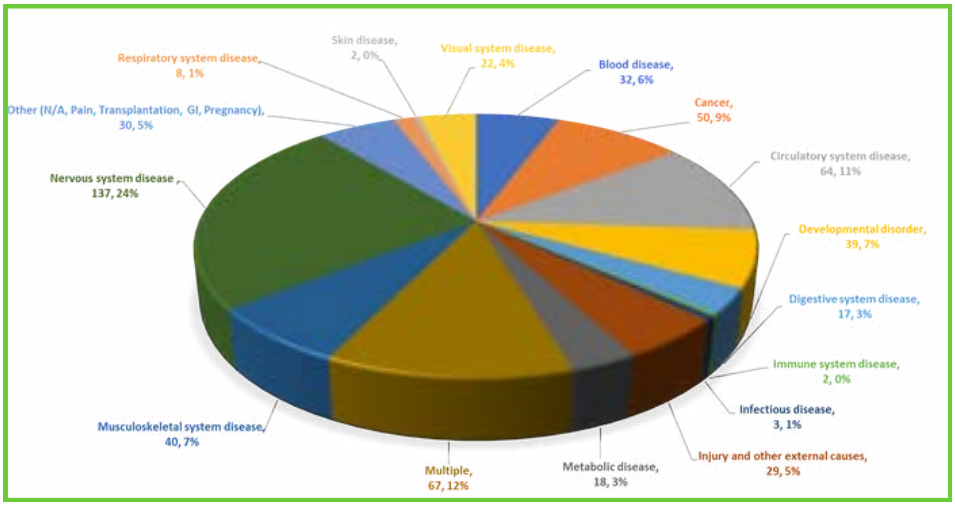
$200M in amount awarded as grants

MSCRF's unwavering dedication, coupled with the state's ongoing support has not only solidified the stem cell therapy research ecosystem in Maryland but has also reverberated globally, leaving an indelible impact.

MSCRF Continues to Support Medical Advances in Maryland

MSCRF's impact transcends geographic boundaries, addressing a spectrum of disease indications that afflict individuals both globally and within the borders of Maryland. This visionary funding has reached far and wide, supporting research initiatives aimed at combating diseases that affect diverse populations. By championing breakthroughs in regenerative medicine, MSCRF's funding has not only contributed to the global pursuit of medical advancements but has also directly addressed the health challenges faced by the people of Maryland.

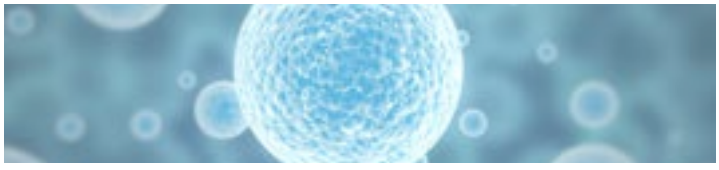
Disease Classifications Funded 2007 - 2023



MSCRF has invested over $50 million to drive groundbreaking advancements in addressing Maryland's most prevalent diseases, including stroke, diabetes, heart disease, cancer, Alzheimer's disease, and Parkinson's disease.

MSCRF Fortifies Biomanufacturing in Maryland

In 2023 in a strategic move toward shaping the future of regenerative therapies, MSCRF awarded grants under the new Manufacturing Assistance Grant Program. **The initiative is designed to mitigate risks in the development and approval of regenerative medicine while fostering the expansion of Maryland's manufacturing workforce.**



$3M **Funds supporting biomanufacturing**

Recognizing biomanufacturing as a cornerstone industry in the Maryland life sciences ecosystem, MSCRF awarded **nearly $3 million to support local companies** in establishing cutting-edge manufacturing facilities and optimizing manufacturing processes.

The Manufacturing Assistance Grant Program aims to strengthen Maryland's position at the forefront of biomanufacturing, significantly contributing to the state's economy

MSCRF Drives Innovation and Transforms Healthcare

The foundation for effective medicine, treatments, and cures lies in robust basic science. MSCRF through our university-based grant programs continues to support high-risk and high-reward ideas at an earlier stage jumpstarting innovations that will form the basis for future medical breakthroughs.

At MSCRF, we proactively seek and identify the next promising technology and act as a catalyst for propelling innovative projects from concept to commercial viability. The consistent funding from MSCRF enables promising ideas to move from bench to bedside.

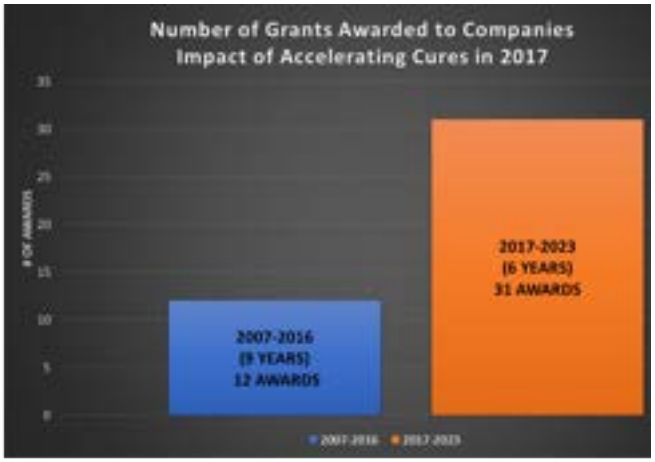
75% **Of portfolio companies still in existence.**

This report underscores the profound impact of MSCRF's unwavering funding, propelling Maryland's researchers and businesses to pioneer advancements in stem cell research.

It has been instrumental in translating groundbreaking ideas from laboratory benches to the bedside, fostering the establishment and growth of stem cell companies within Maryland.

$20M **Awarded to companies since inception**

Furthermore, MSCRF's initiatives have significantly contributed to the expansion of a skilled and innovative workforce throughout the state. In the last **six years**, MSCRF has demonstrated a remarkable surge, almost tripling its awards to companies, driving the commercialization of revolutionary technologies poised for FDA approval. A total of $20 million has been allocated to support 46 research projects at small businesses in Maryland.



Impressively, over 75% of the companies we funded continue to thrive, securing subsequent funding. Notably, a substantial number are either in the midst of clinical trials or on the verge of commencing them.

These portfolio companies not only contribute significantly to Maryland's economic landscape, generating revenue but also play a pivotal role in job creation. This stands as a resounding demonstration of the enduring impact of our program and the successful realization of our mission. (see section on long-term impact)

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
University of Maryland Baltimore County
CaringCross
Johns Hopkins School of Medicine
University of Maryland Baltimore
Kennedy Krieger Institute
University of Maryland School of Medicine Center for Stem Cell Biology and Regenerative Medicine
RENOVATE
Phycin
LIFESPROUT
RoosterBio
Theradaptive
REPROCELL bioserve • stemgent • alvetex • biopta
Secretome Therapeutics
TheraCord Solving Collection + Saving Lives
3Dnamics
seraxis
VITA Therapeutics
Britecyte
KOLON TISSUEGENE
elixirgen scientific
Cartesian Therapeutics
MaxCyte
propagenix
Orgenesis
SR Synaptic Research
GEMSTONE Biotherapeutics
MTI-GlobalStem Acquired by Thermo Fisher Scientific
LONGEVERON Cell-Based Therapies
DOMICELL The Implantable Bioreactor Company
BIOCARDIA
sentien biotechnologies

"We are dedicated to championing Maryland's researchers and companies, propelling the frontier of stem cell research to transform the lives of patients grappling with various diseases and conditions," stated Diane Hoffmann, Chair of the Maryland Stem Cell Research Commission. "Through the innovative Manufacturing Assistance Program, we aim to expedite the creation and deployment of stem cell therapies, ensuring they reach patients promptly and affordably, while also fostering the growth of a skilled manufacturing workforce in the region."

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

By the Numbers

MSCRF since inception

$200M
In awarded grants

$50M
Amount spent on prevalent diseases in Maryland

17%
Funds supporting biomanufacturing in Maryland

39
Number of different entities supported

16
Years since the launch of MSCRF

600
Funded research projects

75%
Supported companies in existence

$20M
Awarded to companies

7
Number of grant programs

Executive Overview

MSCRF has built a vibrant regenerative medicine ecosystem across the state of Maryland that is advancing innovative scientific ideas with an infusion of capital, coaching, and connections. This thriving ecosystem stands as a testament to Maryland's visionary investment in regenerative medicine, now bearing fruit.

The success narrative of MSCRF is one of **vision, resilience, and transformative impact**. The unwavering commitment from the State of Maryland, MSCRF's leadership, and the collective dedication of our team, scientists, companies, and patients have been pivotal to our achievements.

In the last year, we have witnessed and contributed to significant strides in advancing stem cell research and regenerative medicine. As we step into 2024, new strategic initiatives will focus on growth, diversity, health equity, and innovation. MSCRF is poised to broaden its impact, anticipating the revolutionary changes in healthcare driven by the cell and gene therapy industry in Maryland.

The future is promising, and we invite you to join us on this forward path. **"Together We Move the Field and Maryland Forward".**

With hope and gratitude,

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

Diane Hoffmann, MS, JD
Chair, Maryland Stem Cell Research Commission

MSCRF

2023

At-A-Glance

Supporting the Next Frontiers of Medicine

MSCRF Funding Opportunities

Commercialization

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

Validation

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

Discovery

The MSCRF Discovery grant is intended to support stem cell investigators with innovative research ideas that differ from current thinking in the field to advance the stem cell field. . Applicants to these grants may request up to $350,000 for a duration of up to 24 months.

Manufacturing Assistance

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

Clinical

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

Launch

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

Post Doctoral Fellowship

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

2023
A Year of Impact

The year 2023 marked a dynamic and exciting period for MSCRF, characterized by numerous milestones, and notable advancements across various metrics and achievements. The expansion of MSCRF grant programs, with the pivotal introduction of the biomanufacturing grant initiative, was a noteworthy highlight, contributing to the overall excitement of the year. The year witnessed robust growth on multiple fronts.

The surge in the number of grant applications underscored the heightened interest and engagement within the scientific community. This enthusiasm translated into an uptick in the number of grants awarded, showcasing MSCRF's commitment to supporting innovative research endeavors. The substantial **increase in both research and translational grants** reflected the fund's dedication to fostering a comprehensive research ecosystem. Moreover, the expansion reached diverse disease indications, demonstrating MSCRF's responsiveness to the evolving landscape of medical research.

The **significant financial and technological progress made by MSCRF's portfolio companies toward bringing their innovation closer to patients** served as tangible evidence of the positive impact the organization is making in the healthcare sector, within and outside Maryland.

Here are some of the highlights of MSCRF's impactful journey in 2023.

2023 By the Numbers

94
Grant Applications

50
Grants Awarded

56%
New Awardees

Nearly $17 Million in funding awarded

40+
Disease Indications Funded

12%
Increase in number of grant applications

30%
Women Awardees

2023
A Year of Impact

Amount of Funding Awarded in 2023 **$17M**

In the year 2023, the Maryland Stem Cell Research Fund **dedicated around $17 million to support groundbreaking research**, aiming to strengthen and advance stem cell treatments and technologies within the state. This brought MSCRF's total investment to nearly $200 million across almost 600 research projects.

Notably, there was a **12% increase in the number of applications from 2022 to 2023** demonstrating the high demand for MSCRF funding. Among the 50 awardees in 2023, over half, were first-time recipients of MSCRF funding. This expansion and diversification of the MSCRF community not only enriched the innovative ecosystem but also marked a significant contribution to advancing stem cell research in Maryland.

Number of Stem Cell Supported Projects **50**

The first tranche of financing, announced in May 2023, benefitted 38 scientists affiliated with research institutions and companies in Maryland. **The May awards constituted the largest funding amount bestowed upon Maryland-based research institutions and companies since 2010**, made possible by increased financial support from the State.

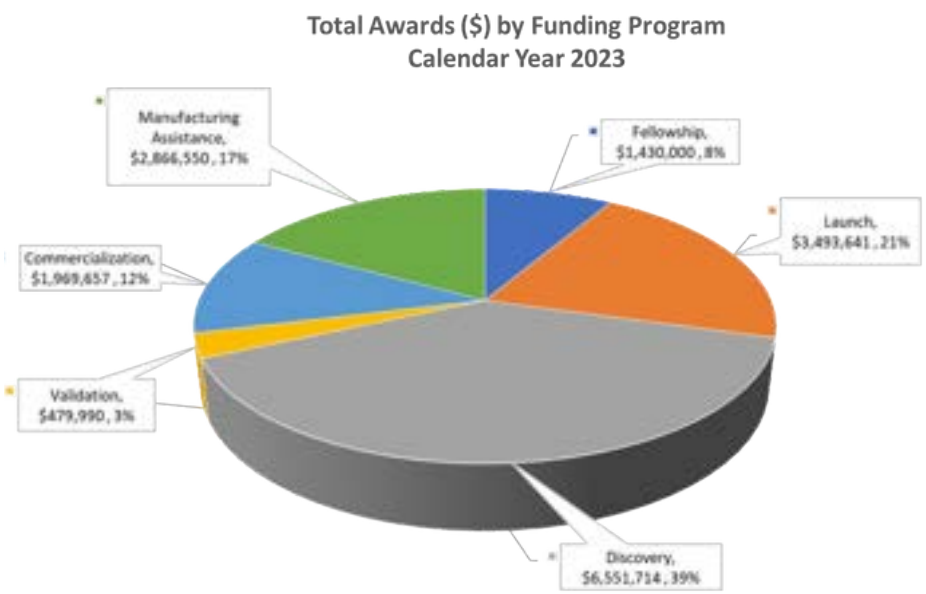
Recipients of these funds included esteemed academic scientists from institutions such as Johns Hopkins University, University of Maryland, Baltimore County, University of Maryland, College Park, University of Maryland, Baltimore, Lieber Institute for Brain Development, Hugo W. Moser Research Institute at Kennedy Krieger, and The Geneva Foundation. Additionally, researchers from the commercial sector, including Vita Therapeutics, Inc., Theradaptive, Inc., Caring Cross, Inc., RoosterBio, Inc., and Reprocell U.S.A., Inc., benefited from these grants.

Increase in number of grant applicants **12%**

Subsequently, a second round of funding was unveiled in November 2023, supporting research endeavors led by 12 Maryland-based scientists. Among the awardees were researchers from esteemed academic institutions such as Johns Hopkins University and the University System of Maryland, along with emerging companies like Secretome Therapeutics, Inc. (formerly NeoProgen), Renovate Biosciences, Inc., and Phycin, Inc.

2023
A Year of Impact

$16,791,552 in total funding



Transformative medicine is most often derived from discoveries made by academic researchers.

In 2023, more than half of MSCRF's grant awards supported innovative research.

MSCRF also allocated nearly $3 million of its awards to support biomanufacturing in Maryland.

"We are immensely grateful to the state of Maryland for its unwavering support of MSCRF. This sustained commitment enables us to push the boundaries of regenerative medicine, fostering groundbreaking advancements and building a stronger, interconnected community."

Ruchika Nijhara, Ph.D., MBA
Executive Director, MSCRF

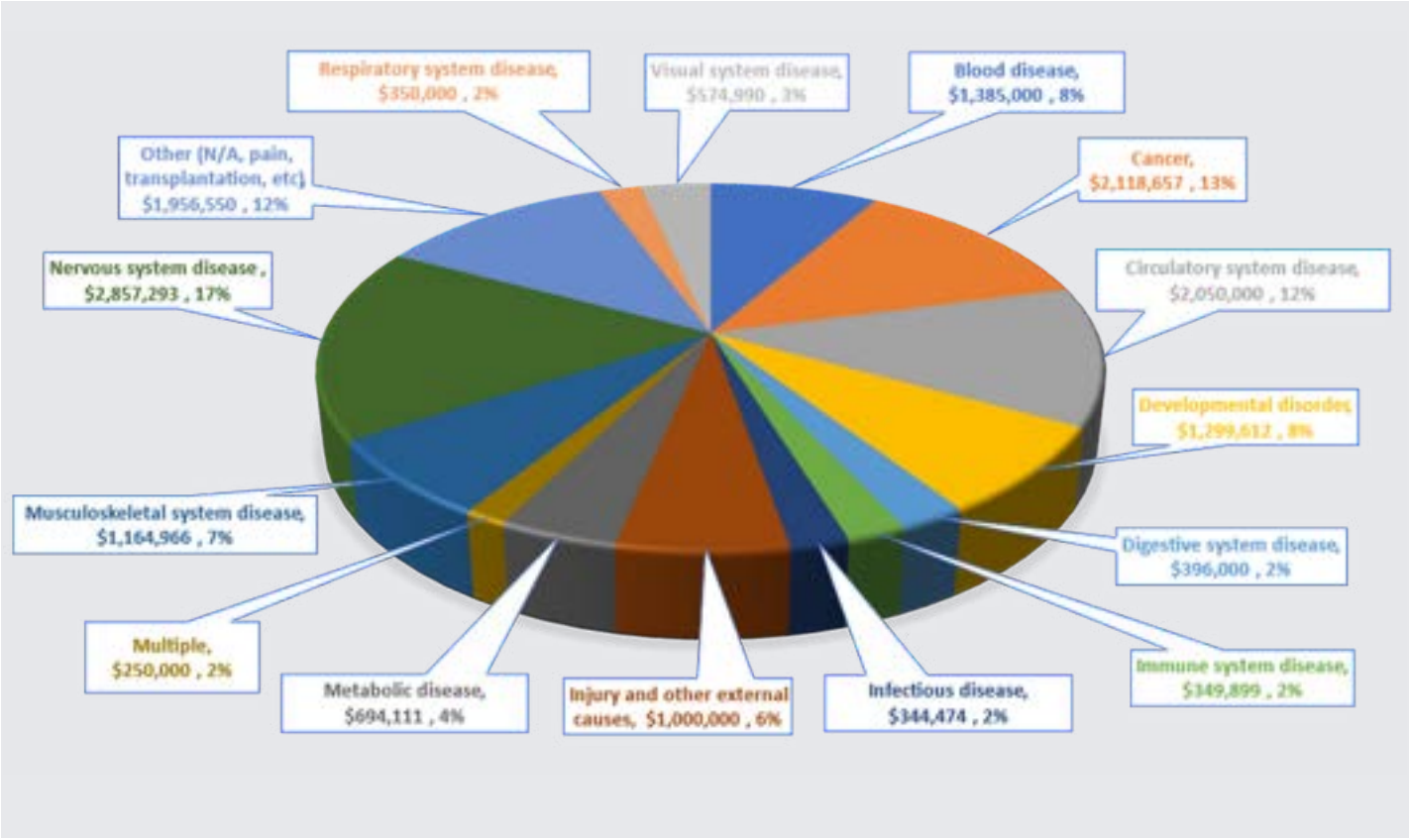
2023
A Year of Impact

Designing Medicines of Tomorrow for the Diseases of Today

This financial support played a crucial role in advancing research across a spectrum of diseases, reflecting, MSCRF's profound commitment to catalyzing impactful advances in therapeutic research. In 2023, our grants were instrumental in supporting potential therapies for over 40 disease indications.

Notably, **medical indications encompassing heart ailments, cancer, and diseases pertaining to the central nervous system such as Alzheimer's and Parkinson's diseases, received paramount funding exemplifying our commitment to advancing research in these critical areas.**

Disease Categories Funded in 2023



New Executive Director

In April 2023, the Maryland Stem Cell Research Fund (MSCRF) welcomed a new chapter with the appointment of Ruchika Nijhara, Ph.D., MBA, as the Executive Director, succeeding Dr. Amritha Jaishankar, who served the organization admirably for five years.

Nijhara brings a wealth of experience in fostering innovation and technology commercialization across federal, public, and private sectors.

Before joining MSCRF, Nijhara held the position of interim Vice President and Senior Director at Georgetown University's Office of Technology Commercialization. She led initiatives related to intellectual property management, licensing, and the formation of strategic alliances, contributing to the university's engagement with the local innovation ecosystem. She played a key role in the successful launch of the university's gap fund, supporting the commercialization of promising technologies.

Before Georgetown, Nijhara served as the Licensing Officer, Commercial Ventures, and Intellectual Property at the University of Maryland, Baltimore. Her expertise spans academic-industrial partnerships, intellectual property protection and management, technology development and commercialization, applied research and development, and technology-led economic development. With these skills acquired throughout her career,

Nijhara is dedicated to ensuring that Maryland's stem cell scientists and companies have the necessary resources to advance their research and deliver cures to patients in need.

In her first few months at MSCRF, she collaborated with the Maryland Stem Cell Research Commission to launch two new initiatives, fostering deliberate collaboration between the public and private sectors—a crucial element in sustaining an innovative ecosystem, according to Nijhara.

Reflecting on her role, she expressed, "Embracing this position at MSCRF allows me to give back to the community I consider my home. Maryland has been my cherished residence since I left India, and I feel a profound sense of gratitude and privilege in contributing to the betterment of my home state."

In September 2023, Nijhara received recognition as one of The Daily Record's 2023 Influential Marylanders, earning a Technology award for her outstanding contributions to supporting stem cell research and technology development in the region. The award acknowledges individuals making significant impacts across various sectors.

"With Ruchika's dynamic leadership and commitment to advancing Maryland's stem cell landscape, MSCRF is poised for continued success under her guidance."

Diane Hoffmann, MS, JD
Chair, Maryland Stem Cell Research Commission

Supporting Innovative Women Researchers

In 2023, 30% of our grant recipients were women who are spearheading research using human stem cells to investigate and combat diseases affecting billions globally.

Whether newly appointed faculty, seasoned researchers, or postdoctoral fellows, these women are driving groundbreaking contributions to medical science.

Their work produces transformative insights into diseases, establishes innovative research technologies, and mentors the next generation of biologists and engineers dedicated to alleviating human suffering.

Here are the remarkable women awardees of 2023. **Heart disease, Cystic fibrosis lung disease, diabetes, Alzheimer's, stroke, schizophrenia, and cancer are just some of the conditions these trailblazing women are collaboratively tackling with creativity and determination**.

We salute them for their invaluable contributions to advancing stem cell research and fortifying the scientific ecosystem.

30% of grant awardees were women

Supporting Innovative Women Researchers

These are the Remarkable Women Scientists and Their Research Projects that we funded in 2023

1. Gila Idelman, PhD
Reprocell, Inc.
"Generation of Immortalized Lymphoid Progenitors and Ready to use NK Cells from Human Induced Pluripotent Stem Cell (hiPSCs)"

2. Hyesoo Kim, DVM, PhD
Johns Hopkins University
"New Gene+Cell Therapy Platform Targeting Diabetes with Engraftable Skeletal Muscle Stem Cells"

3. Linda Smith Resar, MD
Johns Hopkins University
"Developing Stem Cell Technology for Megakaryopoiesis and Platelet Production"

4. Lena Smirnova, PhD
Johns Hopkins University
"Leveraging In-Vitro Organoid Model and Clinical Data to Examine Phenotypic Diversity in SYNGAP1 Intellectual Disability"

5.Mansoureh Barzegar, PhD
Johns Hopkins University
"Investigating the Potential Contribution of Retinoic Acid Receptor-Related Orphan Receptor-Gamma Pathway in Oligodendrocyte Maturation and Myelination"

6.Audra Kramer, PhD
University of Maryland – Baltimore
"Evaluating the Pathogenic Mechanisms Underlying CACNA1A Disorders"

7.Manisha Kumari, PhD
Johns Hopkins University
"Contribution of Oligodendrocytic Cellular Milieu in Generation of A-Synuclein Strains in Multiple System Atrophy"

8.Shuya Li, PhD
Johns Hopkins University
"Spike protein from SARS-CoV-2 Promotes Tauopathy Propagation and Neuroinflammation in Alzheimer's Disease

9.Alejandra Romero Morales, PhD
Lieber Institute for Brain Development
"Investigating the Effects of TCF4 Mutations During Oligodendrocyte Development and Maturation in a Human-Derived Model of Autism Spectrum Disorder"

10.Zanshe Thompson, PhD
Johns Hopkins University
"HMGA1 Chromatin Regulators in Clonal Hematopoiesis and Cardiovascular Disease"

11.Roopa Biswas, PhD
The Geneva Foundation
"Modulation of Rescue Competent MicroRNAs in Cystic Fibrosis Lungs"

12.Jennifer Erwin, PhD
Lieber Institute for Brain Development
"Investigating H3K4 Methylation Pathway Targets in Human Stem Cell Models of Schizophrenia and SETD1A haploinsufficiency"

13.Christina Linnea Nemeth Mertz, Ph.D.
Hugo W. Moser Research Institute at Kennedy Krieger
"LBSL iPSC Brain Modeling: A Prototype for Pediatric Rare Disease"

14.Rachana Mishra, PhD
Secretome Therapeutics, Inc.
"Production of Neonatal Cardiac Mesenchymal Stem Cells for Treating Dilated Cardiomyopathy (DCM)"

15.Xiaoning Han, PhD
Johns Hopkins University
"Engineered MSCs-Derived Extracellular Vesicles as an Effective Microglia/Macrophage-Targeted Therapy for Hemorrhagic Stroke"

New Initiative to Foster Collaborations in Maryland

To strengthen collaborative endeavors within Maryland, **MSCRF has introduced a new initiative that offers supplemental funding** under its Commercialization and Validation grant programs.

The MSCRF's Commercialization and Validation grant programs were established to support translational activities necessary for advancing technologies with significant commercial potential from companies and research universities. Recognizing the inherently collaborative nature of translational research, MSCRF has launched, for the first time, a unique funding opportunity called 'Second-tiered Funding' **offering an additional $100,000, in both grant programs**. This strategic initiative aims to foster collaboration between academic institutions and companies, further propelling technology development.

$100K Supplemental funding for collaborations

"By incentivizing collaboration, we aim to accelerate cures and bolster Maryland's regenerative medicine ecosystem."

Ruchika Nijhara, Ph.D., MBA
Executive Director, MSCRF

Supporting Maryland's Bioindustry

The Maryland Stem Cell Research Fund Manufacturing Assistance Program was created to provide initial resources to enable companies to advance GMP production of cell therapy products within the State of Maryland.

The program aims to accelerate timely and cost-effective manufacturing of cell-based products for patients in need.

In May 2023, the MSCRF announced its first awards supporting manufacturing in Maryland. **The first Manufacturing Assistance grant recipients were** companies with Maryland roots; **RoosterBio, Inc**. (Dr. Jon Rowley), **Theradaptive, Inc**. (Dr. Luis Alvarez), and **Caring Cross, Inc**. (Dr. Boro Dropulic) with **Octomera, Inc.**, (Dr. Sagi Nahum). as a collaborator. The total Manufacturing Assistance grant awards issued in 2023 were nearly $3 million.

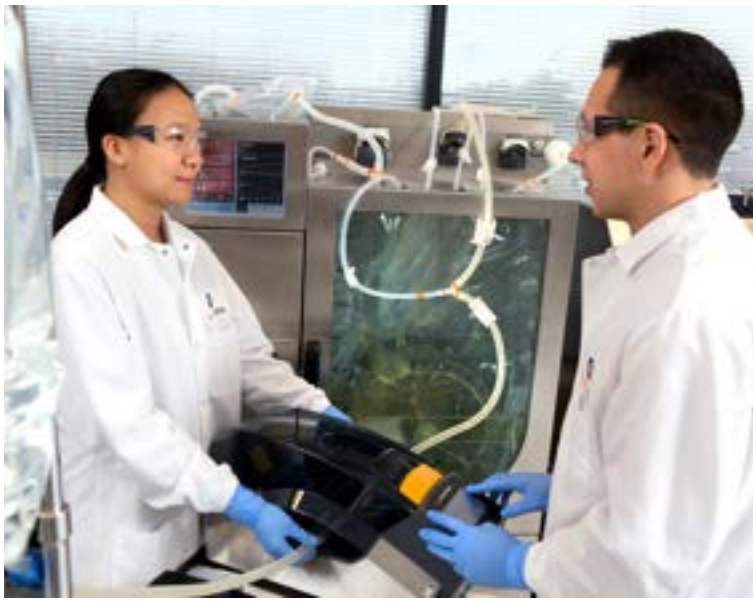
Additionally, **the Manufacturing Assistance Program helps companies located in Maryland to support and retain an advanced manufacturing workforce**.

The grant can help companies build or acquire modular manufacturing facilities, prefabricated clean rooms, closed systems, or similar manufacturing platforms to enable GMP production of cell therapy products within the state.

Expanding Maryland's Bio-Workforce

RoosterBio, Inc.

RoosterBio was a recipient of the MSCRF Manufacturing Assistance Grant. The grant has enabled the forging of a strategic partnership with Cytiv and is also supporting two RoosterBio employees who are focused on exosome manufacturing, downstream exosome bioprocessing, and advanced analytical characterization activities that will result in a fully closed exosome purification process.

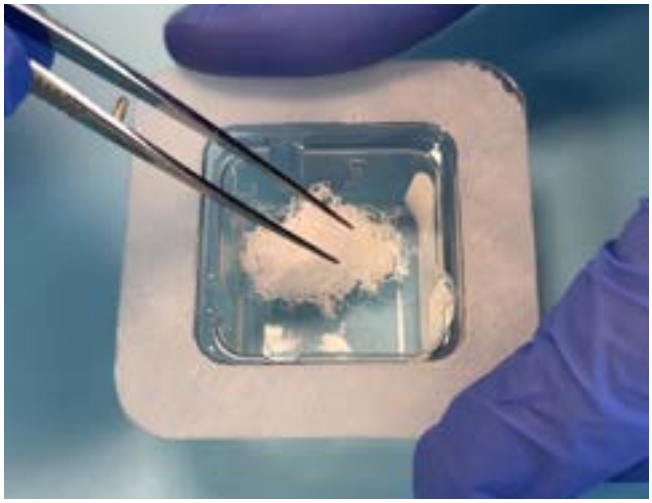


"Being recognized by the MSCRF for our leadership in stem cell and exosome products and services is very gratifying. It validates the hard work, dedication and passion that the RoosterBio team and I invest in our work. More than personal recognition, the awards we have received from the MSCRF reflect the collective effort of a talent and committed group of 'Roosters' striving to accelerate stem cell and exosome product and process development. These awards motivate us to continue pushing boundaries and accelerating our customers' path to clinical and commercial manufacturing," Jon Rowley, founder and chief product officer of RoosterBio says.

Building a Lasting Infrastructure

Theradaptive, Inc.

The first company to receive a MSCRF Manufacturing Assistance Grant, Theradaptive is developing a regenerative therapeutic product for spine and trauma repair. The grant will help Theradaptive construct a GMP Manufacturing Facility for the company's OsteoAdapt regenerative therapeutic product.



"Maryland has one of the most robust stem cell technology support programs in the US, the MSCRF. It is no coincidence that Maryland is one of the top states in the development of new stem cell therapeutics. **"MSCRF investment is an investment in the biotechnology infrastructure of the state, and yields much more value than the amount invested.** MSCRF has stimulated the creation of many new commercial technologies in the state by investing in teams, reducing technical risk, providing validation for private investment, and attracting new jobs to the state," says Luis Alvarez, founder and CEO of Theradaptive.

Octomera, Inc./ Caring Cross

The escalating costs of curative therapies, such as CAR-T cell products, have become a significant concern, hindering accessibility. Through the MSCRF Manufacturing Assistance Fund, Caring Cross and Octomera, a business unit of Orgenesis, aim to establish a low-cost, mobile cleanroom-based manufacturing facility for CAR-T cells, ensuring affordability and accessibility to transformative therapies.

"The MSCRF manufacturing assistance grant allows us to rapidly accelerate the rollout of our proprietary Octomera Mobile Processing Units and Labs (OMPUL) in the United States, prioritizing the state of Maryland. **The manufacturing grant will have a significant impact on bringing technology closer to the clinic by providing us with the resources needed to scale up manufacturing capabilities in Baltimore first, and then throughout Maryland**" says Dr. Sagi Nahum, senior director, cell and gene therapy at Octomera.

"This grant enables the establishment of a cGMP-compliant manufacturing facility so that we can produce cell and gene therapy (CGT) products like CAR-T in a way that meets regulatory standards, which is essential for clinical trials and commercialization of cell and gene therapy products," shared Dr. Rimas Orentas, chief scientific officer at Caring Cross.

The Promise of Stem Cells

In March 2023, state legislators were provided with an eyewitness view to the promise of stem cell therapy and how treatment saved the life of a five-year-old boy. In June 2017, Erin Shelton's son Jackson was born. It was one of the happiest days of her life. However, days later, following a battery of tests, Jackson was diagnosed with Severe Combined Immunodeficiency (**SCID**), a rare, life-threatening genetic disorder that results in a child born with little or no immune system.

Also known as "**Bubble Boy Syndrome**," children born with SCID are unable to fight off infections. That means that typical childhood illnesses can be life-threatening. Without treatment, most SCID babies die within their first year of life.

Following Jackson's diagnosis, he was referred to Johns Hopkins for treatment, specifically **Dr. Elias Zambidis**, a professor of oncology who is an expert in using stem cells to treat various disorders. Once Jackson's particular subtype of SCID was diagnosed. Jackson was treated with a stem cell transplant from a donor's bone marrow.

Hopkins officials reached out to Be The Match, a global leader in bone marrow transplantation that connects patients with donors. Jackson was one of the fortunate ones, there were several donors who were matches to the infant.

Jackson was admitted to the hospital in October 2017. He was three-and-a-half months old. Jackson's immune system was beginning to fail, his little body was already fighting a fungal infection as he entered the hospital. Following treatment for the fungal infection, Jackson was paired with a bone marrow donor from Germany and began his treatment plan under the guidance of **Dr. Zambidis**.

Five years later, a thriving and healthy Jackson walked out of Johns Hopkins for the last time. He was functionally cured of his disorder. He is now enjoying life like any other six-year-old child, free of concern of common illnesses that at one time could have cost him his life.

"Jackson is a prime example and living proof of what stem cell research can do. I want to thank the State of Maryland, Hopkins, and MSCRF for supporting stem cell research. Without this support, I would not have Jackson, and many other children would not be with us today."

Erin Shelton
testimony before the legislature in March 2023.

The Power of Support

In testimony to the Maryland General Assembly, **Dr. Curt Civin**, the Philip A. Zaffere Distinguished Professor in Regenerative Medicine at the University of Maryland School of Medicine and the Director, Center for Stem Cell Biology & Regenerative Medicine, hailed the support for stem cell research provided by the MSCRF, stating that "**the Maryland Stem Cell Research Fund (MSCRF) has been essential to our work to develop stem cell therapies.** Since its inception in 2006, the MSCRF has supported a myriad of research projects across Maryland on a wide range of diseases. As just one current example, in my research on blood-forming stem cells, MSCRF is supporting our collaboration with a biotech startup to enhance isolation of stem cells for transplantation and gene therapies. The new technology to do this is integral to our hopes to expand cell therapies, from CAR-T cell therapies for cancer to corrective gene therapy for genetic diseases.".

"As MSCRF propels science and helps our patients, it also promotes collaborations between research institutes and industry partners and creates jobs," he adds.

As the Associate Dean for Research and Director of the Center for Stem Cell Biology & Regenerative Medicine at UMSOM, I have personally mentored several early-stage investigators who receive funding and support from MSCRF which has helped them become highly productive independent investigators, which in turn builds the stem cell research community.

To close, I want to thank this sub-committee and the State of Maryland for their support of MSCRF. The work that MSCRF funds has transformed Maryland into a leader in stem cell research and life-saving regenerative medicine.

Supporting an Ecosystem

Beyond the gratification of grants, MSCRF emerges as a linchpin in weaving a network of collaboration among stem cell researchers both within Maryland and beyond.

The synergy of scientific minds in collaborative brainstorming sessions becomes the crucible where breakthroughs gestate and thrive, propelling the next wave of medical innovation. **MSCRF stands resolute in its mission to be a regenerative medicine hub, a nexus providing not just financial support but also a wealth of information, expertise, and connections to propel ideas seamlessly from conception to therapeutic fruition.**

Throughout the year, MSCRF actively supports a spectrum of stem cell-related events, a prominent example being the International Society for Stem Cell Research (ISSCR) annual meeting. In the seminal year of 2023, this global congregation unfolded in Boston from June 14-17. As a key sponsor, MSCRF organized a focused session titled "Bench to Bedside."

This exclusive platform provided MSCRF awardees with a stage to unveil their groundbreaking work, spanning the entire spectrum from nascent discoveries to clinical applications, including invaluable biomanufacturing support facilitated by MSCRF. The showcased research not only spotlighted the remarkable progress within the field of regenerative medicine by MSCRF awardees but also underscored the profound impact of MSCRF funding supported by the state of Maryland.

This year also marked the genesis of the MSCRF Inaugural Felicitation Ceremony—a grand event designed to honor the exceptional accomplishments of awardees from Fiscal Year 2023.

This celebratory gathering encompassed awardees from all seven MSCRF grant programs, fostering a potent sense of unity and collaboration within Maryland's vibrant stem cell research community. Beyond a mere acknowledgment of individual triumphs, this event symbolized a collective celebration of Maryland's journey in the realm of regenerative medicine, propelled forward by MSCRF's unwavering support.

In essence, 2023 encapsulated a period of growth and progress for MSCRF, with expanding initiatives, heightened research activity, and tangible advancements affirming its pivotal role in steering Maryland toward positive and impactful directions.

Achievements

MSCRF's Portfolio Companies 2023 Highlights

In 2023, the MSCRF witnessed remarkable growth and success among its funded companies. These enterprises, supported by MSCRF, experienced substantial advancements, contributing to the flourishing landscape of regenerative medicine in Maryland.

BioCardia Inc. announced FDA approval of its Phase III clinical trial of its CardiAMP autologous cell therapy for the treatment of patients with ischemic heart failure. The trial will build on positive clinical data from a previous study of this autologous cell therapy in almost 200 patients. The company also secured a U.S. patent on bone marrow derived neurokinin-1 receptor positive (NK1R+) mesenchymal stem cells for therapeutic applications.

RoosterBio Inc. forged multiple collaborations with companies to support development of its stem cell-derived exosome-based therapies. RoosterBio partnered with Sartorious to advance downstream purification processes for the manufacture of exosomes; a scalable exosome bioprocessing partnership with Repligen; a service provider collaboration with NanoFCM Partners; and a partnership with Cytiva to address exosome manufacturing challenges.

In October of this year, **Seraxis Inc**. a cell therapy company developing a pancreatic organoid cure to transform the lives of patients with Type 1 and Type 2 diabetes announced the closing of a second tranche of its inaugural venture capital round to bring total equity investment in the company to over $50 million. Additionally, the company presented successful completion of IND-enabling preclinical studies of pancreatic organoids from their novel, proprietary, stem cell line at various scientific conferences demonstrating the safety and therapeutic efficacy of their product in preparation for commencing a clinical trial in 2024.

Vita Therapeutics Inc. marked 2023 with a licensing agreement with MilliporeSigma, the U.S. and Canada Life Science business of Merck KGaA, Darmstadt, Germany. Under the agreement, Vita will leverage MilliporeSigma's foundational CRISPR technology to advance the development of its preclinical asset VTA-100 for the treatment of limb-girdle muscular dystrophy.

Cartesian Therapeutics, Inc. began 2023 with the dosing of the first patient in a Phase IIb trial assessing the company's lead asset, Descartes-08 for generalized myasthenia gravis (MG), a debilitating autoimmune neurological disease. In June, the company published a landmark paper in Lancet Neurology, a top-tiered scientific journal, demonstrating a marked and long-lasting clinical improvement in patients with MG. The company closed out the year by merging with Selecta Biosciences. The new company will continue to be known as Cartesian Therapeutics and its combined assets will be used to support the advancement of Descartes-08.

Achievements

Secretome Therapeutics, Inc. formerly known as NeoProgen, secured their first 'Method' patent to treat cardiac conditions using Neonatal Heart-derived Medicinal Signaling Cells (nMSCs) and/or their secretomes/ exosomes from the US Patent Office earlier this year. Importantly, in May of this year, the company received approval from U.S. FDA for a Phase I Study of 'The Safety and Early Efficacy of Intravenous Allogeneic Neonatal Mesenchymal Cells (nMSCs) In Chronic Ischemic Heart Failure'and are currently in preparations to commence their clinical trial.

Theradaptive Inc. began 2023 by winning Maryland Tech Council's Emerging Life Sciences Company of the Year award. The company also closed a $26 million Series A funding round to advance development of its targeted regenerative therapeutics. In March and October of this year, respectively, the company were awarded grants from the Department of Defense; one for $4 million to fund the development of its lead product, OsteoAdapt, and another for $7.4 million to support the company's Phase I/II trial assessing OsteoAdapt for treating musculoskeletal injuries, such as degenerative disc disease. Additionally, Theradaptive forged commercial agreement with South Carolina-based 3D Systems naming that company as its exclusive 3D printing partner.

Reprocell, Inc. entered into multiple collaborative agreements with various companies and research institutions, including Keio University, Vernal Biosciences, and Silo Pharma. In addition, the company continues to provide stem cell services to support stem cell-based clinical trials. The company struck a licensing agreement with Gameto to improve assisted fertility using iPSCs and expanded its presence in the U.K. market through a partnership with BioMavericks Ltd. The company also announced the positive results of its Phase II clinical trial of their regenerative medicine product, Stemchymal® (allogeneic adipose-derived mesenchymal stem cells), for the treatment of spinocerebellar ataxia demonstrating safety and preliminary signals of efficacy.

MaxCyte Inc. signed multiple collaborative agreements with various agreements for the use of their Flow Electroporation® and ExPERT™ technologies. Among those, the company partnered with Curamys to enable cell & gene therapies for the treatment of rare diseases; Vittoria Biotherapeutics to develop a pipeline of highly differentiated cellular therapies for both oncology and immunology indications; Prime Medicine to advance their innovative Prime Editing technology and develop a new class of curative therapies; Catamaran Bio to Support their CAR-NK Cell Therapy Programs; and established a licensing agreement with Lyell Immunopharma in support of their T-cell products targeting solid tumors. In 2023, MaxCyte also joined the Alliance for mRNA Medicines (AMM) as a founding member.

Longeveron Inc. announced positive top-line results from its Phase IIa trial assessing its investigational product Lomecel-B for the treatment of mild Alzheimer's disease. Positive long-term data from a 10 person trial evaluating Lomecel-B for patients with hypoplastic left heart syndrome was also announced in 2023. In October 2023, Longeveron also secured a $4 million secured deposit to support the advancement of its programs.

MSCRF

PATIENT Testimonials

Supporting the Next Frontiers of Medicine

The commitment of MSCRF to improving patient lives is not merely theoretical; it is substantiated by real-world achievements.

Patient testimonials stand as vivid proof of the ripple effect generated by MSCRF funding, bringing hope and improved outcomes to those confronting diverse medical challenges.

These success stories stand as a testament to the foresight and dedication of MSCRF in fostering a healthcare landscape that prioritizes patient well-being.

Providing Hope for Amputees

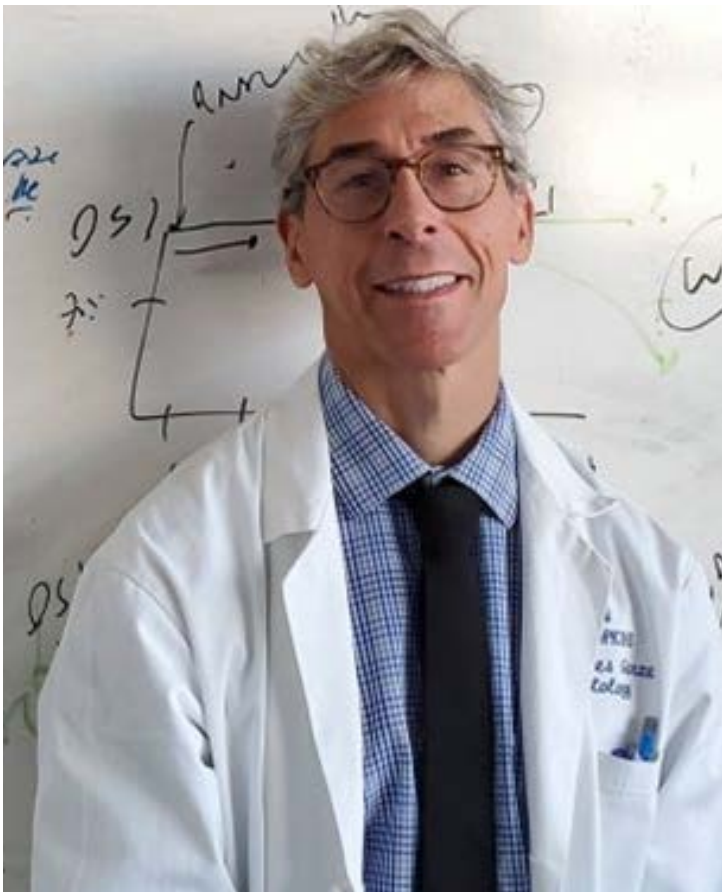
With over 2 million amputees in the U.S. and approximately 200,000 new amputees annually, the number of amputations is rising in Maryland and nationwide, particularly due to the diabetes epidemic. It is estimated that by 2050, 3.6 million people in the U.S. will have lost a limb. Despite advancements in prosthetic design, skin breakdown at the stump site remains a limiting factor.

Currently, there is no therapy to prevent skin breakdown and pressure ulcers at the stump site, leading to long-term issues for amputees, including Vietnam veterans.

Deborah C. Dopkin, an amputee enrolled in a Phase II clinical trial led by Dr. Luis Garza, expresses enthusiasm about the potential of this new cell therapy being tested in the clinical trial,

"**The notion that I may be able to heal using my own cells is empowering**! **Prostheses give an amputee independence – until you cannot wear your devices because your skin breaks down and becomes damaged. Chronically so. Each time a sore appears, it instills a gnawing fear that by being active you may irreparably damage an already damaged limb**," Dopkin says.

"Dr. Luis Garza's study is a source of hope for people like me, as we deal with the emotional and physical challenges of living with parts of our body missing. I believe that the work Dr. Garza and his team are doing will elevate the lives of amputees and will remove one obstacle from our living fully, both mentally and physically," she adds.



Dr. Luis Garza, Professor of Dermatology at Johns Hopkins University and one of current MSCRF's Clinical grant awardees, expresses his gratitude,

"Our team is very grateful for MSCRF support. **With MSCRF grants, we have made breakthrough discoveries in human trials of how cell therapy holds the promise to be the 3rd arm of medicine.** Pills and procedures can help many patients, but cell therapy will likely open entirely new options for diseases that today we cannot cure. **MSCRF support is vital for these discoveries**" added Dr. Garza.

The Phase II Clinical trial is anticipated to be completed in 2024 with the full FDA approval for the autologous product possible well before 2026, according to Dr. Garza. In his ongoing Phase II clinical trial, Dr. Garza injected volar fibroblasts into problematic areas of the skin of eight amputees where skin breakdown is common, demonstrating an increase in average skin firmness in treated patients to date.

Stem Cells Offer Promise in Treating Blood Cancers

Stem cell transplant, a groundbreaking medical procedure, holds tremendous promise in treating leukemia. In this innovative approach, damaged or diseased bone marrow, responsible for abnormal blood cell production, is replaced with healthy stem cells.

These specialized cells possess the remarkable ability to develop into various types of blood cells, effectively restoring normal blood function.

Dr. Suresh Kumar Muttah, a cancer patient, and an internal medicine specialist in Maryland, received a stem cell transplant for leukemia at the University of Maryland Greenebaum Comprehensive Cancer Center (UMGCC) and has been in remission for the last two years.He attributes his recovery to the stem cell transplant and expresses gratitude to his team of oncologists, Drs. Nancy Hardy and Aaron Rapport (both pictured on the right), for curing his medical condition.

"Even though there were ups and downs throughout the course, right from the time of diagnosis, I can say with confidence that stem cell transplant is the way to go for a complete cure of many bone marrow diseases. I am back to my normal life with renewed energy, thanks to the stem cell transplant process," Muttah says.

"I highly recommend stem cell transplant to anyone for the appropriate medical condition as recommended by the physician, because it will pave the way for a complete cure for the illness, and give hope, optimism, and complete confidence to move forward in the journey of life", Muttah further adds.

Dr. Aaron Rapoport emphasizes the collaborative effort, "**It takes a village to provide curative care to patients with blood cancers using stem cell transplants and other forms of cellular therapy for patients with blood cancers. The gratitude and gratification that we all share when patients like Dr. Muttah achieve milestones of long remissions and cures is almost beyond words. The scientific advances and the funding for those advances have been essential to the progress in treatment that has already been achieved and to ensure new lifesaving discoveries in the future**".

Since 1998, Dr. Aaron Rapoport and his colleagues at UMGCCC have achieved success in treating numerous patients with blood cancers through groundbreaking cell therapy.

MSCRF

LONG-TERM Impact

Supporting the Next Frontiers of Medicine

The Maryland Stem Cell Research Fund has been a catalyst for groundbreaking research in stem cell science. By providing funding at various stages, MSCRF aims to bridge the gap between basic research and clinical applications. A key focus has been on advancing research from the laboratory bench to the bedside, translating scientific discoveries into clinical applications.

This section illuminates the enduring impact of continuous funding on researchers and business endeavors, propelling advancements in research and technologies toward commercialization and clinical application. With MSCRF's expansion from an initial three programs in 2006 to a comprehensive set of seven programs in 2023 that covers the entire spectrum from early ideation to product development, the sustained funding's profound effects are examined.

Within this context, the section **features notable awardees**, encompassing Maryland-based businesses and academic researchers, who have effectively **propelled their scientific initiatives from the nascent stages towards commercialization** bringing them closer to clinical translation.

Regenerative Medicine for Spine, Trauma Patients

Frederick-based Theradaptive, Inc., which is pioneering advancements in targeted regenerative therapeutics, was awarded MSCRF's first Manufacturing Assistance Program grant. The grant will help Theradaptive construct a GMP Manufacturing Facility for the company's OsteoAdapt regenerative therapeutic product for spine and trauma repair in preparation for first-in-human clinical trials.

Theradaptive's CEO, Luis Alvarez, a former lieutenant colonel in the US Army, drew inspiration from firsthand experiences witnessing the devastating impact of improvised explosive devices on soldiers' limbs. Despite the advances in saving limbs, the existing gaps in the standard of care prompted Alvarez to develop the technology Theradaptive is now advancing into the clinic, focusing on the targeted delivery of therapeutic proteins to implant sites to enhance patient outcomes.

"MSCRF has stimulated the creation of many new commercial technologies in the state by investing in teams, reducing technical risk, providing validation for private investment, and attracting new jobs to the state," says Luis Alvarez.

Theradaptive, Inc.

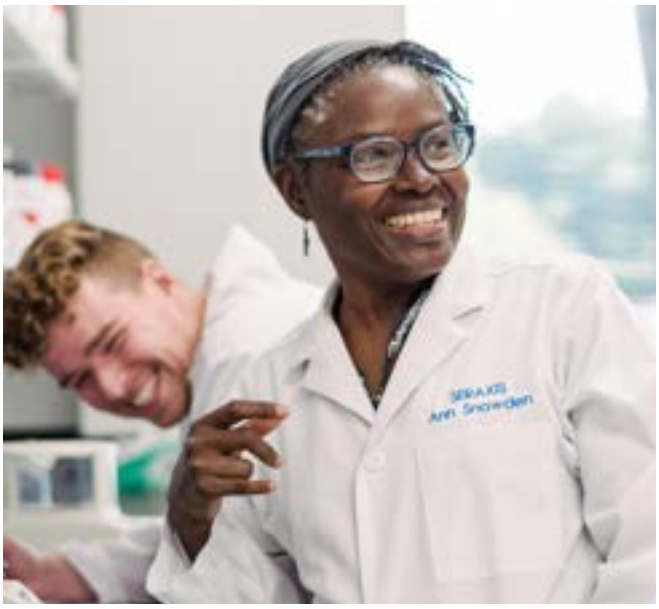
Theradaptive's lead therapeutic, AMP2, is a proprietary material-binding variant of BMP2, a bone-inducing protein that regenerates bone tissue.

The critical need for improved methods of bone and tissue regeneration is evident, with 90% of individuals aged 60 or older exhibiting signs of spinal disc degeneration, resulting in pain and diminished mobility. Additionally, 25% of postmenopausal women endure debilitating vertebral compression fractures.

Current treatments primarily offer structural stability but lack regenerative capacity, often falling short of delivering optimal outcomes.

In addition to the Manufacturing Assistance Grant, MSCRF also provided Theradaptive with two Commercialization Grant awards. Alvarez says these investments allowed the company to move closer to clinical testing of OsteoAdapt. A Phase I/II trial is expected to begin in early 2024.

Bringing Hope to Type 1 Diabetes Patients



Seraxis. Inc.

"For a data-driven company built from the ground up with nothing but good ideas and strong conviction, the recognition from MSCRF is gold. It validates what we do. It's the type of validation we require in order to get bigger exposure," William Rust says

With its proprietary human stem cell line SR1423, Germantown-based Seraxis is developing pancreatic islet replacement therapies that hold the potential for curing Type 1 diabetes, a disease that is historically difficult to manage. Type 1 diabetes is an autoimmune disease that causes an individual's pancreas to stop producing insulin.

The immune system attacks and destroys insulin-producing cells in the pancreas. These patients become insulin-dependent. But, Seraxis' stem cell therapy approach could eliminate the need for such reliance.

Seraxis is **blazing a new trail in "generative medicine," using stem cells to create a new pancreas for these patients.** The company's stem cell-derived pancreatic endocrine cell clusters are designed to form functional pancreatic grafts and potently regulate blood glucose levels.

by a 12-month MSCRF grant to support ongoing research into developing its off-the-shelf pancreatic organoid therapies,

Seraxis is poised to initiate clinical testing of its lead therapeutic candidate SR-02 for Type one diabetes patients with severe recurrent hypoglycemia. Because SR-02 is an allogeneic cell transplant, those patients will require immune suppression to avoid rejecting the implant.

SR-03 is Seraxis' experimental therapeutic also aimed at Type 1 diabetes patients but altered to be unrecognized by the host immune system.

Seraxis Chief Executive Officer William Rust believes SR-03 has the potential to eradicate the need for insulin dependence in the more than 300,000 Type 1 diabetes patients in the United States. He says the grant funds from MSCRF have played a key role in the company's ongoing development of its stem cell programs and anticipates seeking additional grants to support continued research into SR-03.

New Approaches for Heart Failure

Grants supporting the validation of stem cell research in the laboratory of Dr. Sunjay Kaushal at the University of Maryland, Baltimore enabled the 2019 spinout of NeoProgen, a company focused on developing first-in-class therapeutics from neonatal mesenchymal stem cells (nMSC).

In 2022, **NeoProgen rebranded to Secretome Therapeutics** to better align with its vision of revolutionizing cell therapy through the use of neonatal cardiac progenitor cells (nCPCs) and respective secretomes, proteins and molecules released by the cells.

In 2023, Secretome received a Commercial Assistance Grant from MSCRF to advance neonatal cardiac stem cells into a clinical trial for various forms of heart failure. Secretome received clearance from the FDA in 2021 in support of two Phase I clinical trials. Secretome's lead program is STM-01, in development for adult and pediatric forms of heart failure.

The company will **evaluate STM-01 in two different indications** -- Dilated Cardiomyopathy (DCM) and heart failure with preserved ejection fraction (HFpEF).

Both diseases are driven by inflammation, a dysregulated immune system attacking and causing damage to the left ventricle of the heart. Mesenchymal Stem Cells have been shown to promote anti-inflammatory responses.

"Maryland has established a leadership position in supporting the field of regenerative medicine and the development of cell-based medicines. Agencies like MSCRF are rare and critically important in the pathway from innovation to commercialization," Jindal says.

Secretome Therapeutics, Inc.

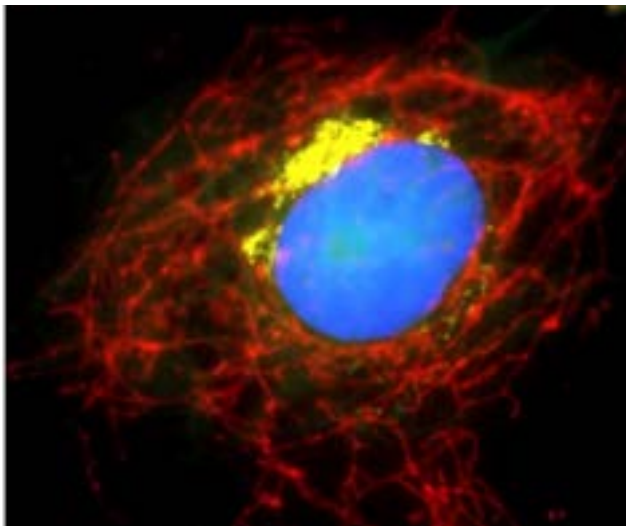
Both clinical trials will assess STM-01 for safety and efficacy. HFpEF is a form of heart failure that affects about 4 million people in the United States. The Phase I DCM trial will assess STM-01 in adult patients but with the ultimate goal being approval in pediatric patients with DCM, Jindal explains, Secretome President and Chief Executive Officer Vinny Jindal says.

The trials will begin in 2024.

Jindal says the MSCRF grant awarded in 2023 was not only instrumental in moving STM-01 forward clinically, it also validated Secretome's platform in the eyes of investors who are considering supporting the company.

Jindal says the funds supporting Secretome's research over the years have been invaluable to the future of the company.

Secretome THERAPEUTICS



Creating New Therapies for Muscular Dystrophy

Vita Therapeutics, Inc.

Baltimore-based Via Therapeutics, founded in 2019 out of the labs of Dr. Gabsang Lee and Dr. Kathryn Wagner at Johns Hopkins University and the Kennedy Krieger Institute, is a cell engineering company that uses induced pluripotent stem cell technology to engineer specific cell types designed to replace those that are defective in patients and cure different forms of muscular dystrophy at their root.

One of the company's cell therapy assets is VTA-100, an autologous treatment that combines gene correction and induced iPSC technology to help repair and replace muscle cells for people with limb-girdle muscular dystrophy 2A (LGMD2A), a subtype of the disease. LGMD2A is a localized form of muscular dystrophy that typically impacts the shoulders, upper arms, pelvic area and thighs.

MSCRF has supported Vita Therapeutics with two commercialization grants. Both of the grants have provided needed funding to help further develop our preclinical therapies and get them closer to the clinic, explains Vita Therapeutics CEO, Douglas Falk. Vita's first cell therapy is expected to enter clinical testing by 2025.

"The MSCRF supported Vita in many ways and helped us further connect to the local community. When we were raising a seed round, it was the MSCRF funding that helped support additional investments. It was also the MSCRF award process that identified the work of Dr. Alan Friedman which then led to a research partnership between his lab and Vita," Falk says.

In addition to VTA-100, the company is also developing VTA-200 an allogenic treatment that combines hypoimmunogenic and iPSC technology to help repair and replace muscle cells while evading the immune system. Both VTA-100 and VTA-200 are **designed to regenerate muscle** in patients who have seen their muscles waste away due to their disease.

The grants provided by MSCRF not only supported Vita's push toward clinical testing of its lead candidate, but also catalyzed additional fundraising. Since the original MSCRF grant awarded to Vita, the company has received more than $45 million in additional funding to support its research.

Low-Cost Approach to CAR-T Therapies

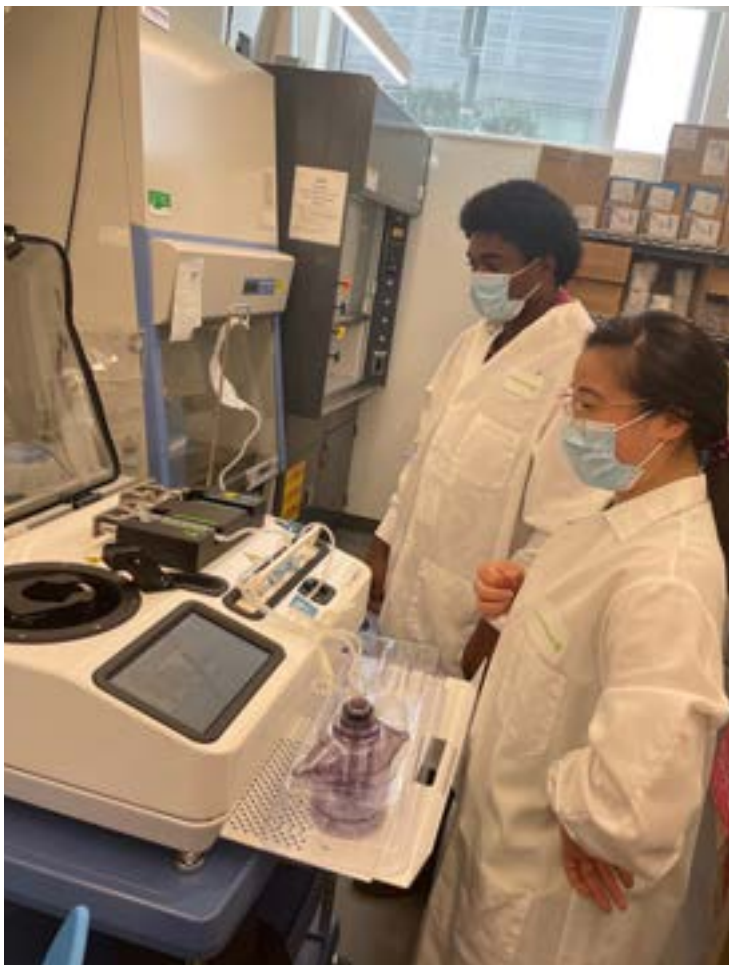
Caring Cross/ Octomera, Inc.

The escalating costs of curative therapies, such as CAR-T cell products, have become a significant concern, hindering accessibility. Through the MSCRF Manufacturing Assistance Fund, Caring Cross, a non-profit focused on creating low-cost workflows for cell and gene therapies and Octomera, business unit of Orgenesis, aim to **establish a low-cost, mobile cleanroom-based manufacturing facility for CAR-T cells,** ensuring affordability and accessibility to transformative therapies.

The grant will allow the companies to accelerate the rollout of proprietary Octomera Mobile Processing Units and Labs (OMPUL) in the United States, prioritizing the state of Maryland. The OMPUL will support the production of cell and gene therapy products like CAR-T.

Both organizations believe the future of autologous gene-modified cell therapy is in a distributed and decentralized model. When considering these factors together it was an easy decision to **partner on this grant, bringing the decentralized production platform to Maryland** to produce their gene-modified cell therapy asset.

"Point-of-care cell manufacturing creates new opportunities for businesses manufacturing the tools needed to deliver these low-cost approaches for creating high-impact therapies like CAR-T cells. Using Octomera's mobile cleanroom technologies, geography will no longer dictate who gets these therapies. MSCRF is building a bridge to the future, and enabling a new approach to cell and gene therapy that will impact Maryland, and beyond.," says Dr. Sagi Nahum, senior director of cell and gene therapy at Octomera.



The assistance grant will bring the technology closer to the clinic by providing the companies with the resources needed to scale up manufacturing capabilities in Baltimore first, and then throughout Maryland.

The grant facilitates workforce expansion and attracts skilled professionals in manufacturing, quality control, and research and development. As the team grows, it not only showcases the talent and expertise of Maryland's economy enhances the region's competitiveness, and diversifies its employment landscape.

Breaking New Ground in Xenotransplantation

RenOVAte Biosciences, Inc.

A precision genome company, RenOVAte Biosciences is a Maryland-based animal biotechnology company founded in 2016.

The company harnesses the power of genome editing and genetic engineering to address critical health priorities. The company is specifically focused on leveraging pig models for regenerative medicine applications.

Since its founding, **RenOVAte Biosciences Inc. has received three grants from MSCRF,** including a $396,000 Commercialization grant awarded in 2023.

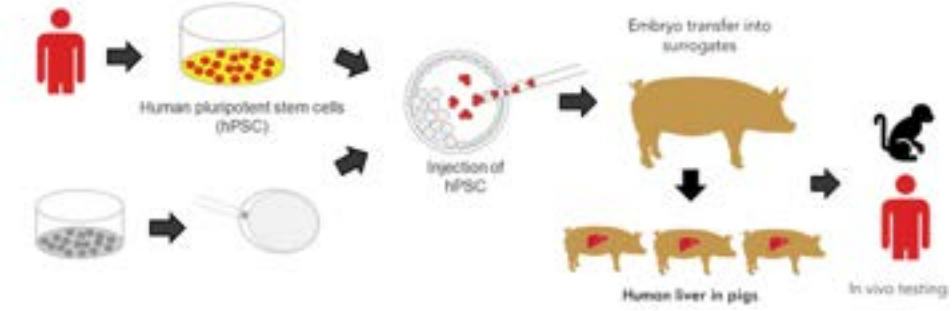
The most recent Commercialization Award will assist the company with the generation of the preliminary dataset for its foundational xenotransplantation technology. The money will also aid in the de-risking process that will lead to future VC/institutional funding.

"We are close to finalizing the genetic engineering efforts in porcine models and are closer to advancing the humanization efforts that will lead to critical animal and in-human trials," Chief Executive Officer Rob Etches says.

Researchers hope to use genetically modified animal organs to prolong the lives of critically ill patients. In the United States alone, more than 123,000 men, women, and children currently need life-saving organ transplants.

The company's work has been funded with a combination of finances from the MSCRF awards, as well as income from its therapeutics division that generates custom genetically engineered pig models for preclinical research.

Renovate Biosciences has been in talks with institutional investors to support a Series A funding round.



"Sadly, an average of 22 people die each day due to lack of available life-saving organs, with the numbers expected to increase every year. In the United States, there are an average of 11,000 patients waiting for a liver transplantation at any given time. Funding from MSCRF is helping us develop technologies to bridge the critical shortfall in the availability of organs (liver) for transplantation.," Etches says

Separating, Sorting Cells for Cell Therapies

Curt Civin

University of Maryland School of Medicine

Stem Cell pioneer Curt Civin has a long history with MSCRF that goes back to the beginning. In 2007 he was one of the first recipients of grant money. Over the years, Civin, as well as members of his lab at the University of Maryland School of Medicine, received numerous grants from MSCRF in support of their research.

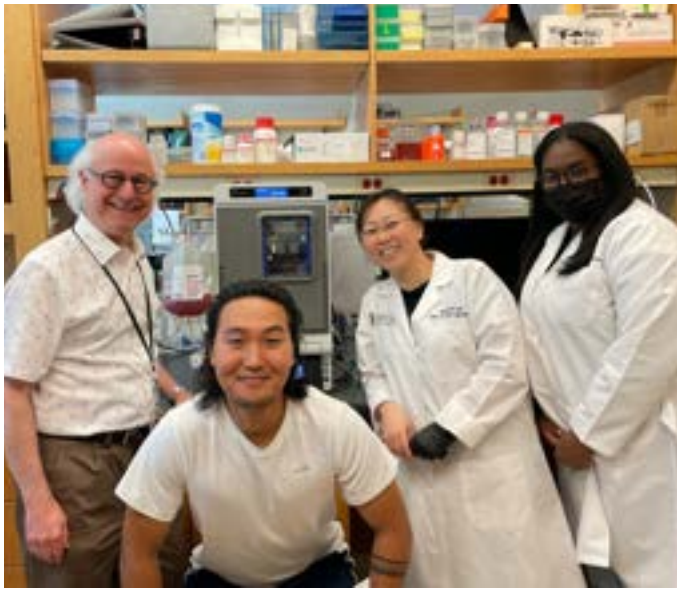
In 2023, Civin **received both a Discovery Grant and a Validation Grant from MSCRF**. The Discovery Grant supported an assessment of DACH as a Novel Human Megakaryopoietic Regulator. Civin's lab specifically studied DACH1, a gene involved with forming eyes, ears and other organs in humans.

The research funded by MSCRF validated a hypothesis that DACH1 can drive Hematopoiesis, the process of creating a wide variety of blood and bone marrow cells.

The findings from this study **open up new possibilities for developing new methods to produce red blood cells.** The implications of this study could have a significant impact on the development of blood-based products essential for numerous medical treatments.

Additionally, Civin secured a Validation grant from MSCRF to support an ongoing collaboration with Curate Biosciences assessing that company's CurateR system designed to harvest Hematopoietic progenitor cells (HPCs) and Hematopoietic stem cells (HSCs) at a high yield. Civin and Curate Biosciences have collaborated on the project for about 12 years.

The partnership is focused on the development of a medical device that can separate cells based on their size and send them on a defined pathway. The project is expected to benefit flow cytometry, a technology that provides rapid multi-parametric analysis of single cells in solution.



Research has shown a "gentle separation of cells," Civin said. There are long-term implications in multiple fields of research, particularly the development of CAR-T cells used in the treatment of some blood-based cancers.

Early data suggests the cells used in the development of CAR-T therapeutics can be manufactured less expensively than current processes and at a higher yield.

A longtime **advocate of MSCRF**, Civin has not only received multiple grants from the organization but so have his protégés.

The different types of MSCRF grants and its efforts to champion stem cell research created a strong foundation for the field across Maryland – a field supported by an intricate network of scientists who can augment each other's research.

Regenerative Medicine to Treat Eye Diseases

Amer Riazuddin

Johns Hopkins University School of Medicine.

"We have come a long way in the past five years from generating pluripotent stem cell-derived corneal endothelial cells to confirming their efficacy as an alternative to human postmortem donor tissue required for corneal transplant surgery in different animal models including non-human primates. This work would not have been possible without MSCRF funds., The support of MSCRF has been indispensable to the success of my research to develop donor tissue-independent treatment of corneal endothelial dysfunction, which undoubtedly will contribute to the global efforts to preserve sight and prevent blindness," says Riazuddin.

Dr. Amer Riazzudin, an associate professor at Johns Hopkins University, is crafting a transformative narrative in the realm of ocular regenerative medicine. His groundbreaking research, fueled by MSCRF grants, aims to revolutionize the treatment landscape for ophthalmologic diseases, particularly corneal edemas caused by a damaged endothelium.

Traditionally, treating this eye condition necessitates healthy tissue sourced from postmortem donor eyes, intensifying the demand for transplantable-grade donor tissue and creating a looming stem-cell-based alternative to donor tissue, a breakthrough that promises to restore corneal transparency and safeguard the eyesight of patients grappling with corneal endothelium dysfunction.

Essentially, the trajectory of advancement continues. T**he Validation grants from MSCRF have fortified Dr. Riazuddin's research, attracting attention and support from additional funding channels**.

Recently, the **Maryland Department of Commerce,** through the Maryland E-Nnovation Initiative (MEI) **awarded $1.5 million** to Dr. Amer Riazuddin, matching dollar-for-dollar the funds raised through qualified donations. This allocation is dedicated to bolstering Dr. Riazuddin's endowment, facilitating the acceleration of his innovative research and entrepreneurial ventures.

A Johns Hopkins startup company, Ocular Biologics, LLC, was formed to support efforts to advance the commercialization of the donor-tissue independent treatment of corneal endothelial dysfunction. The startup will advance studies to confirm the safety and efficacy of pluripotent stem cell-derived corneal endothelial cells as a treatment for corneal endothelial dysfunction in an FDA-approved human clinical trial.

Dr. Riazuddin is eyeing a clinical trial assessing his approach within the next 18 to 24 months bringing his innovative approach closer to patients and positioning Maryland as a forefront leader in ocular regenerative medicine research.

Creating a New Class of hISPCs

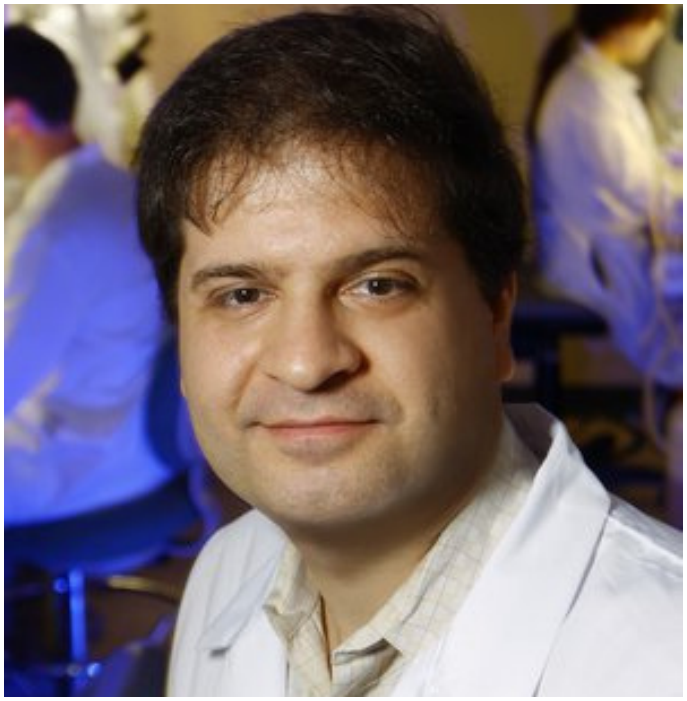
Elias Zambidis

Johns Hopkins University School of Medicine

Dr. Elias Zambidis is a physician-scientist and bone marrow stem cell transplant specialist who cares for children and young adults who suffer life-threatening genetic disorders of the blood and immune system. He aims to develop stem cell therapeutics to treat severe vascular, immunological, hematologic, and neural/retinal degeneration.

His lab at Johns Hopkins ICE focuses on regenerative medicine approaches that employ primitive stem cells called human induced pluripotent stem cells (hiPSC) that will pave the way for cures of currently lethal pediatric diseases using stem cell transplants. To this end, he has developed a multifaceted research program that focuses on human stem cell biology and its translational application to tissue regeneration and transplantation.

Since he opened his lab at Johns Hopkins in 2008, **Zambidis received multiple grants from the MSCRF**. **The awards helped Zambidis' team develop and patent hiPSC-based vascular therapies** for treating retinopathies**.** Other awards led to the discovery of a novel, chemically reprogrammed, class of human pluripotent stem cells that are potentially impactful in repairing damaged blood vessels in stroke and diabetes patients.



In a pioneering leap forward, Dr. Zambidis and his laboratory are generating this new class of hIPSCs that can be used in human trials and can be used to generate mice and pigs with developmentally complete and functional human systems. This innovation promises to revolutionize the realms of oncology, vaccine testing, and transplantation research, offering unprecedented insights and avenues for exploration.

The impact of Dr. Zambidis's work extends beyond his laboratory. The reagents and stem cell lines, generated with support from the MSCRF are now available to the broader research community. The hiPSC lines are not only commercially available and in high demand but have also been cited in numerous publications within high-impact journals by researchers who acquired them.

Accelerating the Promise of Exosome Bioprocessing

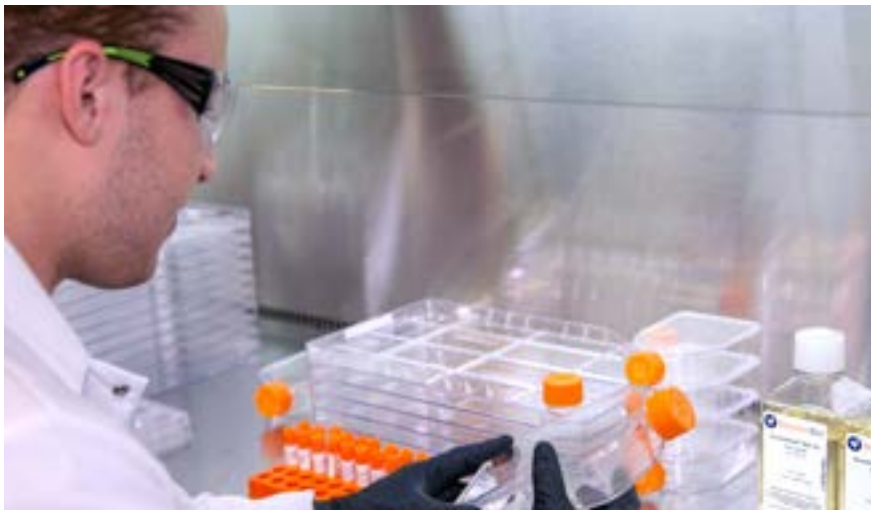
RoosterBio, Inc.

Frederick-based RoosterBio, a privately held cell manufacturing platform technology company, develops high-volume and well-characterized adult human mesenchymal stem/stromal cells (hMSCs) that are paired with highly engineered media systems.

The company is **focused on accelerating hMSC and extracellular vesicle (EV) product and process development** to fuel the rapid commercialization of scalable regenerative cures.

In 2023, the company secured a grant to support the development of a Closed Extracellular Vesicle (EV)/Exosome Drug Substance/Product Manufacturing Process without Sterile Filtration.

Breakthroughs in exosome bioprocessing could potentially translate into new, highly effective therapies for rare and chronic diseases. RoosterBio's efforts to industrialize exosome bioprocessing hold immense promise for the development of new exosome therapies and support the continued growth of the Maryland bio-economy.



"Support from MSCRF's grants bolstered several RoosterBio initiatives, including the development of new stem cell and exosome products and bioprocesses. Our current manufacturing grant is helping improve the efficiency of exosome bioprocessing which could be used both to enable the production of more "off-the-shelf" exosomes for our customers as well as advanced services programs to ensure exosome quality and function are defined," John Rowley says

"The MSCRF grant we received has already begun to accelerate RoosterBio's technical growth in exosome bioprocessing. This grant allows us to dedicate a research unit to this pressing task, developing highly skilled scientists and engineers within Maryland. With this grant, we are **strategically positioning RoosterBio's manufacturing leadership with a highly efficient and scalable exosome bioprocess** to make significant contributions to the Advanced Therapeutic industry," Jon Rowley, founder and chief product officer at RoosterBIo explains.

Developing a workforce with these unique advanced manufacturing skills will foster innovation and expertise growth within the Maryland biopharma community.

As the company's research progresses, it is likely to attract collaborations with other biotechnology companies, research institutions, and healthcare organizations. These **partnerships can lead to the establishment of collaborative manufacturing initiatives** and technology transfer, further boosting the Maryland bio-economy.

Supporting Scientists from Bench to Bedside

With an extensive product catalog developed to support stem cell research and 3D bioengineered tissues, Reprocell's work enables scientists to translate their research into clinical therapies.

The company believes the potential of stem cell reagents and services is growing at a fast pace. The stem cell field is beginning to deliver on its promises in diverse fields of application, from disease modeling to complementing animal studies, and from autologous gene therapy to targeted gene editing.

With MSCRF funding, **Reprocell can develop a portfolio of products and services that will successfully meet the growing market demand for neurodegenerative disease models.** Starting with seed funding, MSCRF has supported Reprocell's efforts to open up the field of stem cell research.

"This funding made a difference in our R&D efforts and helped us secure competent and expert staff members who are strong pillars of economic growth and give us a competitive advantage. In addition, we were also able to procure cutting-edge technology that keeps us at the forefront of research and development," Rama Modali, Chief Executive Officer of Reprocell says.

Because of MSCRF funding, Reprocell has solidified its footprint in Maryland and has secured four additional highly qualified staff members. Each of those employees is increasing the company's expertise and providing new opportunities for Reprocell and Maryland.

Additionally, **the funds validated that Reprocell's research is heading in the right direction**, Modali adds. The grants also allowed the company to solidify its roots in Maryland and plan for future growth.

"The MSCRF funding is instrumental in bridging the gap between academia and industry.

The validation grant helps start-up funding, which is crucial, and helps create partnerships with well-established companies to provide services and stability. Finally, the post-doctoral grants and academic grants encourage innovation at local universities which translate eventually into added value to our local economy."

MSCRF

COMMUNITY Impact

Supporting the Next Frontiers of Medicine

Stem Cell Technology to treat
PEDIATRIC CATARACTS

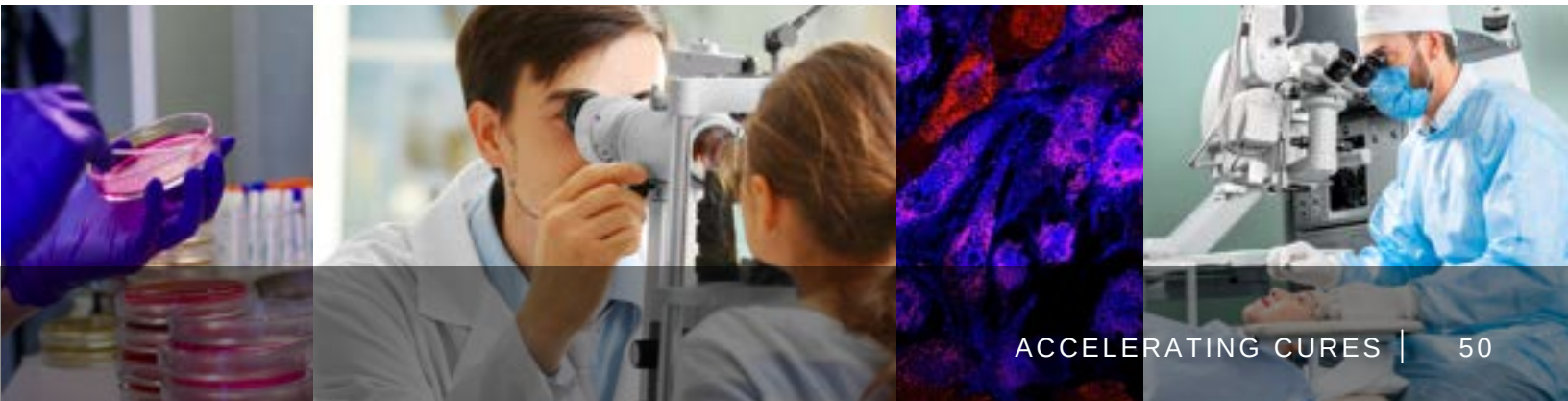
DR. GABSANG LEE

Every year, over one million people lose their vision due to cataracts, a loss of transparency of the lens of the eye. Surgical removal of cataractous lenses is the only effective treatment; however, some risks cause postoperative complications and physical incompatibility, which lead to permanent damage in pediatric patients. It is estimated that 200,000 children worldwide are blind, due to cataracts, and up to 40,000 children are born each year with congenital cataracts. Cataract blindness in children presents an enormous problem in terms of human morbidity, economic loss, and social burden.

The induction of pluripotency in somatic cell types has been one of the great breakthroughs in stem cell biology. After the early pioneering studies by Shinya Yamanaka's lab, human induced pluripotent stem cells became an invaluable source to generate large quantities of otherwise extremely rare cell populations, such as lens cells.

Dr. Gabsang Lee, Professor of Neurology at Johns Hopkins University, is developing lens cells from pluripotent stem cells using specialized differentiation protocols he developed in his lab.

This study will be one of the first steps in the development of a new stem cell-based lens cell therapy for pediatric patients suffering from cataracts. In addition, this stem cell-based human lens technology may be used to elucidate the etiology and pathogenesis of congenital cataracts as well as a platform for drug screening.



Stem Cell-Derived Exosomes as a

THERAPY FOR CYSTIC FIBROSIS

DR. ROOPA BISWAS

Cystic Fibrosis (CF) is a life-limiting, pro-inflammatory genetic disorder due to mutations in the Transmembrane Conductance Regulator (CFTR) gene, leading to severe damage to the lungs, digestive system, and other organs in the body. Cystic fibrosis affects the cells that produce mucus, sweat and digestive fluids, which are normally thin and slippery. In patients with CF, the defective CFTR gene causes the secretions to become sticky and thick, restricting bodily tubes, ducts and passageways, most significantly in the lungs.

Dr. Roopa Biswas, an Associate Professor at the Uniformed Services University of the Health Sciences in collaboration with Dr. Sam Das from Johns Hopkins University, are developing a stem cell-based drug (exosomes) that would not only repair the CF disease phenotype but also attenuate inflammation in the airway by addressing a root problem of CF. Exosomes have emerged as novel regulators of cell-cell communication through release of RNA molecules, including microRNAs (miRNAs, miRs) that are key regulators of gene expression.

"Our goal is to transform this into the "personalized medicine" category in the treatment of pulmonary disorders using the same patient blood cells. This study will not only help elucidate the mechanisms of lung injury in CF, but also serve as a model for developing additional therapeutic strategies that can be extended to help patients suffering from related pulmonary disorders, such as COPD, and Asthma." - Dr. Roopa Biswas

Dr. Biswas aims to develop stem cell derived exosomes as therapeutics for CF. Her lab has previously analyzed miR expression in CF cells and demonstrated that the delivery of miR can rescue CFTR function and is, therefore, a candidate for therapeutic intervention. Importantly, exosomes are rich in miRs are stable, and can be administered by intravenous injection or non-invasively through aerosol-mediated delivery, like an Inhaler.

MSCRF

Development of Novel Stem Cell Therapies for Genetic Bone Disorders

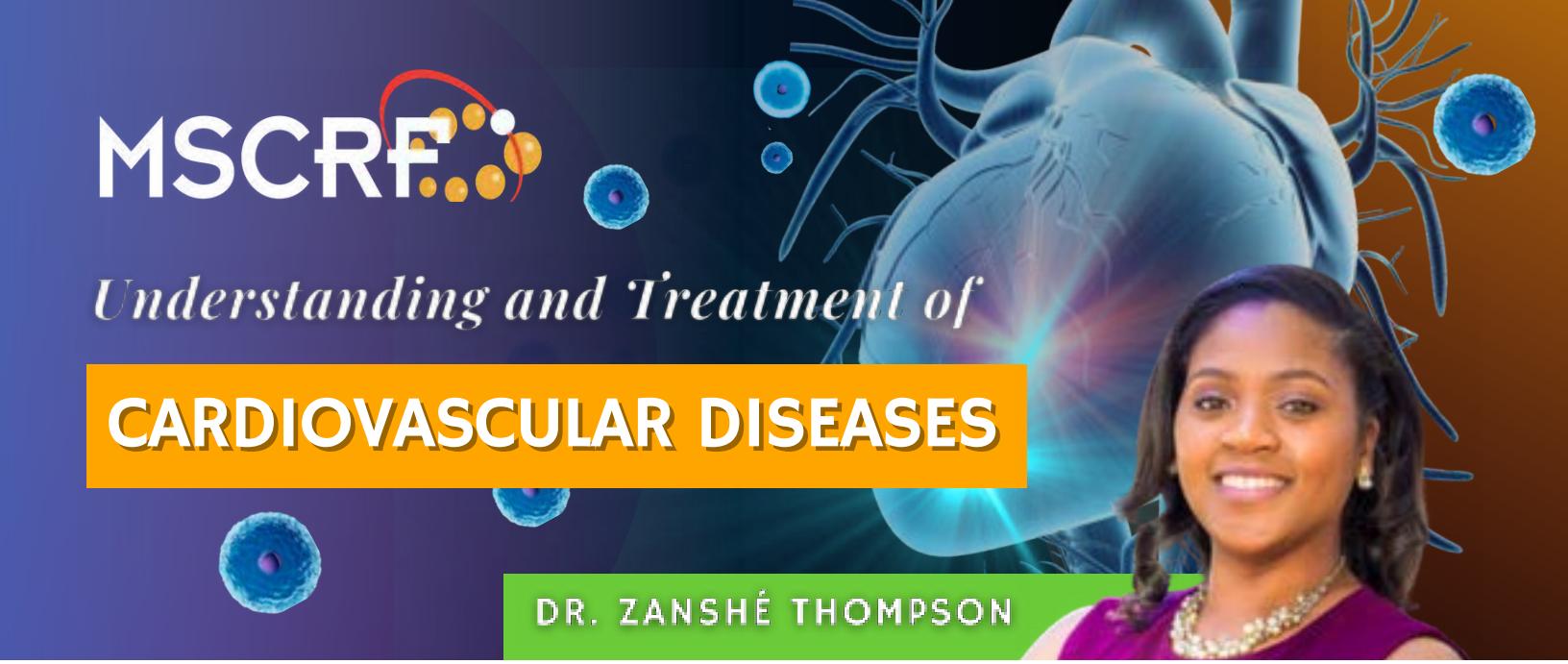
DR. SATORU OTSURU

Osteogenesis imperfecta (OI) is a genetic disorder that causes significant bone fragility. In addition to the fragility, patients suffering from OI exhibit impaired bone growth, which leads to short stature and, in severe cases, often fatal respiratory failure due to immature development of the chest cavity.

Currently, there is no effective treatment for growth deficiency, mainly because the underlying mechanism of OI growth deficiency is poorly understood. Thus, there is a critical need to develop therapies that can reliably stimulate longitudinal bone growth in patients with OI.

Dr. Satoru Otsuru, an Associate Professor of Orthopedics at the University of Maryland, studies the mechanism by which genetic mutations cause bone growth deficiency in patients suffering from OI. His recent work has shown that cell stress is responsible for the short stature in patients with OI. Given that different genetic mutations induce different types of cell stress, the optimal treatment may vary from patient to patient. Therefore, Dr. Satoru's goal is to develop a platform for precision medicine to improve bone growth in each OI patient.

In his current work, Dr. Satoru utilizes tissue-engineered models (growth plate organoids) generated from patients' stem cells to characterize cell stress and to screen various drug candidates for the development of effective treatments to improve bone growth in patients with OI.



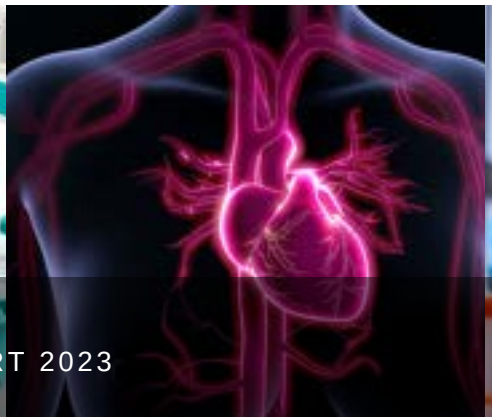
In a groundbreaking initiative, **Dr. Zanshé Thompson**, a postdoctoral fellow at Johns Hopkins University in the laboratory of Dr. Linda Resar, is delving into the intricate connection between clonal hematopoiesis (CH) and cardiovascular disease (CVD). Clonal hematopoiesis, a prevalent blood disorder affecting many people with age, involves mutations in blood stem cells leading to abnormal blood cell generation. This condition elevates the risk of various cardiovascular diseases such as strokes, heart attacks, and deep venous thromboses.

The research project focuses on unraveling the mysteries of CH-related CVD by studying the HMGA1 protein. Acting as a molecular key, HMGA1 unlocks the genome, activating genes associated with inflammation and stem cell function. Particularly, in a form of CH caused by a JAK2 gene mutation, the research team discovered HMGA1 as a crucial regulator of genes involved in inflammatory signaling and clonal expansion.

The hypothesis posits that HMGA1 significantly contributes to CVD development in CH by initiating inflammatory gene networks. To test this, innovative mouse models and technologies were established to dissect underlying gene networks and downstream inflammatory signals. The anticipated outcomes of the project include shedding light on the role of HMGA1 in CH-associated CVD and identifying therapeutic mechanisms that target HMGA1.

What makes this research particularly exciting is its potential to revolutionize the treatment of heart disease.
Dr. Thompson stated: '' Preliminary results indicate that HMGA1 plays a pivotal role in CH pathogenesis and associated inflammatory signals, offering a unique approach to treating CVD in individuals with CH.'

Thompson's groundbreaking work may reveal new ways to manipulate the bone marrow and/or blood stem cells to treat heart disease. By harnessing blood stem cells to counteract inflammatory signals in CH, this novel approach could transform how we address cardiovascular diseases in those with CH. The project not only seeks to deepen the understanding of adult stem cell function in CH but also lays the groundwork for potential applications in patients with clonal hematopoietic disorders.


Artificial Intelligence-Based Systems FOR STEM CELL BIOLOGY

DR. DANIEL LOBO

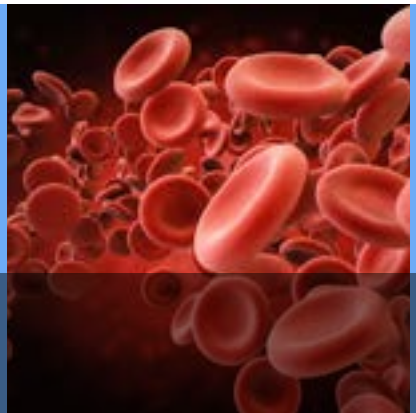
Human hematopoietic stem cells (HSCs) can differentiate into more than 10 different mature cell types to generate and sustain the complete blood-immune system at the outstanding rate of more than one million cells per second. However, the precise genetic regulatory mechanisms that control the biology of human hematopoietic stem cells are still not well understood. Single-cell transcriptomics analyses have revealed that hematopoiesis is continuous and dynamic; yet inferring a mechanistic understanding of its genetic regulation is a current challenge. Extrinsic factors, such as reactive oxygen species (ROS), can cause "stress hematopoiesis" and modulate HSC biology with limited understanding and consensus on the precise effects of ROS on HSC biology.

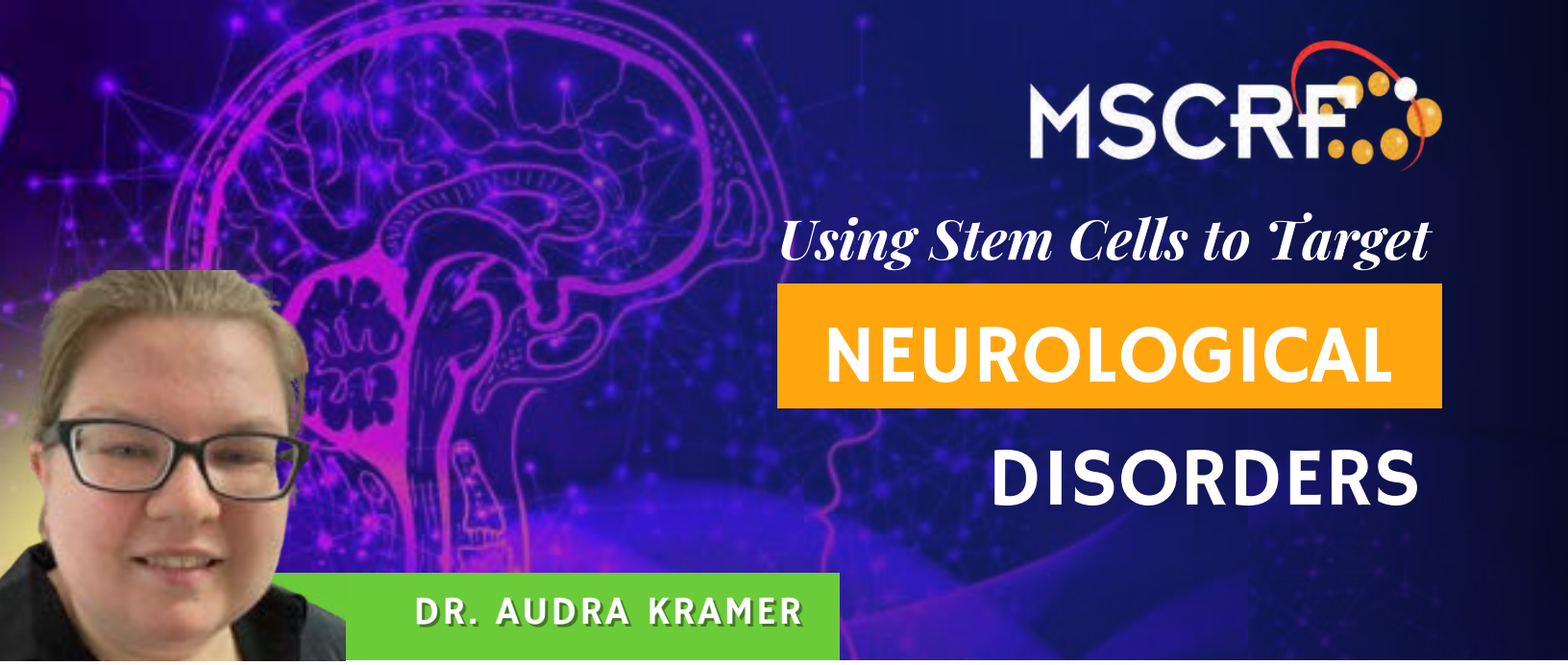
Thus, there is a critical need for advanced computational-molecular approaches that can extract gene regulatory mechanisms from dynamic cell populations and transcriptomic data towards a comprehensive understanding of human HSC biology, under both standard and stress conditions. Artificial Intelligence (AI) and Machine Learning (ML) will likely revolutionize human biology in the 21st century. Although Maryland is poised to be established as a significant hub for such innovation, this effort will require strong collaborations among basic, applied, and computational research.

The work led by **Dr. Daniel Lobo**, an Associate Professor at the University of Maryland, Baltimore County encompasses a collaboration between various institutes, bringing together an interdisciplinary team with clinical, genetics, human stem cell, computational, mathematical, and machine learning expertise.

"The Maryland Stem Cell Research Fund is enabling us to establish new interdisciplinary collaborations across Maryland institutions that will be essential for gaining a mechanistic understanding of human hematopoietic stem cell differentiation." - Dr. Daniel Lobo

Dr. Lobo and colleagues will use novel ML methodology to infer a mechanistic, predictive model of the population/differentiation dynamics of HSCs under normal and stress conditions. The novel computational methods and mechanistic knowledge to be gained in this project have the potential to significantly advance and promote the next generation of AI systems for stem cell biology and directly address the disconnect between computational and translational experts in Maryland.





Using Stem Cells to Target NEUROLOGICAL DISORDERS

DR. AUDRA KRAMER

The CAlcium voltage-gated ChaNnel subunit AIpha 1A (CACNA1A) gene provides instructions for making calcium channels. These channels, which transport calcium ions across cell membranes, play a key role in a cell's ability to generate and transmit electrical signals, primarily in nerve cells. Mutations in the CACNA1A gene cause a range of neurological and developmental disorders that are extremely challenging to treat, including epilepsy, periods of poor balance, rare and severe type of migraines that involves weakness or paralysis on one side of the body, atypical eye movements, developmental delays, and autism spectrum disorder. Currently, there are no treatments that directly target the calcium channel. One can make progress toward improving therapeutic interventions by studying how these mutations affect the calcium channel.

The research focus of **Dr. Audra Kramer**, a Post-doctoral Fellow in the lab of Dr. Ivy Dick at the University of Maryland, Baltimore, has been to elucidate the mechanisms underlying the normal and pathogenic regulation of calcium channel gating. To accomplish this, Dr. Kramer uses stem cells from individuals with specific CACNA1A mutations and induces them to differentiate into nerve cells.

"Thanks to the invaluable support of MSCRF and the CACNAIA Foundation, I am now equipped with the necessary resources and momentum to delve deeper into the unexplored territories of voltage-gated calcium channels. This support also serves as an acknowledgment of the significance of my research, validating the potential impact it may have on the future of medical science." - Dr. Audra Kramer

The utilization of patient-derived iPSCs to study unique mutations in calcium channelopathies will pave the way for personalized medicine to treat this rare disease. Each CACNA1A mutation patient presents differently in the clinic and the channel has biophysical properties that are difficult to predict. The product of her work will not only inform the future medical treatment for these patients but may also create the technology to study other ion channel mutations for related disorders.

Algae- Based Growth Factors for
REGENERATIVE MEDICINE

DR. JUN WANG

Growth factors (GFs) constitute a significant proportion of the expenses associated with stem cell culture. Among these, Basic Fibroblast Growth Factor (bFGF) is crucial for maintaining the undifferentiated and pluripotent state of stem cells. Unfortunately, its inherent instability, with a half-life of less than 9 hours, necessitates daily supplementation of fresh products, resulting in increased costs, labor demands, and the potential for contamination and batch failures.

In response to these challenges, **Dr. Jun Wang**, CEO of Phycin, and the Phycin team developed ebFGF (enhanced bFGF), a thermally stable variant of bFGF created through protein and genetic engineering in green algae. This groundbreaking solution not only enhances stability but also preserves the specific bioactivity of bFGF. Beyond ebFGF, Phycin aspires to revolutionize the regenerative medicine field by providing a comprehensive catalog of top-tier growth factors produced in algae, positioning Maryland as a key player in the transition from early discovery to approved therapies.

All algae-derived growth factors are devoid of animal components and bacterial endotoxins, making them not only cutting-edge but also cost-effective. Harnessing innovative algae technologies, Phycin has unlocked the potential of algae-derived growth factors, extending their impact beyond stem cell research into areas such as cultivated meat and seafood production, as well as regenerative medicine for wound management. This expansion drives transformative advancements in the biotechnology landscape.

"A few years ago, MSCRF funded Phycin to perform the proof of concept with the algae system. Today, MSCRF is funding us to market the first-ever algae-derived growth factor. This is a perfect example of how MSCRF nurtures a startup from inception to commercial success." - Dr. Jun Wang

The realization of Phycin's mission holds profound implications for Maryland's biotechnology sector, propelling scientific and technical progress while fostering economic growth. This success attracts investments, forges collaborations, and generates new opportunities that amplify revenue and job creation within the state. Phycin's steadfast commitment to delivering superior growth factors exemplifies its dedication to supporting the entire stem cell community and driving breakthroughs in regenerative medicine.

MSCRF

A New Method to treat

SCHIZOPHRENIA

WITH STEM CELLS

DR. JENNIFER ERWIN

In a pioneering initiative, **Dr. Jennifer Erwin** spearheads groundbreaking research exploring the therapeutic potential of the H3K4 methylation pathway for schizophrenia. This debilitating neuropsychiatric disorder, affecting approximately 1% of the global population, poses substantial challenges in understanding its mechanisms and developing effective treatments. Characterized by positive symptoms like hallucinations, and negative symptoms such as avolition, withdrawal, and cognitive dysfunction, schizophrenia's heterogeneous nature complicates the identification of precise drug targets and therapeutic interventions.

Dr. Erwin emphasizes the unique challenges in developing drugs for schizophrenia due to its heterogeneity, making it difficult to pinpoint specific drug targets and interventions. The research focuses on exploring neural gene regulation through histone methylation, a process implicated in the risk architecture of schizophrenia and other psychiatric and neurodevelopmental disorders. Notably, loss-of-function mutations in the histone H3K4 methyltransferase SETD1A significantly increase the risk of schizophrenia.

"Drug development for schizophrenia is challenging because the schizophrenic population is heterogeneous. It is difficult to identify a drug target and therapeutic intervention with a defined patient population and clinical trial outcome that are solidly linked to the mechanism of the intervention." - Dr. Jennifer Erwin.

The study's uniqueness lies in the use of induced pluripotent stem (iPS) cell models of well-defined patient populations, such as those with SETD1A mutations. This approach aims to explore the relevance of SETD1A and H3K4 methylation as a therapeutic target for adolescent or adult patients affected by schizophrenia. The research refines specific stem cell assays for drug development, providing valuable insights for the broader schizophrenic population.

Dr. Erwin's research project not only advances our understanding of schizophrenia but also underscores the potential for innovative drug development strategies. This pioneering work brings hope to individuals affected by schizophrenia and signifies a significant step forward in the quest for effective treatments.

Cell Based Therapy for
BONE REGENERATION

DR. AARON JAMES

Advancements in regenerative medicine are unlocking new potential for addressing bone defects through autologous stem cell therapies. The non-adipocytic cellular fraction within adipose tissue has long been celebrated for harboring multipotent progenitor cells crucial in skeletogenic processes. This understanding has driven the adoption of uncultured stromal vascular fraction (SVF) and culture-expanded stromal cells (ASC, adipose stromal/stem cells) in bone tissue engineering. Yet, recent clinical trials deploying adipose-derived cell therapies for bone tissue healing have faced challenges, prompting a pivotal realization within our group. The key to developing a translatable cell-based therapy for bone regeneration lies in identifying a more precisely defined osteoprogenitor subpopulation.

The MSCRF Discovery award enables **Dr. Aaron James** to delve into the 'black box' of the tunica adventitia and leverage this knowledge to enhance skeletal tissue regeneration. Dr. James' groundbreaking discoveries have uncovered an unexpected and functionally relevant cellular diversity within the outermost layer of blood vessels in human white subcutaneous adipose tissue.

The tunica adventitia, often overlooked and considered merely as collagen-rich sheath anchoring vessels, emerges as a crucial focus.

"To our knowledge, no other group has examined the developmental hierarchy within the adventitia, both within and outside a tissue engineering context. We hope that our continued use and reporting on these novel techniques will incite collaborations both within and outside Johns Hopkins University and increase the interest in the development of MSC subset-based regenerative medicine among local biotechnology companies within the State." - Dr. Aaron James

Contrary to previous understanding, his lab's recent work has illuminated that the majority of multipotent mesenchymal cells, including those crucial for vessel remodeling, reside in this stem cell niche. Importantly, adventitial cells can be separated into multiple subsets based on their 'stem' status, and the novel marker CD141 identifies an arterial-restricted population with primitive/undifferentiated cell characteristics.

Stem Cell-Derived Immune Cells for

CANCER THERAPY

DR. GILA IDLEMAN

Since its foundation in 1990, REPROCELL has become an integral part of the biotechnology and research community demonstrating a constant and evolving presence in Maryland. REPROCELL is a manufacturer and supplier of stem cells as well as products for stem cell research specializing in providing biologically and clinically relevant human tissue models for drug discovery and development. REPROCELL offers a range of products and services including biobanking, stem cell line generation and multilineage differentiation.

Using the company's cutting-edge technology and scientific expertise, **Dr. Gila Idleman**, a Stem Cell Scientist at REPROCELL, is developing stem cell-derived immune cells (natural killer [NK] cells) to target the promising and booming NK-CAR research and therapy field.

Using MSCRF funding, the company plans to accelerate its research and development efforts to provide critical support both locally for the small and midsized companies in Maryland and globally to become a one-stop shop for stem cell research.

"I am grateful to be a part of REPROCELL, a longstanding member of the scientific community in Maryland. With the support of MSCRF funding, we are currently advancing a novel research and marketing domain. The realms of NK research and NK-based therapy are continually expanding, and our goal is to provide products that foster the prosperous development of these fields, starting with our local community in Maryland and extending to the broader United States and international arenas," - Dr. Gila Idleman

These new products and services will answer the urgent need of laboratories for an enormous amount of NK cells to facilitate and accelerate the research and development of novel immunotherapies against various diseases, including cancer.

MSCRF

Stem Cells Generated Human Brain Organoids for RARE PEDIATRIC NEUROLOGIC DISEASE

DR. CHRISTINA NEMETH MERTZ

Leukoencephalopathy with Brainstem and Spinal cord involvement and Lactate elevation (LBSL) is a rare pediatric neurological disorder caused by genetic mutations that affect the brain and spinal cord. The disease typically develops early in childhood causing movement problems, abnormal muscle stiffness and difficulty with coordinating movements. In addition, affected individuals lose the ability to sense the position of their limbs or vibrations with their limbs.

Although the patient phenotype is characterized by brain and spinal cord demyelination, little is known about the role or contribution of oligodendrocytes to disease progression, the cells that produce and maintain myelin, the protective sheath of nerve cells.

Dr. Christina Nemeth Mertz, an Assistant Professor at Hugo W. Moser Research Institute at Kennedy Krieger is creating a quantifiable and reproducible model of LBSL that is scalable and appropriate for use in large-scale drug screens.

Her project aims to generate and characterize myelinating human brain organoids (BOs) to model LBSL. The generation of functional BOs can be used in high-throughput drug screens and can be tested using human-specific therapies, some of which have already been developed in her lab.

This model may also provide a rubric from which other rare pediatric diseases may be modeled, providing a method in which highly reproducible, scalable, and accurate disease models can be created and tested to combat these devastating genetic disorders.

Skeletal Muscle Stem Cells Based System for

TARGETING DIABETES

DR. HYESOO KIM

Diabetes, a chronic medical condition affecting the body's ability to process blood sugar, has emerged as a significant global health concern. According to recent reports from leading health organizations, the prevalence of diabetes has reached alarming levels, impacting millions of lives across the globe.

The World Health Organization (WHO) estimates that, as of 2023, approximately 10% of the global population is living with diabetes. This represents a substantial increase over the past decade, underscoring the urgent need for comprehensive strategies to address and manage the disease. In Maryland, diabetes remains a significant public health concern, affecting a substantial portion of the population.

The state grapples with the dual challenges of rising prevalence and the associated health and economic burdens of diabetes. The condition is known to disproportionately impact minority populations, emphasizing the importance of targeted interventions.

Dr. Hyesoo Kim, an Assistant Professor of Neurology at Johns Hopkins University, is developing a new skeletal muscle 'Gene+Cell' therapy platform to target diabetes. Skeletal muscle is the largest tissue in the body, comprising between 40-50% of body mass.

Skeletal muscle is capable of sensing and adapting to metabolic and physiologic changes and is the major site of glucose disposal after a meal, taking up 60-70% of circulating glucose in an insulin-dependent manner, making it an important tissue in normal glucose homeostasis. Her lab's insulin-expressing skeletal muscle stem cell-based system can be an alternative and meaningful strategy for combating diabetes.

MSCRF

PUBLIC Engagement

Supporting the Next Frontiers of Medicine

MSCRF plays a vital role in community engagement, actively fostering connections and collaboration within the vibrant landscape of regenerative medicine. Through initiatives like workshops and panel discussions, MSCRF creates dynamic platforms for stakeholders, researchers, and portfolio companies to come together, exchange ideas, and explore collaborative opportunities. MSCRF actively engages with Maryland legislators providing updates on the progress made in regenerative medicine due to state funding. Through such multiple engagements, MSCRF catalyzes forging meaningful connections contributing consistently to building a strong and interconnected community. Here are some of the highlights of this year.

Inaugural Felicitation Ceremony for Fiscal Year 2023 MSCRF awardees

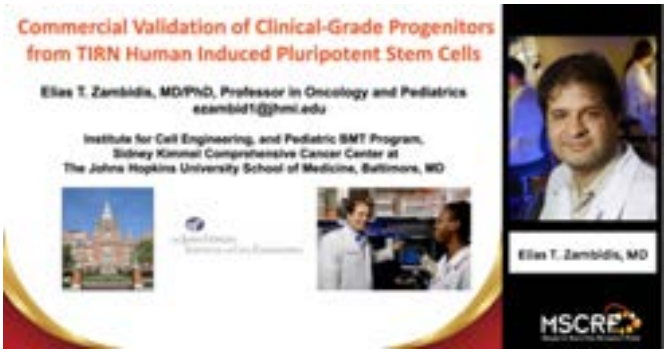
MSCRF held a highly successful event on June 8, 2023, to honor the exceptional achievements of its awardees. The inaugural Felicitation Ceremony celebrated the scientists from Maryland's research institutions and companies who received grants in Fiscal Year 2023, recognizing their significant contributions to advancing stem cell research. The gathering, attended by awardees from all seven grant programs, facilitated vibrant exchanges, connections, and collaborative opportunities. The Felicitation Ceremony not only acknowledged achievements but also cultivated unity and collaboration within the stem cell research community in Maryland

MSCRF Showcases Awardees' Impactful "Bed to Bedside" Work in a Global Event

MSCRF proudly engaged in the renowned ISSCR annual meeting in Boston from June 14 to June 17, 2023, contributing to the dynamic exchange of scientific insights in stem cell research. MSCRF orchestrated the "Bench to Bedside" session, highlighting the impactful outcomes of its funding.

This exclusive platform allowed MSCRF awardees to showcase groundbreaking work, spanning from discovery to clinical applications with biomanufacturing support. The presented research underscored the substantial progress in regenerative medicine. With over 4,000 global participants, the ISSCR annual meeting provided a fertile ground for MSCRF to promote its funding opportunities, programs, and mission.

The active involvement in this global event reinforces MSCRF's dedication to fostering excellence in stem cell science and its applications for enhancing human health

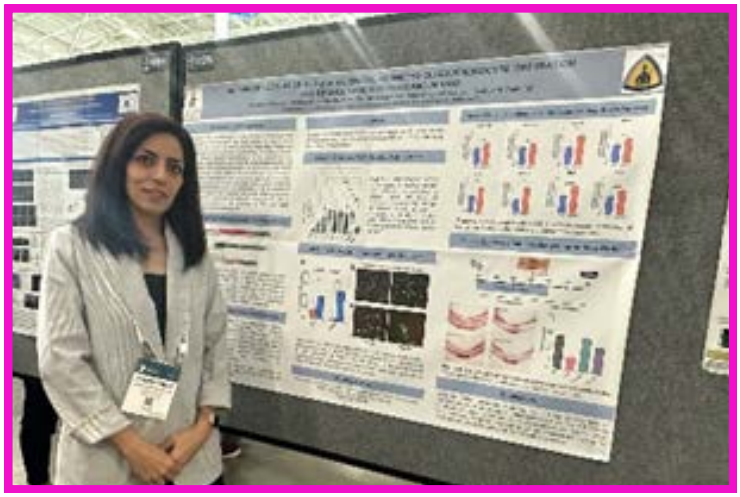


MSCRF's Insightful Presence at ARM Workshop: Shaping Tissue Engineering Discourse

MSCRF played a key role at the Alliance for Regenerative Medicine (ARM) workshop on September 6, 2023, where our Executive Director, Dr. Ruchika Nijhara, provided valuable insights in a panel discussion. Focused on Tissue Engineering and Therapeutics, this workshop marked ARM's inaugural event dedicated to advancing this rapidly evolving field within regenerative medicine.

Our active participation showcased our commitment to influencing discussions on tissue engineering trends and boosting the fundability of research in this crucial domain. The workshop, uniting stakeholders from the tissue-engineering industry, facilitated discussions on cutting-edge advances, manufacturing challenges, regulatory considerations, and funding/partnership opportunities. This engagement not only allowed us to share guidance but also served as a dynamic platform for MSCRF portfolio companies to exchange ideas and explore collaborative opportunities within the vibrant tissue-engineering community.

Expanding Reach in Scientific Endeavors

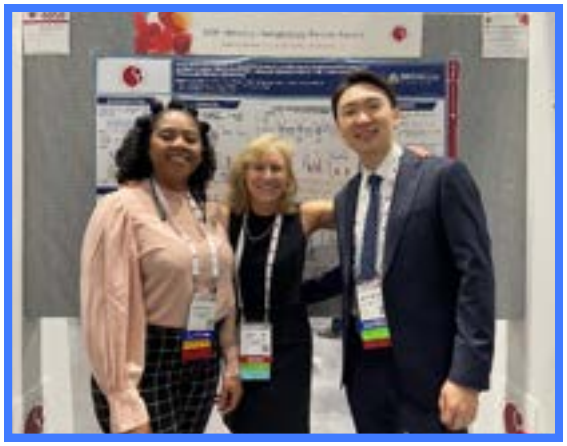


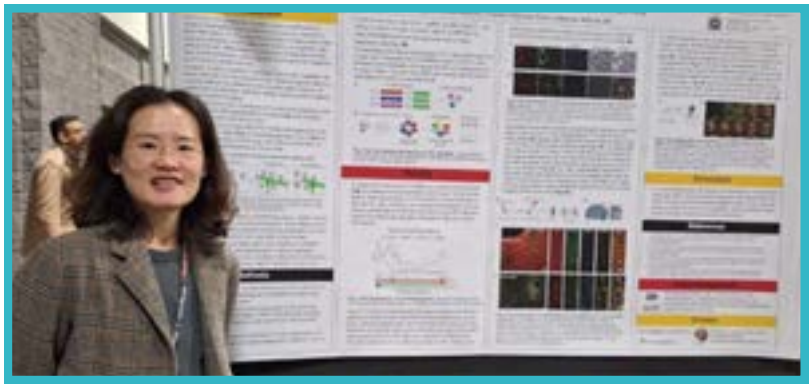
Scientific conferences serve as crucial platforms for researchers to showcase their work, receive mentorship, and foster collaborations. Unfortunately, the associated costs can be a significant obstacle, especially for early-career scientists. MSCRF has played a pivotal role in overcoming this barrier, enabling scientists from various backgrounds to attend conferences.

This support not only facilitates the sharing of cutting-edge research but also promotes global collaboration among emerging scientific leaders.

MSCRF REACHES OUT: Strengthening the Ecosystem

MSCRF REACHES OUT: Strengthening the Ecosystem





Meetings with Maryland Lawmakers

Meetings with Maryland State Legislatures: Governor, Wes Moore, Lieutenant Governor, Aruna Miller and Secretary of State, Susan Lee. MSCRF says **"THANK YOU FOR YOUR SUPPORT"**

MSCRF

FUNDED
Grant Awards

Supporting the Next Frontiers of Medicine

50 MSCRF AWARDS

COMMERCIALIZATION:
Peter Andersen, PhD | Vita Therapeutics, Inc.
Gila Idelman, PhD | Reprocell, Inc.
Rachana Mishra, PhD | Secretome Therapeutics, Inc.
Bhanu Telugu, PhD | RenOVAte Biosciences Inc
Jun Wang, PhD | Phycin, Inc.

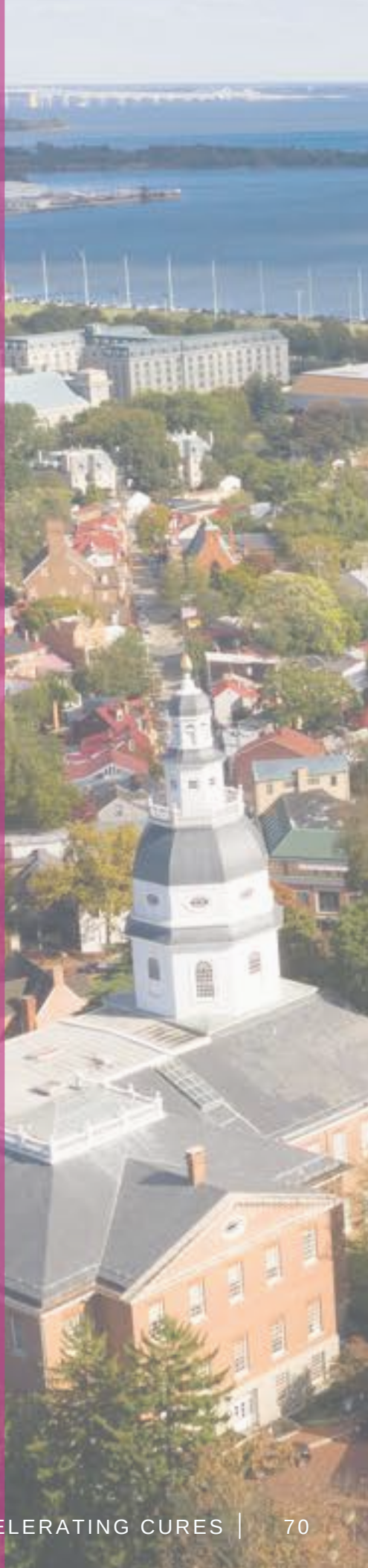
MANUFACTURING ASSISTANCE:
Luis Alvarez, PhD | Theradaptive, Inc.
Boro Dropulic, PhD, MBA | Caring Cross, Inc.
Sagi Nahum | Octomera, Inc.
Jonathan Rowley, PhD | RoosterBio, Inc.

VALIDATION:
Sheikh Amer Riazuddin, PhD | Johns Hopkins University
Elias Zambidis, MD, PhD | Johns Hopkins University

LAUNCH:
Roopa Biswas, PhD | The Geneva Foundation
Jennifer Erwin, PhD | Lieber Institute for Brain Development
Luo Gu, PhD | Johns Hopkins University
Xiaoning Han, PhD | Johns Hopkins University
Sangmoo Jeong, PhD, MS | Johns Hopkins University
Alexander Ksendzovsky, MD, PhD | Johns Hopkins University
Daniel Lobo, PhD | University of Maryland, Baltimore County
Christina Mertz, PhD | Hugo Moser Research Institute at Kennedy Krieger
Mark Ranek, PhD | Johns Hopkins University
Ali Shakeri-Zadeh, PhD | Johns Hopkins University

DISCOVERY:
Kenneth Boheler, PhD | Johns Hopkins University
Jeff W.M. Bulte, PhD, MS | Johns Hopkins University
Hee Cheol Cho, PhD | Johns Hopkins University
Curt Civin, MD | University of Maryland, Baltimore
Hariharan P. Easwaran, PhD, MS | Johns Hopkins University
Ricardo A. Feldman, PhD | University of Maryland, Baltimore
Alan David Friedman, MD | Johns Hopkins University
Warren L. Grayson, PhD | Johns Hopkins University
Maged Harraz, MB,BCh, MS, PhD | University of Maryland, Baltimore
Norman James Haughey, PhD | Johns Hopkins University
Aaron Watkins James, MD, PhD | Johns Hopkins University
Steven Jay, PhD | University of Maryland, College Park
Hyesoo Kim, DVM, PhD | Johns Hopkins University
Chulan Kwon, PhD, MS | Johns Hopkins University
Gabsang Lee, PhD | Johns Hopkins University
Nicholas John Maragakis, MD | Johns Hopkins University
Satoru Otsuru, MD, PhD | University of Maryland, Baltimore
Linda Smith Resar, MD | Johns Hopkins University
Lena Smirnova, PhD | Johns Hopkins University

POST-DOCTORAL FELLOWSHIP:
Mansoureh Barzegar, PhD | Johns Hopkins University
Arthur Feltrin, PhD | Lieber Institute for Brain Development
Audra Kramer, PhD | University of Maryland, Baltimore
Manisha Kumari, PhD | Johns Hopkins University
Shuya Li, PhD | Johns Hopkins University
Alejandra Romero Morales, PhD | Lieber Institute for Brain Development
Joe Kodama, PhD | University of Maryland, Baltimore
Sean Murphy, PhD | Johns Hopkins University
Milton Roy, PhD | Johns Hopkins University
Alejandra Romero Morales, PhD | Johns Hopkins University
Hongjun Zhang, PhD | Johns Hopkins University



MSCRF

COMMERCIALIZATION

GRANT AWARDS

2023

Peter Andersen, PhD

Vita Therapeutics, Inc.
Awardee Amount: $400,000
Disease Target: Solid Cancer

Gila Idelman, PhD

Reprocell, USA.
Awardee Amount: $373,657
Disease Target: Cancer

Developing a Hematopoietic Stem Cell-Derived Myeloid Cell Therapy for Solid Cancer

Cancer is the second leading cause of death in the US, with solid cancer accounting for nearly 90% of all cases. Despite success in treating hematological cancers, chimeric antigen receptor (CAR) T-cell therapy has not yet demonstrated any major success in treating solid cancer. Consequently, there is a high demand to develop effective cell therapies that are capable of overcoming the unique hurdles posed by solid cancer, including the lack of tumor penetration by CAR-T cells, an immunosuppressive tumor microenvironment (TME), and inherent tumor cell heterogeneity. Compared to CAR-T cells, myeloid cells have a natural ability to penetrate solid tumors and are the most abundant immune cells in the TME. Vita Therapeutics is developing a novel hematopoietic stem cell-derived and genetically modified myeloid cell therapy to treat solid cancer using two approaches: 1) engineering myeloid cells to be deficient of the inhibitory NFB1 (p50) subunit which diminishes formation of myeloid derived suppressor cells (MDSCs) and locks the myeloid cell fate into becoming pro-inflammatory, tumor targeting M1-type macrophages, and 2) expressing a CAR-like Immune Receptor (CARIR) that outcompetes with endogenous inhibitory PD1 receptor and triggers anti-tumor functionality upon interacting with tumor cells that overexpress PD-L1. Using syngeneic mice with an established solid tumor, Vita has demonstrated that the murine analogue of either p50 knockout immature myeloid cells (p50-/--IMCs) or CARIR-expressing immature myeloid cells (CARIR-IMCs) significantly slows tumor growth and prolongs survival. The current proposal aims to further validate Vitas myeloid cell therapy approach on PD-L1+ solid cancer with quantifiable milestones. In the specific Milestones 1, we are going to comprehensively characterize our IMC cell products. In the specific Milestones 2, we will validate the anti-tumor efficacy of p50-/--CARIR-IMC in vivo using both syngeneic and xenograft tumor mice models, with potential mechanism of action examined.

Generation of Immortalized Lymphoid Progenitors and Ready to Use NK Cells from Human Induced Pluripotent Stem Cell (hiPSCs)

NK cells have recently gained great attention due to their safety in clinical use and unique mechanism of target cell recognition. However, their usage relies on the availability of substantial numbers of cells with optimal cytotoxic activity. Currently the main sources of NK cells are peripheral blood (PB), umbilical cord blood (UCB), and invitro cell lines. NK cells isolated from PB have the advantage of safety and high cytotoxicity but show low purity and proliferation. UCB-derived NK cells can be expanded with acceptable clinical outcomes but carry the risk of incomplete differentiation, lethality, and tumorigenicity. A major disadvantage of some invitro cultured NK lines is their aneuploidy, as these must be irradiated prior to being administered to patients. In contrast, hiPSC-derived NK cells are homogenous and can be generated in sufficient cell numbers for allogeneic clinical use. To generate considerable amounts of mature hiPSC-derived NK cells, a large amount of CD34+ progenitors are needed, as hiPSC-derived NK cells have a limited ability to proliferate. REPROCELL is a manufacturer and supplier of cells and products for stem cell research specializing in providing biologically and clinically relevant human tissue models for drug discovery and development. REPROCELL offers a range of products and services including biobanking, iPSC line generation and multilineage differentiation. Using our cutting-edge technology and scientific expertise, we propose to expand REPROCELLs current portfolio to include immortalized hiPSC-derived hematopoietic and lymphoid progenitors, along with mature NK cells showing extended proliferative capacity. Furthermore, we will offer a service to generate immortalized NK cells from client provided tissues. These new products and services will answer the urgent need of laboratories for an enormous amount of NK cells to facilitate and accelerate the research and development of novel CAR-NK immunotherapies against cancer.

Rachana Mishra, PhD

Secretome Therapeutics, Inc.
Awardee Amount: $400,000
Disease Target: Dilated Cardiomyopathy
(2024 1st Funding Cycle)

Bhanu Telugu, PhD

RenOVAte Biosciences, Inc.
Awardee Amount: $396,0000
Disease Target: Liver Insufficiency
(2024 1st Funding Cycle)

Production of Neonatal Cardiac Mesenchymal Stem Cells for Treating Dilated Cardiomyopathy

Dilated cardiomyopathy (DCM) is a clinical diagnosis characterized by left ventricular dilatation and systolic dysfunction which results in decreased left ventricular ejection fraction. The prevalence of DCM in the U.S. is 1:250-400, equaling a population of roughly one million patients. Current pharmacological agents and medical devices are modestly effective, and heart transplantation remains the only definitive therapy. Mesenchymal stem cells (MSC) derived from adult human donors have shown substantial promise in clinical studies in DCM but have suffered from low and variable potency. In comparison, neonatal heart-derived cardiac mesenchymal stem cells (nMSCs) have proven significantly more potent in animal models of heart failure compared to all clinically-relevant, adult human-derived MSCs. Our proprietary product STM-01 is an allogenic culture-expanded formulation of nMSCs, and is already approved by FDA for a Phase I/IIb clinical trial in heart failure (IND #27548). Our hypothesis is that nMSCs will improve left ventricular function more and more consistently than adult MSCs have in patients with DCM given their greater potency in reducing inflammation, promoting immunomodulation, and decreasing fibrosis. The overarching goal of our application is to manufacture clinical-grade STM-01 drug product under Good Manufacturing Practice (GMP) standards to administer to patients with DCM in a fully-funded Phase I multiple-ascending dose study. Specific Aim 1: Generation of parental cell bank (PCB) of nMSCs and comparison of donor-to-donor reproducibility. Specific Aim 2: Support transfer of manufacturing to a good manufacturing practices (cGMP)-certified contract organization for scale-up and production of a master cell bank (MCB) from parental cell bank. Specific Aim 3: Production of a working cell bank (WCB) that meets cGMP standards and QC testing for release. Expected Outcomes: By the end of the grant, Secretome Therapeutics will generate a fully qualified STM-01 for use in an FDA-approved Phase I/II clinical trial in patients with DCM.

Growing Human Liver in Pigs

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

Jun Wang, PhD

Phycin, Inc.
Awardee Amount: $400,000
Disease Target: General Regenerative Medicine
(2024 1st Funding Cycle)

Commercialization of an Affordable, Robust, and Biosafe bFGF Produced in Algae

Growth factors (GFs) drive a significant portion of the costs associated with stem cell culturing. Phycin presents an innovative solution to substantially reduce these expenses by leveraging the efficient expression of proteins in green algae within an Animal Origin Free (AOF) production system. Unlike conventional platforms like CHO cells and bacteria, algae offer enhanced safety as they do not produce endotoxins or harbor human pathogens. Among the GFs commonly used in stem cell culture, Basic Fibroblast Growth Factor (bFGF) plays a pivotal role in maintaining the undifferentiated and pluripotent state of stem cells. Unfortunately, the inherent instability of wildtype bFGF at 37C, with a half-life of less than 9 hours, necessitates daily supplementation of fresh product. This practice drives up costs, increases labor requirements, and poses the risk of contamination and batch failures. To overcome these challenges, Phycin has developed a thermally stable variant of bFGF called ebFGF (enhanced bFGF), generated through protein and genetic engineering in green algae. This innovative solution preserves the specific bioactivity of the protein without compromising its functionality (patent pending). By establishing a scaled-up production system centered around ebFGF as the initial product, Phycin aims to facilitate the commercial production of other widely used, cost-effective GFs, such as TGF[B]-1 family proteins. Phycin's commercialization strategy commences with the production of a research-grade version of stable ebFGF. This involves iterative design optimization, scaling up the production process, and generating prototype samples. Once customers validate the product's biosafety and potency, Phycin will proceed to manufacture optimized ebFGF in a cGMP quality system, ensuring compliance with rigorous quality standards for customer applications. Through this strategic approach, Phycin aims to revolutionize the production of GFs for stem cell culture, enabling cost reduction and improved reliability in a range of applications.

Maryland stands as a prominent biotechnology hub, leading the charge in regenerative medicine with its array of stem cell-focused companies and research institutions, and Phycin aims to revolutionize the field by supplying top-tier growth factors, positioning Maryland as an influential player in the journey from discovery to disease cure. Leveraging innovative algal technologies, Phycin has harnessed the potential of algae-derived growth factors, extending their impact beyond stem cell research into domains like cosmetics, cultivated meat and seafood production, and regenerative medicine for wound management, driving transformative advancements in the biotechnology landscape. The successful realization of Phycin's mission will yield profound effects on Maryland's biotechnology sector, propelling scientific and technical progress while fostering economic growth, attracting investments, forging collaborations, and generating new opportunities that amplify revenue and job creation within the state. Phycin's unwavering commitment to delivering superior growth factors epitomizes their dedication to supporting the entire stem cell community and propelling breakthroughs in regenerative medicine, ultimately solidifying Maryland as an innovation hub of unrivaled excellence in biotechnology, leaving an enduring imprint by advancing scientific knowledge and revolutionizing lives through the development of pioneering treatments and therapies.

MSCRF

MANUFACTURING ASSISTANCE

GRANT AWARDS

2023

Luis Alvarez, PhD

Theradaptive, Inc.
Awardee Amount: $1,000,000
Disease Target: Spine Injury

Boro Dropulic, PhD, MBA
Sagi Nahum

Caring Cross & Octomera .
Awardee Amount: $1,000,000
Disease Target: Leukemia and Lymphoma

Theradaptive GMP OsteoAdapt Manufacturing Facility 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) 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 late 2023 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 will build a GMP manufacturing facility for OsteoAdapt at our Frederick headquarters. Additionally, we are establishing a qualified Quality Control analytical testing laboratory 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.

Mobile Cleanroom-Based Manufacturing of Genetically Engineered Cellular Products

Caring Cross was founded to enable access to affordable gene medicines. Specifically, genetically engineered cell products that are curative for human disease, such as lymphocytes engineered with chimeric antigen receptors (CAR-T) and stem cells engineered to overcome inherited disorders such as sickle cell disease. Access to these therapies, especially for underserved populations, is directly related to cost. Costs can be brought down through local manufacturing. A local or place-of-care manufacturing approach requires a standardized manufacturing platform featuring regulated cleanroom space, uniform equipment, and established workflows. Such an environment can be created in a mobile cleanroom, as enabled by Orgenesis, Inc. Caring Cross has expertise in the creation of high impact CAR-T cells. A Caring Cross vector specific for HIV is currently in phase I clinical trials. Caring Cross has also developed a next generation CAR-T cell technology applicable to leukemia and lymphoma, expressing three specificities at the same time CD19, CD20, and CD22. By combining a mobile cleanroom shell with workflows developed by Caring Cross, a standardized cell manufacturing facility that could be replicated throughout the state would be enabled. Moreover, it would establish both organizations as global leaders in the design and manufacture of cell engineering facilities. Manufacturing of a cell therapy product begins with entry of a patient-derived cell product into the facility, purification of target cells, activation preparation of the cells for transduction with a gene vector, and subsequent culture steps to expand the engineered cell product. Finally, quality and safety testing must be carried out prior to infusion back into the patient. In this application we will use a CAR-T vector designed by Caring Cross to demonstrate effective workflows in a mobile cleanroom environment, the Orgenesis OMPUL, thus enabling manufacturing of a high impact cell therapy in a standardized facility.

Jonathan Rowley, PhD

RoosterBio, Inc.

Awardee Amount: $866,550

Disease Target: N/A

Development of a Closed Extracellular Vesicle (EV) / Exosome Drug Substance Product Manufacturing Process without Sterile Filtration

Extracellular vesicles (EVs) and exosomes are lipid bilayer membrane bound particles released from cells. Research shows that EVs derived from human mesenchymal stem/stromal cells (hMSC-EVs) can be used for therapeutic applications. Specifically, hMSC-EVs have been studied in over 200 preclinical applications and dozens of clinical trials. A major upstream bottleneck for hMSC-EV therapies is the manufacturing requirement for a large number of cells to produce a clinically relevant hMSC-EV dose. RoosterBio is a leader in rapid hMSC expansion, allowing us to move beyond this bottleneck and solve new challenges in hMSC-EV manufacturing. A major downstream challenge remains: high product cost and low supply due to poor yields (often <5%) given the use of 0.2 micron membrane filtration. This common practice ensures sterility but excludes the majority of EVs > 0.1 microns from doses. Many of these EVs are potent and so could be included in the product if 0.2 micron filters could be avoided.

This is only possible with a fully closed (aseptic) manufacturing process, which has existed for several years for cell therapies, but not for soluble product species requiring high purities (e.g. proteins, mabs, viral vectors) and so manufacturing processes with several unit operations. Such biologics might employ depth and membrane filtration for clarification, Tangential-Flow Filtration (TFF) for concentration and buffer exchange, and chromatography for purification. The imperative to minimize the risk of microbial contamination and decrease the cost of establishing manufacturing facilities has led to the widespread adoption of single-use plastic consumables (e.g. bioreactor bags, tubing, filters, membranes, etc.). Only recently have commercial products become available that enable a closed manufacturing process from the production bioreactor to drug substance/product: Cytiva's AKTA Ready chromatography and readyflux TFF systems. This proposal will incorporate these systems to develop a closed EV manufacturing process without 0.2 micron filtration, thereby maximizing yield.

MSCRF

VALIDATION

GRANT AWARDS

2023

Sheikh Amer Riazuddin, PhD

Johns Hopkins University
Awardee Amount: $229,990
Disease Target: Corneal Endothelial Dysfunction
(2024 1st Funding Cycle)

Elias Zambidis, MD, PhD

Johns Hopkins University
Awardee Amount: $250,000
Disease Target: Immunotherapies
(2024 1st Funding Cycle)

Validating the Utility of Pluripotent Stem Cell-Derived Corneal Endothelial Cells for Regulatory Approval by FDA

Cornea is the outermost, transparent tissue of the eye with corneal endothelium (CE) being the innermost layer essential for maintaining corneal clarity. Corneal endothelial dystrophies are the leading cause of corneal transplantation performed in the United States. Although keratoplasty has been successful in treating corneal edema, the worldwide shortage of transplantable-grade donor CE remains an insurmountable obstacle in reducing corneal blindness. We previously reported (Ali et al., 2021) that injected hESC-derived CECs can form a functional CE on denuded Descemets membrane (DM) in rabbits and monkeys (mimicking treatment for a mild phenotype). We have now further confirmed that hESC-derived CECs and hESC-derived CEC sheets can form a functional CE and DM on denuded stroma in rabbits and monkeys (mimicking treatment for a moderate-to-severe disease where the pathology extends beyond the CE but extends to DM). We recently had a pre-IND (Investigational New Drug) meeting with the FDA. The meeting package was reviewed by FDAs Center for Biologic Evaluation and Research (CBER), and Center for Drug Evaluation and Research (CDER). Advisors from both centers comprehensively responded to our queries and provided much-valued suggestions for the completion of the IND application. We have successfully addressed many concerns of the FDA, especially those relating to the production and testing of hESC-derived CECs, and the genotoxicity of Rho Kinase (ROCK) inhibitor, Y-27632.. However, there are still a few parameters in our pre-clinical studies that need validation as per the recommendations of the FDA. These include, 1) to validate the genomic stability of hESC-derived CECs following differentiation, 2) to validate a two-arm pre-clinical study exploring the escalating doses of hESC-derived CECs, and 3) to validate the distribution of injected hESC-derived CECs in ocular and non-ocular tissues. The completion of these studies will help complete the IND application seeking FDAs approval for a human clinical trial.

Validation of MoroPLUR TIRN-hiPSC for Generating Animals with Human Lympho-Hematopoietic Systems

We have derived a new class of human stem cells termed Tankyrase/PARP (poly (ADP-ribose) polymerase) inhibitor-regulated nave (TIRN) human induced pluripotent stem cells (hiPSC). PARP-regulated proteogenomic reprograming of primed, conventional hPSC with the TIRN method generates human stem cells that are enriched in expression of pioneer transcription factors that activate and sustain the eight-cell and cleavage human embryonic stages. TIRN-hiPSC possess dramatically high differentiation performance; including a functional capacity to contribute differentiating human cells into developing mouse embryos. In this project, we will forge an academic-commercial collaboration with RenOVAte Biosciences, Inc. to exploit TIRN-hiPSC to generate interspecies chimeric SCID pigs with fully functional human immune systems. We propose to commercially validate our patent-pending TIRN-hiPSC technology for this goal by first generating mice with fully humanized immune systems in a novel interspecies blastocyst complementation (IBC) approach in mutant SCID mice that are incapable of generating endogenous hematolymphoid tissues. Once licensing agreements with RenOVAte are in place for the TIRN method, we will collaborate with RenOVAte to similarly test the human lympho-hematopoietic chimera potential of TIRN-hiPSC following injection into SCID pig blastocysts derived with their proprietary methods. Validation of TIRN-hiPSC technology in mice will also further determine the commercial feasibility of generating pigs with patient-specific human organs and tissues for clinical transplantation with our partners at RenOVAte. If successful, commercially available TIRN-hiPSC-derived mice and pigs with comprehensive human lymphohematopoietic systems will be a disruptive technology that will enable the research community to utilize a more reliable pre-clinical testing of vaccines, and cancer immunotherapies that were previously hampered by a lack of reliable animal models for testing human immune responses.

MSCRF

LAUNCH

GRANT AWARDS

2023

Roopa Biswas, PhD

The Geneva Foundation
Awardee Amount: $350,000
Disease Target: Cystic Fibrosis

Jennifer Erwin, PhD

Lieber Institute for Brain Development
Awardee Amount: $349,633
Disease Target: Schizophrenia

Modulation of Rescue Competent MicroRNAs in Cystic Fibrosis Lungs

Induced pluripotent stem cells (iPSC) secrete extracellular vesicles (EV) known as exosomes. Exosomes have emerged as novel regulators of cell-cell communication through release of RNA molecules, including microRNAs (miRNAs, miRs) that are key regulators of gene expression. The expression of specific miRNAs is altered in many diseases including Cystic Fibrosis (CF). CF is a life-limiting, pro-inflammatory genetic disorder due to mutations in the Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) gene. CF is associated with failure of the mutant F508del-CFTR to traffic to the plasma membrane, with concomitant loss of cAMP-activated chloride conductance, and hypersecretion of pro-inflammatory chemokine IL-8, leading to progressive lung damage. To identify novel miRNA-based anti-inflammatory therapeutic targets for CF, we have previously analyzed miR expression in CF cells compared to controls, both in culture and in ex vivo lung biopsies. Our studies have demonstrated that the delivery of miR-16 can rescue F508del-CFTR function and is therefore a candidate for therapeutic intervention. Our goal is to modulate the expression of miRNAs in CF lungs in order to repair the disease phenotype using human iPSC-derived exosomes (hiPSC-exo). Interestingly, human iPSC-exo express significant endogenous levels of miR-16. Our preliminary data also demonstrated that hiPSC-exo can deliver its cargo into CF lung epithelial cells. Thus, we hypothesize that delivery of hiPSC-Exo containing rescue-competent miR-16 will enhance gene transfer to CF lungs and provide novel therapeutics for CF. 1. We will determine the functional efficacy of delivering rescue-competent miR-16 through hiPSC-derived exosomes to CF lung epithelial cells. We will deliver hiPSC-exo to CF lung epithelial cells. We will use RNA deep sequencing, qPCR, and multiplex ELISA to analyze the alteration in gene expression profile (including inflammatory genes as well as CFTR) in these cells. We will validate potential CF-specific targets of miR-16 in CF lung epithelial cells (CFBE410-) and perform functional analyses in cultures of primary CF HBE systems. Identifying novel target mRNAs will not only help understand miR-mediated regulation of CF but will also lead to novel candidates for therapeutic intervention of CF. . We will deploy rescue-competent miR-16 to in vivo CF mouse models and repair CF lung disease. We will deliver hiPSC-Exo to the lungs of chronically infected F508del-CFTR mice. F508del-CFTR CF mouse models challenged with Pseudomonas aeruginosa will be administered with hiPSC-exo via retro-orbital injection. Subsequently, we will analyze the in vivo functional effect of miR-16 expression on inflammatory disease phenotype as well as F508del-CFTR function in these animals. Successful completion of the proposed experiments will lead to the development of novel hiPSC-based therapeutic strategies for CF and related pulmonary disorders.

Investigating H3K4 Methylation Pathway Targets in Human Stem Cell Models of Schizophrenia and SETD1A Haploinsufficiency

Neural gene regulation by histone methylation plays an important role in the risk architecture of schizophrenia, psychiatric disorders and neurodevelopmental disorders more broadly. Loss-of-function mutations in the histone H3K4 methyltransferase SETD1A confer a large increase in disease risk for schizophrenia. More broadly, histone methylation showed the strongest statistical enrichment of biological pathways in genome-wide association study (GWAS) data of psychiatric disorders, suggesting that histone H3K4 methylation could be an important therapeutic target. Mutations in both methyltransferase enzymes and demethylase enzymes confer risk for neurodevelopmental disorders and cancer. Here we propose to use iPS models of neural development to explore the relevance of H3K4 methylations pathway as a therapeutic target for future small molecule drug development efforts. Successful completions of these aims will determine the relevance of SETD1A and H3K4 methylation as a therapeutic target in adolescent or adult patients affected by schizophrenia and refine specific stem cell assays for drug development. We propose to evaluate the relevance of H3K4 methylation pathway as a potential therapeutic target for SETD1A haploinsufficiency and schizophrenia more broadly in our previously generated cohort of dura-derived iPS from postmortem neurotypical and Schizophrenia patient tissue and five isogenic heterozygous Loss of Function SETD1A iPSC edited in two control iPSC genomic backgrounds. Two of the SETD1A lines contain the most common patient mutation, a heterozygous two-base deletion at the exon 16 splice acceptor. The iPS lines are derived from dura of individuals with SZ and controls from the psychENCODE and BRAINSEQ consortium cohort with matched postmortem brain tissue and neurogenomic datasets. Subjects were selected for maximal contrast of common variant SZ risk and SETD1A expression in the postmortem tissue. Our preliminary data and data from others demonstrate perturbed neurodevelopment in isogenic SETD1A models, including premature commitment to neurogenesis, increased genomic instability and hyperexcitability. Here, we propose to test if normalizing H3K4 methylation levels in SETD1A haploinsufficient neural progenitor cells and neurons rescues these previously identified cellular endophenotypes. Second, we propose to determine if a subset of common variant SZ iPS lines with compromised SETD1A phenocopy SETD1A haploinsufficiency. Successful completion of the aims will inform the therapeutic potential for H3K4 methylation targets in patients affected by schizophrenia and SETD1A haploinsufficiency.

Luo Gu, PhD

Johns Hopkins University
Awardee Amount: $349,899
Disease Target: Graft Versus Host Disease (GVHD)
(2024 1st Funding Cycle)

Tunable Viscoelastic Hydrogels to Enhance the Production and Immunomodulation of Mesenchymal Stem Cell-Derived Extracellular Vesicles

Graft versus host disease (GvHD) is a major cause of morbidity and mortality in hematopoietic stem cell transplantation (HSCT). Despite recent advances in prophylaxis and treatment, about 3070% of allogeneic HSCT recipients will develop GvHD. Mesenchymal stem/stromal cells (MSCs) are potent regulators of immune cells. MSC products including Prochymal and TEMCELL have recently been approved for GvHD treatment in Canada, New Zealand, or Japan. However, MSCs have not always shown consistent efficacy in clinical trials. This is in part due to the challenges of manufacturing MSCs with consistent, high therapeutic potency. MSC-derived extracellular vesicles (EVs) have been shown to play a crucial role in MSCs therapeutic function. Recent preclinical studies show that hMSC-EVs can control GvHD progression and improve animal survival, and clinical cases also reported that hMSC-EVs could decrease the symptoms caused by GvHD. The use of MSC-EVs, as an alternative to MSCs, confers a number of advantages, including higher safety profile, lower immunogenicity, and potentially easier to be characterized and regulated as compared to cellular products. Therefore, there has been a growing interest in developing MSC-EVs as an alternative therapeutic option. The goal of this project is to develop new technologies to improve the manufacturing of MSC-EVs and enhance their efficacy for GvHD treatment. Past works on priming MSCs to improve EV production and enhance their EVs immunosuppressive capacity have only focused on using biochemical factors such as inflammatory cytokines and small molecules. However, studies from us and others have shown that microenvironment mechanical cues such as matrix stiffness and viscoelasticity play important roles in regulating various behaviors of MSCs. Through collaboration with colleagues at Johns Hopkins and FDA, we have recently discovered that substrate stress relaxation, a viscoelastic property, has a strong effect on MSCs immunosuppressive activity and that substrate stiffness regulates cell EV production. In light of these findings, we hypothesize that substrate viscoelasticity can be exploited as a new class of mechanical cues to prime MSCs to improve the manufacturing of MSC-EVs and enhance their immunosuppressive efficacy for therapeutic applications. The specific aims of this project are: Aim 1: Establish the role of substrate viscoelasticity in regulating the production and characteristics of human MSC-EVs. Aim 2: Interrogate the effects of substrate viscoelasticity on the immunosuppressive capacity of hMSC-EVs. Aim 3: Evaluate the efficacy of hMSC-EVs primed by viscoelastic hydrogels for GvHD treatment in an animal model. We have developed tunable viscoelastic hydrogels as a crucial tool for this project, and have assembled a team with expertise in biomaterials and MSC mechanobiology (Dr. Luo Gu), in the translation and regulation of MSC products (Dr. Kyung Sung at FDA), and in clinical GvHD treatment (Dr. Kenneth Cooke at JHU School of Medicine). This multidisciplinary project aligns with the objective of the MSCRF new faculty Launch Program that targets innovative hypotheses or approaches. Successful completion of the project will have significant impact in understanding how mechanical cues regulates the production and immunosuppressive capacity of MSC-EVs, with the findings potentially leading to new strategies for GvHD treatment.

Xiaoning Han, PhD

Johns Hopkins University
Awardee Amount: $350,000
Disease Target: Intracerebral Hemorrhage
(2024 1st Funding Cycle)

Engineered MSCs-Derived Extracellular Vesicles as an Effective Microglia/Macrophage-Targeted Therapy for Hemorrhagic Stroke

Hemorrhagic stroke is a devastating disease caused by bleeding in the brain and remains the most lethal type of stroke and a major cause of disability worldwide. Despite recent advances in medical treatments, the prognosis for hemorrhagic stroke remains poor. Treatments that can effectively limit hematoma expansion and accelerate hematoma resolution are desperately needed but remains an unmet need. Recent research has shown that human mesenchymal stem cell (MSC)-derived extracellular vesicles (EVs) may effectively protect the impaired brain and improve and recover neurological functions through various mechanisms. Motivated by the immunomodulatory effects and blood-brain barrier (BBB)-penetrating ability of human MSC-EVs, herein we propose to develop an even more effective immunomodulatory therapy for optimized attenuation of the intracerebral hemorrhage (ICH) induced inflammatory injury and hematoma-caused damage. Our design is composed of MSC-EVs and interleukin 10 (IL-10)-carrying phosphatidylserine (PS)-modified liposomes (PSL-IL10). The deliverable of our study, hybrid EVs, is not just a combination of effects. Rather, it is a synergy of advantages from both sides that overcomes the disadvantages of the other side. While IL-10 has been shown effective in accelerating hematoma resolution, its clinical application is hampered by poor intracerebral delivery. Our hybrid EV design can deliver a much higher quantity of IL-10 to the affected areas. On the other hand, the direct benefits of hMSC-EVs on the M/M-modulation may not be sufficient for the acute brain damage following ICH, which can be leveraged by IL-10 (and many other potential drugs). To make our hybrid EVs more M/M targeting, we also adapt a liposome formulation composed of phosphatidylserine (PS) that is a well-known eat me signal for M/M to recognize. The optimization and future application will be guided by multimodal imaging, including our proprietary MRI tracking method (patent pending) as well as positron emission tomography (PET). The imaging capability of tracking, guiding, and optimizing is unprecedented compared to previous studies. Because the poor outcome post-ICH is largely attributed to the inflammatory cascade caused by the activation and phenotypic polarization of M/M, we hypothesize that an improved ICH outcome can be achieved by regulation of M/M using a hybrid nanotherapeutic system composed of BBB-penetrating M/M-modulating hMSC-EVs and drug-carrying, M/M-targeted liposomes. To test this hypothesis, we propose to carry out two specific aims. Aim 1: Constructing, characterizing, and optimizing hybrid EVs, in which we will construct hybrid EVs using a polyethylene glycol (PEG)-mediated fusion method, characterize their particle properties, cargo quantities, biological effects on M/M, and intracerebral delivery efficiency, and optimize the liposome formulation, EV/liposome ratios, and fusion conditions. Aim 2: Evaluating and optimizing hybrid EVs treatment on acute ICH, in which we will evaluate the effectiveness of hybrid EVs to promote the treatment outcomes post-ICH, and optimize treatment schedule and dose. The proposed study will broaden the therapeutic scope of human stem cells and lead to a new type of engineered EV therapy for combating ICH, which will be ready to be tested in large animal models and potentially in clinical trials upon the completion of the project.

Sangmoo Jeong, PhD, MS

Johns Hopkins University
Awardee Amount: $350,000
Disease Target: Heart Transplantation

Alexander Ksendzovsky, MD, PhD

University of Maryland, Baltimore
Awardee Amount: $349,999
Disease Target: Epilepsy

Use of Extracellular Vesicles from Metabolically-Engineered Mesenchymal Stem Cells for Prolonged Organ Preservation in Heart Transplantation

Advancement in organ transplantation has benefited about 40,000 patients with end-stage organ failure in the US annually. However, the number of patients on the transplantation waitlist is currently over 133,000, and about 20 patients die every day while waiting for a transplant. Given that the waitlist is expected to increase continuously, more patients will likely die or become too sick before having the life-saving transplant. Therefore, the significant disparity between organ supply and demand must be urgently addressed. The major bottleneck for solving this problem is ischemia time between organ retrieval and implantation, during which a donor organ experiences oxygen depletion due to lack of blood flow. This inevitable stress stops oxidative phosphorylation in the mitochondria, causing unfavorable molecular and metabolic changes. One of the most detrimental effects is NAD+ depletion, which hampers glycolysis and ATP generation, initiating cell death programs. Also, it leads to excessive accumulation of succinate, driving the reactive oxygen species (ROS) production in the mitochondria upon reperfusion. Furthermore, NAD+ is required for DNA repair processes, and its depletion after ischemia makes a graft vulnerable to ROS-induced DNA damage. Therefore, the longer ischemia time leads to more organ damage and, consequently, a higher risk of graft failure. If we can extend tolerable ischemia time during organ transport, we will find donor organs from larger geographical areas, markedly increasing donor organ availability. We propose to use extracellular vesicles (EVs) from metabolically engineered mesenchymal stem cells (MSCs) for prolonged organ preservation after harvest. MSCs are multi-potent stromal cells found in various tissues, including bone marrow, and they can provide therapeutic benefits of tissue regeneration or immune modulation. Thus, many clinical trials are ongoing to use MSCs in organ transplantation to foster recovery from ischemia-reperfusion injury and diminish pro-inflammatory responses. Recent studies have shown that the therapeutic effects of MSCs in ischemic organs could be achieved via EVs secreted from MSCs. EVs are membrane-bound vesicles with a diameter of 50-200 nm, and they carry specific molecules from their parental cells and deliver them to other cells locally or systemically. EVs from MSCs have been shown to mitigate oxidative damages, suppress cytotoxic immune responses, and improve organ functions in various models of ischemia-reperfusion injury. We hypothesize that EVs from MSCs can reduce ischemia damage in a donor organ when administered during organ preservation. To amplify the benefits of the EVs, we will engineer MSCs to overexpress nicotinamide phosphoribosyl-transferase (NAMPT), a key enzyme for NAD+ generation. NAMPT-overexpression (OV) increases NAD+ levels, reducing oxidative damage and cell death; for example, cardiac-specific NAMPT-OV resulted in considerably lower damage from myocardial infarction in a preclinical model. We expect that EVs from NAMPT-OV MSCs will deliver NAMPT to a donor organ and increase its NAMPT level when the EVs are administered to organ preservation solution, significantly reducing ischemia-reperfusion injury and extending graft survival after transplantation. To test our hypothesis, we propose the following Specific Aims: Aim 1. Determine whether EVs from NAMPT-OV MSCs help EV-recipient cells recover from ischemia-reperfusion injury. 1.1) Develop a sensitive EV molecular.

A High-Throughput Drug Screening Assay for Focal Cortical Dysplasia

Epilepsy affects 1 in 26 people in the United States, making it the most common neurological disorder. Approximately 70-80% of epilepsy cases are caused by genetic variants, the most common of which are found within the mTOR pathway and result in cortical malformations including Focal Cortical Dysplasia (FCD). FCD is particularly challenging due to the presence of highly epileptogenic lesions within the cortex that are often resistant to medication and/or difficult to resect. The most common genetic variants causing FCD are found in genes that encode the protein subunits that form the GATOR1 complex: DEPDC5, NPRL2, and NPRL3 (GATORopathies). Current pharmacological trial-and-error treatment approaches result in life-long polypharmacy, associated with childhood developmental delay and poor quality of life. Precision therapies are needed, and small molecule inhibitors of the mTOR pathway are thought to have great potential. However, advancing these therapies is blocked by two critical gaps in knowledge. First, there is incomplete understanding of the causal mechanisms from FCD variants to mTOR pathway hyperactivation to neuronal hyperexcitability. Second, it has not been established whether disease-relevant cellular consequences of FCD mutations can be rescued by mTOR inhibitors, and since different doses and types of mTOR inhibitors can produce different mTOR signaling changes and clinical outcomes, there is a need to test these pharmacological responses using multiple mTOR inhibitors with distinct pharmacological characteristics. Here, we propose to address both knowledge gaps and make progress toward FCD precision therapy utilizing human induced pluripotent stem cell (iPSC)-derived neurons (iPSNs). In preliminary work, we have established iPSC lines from FCD patients with mutations in the two most common FCD genes, DEPDC5 and NPRL3, and we developed protocols using iPSNs to assess several of the core phenotypes of FCD. With funding from MSCRF, we will define the consequences of each FCD mutation on mTOR pathway signaling and neuronal excitability and test the mTOR-dependence of these phenotypes using therapeutically-relevant mTOR inhibitors. These studies will establish proof-of-principle for precision therapies and set the stage for broader mechanistic and drug screening applications. We propose to accomplish this in two Aims where we establish effects of GATORopathy mutations on mTOR pathway signaling in hiPSNs (Aim 1) and Establish effects of GATORopathy mutations on neuronal excitability in iPSNs (Aim 2).

Daniel Lobo, PhD

University of Maryland, Baltimore County
Awardee Amount: $350,000
Disease Target: Blood Diseases & Disorders

Christina Linnea Nemeth Mertz, PhD

Hugo W. Moser Research Institute at Kennedy Krieger, Inc.
Awardee Amount: $349,111
Disease Target: LBSL

Inferring the Mechanistic Regulation of Human Hematopoietic Stem-Progenitor Cells During Standard and ROS-induced States

The precise genetic regulatory mechanisms that control the biology of human hematopoietic stem-progenitor cells (HSPCs) is still not well understood. While human hematopoietic stem cells (HSCs) can differentiate into more than 10 different mature cell types to generate and sustain the complete blood-immune system at the outstanding rate of more than one million cells per second, a homeostatically balanced pool of stem, progenitor, precursor, and mature cells is maintained at most times during a persons life. Single-cell transcriptomics analyses have revealed that hematopoiesis is continuous and dynamic; yet inferring a mechanistic understanding of its genetic regulation is a current challenge due to the complexity and feedback loops characteristic of regulatory mechanisms. Furthermore, beyond HSPC-intrinsic mechanisms, hematopoietic niche-dependent extrinsic factors can modulate HSPC biology. Among the multiple types of extrinsic hematopoietic factors, reactive oxygen species (ROS) levels provide an oxidative stress shown to significantly alter human HSPC biology. However, so-called stress hematopoiesis is even more poorly understood than standard hematopoiesis, and there is no strong consensus on the precise effects of ROS on HSPC biology and gene expression. Thus, there is a critical need for advanced computational-molecular approaches that can extract gene regulatory mechanisms from dynamic cell population and transcriptomic data towards a comprehensive understanding of human HSPC biology, under both standard and stress conditions.The central hypotheses of this proposal are that ROS will substantially modulate gene expression dynamics and thereby HSPC biology, and that a novel machine learning methodology will be able to infer a mechanistic, predictive model of the population/differentiation dynamics of HSPCs. Our rationale is that temporal datasets of cell populations and single-cell transcriptional states of human HSPCs under in vitro standard vs. drug-induced high ROS states will reveal the key regulatory genes responsible for HSPC differentiation and population dynamics. Furthermore, applying a machine learning methodology to these datasets will result in a mechanistic and predictive understanding of the interactions among intrinsic and extrinsic regulators. Our collaborative team is ideally-prepared to undertake the proposed research based on our deep expertise in computational and machine learning methods for building systems-level mechanistic regulatory models and rich expertise in human HSPCs and their genetic regulation. This project is expected to reveal major molecular regulatory mechanisms controlling human HSPC biology via two main aims: (1) Identify the dynamic transcriptional regulation of in vitro cultures of human HSPCs. (2) Validate the dynamic effects of ROS-inducing drugs on human HSPC cultures. The proposed research is innovative based on its deep integration of cell, molecular, and computational methods with temporal scRNA-seq and population data in a novel approach to reconstruct the regulatory mechanisms controlling human HSPC differentiation and population dynamics. These advancements will transform our general understanding of how HSPCs integrate intrinsic and extrinsic (i.e. ROS) signals to balance quiescence with differentiation. We predict significant impact by (a) providing a multi-level predictive dynamic model of human HSPC fate and (b) advancing the use of systems-level methodologies to understand how human HSPCs quiesce and differentiate into progenitor-precursor and mature cells under standard and stress conditions.

LBSL iPSC Brain Modeling: A Prototype for Pediatric Rare Disease

LBSL (Leukoencephalopathy with Brainstem and Spinal cord involvement and Lactate elevation) is a rare pediatric neurological disorder caused by mutations in the gene DARS2, which encodes the mitochondrial aspartyl-tRNA synthetase. Patients usually present in childhood with progressive gait difficulty secondary to spasticity, ataxia, and proprioceptive deficits, though more severe, fatal, antenatal onset cases are also observed. The overall goal of this project is to create a quantifiable and reproducible model of LBSL that is scalable and appropriate for use in large-scale drug screens. Although the patient phenotype is perpetuated by brain and spinal cord demyelination, little is known about the role or contribution of oligodendrocytes to disease progression as LBSL disease models have failed to recapitulate disease mutations within oligodendrocytes (mouse) and in vitro monolayer cultures have focused on neuronal phenotypes or peripheral cell constructs. In collaboration with the lab of Dr. Lena Smirnova at the Johns Hopkins School of Public Health, the work proposed within this application introduces novel 3D human brain models, or brain organoids (BO). BOs better mimic the developing brain compared to traditional monolayer cultures and contain different types of neurons, astrocytes, and importantly, also express myelinating oligodendrocytes. The BOs described within this application will be generated from LBSL patient derived induced pluripotent stem cells (iPSCs), generated from blood collected from patients at the Kennedy Krieger Institute. BOs model the complexity of both in vivo neurite networks and brain cell architecture and because they mimic the developing brain in their structure and gene expression profile, they are an ideal system for studying neurodegenerative disorders. To further aid in the analysis and quantification of oligodendrocytes and oligodendrogenesis within LBSL BOs, we will use CRISPR/Cas9 to induce an oligodendrocyte-specific reporter, which will fluorescently tag oligodendrocytes for the clear and specific identification of this myelinating cell type. We will monitor growth and development of LBSL and isogenic control BOs and characterize function in terms of cell growth, gene expression, and electrophysiological and metabolic activity. Following this work's successful completion, LBSL BOs will be used for high-throughput drug screens to discover and develop therapeutic strategies for treating LBSL. Furthermore, within our lab, we have developed gene targeted therapies including antisense oligonucleotide (ASO) and AAV9 technology that require testing in human-derived models. We plan to test these therapies in this highly disease- and patient-relevant model. It is our hope that the work proposed here accelerates our testing of these therapies, and expedites the involvement of commercial partners. We have garnered a community of LBSL patients and families who are active supporters of these research endeavors. This project directly impacts these families and the development of therapies that may preserve the ability of LBSL patients to live independently.

Mark Ranek, PhD

Johns Hopkins University
Awardee Amount: $350,000
Disease Target: Transthyretin Cardiac Amyloidosis
(2024 1st Funding Cycle)

Ali Shakeri-Zadeh, PhD

Johns Hopkins University
Awardee Amount: $345,000
Disease Target: Multiple Sclerosis (MS)
(2024 1st Funding Cycle)

New Human Cardiac Amyloidosis iPSC Lines Provide Novel Insight and Therapeutic Opportunities

Heart disease is the most common cause of morbidity and mortality in the US and worldwide. This proposal studies the mechanistic underpinnings of myocardial deposition of transthyretin (TTR) and tests new thereapetuic strategies. Myocardial TTR accumulation causes amyloid TTR cardiomyopathy (ATTR-CM), a restrictive heart disease found in over 6% of patients with heart failure and ejection fraction more than 50% (~1M worldwide).1-3 The pathogenesis of ATTR-CM involves both extracellular and intracellular TTR deposition. Cardiomyocytes can take up TTR, leading to intracellular protein aggregation, oxidative stress, cytotoxicity, and worsened heart failure.4-8 Lowering intramyocyte TTR may attenuate heart disease, though current therapies only reduce the formation of TTR monomers, slowing their cardiac uptake, but not reducing intracellular TTR. ATTR-CM can be hereditary (mutations in TTR) or by the deposition of wild type (WT) TTR. The mechanisms underlying to and contributing to all forms of ATTR-CM remain unknown. Moreover, cardiomyocytes degrade TTR via the lysosome4,5 by an undefined pathway. We now show that intracellular TTR is cleared by cGMP-activated kinase (PKG) phosphorylation of the E3 ligase/co-chaperone Chip (carboxyl terminus of Hsc-70 interacting protein) at Serine 19 (pS19, human). Chip is intracellular and expressed in cardiomyocytes, so this modulation could increase clearance of intracellular TTR. We focus on our newly uncovered PKG/Chip pathway as a novel therapeutic strategy, along with searching for additional ATTR-CM predispositions. In new exciting preliminary data from human iPSC derived cardiomyocytes (hiPSC-CMs) and ATTR-CM myocardial biopsies, we find reduced PKG activity and pS19-Chip, along with greater phosphodiesterase 5 (PDE5) expression, known to reduce PKG activation and raise oxidative stress. Myocytes from ATTR-CM tissue have reduced myofibrillar force upon calcium activation, similar to heart failure with reduced ejection fraction (HFrEF), supporting major intracellular defects. Importantly, we find TTR clearance is increased by activating PKG to enhance Chip pS19, and the consequent TTR degradation reduces intramyocyte TTR and cytotoxicity. Mechanistic insights of ATTR-CM have been historically impeded by lack of access to human tissue and translational models. We have now overcome both limitations and so are uniquely positioned to address this important disease. First: we created 3D engineered heart tissue (EHT) and cardiac organoid models of ATTR-CM with hiPSC-CMs. Cardiac organoids are coupled to hepatic organoids, both from a single iPSC line, that excrete TTR in a micro-physiological system, allowing us to perform the first precision medicine approach for ATTR-CM. Mutating Chip to a phospho-mimetic S19E (SE) or treatment with a PDE5 inhibitor (activates PKG) lowers cardiac organoid TTR aggregation and cytotoxicity, whereas a phospho-null Chip S19A (SA) abrogates PKG-coupled protection. Second: we developed a Chip-TTR proteolysis targeting chimera (PROTAC) so Chip only targets TTR for degradation. Our PROTAC enhances TTR removal in hiPSC-CMs and may be developed as a therapy. Third: we have access to human ATTR-CM myocardium and blood to test translational relevance. We hypothesize impaired TTR degradation underlie and exacerbates ATTR-CM pathogenesis, presenting a novel treatment strategy.

Dynamic MPI Cytometry of Mesenchymal Stem Cells in a Mouse Model of Multiple Sclerosis (MS)

There is a wide spectrum of immunologically non-specific drugs to reduce or stop the progression of multiple sclerosis (MS). In this context, the use of cell therapy shows promise as an alternative treatment option. Among cell-based methods with a potent immunomodulatory effect, mesenchymal stem cells (MSCs) have been found to be safe and well tolerated by MS patients, with no clinically significant side effects. In addition to numerous pre-clinical studies, clinical trials have reported beneficial effects of MSCs through a decrease in relapse rate or disability. These promising results encourage MSC therapy for MS patients. More than 20 MSC-related MS trials are currently listed on ClinicalTrials.gov. However, most studies have been unable to draw conclusions about the efficacy of this treatment due to protocol inconsistencies (routes of administration, doses, frequencies, etc.). Non-invasive and quantitative monitoring of cells administered by different routes would help neurologists adjust cell delivery parameters and monitor immediate cell dispersion to achieve optimal biodistribution profiles. Using magnetic particle imaging (MPI), our lab has recently developed a new fast dynamic image-based cytometry approach to quantify administered cells in different organs within a minute. This approach was initiated when the PI was a postdoctoral researcher in the cellular imaging section at the Johns Hopkins Institute for Cell Engineering (ICE). In this Launch Grant application, the PI (now a tenure-track Assistant Professor in ICE) intends to investigate the use of dynamic MPI for tracking administered human MSCs in a mouse model of MS. Using magnetic labeling of MSCs and in combination with MRI, quantitative dynamic MPI may assist in addressing fundamental queries about the fate of MSCs administered using intravenous (IV), intraarterial (IA), and intracerebroventricular (ICV) routes. MRI and MPI are two complementary techniques to detect the MSCs labeled with superparamagnetic iron oxide (SPIO) nanoparticles. When performing an MRI scan, it is possible that multiple hypointense spots get misinterpreted as labeled cells. Therefore, MPI is useful to provide specific and quantitative information on the same labeled cells. In this proposal, we will utilize an experimental autoimmune encephalomyelitis (EAE) mouse model, which is a widely-accepted model for MS proof-of-concept research and with which our lab has extensive expertise. We will label MSCs with Resovist, a commercial SPIO formulation, and then administer them into EAE mice via IV, IA, and ICV routes (Fig. 1). At the end of our studies, after identifying the most promising delivery route, unlabeled MSCs will be administered and tracked using mannose-weighted chemical exchange saturation (CEST) MRI, a new technique that has recently been developed in our lab. By analyzing non-invasive imaging data, neurological function tests, and post-mortem examinations for early and late time periods, it will be possible to determine how different MSC administration routes affect immunomodulation and remyelination. This proposal has translational potential to advance the current clinical techniques for cell tracking, as the company that built our MPI scanner is producing a clinical MPI machine. MRI is available and already routinely used for stratification of MS patients. The use of MSCs for MS has already been evaluated clinically.

MSCRF

DISCOVERY

GRANT AWARDS

2023

Kenneth Boheler, PhD

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

Jeff W.M. Bulte, PhD, MS

Johns Hopkins University
Awardee Amount: $342,501
Disease Target: Stroke, MS, AD

FBN1-Deficiency destabilizes Endothelial Cell-Cell Attachment & Mechanosensing in Marfan Syndrome

Marfan Syndrome (MFS) is a genetic disorder of the connective tissue caused by FBN1 (fibrillin-1) gene mutations that may lead to catastrophic aortic lesions and death.1,2 Mechanistically, FBN1 protein deficiency in the extracellular matrix (ECM) leads to excess extracellular TGF-1 availability, promotes vascular smooth muscle cell (vSMC) apoptosis, increases matrix metalloproteinase abundance, leads to collagen overexpression, and causes immature elastin formation and degeneration in the medial layer of the aorta.3 Of relevance to this application, the use of human induced pluripotent stem cell (hiPSC) lines from MFS patients has been instrumental in resolving some discrepancies in intracellular signaling, phenotypic plasticity, and therapeutic efficacy between vSMC primary explants from human MFS aneurysmal tissues and mouse MFS model aortic tissues.1,3-8 Despite these advances, one critical component of the vasculature that has not been examined at any great depth is the effect of FBN1 deficiency on endothelial cells (ECs) present in the intima.9-13 Structurally, FBN1 binds ECM components that underlie basement membranes critical to EC function, including but not limited to ADAMTS family proteins, heparin-sulfate proteoglycans, and fibronectin (FN).14 To determine whether FBN1-deficient ECM in an hiPSC model can disrupt EC structure or function, we performed pilot studies using an established MFS line with a heterozygous nonsense FBN1 mutation to examine ECs in vitro. Relative to control cells with normal FBN1, we found that cell-cell cohesion is disrupted among ECs by the loss of FBN1, particularly when the cells are subjected to cyclic stretch. Moreover, the mechanisms whereby EC cells adapt to mechanical stress (adherens junctions and stress fibers) appear to be affected adversely. These preliminary results suggest that disrupted EC function may contribute to the manifestation of MFS, perhaps through altered vSMC regulation or exposure to shear stress. Here we propose to validate our potentially transformative discovery and extend these findings to isogenic control lines and newly created MFS hiPSC lines differentiated into ECs. Experimentally, we will test our hypothesis using normal and FBN1 deficient ECs subjected to dynamic stretch in 2D to mimic in vivo physiological mechanical forces. The goals of this project are 1) to confirm and extend our analyses with normal and MFS hiPSC lines differentiated into ECs evaluated under control and cyclic stretch conditions and using more quantitative assessments; and 2) to determine whether the effects on ECs are specific to the point mutation analyzed or a general feature of FBN1 deficiencies in MFS. If the pilot data on which this grant is based are proved to be correct, this discovery will have defined an EC-centric mechanism that may underlie a novel etiology of MFS. Such a finding would prove critical to future patient prognoses and to the development of new therapies.

In Vivo Tracking of Unlabeled MSCs and MSC-EVs for Rapid Clinical Translation

Human mesenchymal stem cells (hMSCs) and their extracellular vesicles (EVs) have been used as cellular therapeutics for treating various diseases, but little is known about their fate in vivo once administered. Available magnetic resonance imaging (MRI) cell tracking techniques use either paramagnetic metals, superparamagnetic iron oxides, or fluorinated nanoparticles as imaging agents, which have several major hurdles for clinical translation. We now aim to track unlabeled hMSCs and EVs in vivo with MRI based on their natural high-mannose-type N-glycan expression on the cell membrane. Such label-free tracking of stem cells using chemical exchange saturation (CEST) MRI avoids potential adverse effects of label on cell survival or function, transfer of label to host cells upon cell death, dilution of label due to cell division, and the need for regulatory approval of label for clinical use and associated high cost of GMP production. Since the membrane envelope of EVs mimics that of the parent cell itself, we hypothesize that we can also track unlabeled hMSC-EVs in a similar fashion, as supported by our preliminary data. To this end, we will use an ischemic stroke model (middle cerebral artery occlusion or MCAO) as an example of a target disease, where injected hMSCs and hMSC-EVs have been reported to have therapeutic beneficial effects. We will study hMSCs with and without VLA-4 transfection as we have shown that VLA-4/VCAM-1 docking can lead to diapedesis and increased accumulation on the brain parenchyma. Since MSCs and MSC-EVs can be used off the shelf without the need of further manipulation, our approach could be readily clinically translatable for ongoing and future clinical trials. To this end, we will perform cell phantom imaging studies at 3T using a clinical scanner, as part of proof-of-concept given the recently expressed interest from ongoing clinical trials in other neurodegenerative diseases (e.g., Alzheimers disease).

Kenneth Boheler, PhD

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

Jeff W.M. Bulte, PhD, MS

Johns Hopkins University
Awardee Amount: $342,501
Disease Target: Stroke, MS, AD

FBN1-Deficiency destabilizes Endothelial Cell-Cell Attachment & Mechanosensing in Marfan Syndrome

Marfan Syndrome (MFS) is a genetic disorder of the connective tissue caused by FBN1 (fibrillin-1) gene mutations that may lead to catastrophic aortic lesions and death.1,2 Mechanistically, FBN1 protein deficiency in the extracellular matrix (ECM) leads to excess extracellular TGF-1 availability, promotes vascular smooth muscle cell (vSMC) apoptosis, increases matrix metalloproteinase abundance, leads to collagen overexpression, and causes immature elastin formation and degeneration in the medial layer of the aorta.3 Of relevance to this application, the use of human induced pluripotent stem cell (hiPSC) lines from MFS patients has been instrumental in resolving some discrepancies in intracellular signaling, phenotypic plasticity, and therapeutic efficacy between vSMC primary explants from human MFS aneurysmal tissues and mouse MFS model aortic tissues.1,3-8 Despite these advances, one critical component of the vasculature that has not been examined at any great depth is the effect of FBN1 deficiency on endothelial cells (ECs) present in the intima.9-13 Structurally, FBN1 binds ECM components that underlie basement membranes critical to EC function, including but not limited to ADAMTS family proteins, heparin-sulfate proteoglycans, and fibronectin (FN).14 To determine whether FBN1-deficient ECM in an hiPSC model can disrupt EC structure or function, we performed pilot studies using an established MFS line with a heterozygous nonsense FBN1 mutation to examine ECs in vitro. Relative to control cells with normal FBN1, we found that cell-cell cohesion is disrupted among ECs by the loss of FBN1, particularly when the cells are subjected to cyclic stretch. Moreover, the mechanisms whereby EC cells adapt to mechanical stress (adherens junctions and stress fibers) appear to be affected adversely. These preliminary results suggest that disrupted EC function may contribute to the manifestation of MFS, perhaps through altered vSMC regulation or exposure to shear stress. Here we propose to validate our potentially transformative discovery and extend these findings to isogenic control lines and newly created MFS hiPSC lines differentiated into ECs. Experimentally, we will test our hypothesis using normal and FBN1 deficient ECs subjected to dynamic stretch in 2D to mimic in vivo physiological mechanical forces. The goals of this project are 1) to confirm and extend our analyses with normal and MFS hiPSC lines differentiated into ECs evaluated under control and cyclic stretch conditions and using more quantitative assessments; and 2) to determine whether the effects on ECs are specific to the point mutation analyzed or a general feature of FBN1 deficiencies in MFS. If the pilot data on which this grant is based are proved to be correct, this discovery will have defined an EC-centric mechanism that may underlie a novel etiology of MFS. Such a finding would prove critical to future patient prognoses and to the development of new therapies.

In Vivo Tracking of Unlabeled MSCs and MSC-EVs for Rapid Clinical Translation

Human mesenchymal stem cells (hMSCs) and their extracellular vesicles (EVs) have been used as cellular therapeutics for treating various diseases, but little is known about their fate in vivo once administered. Available magnetic resonance imaging (MRI) cell tracking techniques use either paramagnetic metals, superparamagnetic iron oxides, or fluorinated nanoparticles as imaging agents, which have several major hurdles for clinical translation. We now aim to track unlabeled hMSCs and EVs in vivo with MRI based on their natural high-mannose-type N-glycan expression on the cell membrane. Such label-free tracking of stem cells using chemical exchange saturation (CEST) MRI avoids potential adverse effects of label on cell survival or function, transfer of label to host cells upon cell death, dilution of label due to cell division, and the need for regulatory approval of label for clinical use and associated high cost of GMP production. Since the membrane envelope of EVs mimics that of the parent cell itself, we hypothesize that we can also track unlabeled hMSC-EVs in a similar fashion, as supported by our preliminary data. To this end, we will use an ischemic stroke model (middle cerebral artery occlusion or MCAO) as an example of a target disease, where injected hMSCs and hMSC-EVs have been reported to have therapeutic beneficial effects. We will study hMSCs with and without VLA-4 transfection as we have shown that VLA-4/VCAM-1 docking can lead to diapedesis and increased accumulation on the brain parenchyma. Since MSCs and MSC-EVs can be used off the shelf without the need of further manipulation, our approach could be readily clinically translatable for ongoing and future clinical trials. To this end, we will perform cell phantom imaging studies at 3T using a clinical scanner, as part of proof-of-concept given the recently expressed interest from ongoing clinical trials in other neurodegenerative diseases (e.g., Alzheimers disease).

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Awardee Amount: $345,000
Disease Target: Cardiac Arrhythmias

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Awardee Amount: $345,000
Disease Target: Thrombocytopenia, Platelet Functional Disorders

Bioengineered Cardiac Pacemaker Organoids

The goal of this translational project is to understand the efficacy, durability and safety of stem cell-derived cardiac biological pacemaker organoids as potential therapeutic alternatives to electronic pacemaker devices. Background: Human iPSC-derived cardiomyocytes are the most immediate source of de novo human cardiomyocytes for cell replacement therapies for heart diseases. However, two major problems give pause to their potential therapeutic utility. First, the de novo cardiomyocytes are immature in their electrophysiology and contractility. Indeed, virtually all ventricular or atrial chamber-like hiPSC-derived cardiomyocytes beat spontaneously. Indeed, transplantation of hundreds of millions of hiPSC-derived cardiomyocytes into large animal models of myocardial ischemia has been shown to elicit cardiac arrhythmias that could precipitate to sudden cardiac death. Secondly, the de novo cardiomyocytes are heterogeneous in their cell type. Any given cardiac differentiation protocol gives rise to myocytes and nonmyocytes of cardiac lineage, as well as an obligatory mixture of atrial, ventricular and nodal-like cardiomyocytes. All putative cell replacement therapies for ischemic heart diseases are built on the a priori ability to purify either ventricular or atrial myocytes, and exclude nodal-like cardiac conduction system myocytes to decrease arrhythmia propensity. Rationale: In contrast to ischemic heart diseases, heart rhythm diseases demand a distinct set of criteria for stem cell therapies. Particularly for cardiac pacing, therapeutic products would need to generate spontaneously oscillating electrical activity, but not the contractile force of mature ventricular myocytes. The biological pacemaker cell products may not need to attain pure or highly homogenous population of nodal pacemaker cells as long as they can achieve cardiac pacing function. Indeed, the sinoatrial node, the native pacemaker tissue of the heart, is a minuscule and highly heterogeneous structure harboring only a few tens of thousands of nodal pacemaker cells, intermingled with atrial chamber cardiomyocytes and nonmyocytes. Significance: Electronic pacemaker devices represent the only treatment for symptomatic bradycardia. While effective, the device therapy are increasingly correlated with complications that are inherent to indwelling hardware. These include device malfunction, repeat surgeries for hardware replacement /repositioning and infected device. For pediatric patients with congenital heart defects, pacemaker implantation and device pacing are far from ideal and largely inadequate. In this proposal, we will develop and characterize human pluripotent stem cell-derived cardiac pacemakers (stemPace). The proposed work will test whether hiPSC-derived cardiac organoids could serve as biological pacemakers for temporary pacing indications that last up to one month.

DACH as a Novel Human Megakaryopoietic Regulator

With previous MSCRF support, we reported that SIX1 overexpression (OE) stimulated erythropoiesis in human primary CD34+ hematopoietic stem-progenitor cells (HSPCs). SIX1-stimulated erythropoiesis required the master erythroid transcription factor GATA1, and SIX1 can associate with GATA1 to simulated GATA1-mediated gene expression. Thus, the SIX1-GATA1 interaction is a novel regulatory mechanism functioning in human HSPCs. Since our findings revealed the previously unknown functional interaction between two highly-conserved major developmental transcriptional networks, the PSEDN and GATA networks, to regulate human hematopoiesis, we continue to investigate the cellular effects and molecular mechanisms of SIX1 and additional PSEDN molecules in human hematopoiesis. In our Preliminary Results, DACH1 OE in HSPCs stimulated megakaryopoiesis in the presence and absence of thrombopoietin (TPO), whereas DACH1 OE inhibited erythropoiesis in the presence of erythropoietin (EPO). DACH1 OE HSPCs cultured in media containing SCF+EPO+TPO generated greater numbers of megakaryocytic lineage (meg) cells with fewer erythroid cells, suggesting that enforced DACH1 expression in HSPCs may drive megakaryopoiesis at the expense of erythropoiesis. Generation of meg cells via ex vivo culture of HSPCs is under investigation as an alternative to simple blood donation, but clinical use of ex vivo-cultured meg cells is still challenged by low meg cell yields. We believe that coordinated manipulation of several meg regulatory molecules will be required to enhance current ex vivo protocols sufficiently to produce the huge numbers of meg cells needed for clinical transfusions, and that elucidation of novel mechanisms regulating megakaryopoiesis has the potential to provide the necessary new approaches. In this proposal, we plan to investigate the cellular effects and molecular mechanisms of DACH as a novel meg regulator (in Aim 1), and to investigate the use of DACH to enhance expansion of meg cells in vitro.

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Awardee Amount: $345,000
Disease Target: Hematologic Disorders, Age-related changes in Hema

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Awardee Amount: $345,000
Disease Target: GBA1-Associated Neurodegeneration

Establishing the Role of DNA Methylation Changes in Aged Human Blood Stem Cells by Xenotransplants

How aging impacts the epigenome of stem cells is a central question in aging biology that is relevant to normal human health as well as various disease states. The consistency of age-related epigenetic changes, particularly the DNA methylation alterations, across individuals has allowed development of molecular clocks of aging based solely on DNA methylation patterns. The most widely used clocks are based on methylation profiles of peripheral blood mononuclear cells (PBMCs). These clocks have been shown to accurately predict age in a variety of species and tissues and have even been correlated to biological function. As PBMCs are derived from hematopoietic stem and progenitor cells (HSPCs), understanding the age-related methylation changes in HSPCs will provide a better understanding of the relationship between epigenetic changes, HSPC function, and their relationship to methylation clocks. During aging, HSPCs display altered phenotypes that include loss of functional capacity, altered clonal composition, and changes in lineage contribution; all of which predispose to a variety of age-related diseases and malignancies. It is unknown if the age-related epigenetic alterations in HSPCs, and therefore the widely used epigenetic aging clocks, are driven by factors "intrinsic" to the age of a cell or "extrinsic" microenvironmental factors dependent on organismal aging. Our central hypothesis is that a primary driver of HSPC decline during aging is an altered epigenetic landscape, and that rejuvenation of stem cells is possible by manipulating or resetting their epigenetic profiles. Restoring functional potential to the primitive stem cells would be, at least in part, reflected in the epigenetic clock. Therefore, our long-term goal is to achieve a better understanding of the epigenetic changes that occur with age in HSPCs and how to manipulate them. This will provide means to reduce age-related stem cell dysfunction, with direct therapeutic implications on HSPC transplants and mitigating aging defects. A major objective of this grant is to define alterations in aged human HSPCs driven by cell-extrinsic factors from the organismal aging environment or cell-intrinsic factors. To accomplish this, we will use an innovative approach of xenotransplantation, wherein immune-deficient mice carrying cKit mutations enable robust engraftment of human HSPCs and provide a model to test for the influence of cell "intrinsic" vs. "extrinsic" factors in the age-related epigenetic changes. The outcomes of this study will help determine the functional impact of interventions on human stem cells in an in vivo environment that is decoupled from the clinic. The proposal will utilize the complementary expertise of the Easwaran and Beerman groups to successfully address the following Specific Aims: Aim-1: Determine the role of intrinsic or extrinsic factors influencing age-related DNA methylation changes and the epigenetic aging clock: As humans and mice age at different rates, we will test the hypothesis that in a mouse chimera harboring blood cells derived from human HSCs, the epigenetic clock moves at a rate proportional to the donor species' aging rate. Aim-2: Establish the impact of manipulating the DNA methylome in HSPCs on their function and towards resetting of the epigenetic clock: We will determine the effects of inhibiting DNA methylation using FDA approved drugs to test impact of reversing age-related methylation gains on the epigenetic clock and restoration of HSPC function. Impact: Upon completion, our studies will: (i) provide broader understanding of the drivers of age-related DNA methylation changes in human stem cells; (ii) improve understanding of the intrinsic and extrinsic drivers of HSC aging; (iii) advance our ability to utilize epigenetic aging clocks in a meaningful way and (iv) define critical genetic loci to target in future interventions. As HSPCs are the critical units for long term success of transplants, our results will lead to more robust engraftment and better clinical outcomes from a wider range of donor ages.

Novel use of Peripheral Blood-Derived Cells to Identify Individuals at High Risk of Developing PD

Bi-allelic mutations in GBA1, a gene that encodes the lysosomal enzyme glucocerebrosidase, causes Gaucher disease (GD). GD patients and GD carriers have a 5 to 20-fold similarly increased risk of developing Parkinsons disease (PD), and 7% of all PD patients harbor GBA1 mutations. Due to the small penetration of the GBA1 mutations, this risk is still small, and other factors, including genetic background, age and epigenetic alterations become important contributors to PD. As not all GD patients or carriers develop PD, there is an unmet need to identify the individuals at highest risk of developing PD. Because dopaminergic (DA) neurons are particularly susceptible to GBA1 mutations, these cells are likely to undergo pathognomonic alterations long before clinical manifestations are evident. Microglia activation is believed to play a key role in GBA1-associated PD (GBA1-PD) and idiopathic PD. While access to patient-derived neurons and microglia would be desirable, these cells can only be obtained through laborious reprogramming technology; in addition, all the epigenetic changes that accumulate over time and may contribute to PD are lost during reprogramming. Our laboratory has developed a robust protocol to derive microglia-like cells (MLCs) from patient-derived monocytes in 3-D co-cultures with iPSC-derived DA neurons. In these organoids, GD MLCs induced an elevation of phospho-a-synucleinS129 in WT iPSC-derived DA neurons compared to control MLCs. We hypothesize that peripheral blood mononuclear cell (PBMC)-derived MLCs from GD patients and carriers at high risk of developing PD, can induce pathogenic a-synuclein alterations in sentinel WT DA neurons. PBMC-MLCs are a good microglia surrogate to use. In addition to the loss- and gain-of-function alterations of mutant GBA1, these cells retain all the other unknown genetic and epigenetic risk factors for PD. In Aim 1, we will determine if PBMC-MLCs derived from GD patients and carriers with PD, induce higher accumulation of a-synuclein in DA neurons than those without PD. In Aim 2, we will investigate if mutant PBMC-MLCs from patients with GBA1 associated PD deregulate key nodes of the autophagy lysosomal pathway (ALP) that regulate a-synuclein clearance. We will examine autophagy regulators, TFEB, and cathepsins B and D. We will analyze 10 subjects in each experimental group. The proposed experiments will enable us to rigorously determine if disease PBMC-MLCs can induce pathogenic alterations in DA neurons compared to control MLCs. Importantly, the donors of the PBMCs to be used are clinically very well characterized by our collaborators. In this biological system, DA neurons and MLCs interact with each other, enabling bi-directional pathogenic signals to be amplified. This model is flexible, as astrocytes can be introduced at later stages of the project. Procurement of the critical PBMC-MLC reagents is non-invasive, and blood samples can be used to follow the subjects over time, helping to decide when clinical intervention should be started. The proposed studies will help identify individuals at high risk of developing PD, and will lead to clinical trials focused on preventing GBA1-associated PD. This system is also applicable to PD induced by other genetic risk factors and to idiopathic PD.

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Awardee Amount: $345,000
Disease Target: Pancreatic Cancer

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Awardee Amount: $345,000
Disease Target: Volumeric Muscle Loss

Human Marrow Stem-Cell Derived Pro-Inflammatory Myeloid Progenitors as Immunotherapy for Pancreatic Cancer

Pancreatic ductal carcinoma (PDC) has a dismal prognosis, with median survival 10-12 months with standard chemotherapy. We seek to optimize pro-inflammatory immature myeloid cells (IMC), derived from human marrow hematopoietic CD34+ stem/progenitor cells, as a component of PDC treatment to improve outcomes. Adoptive transfer of IMC activated by absence of the repressive NF-kB p50 subunit (p50-IMC), following a dose of myelo-depleting 5-FU, demonstrate efficacy against PDC and other murine cancers in immune-competent, syngeneic hosts. p50-IMC macrophage and dendritic cell progeny may phagocytose tumor cells and then present multiple tumor antigens to induce a robust anti-tumor T cell response, while also secreting T and myeloid cell-activating cytokines. p50-IMC more readily reach tumors than do mature macrophages, which largely localize to the liver. NF-kB is a key transcription factor favoring pro-inflammatory, M1 gene expression. In myeloid cells lacking p50, M1 genes are de-repressed. STAT6 is a key transcription factor mediating tumor-suppressive M2 gene expression while repressing M1 gene expression, activated by JAK2 kinase downstream of IL-4 and IL-13. We hypothesize that simultaneous knockout of p50 and STAT6 will increase efficacy of IMC against PDC. Our specific aims are to: Aim 1. Develop human p50/STAT6DKO-IMC suitable for clinical translation and evaluate their macrophage gene expression in vitro and in human pancreatic cancer tumors in immune-deficient mice. Aim 2. Compare murine p50/STAT6 double knockout (DKO)-IMC with p50KO-IMC and STAT6KO-IMC for macrophage gene expression, efficacy vs syngeneic pancreatic cancer, and tumor T cell activation. Multiple solid tumors secrete chemokines and cytokines (such M-CSF) that attract monocytes and induce their maturation into M2-polarized tumor macrophages. Tyrosine kinase inhibitors targeting signaling from the M-CSF Receptor are under clinical investigation; however, resistance to such agents develops due to tumor secretion of alternative M2-polarizing cytokines. In contrast, targeting transcription factors such as p50 (to favor M1 gene expression) and STAT6 (to prevent M2 gene expression) is expected to genetically lock tumor myeloid cells in a pro-inflammatory state. p50 and STAT6 KO will be accomplished by gene-editing of human or murine marrow cells. Gene expression will be analyzed by qRT-PCR, RNAseq, ELISA, and flow cytometry. Phagocytosis will be evaluated by combining control and gene-edited macrophages with CSFE-labeled PDC cells. Anti-tumor efficacy will be evaluated against orthotopic PDC tumors in syngeneic murine hosts. Total and activated tumor cells will be enumerated by flow cytometry. Proposed studies are intended to demonstrate substantial efficacy of murine p50/STAT6DKO-IMC against pancreatic ductal carcinoma in immune-competent hosts and to develop and validate corresponding human marrow stem cell-derived IMC for rapid clinical evaluation.

Human Pluripotent Stem Cell-Derived MYOrganoid for the Treatment of Volumetric Muscle Loss

Skeletal muscle possesses inherent capacity for regeneration and repair following injury. However, this intrinsic potential is compromised in cases of volumetric muscle loss (VML) where extensive damage results in permanent functional deficits. Our labs have recently established several strategies that will be combined to effectively regenerate the lost muscle and to overcome the lack of re-innervation, reduced contractility, and limited function that follows VML. Tissue engineering strategies using a combination of human pluripotent stem cells (hPSCs) have unprecedented potential to provide effective therapies for VML but are typically limited by scale-up considerations, low differentiation efficiencies, and challenges inducing their structural organization into contractile, 3D skeletal muscle grafts. Recently, we have developed a myogenic specification protocol to direct hPSCs into functional skeletal muscle cells in a highly defined, fast, and efficient manner. To attain comprehensive characterization of the myogenic population derived from hPSCs, and to aid subsequent in vivo studies toward meaningful cellular therapy, we established a genetic PAX7 reporter line (PAX7::GFP) as putative skeletal muscle stem/progenitor cells. Our hPSC-derived PAX7::GFP+ cells show robust in vitro myogenic potential and form myofibers in the myotoxin-treated muscle tissues in immuno-deficient mice. However, transplanted hPSC-derived PAX7::GFP+ cells under mouse VML environment show only limited cell survival and poor integration into host tissues. To overcome this issue, our group has developed a new methodology to direct hPSCs into 3-dimensional (3D) skeletal muscle organoids (named as MYOrganoids). Our hPSC-derived MYOrganoids are composed mostly of myoblasts, multinucleated myofibers, and PAX7+ skeletal muscle stem cells. Interestingly, our preliminary data show that the transplanted MYOrganoids survived in our mouse VML model. Here, we propose to characterize the cellular diversity of MYOrganoids and properties of PAX7+ cells, and to evaluate if the transplanted MYOrganoids can develop a new vascularized and functionally innervated skeletal muscle tissues in the VML mouse model. Our Specific Aims are: Sp. Aim 1: Characterize the cellular diversity and molecular properties of the MYOrganoids as an in vitro surrogate model for stem cells and niche interaction. The goal of this aim is to characterize the PAX7+ cells of MYOrganoids and located in stem cell niche areas. We will perform single cell RNA-seq analyses to dissect molecular identities of the MYOrganoids, extensive immuno-staining to quantify PAX7+ cells in niches, and cell cycle analysis. In addition, we will quantitatively evaluate ultrastructural morphology, proliferation, multi-nucleation, and myogenic differentiation during the MYOrganoid specification. Sp. Aim 2: Utilize MYOrganoids to regenerate skeletal muscle following VML in vivo. Here we will implant MYOrganoids into VML injuries created in the tibialis anterior (TA) muscle of immuno-deficient mice. Two-weeks post-transplantation, we will harvest the muscle and assess survival and integration of the entire MYOrganoids as well as PAX7::GFP+ cells. Secondly, we will implant the optimized dosing of MYOrganoids into VML defects to assess their regenerative potential with and without inducing the mice to exercise. We will quantitatively assess cell survival, vascular and neural infiltration, the presence, and 3D morphology (i.e. maturity) of neuromuscular junction (NMJ), skeletal muscle regeneration and functional recovery at 2 months post-transplantation.

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Awardee Amount: $345,000
Disease Target: Drug Addiction

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Awardee Amount: $344,474
Disease Target: Viral Infections

Targeting a Novel Cocaine Receptor to Mitigate Cocaine Neurotoxicity

Cocaine abuse is a significant public health problem in Maryland. Maryland's drug overdose death rate is nearly double that of the national average. In Maryland, cocaine-related drug overdose deaths have been surging over the last few years. Despite decades of intensive research, there's no current FDA-approved treatment for cocaine abuse. Previous research suggests the presence of a yet-to-be-discovered high-affinity cocaine receptor. However, the identity of such a receptor is still unknown. Our research reveals two significant discoveries. Our published work demonstrates that the nerve terminal-enriched brain acid-soluble protein 1 (BASP1) is a high-affinity receptor for cocaine. We also show that the cocaine locomotor stimulant effect depends on autophagic degradation of the dopamine transporter (DAT). Furthermore, our preliminary findings suggest that BASP1 mediates DAT depletion by cocaine in dopaminergic nerve terminals. Hence, further research into BASP1 as a novel high-affinity cocaine receptor enables drug discovery and development for cocaine abuse. HiPSCs are a powerful tool for disease modeling in a dish and personalized medicine drug discovery. We propose to validate BASP1 as a cocaine receptor in human stem cell-derived neurons. In this proposal, we will validate our discovery that BASP1 is a putative high-affinity cocaine receptor in hiPSC-derived neural cells. The central hypothesis for this proposal is that: BASP1 is a cocaine receptor that mediates its neurotoxic actions through autophagy of the dopamine transporter in human-induced pluripotent stem cells-derived dopaminergic neurons. We will test this hypothesis through the following aims: Aim-1: Validate BASP1 as a high-affinity cocaine binding protein in human-induced pluripotent stem cells-derived dopaminergic neurons. Our research demonstrates that high affinity [3H]cocaine binding in rodent striata is dependent on BASP1. Moreover, 40% of BASP1 in the striatum is in dopaminergic nerve terminals. Hypothesis: BASP1 is an endogenous high-affinity cocaine binding protein in human-induced pluripotent stem cells-derived dopaminergic neurons. Specifically, we will use shRNA to knock down BASP1 in hiPSC-derived dopaminergic neurons and test the effect on [3H]cocaine binding to dopaminergic nerve terminals. Aim-2: Interrogate the effect of BASP1 on cocaine-induced dopamine signaling in human-induced pluripotent stem cells-derived dopaminergic neurons. Our previous work demonstrates that cocaine-induced locomotor stimulation is dependent on the autophagic degradation of DAT. Hypothesis: BASP1 mediates cocaine-induced dopamine reuptake inhibition through autophagic degradation of the dopamine transporter. We will overexpress or knockdown BASP1 in hiPSC-derived dopaminergic neurons and test the effect on (1) cocaine-induced autophagy using biochemical and morphologic approaches, (2) cocaine-induced DAT degradation by autophagy using biochemical, enzymatic, and morphologic approaches, and (3) electro-physiological function of hiPSC-derived dopaminergic neurons. Impact: Validating BASP1 as a high-affinity cocaine receptor in hiPSC-derived dopaminergic neurons constitutes a milestone in understanding the molecular mechanisms of cocaine neurotoxicity and discovering novel anti-cocaine medications. By defining the role of BASP1 in cocaine signaling in hiPSC-derived dopaminergic neurons, we will gain in-depth knowledge of how it mediates the actions of cocaine in human cells, lending strong support that it is indeed a clinically relevant receptor. Also, this work enables interrogating the role of BASP1 in cocaine neurotoxicity in other components of the reward circuit derived from hiPSCs. Besides, this project facilitates the exploration of new therapeutic options that specifically target BASP1 for the treatment of cocaine and other substances of abuse.

Microglial Infused Cerebral Organoid Cultures to Study Viral Infections of the CNS

HIV infection of the brain: While the development of antiretroviral therapies (ART) produced dramatic declines in the number of HIV/AIDS-related deaths, a considerable amount of morbidity remains in this population that includes an increased prevalence of cognitive impairments (CI). There is a considerable need for a human model system that recapitulates key aspects of the neuroimmune response to study the effects of HIV and other neurotrophic viruses on brain, and to develop interventions that can protect the brain in people infected with HIV. Development of microglial colonized forebrain organoids: Advancements in the use of pluripotent stem cell (iPSCs) derived cerebral organoids have resulted in the development of three-dimensional (3-D) model systems that recapitulate many of the physiological and biochemical interactions between multiple different types of brain cells34-3738-41. However, it has been difficult to develop brain organoid systems that include microglia, as these cells are derived from erythro-myeloid progenitor cells originating from the yolk-sac during embryonic development. The presence of microglia in brain organoids is especially important for the study of neurotrophic viruses. Microglia are the immune sentinels of the brain and express a variety of pattern recognition receptors. Without microglia it is not possible to accurately study the neuroimmune response to viral infections. In the case of the human immunodeficiency virus (HIV), it is not possible to study brain infection without the presence microglia as these are the only brain resident cell productively infected with HIV. To address this gap we have developed a robust, and reproducible microglia engineered human forebrain organoid (FOR-organoids) system in which microglia are derived from iPSCs using a yolk-sac differentiation protocol. These human microglia colonize FOR-organoids (FOR-organoids+Micro) and actively survey the organoid environment by extending and retracting processes. These FOR-organoids+Micro can successfully support HIV infection (FOR-organoids+Micro+HIV). Specific to HIV research, these FOR-organoids+Micro+HIV allow us for the first time to identify mechanisms for neuronal damage in the setting of ART-induced suppression of HIV replication. In this application we propose to test the hypothesis that antiretroviral therapy (ART) reduces but is unable to eliminate the neuroimmune response and associated neuronal damage associated with HIV infection. We decided to focus our initial development on the innate immune system and microglial infection as this is the predominant cell type infected in brain that by far out numbers the number of HIV infected (or uninfected) leukocytes in brain. However, we do recognize the importance of the adaptive immune response in a chronic viral infection and in this study we will measure a number of cytokines and chemokines that are involved in the transmigration of leukocytes into brain parenchyma. Future studies/development will extend these findings to incorporate the adaptive immune response to chronic HIV infection.

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Awardee Amount: $345,000
Disease Target: Bone Defects

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Awardee Amount: $345,000
Disease Target: Wound Repair

CD141 Identifies Novel Mesenchymal stem/Progenitor Cell Subsets for Skeletal Tissue Engineering

Autologous stem cell therapies hold promise for the treatment of bone defects. The non-adipocytic cellular fraction of adipose tissue has long been known to house multipotent progenitor cells6, including skeletogenic cells7. This has spurred use of the uncultured stromal vascular fraction (SVF) or culture-expanded stromal cells (ASC, adipose stromal/stem cells) for bone tissue engineering. However, recent clinical trials using adipose-derived cell therapies for bone tissue healing have demonstrated suboptimal results8. This has led our group to the overarching tenant that development of a translatable cell-based therapy for bone regeneration hinges 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 skeletal engineering and regeneration (reviewed in 12), having been first described by our group9-14. The vascular tree is exquisitely organized into concentric layers, the outermost layer termed the tunica adventitia. The bulk of multipotent progenitor cells within fat lie within the adventitia (termed adventitial cells or adventicytes)10,40, yet the cellular diversity within this stem cell niche is only now being uncovered. When examined in a clonal fashion, adventicytes represent a mixed population, with single cells of unipotent, bipotent, or multipotent properties. Recent single cell molecular analysis by our group confirms this heterogeneity14, which has also been confirmed by others using alternative -omics approaches15. In a series of new observations, we recently employed several high throughput approaches, including single cell RNA sequencing and spatial transcriptomics to further define the stem cell hierarchy of CD34+ human adventitial progenitor cells. Our findings suggest that: 1) a continuum of cell types exist within this microanatomical stem cell niche, 2) several uncharacterized cell surface markers demonstrate restricted expression profiles among adventicytes within human blood vessels, and 3) the novel marker CD141 identifies a primitive / undifferentiated mesenchymal progenitor cell type. CD141 (Thrombomodulin (THBD) or Blood dendritic cell antigen-3 (BDCA3)) is a cell surface glycoprotein studied in the context of endothelial and dendritic cell function23-25, and until now has no known significance in mesenchymal stem cell biology. In aggregate, our findings have uncovered an unexpected and functionally relevant cellular diversity within the outermost layer of blood vessels in human white subcutaneous adipose tissue. These markers procure cells in sufficient numbers for a tissue engineering approach. The current MSCRF discovery proposal capitalizes on this series of new observations. In an exploratory approach, we seek to further characterize the cellular hierarchy of adventitial subpopulations across vascularized human tissues (Aim 1), and leverage this knowledge for improved skeletal tissue regeneration.

Induced Pluripotent Stem Cell-Derived Extracellular Vesicles for Wound Healing

Extracellular vesicles (EVs) have emerged as a promising therapeutic platform with substantial potential for treatment of a variety of diseases and injuries, including wound repair. EVs are natural mediators of intercellular communication through transfer of proteins, lipids, and nucleic acids, and may overcome some of the limitations associated with molecular and cell-based wound healing therapies. Specifically, compared to molecules, EVs are multifactorial vectors capable of stimulating multiple signaling and gene regulation pathways, critical to effective wound repair. Compared to cells, EVs may be: (1) delivered more frequently without inducing an immune response leading to longer effect durations, (2) less prone to damage in the delivery process, (3) have defined half-lives and clearance pathways and are thus more drug-like, and (4) are not capable of uncontrolled division or differentiation. Many exciting results pervade the scientific literature, including clinical trials and the successful use of EVs in non-human primates and humans. In particular, EVs from mesenchymal stem cells (MSCs) have been shown to be promising wound healing therapeutics due to their pro-vascularization and anti-inflammatory properties. Unfortunately, translation of MSC-based therapies, including MSC EVs, is hindered by donor heterogeneity effects and biomanufacturing constraints To address these critical limitations, our group has explored alternative cell sources and developed novel biomanufacturing approaches. Specifically, we have examined the potential of induced pluripotent stem cell (iPSC)-derived EVs for wound repair, as a single iPSC-derived source can be cultured indefinitely. We have shown in preliminary experiments that EVs from iPSC-derived MSCs (iMSCs) perform similarly to or better than EVs from human bone marrow-derived MSCs (bmMSCs) with respect to angiogenic and anti-inflammatory activity, both of which are relevant for wound healing. Interestingly, EVs from undifferentiated iPSCs have similar angiogenic activity combined with superior anti-inflammatory activity compared to donor-matched iMSC EVs while requiring less steps to generate. Thus, In Aim 1, we will compare the potential of EVs from donor-matched iPSCs and iMSCs in a mouse model of wound healing to assess which source is most promising for further development. Additionally, our team has utilized perfusion bioreactors with customizable 3D-printed cell culture chambers for EV production 9. These devices match the ability of commercially-available bioreactors to increase EV production by 10-100+ fold compared to conventional cell culture flasks. However, they offer unique potential to easily and reproducibly customize the cell culture environment, which is critical in defining both EV quantity and biological activity, enabling knowledge of cell-biomaterial interactions and cellular mechanobiology to be applied towards improving EV therapeutic potency. Our preliminary data show that bmMSC EV pro-vascularization and anti-inflammatory bioactivity (critical for wound repair) are enhanced via dynamic culture in these bioreactor systems, as is the overall wound healing potential of the EVs in vivo. In Aim 2, we will examine the mechanism of the effects of bioreactor culture on stem cell-derived EV potency with respect to wound repair. The overall goal of the project is to identify a renewable source and scalable biomanufacturing platform to bring stem cell-derived EVs closer to clinical translation for wound healing therapy.

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Disease Target: Diabetes

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Awardee Amount: $345,000
Disease Target: Heart Disease

New Gene+Cell Therapy Platform Targeting Diabetes with Engraftable Skeletal Muscle Stem Cells

Diabetes is a peptide hormone deficient disorder, characterized by destruction and/or loss-of-function of beta-cells in the pancreas and the absence of insulin. In addition to daily injection of exogenous insulin formulations, several other treatments are under development, although many still remain experimental or have achieved limited clinical success. These include cell therapy by islet and stem cell transplantation. However, many of these approaches have limitations, for example, insufficient [B]-cell mass and incomplete engraftment in their encapsulation device by the infiltrating myofibroblasts as shown in a recent clinical trial. Another strategy is the use of viral or non-viral gene therapy to replace missing insulin function either by directly targeting the pancreas or by indirectly targeting other tissues such as skeletal muscle. Skeletal muscle is the largest tissue in the body, comprising between 40-50% of body mass. Skeletal muscle is capable of sensing and adapting to metabolic and physiologic changes, and the major site of glucose disposal after a meal, taking up 60-70% of circulating glucose in an insulin-dependent manner, making it an important tissue in normal glucose homeostasis. However, previous gene therapy in skeletal muscle did not have a long-term efficacy due to fast turn-over time and insufficient gene delivery efficacy. To achieve a meaningful gene therapy via skeletal muscle tissues, it is important to have an engraftment of skeletal muscle stem cells (satellite cells), although adult skeletal muscle stem cells have limited in vitro growth and/or there is no gene-delivery system specific for satellite cells. In this proposal, we will develop a new Gene+Cell therapy model for diabetes, by utilizing our hPSC-derived PAX7::GFP+ cells. Insulin-expressing constructs under control of myofiber-specific promotor or glucose-sensing promotor will be knock-in into the PAX7::GFP hPSCs, followed by PAX7+ satellite cell isolation and detailed characterization. In addition, we will test in vivo therapeutic effects of the insulin-expressing PAX7+ satellite cells in an immune-deficient diabetic mouse model. In combination of the hypoimmunogenic hPSC concept in future, our insulin-expressing PAX7+ satellite cells will be a better (or at least alternative) solution for diabetes patients. Our study will enable us to establish a new skeletal muscle Gene+Cell therapy platform targeting for diabetes. Our insulin-expressing skeletal muscle stem cell-based system can be an alternative but meaningful strategy for the patients. In addition, this approach can be readily applicable for other metabolic disorders by providing a persistent and long-term enzyme delivery.

Transcriptomic Maturation of Human iPSC-Cardiomyocytes

Cellular immaturity is a universal problem and one of the most pressing challenges in pluripotent stem cell (PSC) medicine. Its been more than fifteen years since the Yamanaka lab demonstrated that adult cells can be reverted to a pluripotent state by overexpressing a combination of transcription factors (TFs) regulating pluripotency. The resulting PSCs can differentiate into any type of body cell, including cardiomyocytes (CMs), which have been utilized widely to study heart muscle disease (i.e., cardiomyopathy) and repair. Yet, PSC-derived CMs (PSC-CMs) remain fetal-like in phenotype and function, and the lack of maturity has emerged as a major concern in their biomedical application. However, it remains unknown why PSC-CMs are mired in an immature state. To answer this question, a direct comparison with endogenous CMs in vivo is required, which is the gold standard for identifying factors that can instruct PSC-CM maturation in vitro. In order to make a valid comparison, it is crucial to analyze CMs on a single-cell level. This is due to the fact that CM maturation happens in a heterogeneous and continuous fashion over time. However, conventional single cell RNA-sequencing (scRNA-seq) platforms do not work for mature CMs due to their size and morphology. To overcome this technical hurdle, we developed a large particle sorting method that enables high-quality, high-resolution scRNA-seq of postnatal/adult CMs. With this new platform, we generated a comprehensive, high-resolution scRNA-seq reference map of CM maturation in vivo, encompassing 15 time points from embryonic to adult stages. Trajectory reconstruction revealed a perinatal window during which a majority of genes change their expression. We refer to these genes as maturation genes (MGs). We directly integrated these with a reference created using isogenic PSC-CMs and found 550 abnormally regulated MGs in PSC-CMs. To define TFs regulating the dysregulated MGs, we have performed a series of computational analysis, including affinity propagation, chromatin accessibility, and upstream regulator analysis, and identified a gene regulatory network of nine dysregulated TFs (dTFs) that underlie maturation failure in PSC-CMs. Based on this finding, we hypothesize that activating dTF expression enables PSC-CMs to overcome the perinatal arrest. Aim 1: To determine if dTF expression affects the structural, functional, and metabolic maturation of PSC-CMs. We will increase dTF levels with modified RNAs and analyze changes in morphology/structure, calcium handling, and force generation at the single cell level. Metabolic and electrophysiological maturation will be assessed as well. Aim 2: To determine if dTF expression allows for the modeling of late-onset cardiomyopathy in PSC-CMs. We will use PSC-CMs derived from patients with arrhythmogenic right ventricular cardiomyopathy (ARVC), an inherited form of cardiomyopathy that manifests in adolescence and adulthood and determine if dTFs can promote disease manifestation in a dish. Aim 3: To identify the minimal, most effective dTF combinations for PSC-CM maturation. We will use our entropy metric and determine which combinations of dTFs have the most significant effects on promoting maturation of PSC-CMs, as some TFs may have regulatory redundancy. By completing these aims, we expect to decode the function of dTFs mediating PSC-CM maturation, which would provide novel insights into the premature arrest and maturation of PSC-CMs. The knowledge will help us develop a TF-based method for PSC-CM maturation, which will be leveraged towards improving clinical viability of PSC-CMs.

Gabsang Lee, PhD

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

In Vitro and in Vivo Characterization of Human PSC-Derived Lens Cells

Every year, over one million people lose their vision due to cataracts, a loss of transparency of the lens of the eye. In addition, congenital cataracts cause approximately one-third of all cases of blindness in infants worldwide. Surgical removal of cataractous lenses is the only effective treatment; however, some risks cause postoperative complications and physical incompatibility, which lead to permanent damage in pediatric patients. It is estimated that 200,000 children worldwide are blind, due to cataracts, and that 20,000 to 40,000 children are born each year with congenital cataracts. Cataract blindness in children presents an enormous problem in terms of human morbidity, economic loss, and social burden. Lens development is initiated from the presumptive lens ectodermal cells, which become the lens placode that subsequently invaginates to shape the lens vesicle, and ends with terminal differentiation of the epithelial cells into elongated fiber cells in lens tissue. Then, protein synthesis and turnover in the mature fiber cells are halted so that the proteins of the lens can remain soluble and transparent throughout life. In the lens, A-crystallin (CRYAA) and B-crystallin (CRYAB) constitute 30% of the protein content of the lens, and they help maintain the solubility of the other lens proteins, such as [B]- and -crystallins. It is generally believed that genetic mutations in crystallins and/or unknown environmental factors induce crystalline protein misfolding and aggregation into insoluble amyloids, which forms aggregated crystallin proteins, a key pathogenic characteristic of cataracts. The current surgical treatment (removal of the existing cataractous lenses and implantation of artificial lens) has been used for adult patients but has not been unsuccessful for pediatric patients. A recent study described the existence of adult lens stem cells, which can be isolated and transplanted into cataractous lenses and/or animal models, showing significant rescue effects. This study showed the possibility of developing a stem cell-based treatment for cataract patients; however, the adult lens stem cells did not proliferate enough in a large number of patients. The induction of pluripotency in somatic cell types has been one of the great breakthroughs in stem cell biology. After the early pioneering studies by Shinya Yamanakas lab, human induced pluripotent stem cells (hiPSCs) became an invaluable source to generate large quantities of otherwise extremely rare cell populations. While the approaches used in these studies show the promise of utilizing hESCs and hiPSCs for lens development, their lens differentiation protocols may not follow the natural placode development. In addition, it is still unclear how a pure lens cell population can be isolated for detailed characterization, as well as in vivo efficacy studies. In this proposal, we will use our established protocol and lens-specific genetic reporter CRYBB1::GFP hESC clones to generate and isolate lens cells from hESCs and hiPSCs, followed by in vitro molecular and cellular characterization and in vivo transplantation. Our proposed study will be one of the first steps in the development of a new hiPSC-based lens cell therapy for cataract patients.

Nicholas John Maragakis, MD

Johns Hopkins University
Awardee Amount: $344,794
Disease Target: Amyotrophic Lateral Sclerosis

A Versatile Human iPSC Platform for Modeling Corticospinal Motor Neuron Connectivity

Dysfunction in cell signaling between corticospinal motor neurons (CSMN) and spinal motor neurons (SPMN) is a central theme in the pathobiology of a number of neurodegenerative disorders, including amyotrophic lateral sclerosis (ALS). There is also an increasing appreciation regarding neuronal and astrocyte subtype heterogeneity which adds a layer of complexity to the etiology and cell specificity of neurodegenerative diseases. CSMNs synapse on SPMNs, thus communicating signals from the brain to the spinal cord. Despite their involvement in ALS pathogenesis, CSMNs are vastly understudied compared to SPMNs and almost all proposed therapeutics for this disease have targeted SPMN-specific biological pathways. Almost all of these therapeutics have failed. However, it has been increasingly recognized that cortical dysfunction is an early symptom in ALS, including in pre-symptomatic familial ALS patients harboring disease-causing mutations. This bias has occurred, in part, because modeling CSMN pathobiology in neurodegenerative disorders, like ALS, is particularly challenging. Transgenic mouse models are important for understanding pathogenic mechanisms of ALS but mouse CSMNs have different connectivity to SPMNs than human CSMNs, which may mask the observable pathology stemming from CSMNs in these models. This proposal seeks to create an innovative human induced pluripotent stem cell (hiPSC)-based platform for understanding CSMN and SPMN connectivity, key contributors to the corticospinal tract (CST). Because the incorporation of not only corticospinal and spinal motor neurons but regionally-specific astrocyte subtypes (cortical and spinal) in a newly designed microfluidic chamber is proposed, the development of this model will lend itself to accurately modeling the complexity of these cell-subtype interactions. The proposal seeks to partner these cell subtypes with innovative technologies including microfluidic apparati, designed by our team, that can separate cortical and spinal cord compartments while allowing unidirectional axon growth from the cortical to the spinal compartmentmimicking CST connectivity. This will afford us an opportunity for understanding morphological and pathological relationships amongst different cell types. The use of multielectrode array (MEA) electrophysiology adds to the models utility and versatility in understanding the functional characteristics of CSMN and SPMN in isolation as well as their synaptic connectivity through the extension of axons via microfluidic channels. Specifically activating CSMN via optogenetic approaches in this proposal allows for a temporal and cell-specific understanding of these networks. Providing real-time functional analysis following the administration of various proposed therapeutic compounds complements the neuroanatomical and neuropathological elements of the platform. The initial aims of the study seek to validate this system using healthy control hiPSCsdefining the temporal maturation of this corticospinal connectivity. However, in a proof of concept, the incorporation of hiPSC derived from patients with amyotrophic lateral sclerosis (ALS) will also be undertaken. This will provide an opportunity to examine how hiPSC-CSMNs and SPMNs harboring specific ALS mutations behave pathologically and electrophysiologicallyan opportunity to understand how ALS CSMN influence SPMN function. Taken together, the development of this versatile humanized model of the CST will provide insights into CSMN connectivity with implications for understanding cell subtype-specific contributions to neurodegenerative diseases and providing a platform for testing targeted therapeutics.

Satoru Otsuru, MD, PhD

Johns Hopkins University
Awardee Amount: $344,966
Disease Target: Osteogenesis Imperfecta

Linda Smith Resar, MD

Johns Hopkins University
Awardee Amount: $345,000
Disease Target: Thrombocytopenia, Myeloproliferative Neoplasms

Developing Novel Therapies for Osteogenesis Imperfecta using Growth Plate Organoids from Patient-Derived iPSCs

Osteogenesis imperfecta (OI) is a genetic disorder most commonly caused by autosomal dominant mutations in genes encoding type I collagen (Col1). Besides bone fragility, patients suffer from impaired bone growth, which leads to short stature and often fatal respiratory failure secondary to the immature development of the chest cavity. While bisphosphonates are the standard treatment for bone fragility, no such treatments currently address longitudinal growth deficiency. Elucidation of the mechanism by which mutations in Col1 genes result in growth deficiency will lead to the development of effective treatments for the improvement of skeletal growth. The growth plate (GP) plays a major role in longitudinal bone growth. In the GP, chondrocytes undergo progressive steps of maturation to become hypertrophic chondrocytes (HCs). In dominant forms of OI, mutations in Col1 induce misfolding of Col1, resulting in the accumulation of mutant Col1 in the endoplasmic reticulum (ER). The retention of misfolded collagens induces ER disruption/cell stress leading to cellular dysfunction, which is the major pathogenesis of brittle bones in OI. Using a murine model of OI (G610C OI mice), we have recently found that chondrocytes begin to express Col1 as they become hypertrophic chondrocytes (HCs) in the GP and OI HCs exhibit the remarkably dilated ER, suggesting that OI HCs are exposed to ER disruption similar to OI osteoblasts. Treatment with 4-phenylbutyrate (4PBA, cell stress reducer) substantially restored ER size in HCs and improved bone length in G610C OI mice. Interestingly, 4PBA treatment failed to rescue ER size in osteoblasts and strengthen bones. Given that previous studies showed that treatments with an anti-sclerostin antibody to improve osteoblast activity did not enhance bone length in OI mice, our findings suggest that HC dysfunction induced by ER disruption is responsible for growth deficiency in G610C OI mice and can be a potential drug target to enhance bone growth. Considering the translational approach of these findings, it is essential to clarify if a similar mechanism can apply to OI patients and whether the cellular response to ER disruption in HCs varies between patients with distinct mutations. These questions will be addressed by the following specific aims using iPSCs derived from OI patients. Specific Aim1. To characterize stress response in HCs differentiated from iPSCs of OI patients In general, cells under ER disruption/cell stress utilize unfolded protein response (UPR) to rescue ER homeostasis. It has been demonstrated that different mutations in Col1 induce different stress responses. Additionally, our data indicate that HCs utilize canonical UPR while osteoblasts do not in G610C OI mice, suggesting that stress responses can be specific to mutations and cell types. Specific Aim2. To determine the effects of cell stress reducers on ER homeostasis 4PBA had different effects on HCs and osteoblasts in G610C mice and our follow-up studies demonstrated that HCs and osteoblasts had different types of cell stress/stress response even with the same G610C mutation. Moreover, previous studies showed that fibroblasts from OI patients with different mutations exhibited different cell stress and different responses to 4PBA, suggesting that cells with different cell stress and mutations utilize different stress response pathways to maintain ER homeostasis. Thus, to develop precision medicine for OI growth deficiency, it is critical to establish a method to examine stress response pathways in each patients HC and evaluate the effects of drugs on HC ER homeostasis. If successful, we will be able to determine whether this GP organoid system can be utilized to examine stress response and drug response in individual patients HCs. This system can be used for drug screening to search for new drugs and has the potential to lay the foundation to develop precision medicine to find the most effective treatment for OI growth deficiency in each patient.

Developing Stem Cell Technology for Megakaryopoiesis and Platelet Production

We propose to develop innovative stem cell technology using bone marrow organoids for megakaryopoiesis. Our long term goal is to establish hematopoietic organoids as personalized platforms of cell-based therapy for platelet production. We focus on the High Mobility Group A1 (HMGA1) chromatin regulator in fostering regenerative potential of hematopoietic stem cells (HSCs), differentiation to megakaryocytes (megakaryopoiesis), and platelet production. Our scientific premise that HMGA1 enhances HSC function and megakaryopoiesis is based on our exciting published work and more recent, unpublished research, including: 1) HMGA1 fosters proliferation and self-renewal in embryonic stem cells by inducing specific transcriptional networks involved in pluripotency (Resar et al, Cancer Res 2018). 2) In intestinal epithelium, Hmga1 promotes expansion of stem cells by amplifying Wnt signals (Xian et al, Nature Commun 2017). 3) Surprisingly, Hmga1 also builds a stem cell niche by up-regulating Sox9 to drive terminal differentiation of intestinal stem cells to Paneth cells; Paneth cells, in turn, secrete Wnt to nourish the stem cells. 4) In mouse models of JAK2V617F mutant myeloproliferative neoplasms (MPN), loss of just a single Hmga1 allele decreases platelet counts and the frequency of HSC, megakaryocyte-erythroid progenitors (MEP), and megakaryocytes (Mk), while preventing bone marrow fibrosis (Li et al, Blood 2022; featured in a Commentary and on the cover). 5) In patients with JAK2V617F clonal hematopoiesis, HMGA1 increases in HSC and progenitors with rising platelet counts and disease progression. 6) Hmga1 deficiency in mice causes thrombocytopenia with aging. 7) Loss of Hmga1 within mouse HSCs: a) disrupts regenerative function of HSC and Mk development in clonogenicity assays in vitro, and, b) decreases platelet counts and repopulating ability in bone marrow transplantation assays. 8) Preliminary transcriptomic analyses at the level of single cells using RNA sequencing (scRNAseq) reveal that Hmga1 governs networks involved in proliferation and Mk development. 9) Recent advances in bone marrow organoids demonstrate robust Mk differentiation which recapitulate salient features of human megakaryopoiesis, both in normal and diseased states. Together, these exciting results led us to the following hypotheses: 1) HMGA1 fosters megakaryopoiesis from HSC through specific enhancers that fuel Mk development and platelet production, and, 2) Optimizing HMGA1 function in human bone marrow organoids will enhance HSC differentiation to Mks and platelet production. To test this, we propose to harness organoid technology with human CD34+ hematopoietic stem and progenitor cells (HSPC) for megakaryopoiesis with the following Specific Aims: 1) To define the role of HMGA1 in HSC regenerative function and megakaryopoiesis using innovative models, and, 2) To deploy bone marrow organoid technology and CRISPR to modulate HMGA1 levels in human blood cells for megakaryopoiesis and platelet production. At the completion of this project, we expect to compare megakaryopoiesis from human CD34+ stem and progenitor cells with varied levels of HMGA1 in bone marrow organoids, elucidate key HMGA1 enhancers and transcriptional networks, and identify pathways that foster platelet production. Together, these studies will provide the groundwork necessary to develop cell-based therapies for individuals with platelet disorders.

Lena Smirnova, PhD

Johns Hopkins University

Awardee Amount: $344,979

Disease Target: SYNGAP1-Intellectual Disability

Leveraging In-Vitro Organoid Model and Clinical Data to Examine Phenotypic Diversity in SYNGAP1 Intellectual Disability

SYNGAP1-Intellectual Disability (SYNGAP1-ID) is a rare neurodevelopmental disorder resulting from defects in SYNGAP1, a gene that encodes a Ras-GAP-GTPase is expressed in excitatory and inhibitory neurons and is important for AMPA (-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor) receptor trafficking, synaptic plasticity, learning, and memory. The clinical spectrum and disease phenotypes are quite variable with some individuals having well-controlled epilepsy and mild cognitive difficulties while others have intractable epilepsy with significant cognitive and behavioral impairments that have profound consequences and significant morbidity across the lifespan. There are no disease-specific treatments for SYNGAP1-ID and the current approach has focused on symptomatic care. Several gaps limit the discovery of novel therapeutics. First, there are no high-throughput models with predictive validity for testing novel therapeutics. The second gap is in our knowledge of the basic pathophysiology, this limits our ability to identify secondary treatment targets. For example, it is not known whether genetic variants differentially signal through the Ras/Rap/Rac pathways and their impact on network function. The third gap is our understanding of the molecular mechanisms that lead to phenotypic diversity and the contribution of temporal and spatial expression of SYNGAP1 protein isoforms in this process. Since pathogenic variants that lead to SYNGAP1-ID are distributed across the gene, the variant location may disrupt the normal balance of protein isoforms with the resulting impact on neuronal development, function, and physiology. In this collaborative project we propose to address the limitations presented by the lack of high-throughput models and understanding the phenotypical diversity by generating several distinct patient-derived iPSC lines and brain organoids therefrom.

We used knowledge gained from our interdisciplinary studies to select five SYNGAP1 variants from persons with distinct clinical phenotypes and deep phenotyping data. We will generate iPSC lines from patients with SYNGAP1-ID, and familial controls and then differentiate them into brain organoids. RNAseq, in addition to extensive image analysis of different cell types and synapses within the organoids, will be performed to fully characterize the model and narrow down the molecular network potentially underlying the phenotypical diversity and to assess the effects of SYNGAP1 mutations on neural differentiation. The functionality of brain organoids will be assessed with Ca^{2+} imaging and neurite outgrowth assay. Since seizures are a common feature of SYNGAP1-ID we will use high-density multielectrode arrays (HD-MEAs) as a functional readout of network activity to lay the ground for future researcH: developing a functional readout assay, which should enable 1) the comparison of in vitro data with human phenotypic data, such as EEG recordings, 2) modeling seizures and cognitive impairment in vitro and correlating it with human EEG outcomes. This will allow us to develop a screening tool for drug development for this rare neurodevelopmental disorder with no targeted treatments. We expect that this model will provide foundational knowledge and will serve as a high-throughput model for use in preclinical trials. These tools, once established, will be an invaluable resource to scientists studying SYNGAP1 and the wider community of scientists studying AMPA receptor-mediated processes and the mechanistic impact of genetic variants on disease phenotypes.

MSCRF

POST-DOCTORAL FELLOWSHIP

GRANT AWARDS

2023

Mansoureh Barzegar, PhD

Johns Hopkins University
Mentor: Xitiz Chamling, PhD
Awardee Amount: $130,000
Disease Target: Multiple Sclerosis

Arthur Feltrin, PhD

Lieber Institute for Brain Development.
Mentor: Jennifer Erwin, PhD
Awardee Amount: $130,000
Disease Target: Schizophrenia (SCZ)

Investigating the Potential Contribution of Retinoic Acid Receptor-Related Orphan Receptor-Gamma Pathway in Oligodendrocyte Maturation & Myelination

Multiple Sclerosis (MS), the most common myelin disorder disease, can occur from autoimmune insult leading to severe disability. Available treatment options are limited to modulation of immune responses to slow down the progression of the disease, but do not directly promote remyelination. Several studies have reported that following myelin injury, oligodendrocytes progenitor cells (OPCs) migrate towards the site of injury, where they show some ability to differentiate into mature oligodendrocytes (OLs) capable of remyelination of the demyelinated axons. However, endogenous progenitors around a CNS lesion appear to be limited in both their mitotic competence and differentiation potential, and they progressively lose their remyelination capacity with aging. To identify compounds and biological pathways that could promote OL-myelin repair, our lab developed a screening platform to assess OPC maturation to OLs capable of expressing myelin components. Using hOPCs derived from this reporter cell line, we performed drug screening of about 2500 bioactive molecules to identify the small molecules that can enhance maturation of hOLs from hOPCs. One of the bioactive compounds we identified was SR2211, an inverse agonist for retinoic acid receptor-related orphan receptor g (ROR-g). Interestingly, the expression of ROR-g has been shown to be crucial in differentiation of TH17 cells, a subset of proinflammatory helper T cells, and the expression of IL-17, both of which have been implicated in pathogenicity of MS. Our data with SR211 suggests that modulation of the ROR-g pathway may also contribute to myelination. This MSCRF aims to investigate the contribution of ROR-g pathway in hOPC differentiation and maturation to hOLs with remyelination capacity. We hypothesize that the ROR-g pathway may be a promising new potential target for the development of remyelination-based therapies in neurodegenerative diseases.

Neural Mechanisms of Schizophrenia using Variational Autoencoder Analysis of Induced Pluripotent Stem Cell-Derived Neural Networks

Schizophrenia (SCZ) is an inherited neuropsychiatric disorder that affects about 1% of the population. The underlying biology of SCZ is not well understood due to its diverse symptoms and the complex combination of low-penetrance common alleles and high-penetrance ultra-rare variants, many of which have not yet been identified. High-penetrance variants can provide valuable insights into the disease's biology and inform the development of therapeutics. Heterozygous loss-of-function mutations in the histone H3K4 methyltransferase SETD1A is one of the few single gene variants that is strongly linked to SCZ risk. Previous research and our preliminary data support the idea that there are shared genetic and molecular features between SETD1A and SCZ. However, like most rare variants, heterozygous SETD1A LOF are pleiotropic and are causal variants in both SCZ and early onset neurodevelopmental syndrome. We propose to study the biology of SETD1A LOF pathogenesis and its relationship to neuropsychiatric disorders in human systems: postmortem DLPFC from SCZ patients, 2D neurodevelopmental induced pluripotent stem cell (iPSC) models derived from the same individuals in the postmortem cohort, and isogenic SETD1A LOF heterozygous lines edited in those same dura-derived iPSC. We will study five previously generated isogenic heterozygous LOF SETD1A iPSC in two control iPSC genomic backgrounds, including two lines that contain the most common patient mutation, a heterozygous two-base deletion at the exon 16 splice acceptor (c.4582- 2delAG>-). The postmortem cohort is the deeply characterized large LIBD Brain collection with corresponding genomics datasets from DLPFC, hippocampus, and caudate. Our overarching hypothesis is that SETD1A and SCZ in general share a causal structure and that iPSC derived from postmortem brain recapitulates this structure. Linking datasets with iPSCs from the same control individuals edited to contain SETD1A mutations will inform the causal mechanisms involved in SCZ pathogenesis that are both shared and specific for SETD1A and SCZ.

Joe Kodama, PhD

University of Maryland, Baltimore
Mentor: Satoru Otsuru, MD, PhD
Awardee Amount: $130,000
Disease Target: Osteogenesis Imperfecta

Audra Kramer, PhD

University of Maryland, Baltimore
Mentor: Ivy E. Dick, PhD
Awardee Amount: $130,000
Disease Target: CACNA1A

Investigating the Changes in Mitochondrial Function and Energy Metabolism in Osteoblasts in Osteogenesis Imperfecta

Osteogenesis Imperfecta (OI) is a genetic disorder that is most commonly caused by mutations in genes encoding type I collagen (Col1). Osteoblasts (OBs) in OI patients have impaired osteogenic differentiation capability, leading to low bone accrual and contributing to the "brittle bone" phenotype. Studies have shown that ER stress caused by the accumulation of mutant Col1 impairs OB function. However, despite multiple efforts to reduce cell stress, OB function has not been fully restored, indicating that there may be additional mechanisms at play in the impaired osteogenesis in OI. Our previous research and new preliminary data have identified potential mitochondrial dysfunction in OBs from a murine OI model and in Osteoblasts derived from bone marrow stem cells (BMSCs) of OI patients. Given that mitochondrial activation is required for cells to handle early ER stress, we hypothesize that cell stress in OI osteoblasts is aggravated due to altered mitochondrial function. To test this, we will use engineered BMSCs expressing the transcription factor A, mitochondrial (TFAM) that are activated mitochondrial function and examine if enhanced mitochondrial function can mitigate ER stress and improve osteogenesis. Upon completion of this project, we will be able to determine if mitochondrial dysfunction plays an important role in pathogenesis of OI. This will lead to the development of novel therapies targeting mitochondrial function for OI. The underlying mechanism of osteopenia in osteogenesis imperfecta (OI) patients, caused by a deficiency in osteoblast (OB) differentiation, is not well understood. Our and others previous works have identified an ER stress caused by the misfolded mutant collagen produced in OI OBs. However, either ER reducer or autophagy enhancer had limited benefits in rescuing the osteogenesis, suggesting an unknown mechanism underlying the unique ER stress in OI OBs. Our novel finding that mitochondrial function is potentially impaired and therefore fails to support the ER response to the misfolded proteins provides us with a not-yet-known mechanism of the cellular dysfunction in OI OBs, which might fill the gap. We will investigate the benefits of genetically manipulating mitochondrial function on osteogenesis in human bone marrow stem cell-derived OBs. Our ultimate goal is to treat OI patients by improving mitochondrial dysfunction. To achieve this goal and complete the bench-to-bedside translation, we will have to further clarify the mechanism by which the mutant collagen causes mitochondrial dysfunction and find the precise molecular targets to develop practical clinical treatments.

Evaluating the Pathogenic Mechanisms Underlying CACNA1A Disorders

Mutations in CACNA1A lead to a wide spectrum of neurological conditions, including familial hemiplegic migraine type 1, episodic ataxia type 2, and cerebellar atrophy. CACNA1A encodes the voltage-gated calcium channel CaV2.1 which is highly expressed in the Purkinje neurons of the cerebellum and at the neuromuscular junction. Unfortunately, there currently are no drugs available to directly target CaV2.1, and the therapeutic interventions that are often used do not have universal efficacy. This proposal aims to study four novel mutations that have been obtained through a collaboration with a child neurologist at Johns Hopkins Hospital. The clinical phenotype for these mutations is complex, including nontraditional symptoms such as congenital ataxia, hypotonia, headache, and epilepsy. The combination of symptoms makes it difficult to predict the effect of mutations on the channel. I have recently use HEK cells to study the gating of two mutations, R1667P and Q1674, which showed a mixed gain/loss-of-function and complete loss-of-function, respectively. Here I propose to study the electrophysiological characteristics of CaV2.1 gating and protein trafficking of CACNA1A mutations in HEK cells. While HEK cells are a promising model to study basic biophysical properties, they do not allow for an understanding of how the mutated calcium channel functions in the context of neurons, a very specialized cell type. For this reason I will use two patient-derived iPSC lines to derive iPSC-neurons (iPSC-N) and Purkinje neurons. These cells are ideal study channel trafficking, Ca^{2+} signaling, and action potential characteristics in the CACNA1A rare disease.

Manisha Kumari, PhD

Johns Hopkins University
Mentor: Ted M. Dawson, MD, PhD
Awardee Amount: $130,000
Disease Target: Multiple System Atrophy (MSA)

Shuya Li, PhD

Johns Hopkins University
Mentor: Xiaobo Mao, PhD
Awardee Amount: $130,000
Disease Target: COVID-19 and Alzheimer's Disease

Contribution of Oligodendrocytic Cellular Milieu in Generation of A-Synuclein Strains in Multiple System Atrophy

Multiple system atrophy (MSA) is a neurological disorder characterized by the accumulation of aggregated a-synuclein protein within the oligodendrocytes in the form of glial cytoplasmic inclusions (GCIs) (1). Although to a lesser extent, cytoplasmic inclusions can also be observed in the neurons of MSA patients (2). Oligodendrocytes are the glial cells which generate myelin sheath around axons of neurons and help in propagation of fast saltatory impulse (3). It is evident that accumulation of a-synuclein, a natively unfolded protein loaded GCIs within oligodendrocytes have direct relation with the neurodegeneration. a-Synuclein also plays vital role in pathogenesis of other neurodegenerative diseases such as Parkinsons disease (PD), Dementia with Lewy bodies (DLB), etc. (4). In MSA, the strain of a-synuclein is characteristically different from the a-synuclein strain in all a-synucleinopathies (5). However, curtain questions such as, which factors drives the conformational variations within a-synuclein fibrils and how their transmissibility is majorly confined to oligodendrocytes, are still unanswered. Therefore, we are proposing important hypotheses along with identification and characterization of the pathological MSA strain of a-synuclein, and how this will provide insights in the mechanism of a-synuclein aggregation and its contribution in MSA disease pathology. We hypothesize the involvement of unique interacting partners of a-synuclein that contribute to the generation of a pathogenic MSA fibrillar strain within oligodendrocytes. Mapping down the interacting partners of monomeric and aggregated a-synuclein within oligodendrocytes versus neurons will contribute and enhance the understanding of cell specific and conformational differences of the MSA strain. Moreover, identification of novel interacting partners can serve in the development of diagnostic tools. Executing an exploration of these hypotheses is dependent on appropriate cellular models for MSA. Along these lines we propose to work with a synchronized population of oligodendrocytes and neurons differentiated from human induced pluripotent stem cells (iPSC)s. iPSCs derived oligodendrocytes and neurons will be used to provide a stable environment for a-synuclein aggregation and identification of interacting protein partners that contribute to the development of the MSA strain and ultimately its pathogenesis. Through the use iPSCs derived oligodendrocytes and neurons, we will elucidate that importance of the oligodendrocyte cellular environment in the formation of a-synuclein strains and the transmission of pathologic a-synuclein.

Spike Protein from SARS-CoV-2 Promotes Tauopathy Propagation and Neuroinflammation in Alzheimers Disease

Neurological and psychiatric complications are commonly reported in coronavirus disease 2019 (COVID-19) patients infected by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). The increased risk of neurological and psychiatric outcomes persisted throughout as reported by the 2-year retrospective cohort studies of 1,284,379 COVID-19 patients infected with SARS-CoV-2. Even with omicron, which had a lower death rate than just before the emergence of the variant, the risks remained similar. However, the potential association between COVID-19 and the long-term progressive Alzheimer's disease (AD) and related dementias, is still a knowledge gap that may hinder dementia prevention and treatment in people that had SARS-CoV-2 infection. Tauopathy is one of the key components in the pathogenesis of AD, which is driven by abnormally aggregated hyperphosphorylated tau protein. These pathogenic tau proteins act as a prion-like protein spreading among brain cells and cause neuroinflammation that further exacerbates tauopathy. SARS-CoV-2 has been reported to persist in the human brain after infection and target neurons of brain organoids to cause tauopathies. It is important and feasible to use pre-clinical models, human induced pluripotent stem cell (iPSC)-derived organoid models including neurons and microglia, to study whether and how SARS-CoV-2 infection promotes the pathogenesis of tauopathy. The SARS-CoV-2 spike protein (S protein) can induce NLRP3 (NOD-, LRR-, and pyrin domain-containing protein 3) inflammasome activation, which is a key driver of tau pathology. Meanwhile, COVID-19 patients showed a higher expression of lymphocyte activation gene 3 (LAG3), which has been proven to mediate tau spreading in our preliminary data. Therefore, we propose to study the impact of S protein exposure in tauopathy using human brain organoid cultures and reveal the underlying mechanism involving NLRP3 inflammasome activation and LAG3 upregulation in mediating COVID-19-associated tauopathies. Furthermore, we propose to use pharmaceutical targeting of these two essential molecular mediators for inhibiting COVID-19-associated dementias.

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Investigating the Effects of TCF4 Mutations During Oligodendrocyte Development and Maturation in a Human-Derived Model of Autism Spectrum Disorder

Pitt-Hopkins Syndrome (PTHS) is a rare form of autism spectrum disorder (ASD) characterized by developmental delay, breathing abnormalities, seizures, lack of speech, and distinctive facial features. PTHS is caused by de novo mutations in the Transcription Factor 4 (TCF4), a key transcription factor that orchestrates multiple neurodevelopmental programs. Previous studies from our laboratory using PTHS mouse models showed dysregulation in the density of oligodendrocyte progenitor cells (OPCs) and maturity of functional oligodendrocytes (OLs) which resulted in myelination deficits, however, these deficiencies have not been model in a human system. The current proposal centers on exploring the effects of TCF4 mutations in human OLs generated from patient-derived induced pluripotent stem cells (iPSCs). Our laboratory has generated a collection of iPSCs that harbor various types of disease-causing mutations in TCF4 that result in the expression of putative dominant-negative proteins or haploinsufficiency. 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.

The Role of Splicing Factor Sf3b2 in Cardiomyocyte Maturation

Endogenous cardiomyocytes (CMs) increase their volume and contractility after birth; however, cultured CMs derived from pluripotent stem cells (PSC-CMs) do not advance beyond perinatal stages of development. This immaturity limits the scientific and therapeutic applications of hPSC-CMs. For this reason, extensive biological and engineering efforts are underway to promote maturation of PSC-CMs with features similar to those found in adult hearts. I previously performed high-quality scRNA-seq analysis and identified the metabolic factors PGC1/PPARa as key regulators of CM contractility. To investigate how metabolic factors influence the functional maturation, we performed an siRNA screen of genes regulated by PPARa using high throughput calcium imaging that measures calcium transient duration in PSC-CMs. Intriguingly, the novel RNA splicing factor Sf3b2 had the most potent effects on PSC-CM contractility among the positive hits. Sf3b2 encodes a component of the U2 small nuclear ribonucleoprotein complex, which removes introns from mRNA transcripts in constitutive and alternative splicing. While alternative splicing is also observed in cardiac development and disease, the function of RNA splicing factors remain largely unknown. I hypothesize that Sf3b2 is a key RNA splicing factor essential for functional maturation of cardiomyocytes in vivo. Specific Aim 1: To determine if Sf3b2 is required for CM maturation in vivo I will generate Sf3b2-deficient mosaic mouse CMs at postnatal stages using CASAAV at birth. I will analyze these CMs at the structural, functional, and metabolic levels to assess maturation levels. I will also quantify the maturation status using single cell RNA-seq and trajectory analysis. Specific Aim 2: To identify mRNA bound and spliced by Sf3b2 in human PSC-CMs I will identify mRNAs spliced by Sf3b2 through RNA-seq quantification of alternative splicing in the Sf3b2 knockdown hPSC-CMs. I will also use RNA immunoprecipitation sequencing to find mRNA bound by Sf3b2 in vitro.

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Role of MIF Nuclease in Neuroinflammation and Pathogenesis of Alzheimer's Disease

Oxidative, nitrosative, genotoxic, and aggregate stress can cause or amplify DNA damage and activate poly (ADP-ribose) polymerase 1 (PARP1). DNA damage-activated PARP1 promotes PAR polymer synthesis, which recruits DNA damage repair proteins. However, excessive PAR polymer causes mitochondrial AIF release and binding to MIF. The AIF-PAR-MIF complex translocates to the nucleus leading to pathogenic MIF nuclease activation culminating in regulated cell death, known as parthanatos. Markers of parthanatos, including DNA breaks, PARP1, and PAR, have been observed in Alzheimers disease (AD) patients and animal models. These observations are consistent with the activation of parthanatos, but its role in AD is not explored. According to the amyloid cascade hypothesis, A deposition and plaque formation are early and critical events in AD pathogenesis. A-induces microglial activation and inflammasome-dependent tau hyperphosphory-lation and tau pathology. Interestingly, nucleic acid (NA) is found in amyloid plaques, Neurofibrillary tangles (NFTs), and Lewy bodies (LBs). NA binding can convert protein oligomers to amyloid and induce nucleation of A[B] fibrils, suggesting NA may have a direct role in AD pathology by modulating plaque pathology. However, how these NA+ plaques are formed remains unknown. We hypothesize that MIF-mediated parthanatos and large-scale DNA degradation may promote the formation of NA+ plaques and augment microglia-dependent neuroinflammation, tau pathology, and AD pathogenesis. Therefore, we plan to develop a human iPSC-derived neuron, astrocyte, and microglia triculture model to monitor NA+ plaque formation and investigate the role of MIF nuclease in AD pathophysiology. The recent success of Lecanemab's phase III trial brings hope and excitement to the scientific community for developing therapies for AD59. This warrants investigations on the amyloid cascade theory of AD for developing efficient and safe therapeutics. The proposed study focuses on stem cell-based modeling of AD pathology and investigates the hitherto unknown role of Parthanatos regulator MIF in AD. Modeling NA+ plaque formation and AD pathology in vitro will help expedite more efficient drug screens for AD. In addition to uncovering molecular mechanisms, the study also fills the gap in the field by systematically assessing cell type-specific contribution in plaque formation and its consequences. Targeting MIF's nuclease activity has tremendous potential as it selectively inhibits a unique mode of cell death known as parthanatos. The initial success of pharmacological MIF nuclease inhibitor in PD33 and genetic ablation of MIF in 5xFAD mice showing improved memory and learning performance (Figure 4) encourages mechanistic investigation that may lead to drug development for AD. Extending the test of PAANIB-1 (blood brain barrier permeable MIF nuclease inhibitor) in pre-clinical AD models will be important for evaluating efficacy of the drug in alleviating AD pathology. Lessons learned during this project will inform and improve the usage of stem cell models in studying AD and related dementia (ADRD) and other neurodegenerative conditions.

HMGA1 Chromatin Regulators in Clonal Hematopoiesis and Cardiovascular Disease

The goal of this project is to understand why people with clonal hematopoiesis (CH) develop cardiovascular disease. CH is a common blood disorder that occurs in older people when blood stem cells acquire a mutation that allows this clone to expand and generate abnormal blood cells. Unfortunately, individuals with CH are at risk for a variety of cardiovascular diseases (CVD), including strokes, heart attacks, or vein thromboses (deep venous thromboses). While the causes for CVD in CH remain poorly understood, recent research suggests that excessive inflammatory signals from mutant blood cells help to fuel the development of CVD. We are studying a protein, called HMGA1, which acts as a molecular key that unlocks the genome to turn on genes involved in inflammation and stem cell function. In a form of CH caused by a mutation in the JAK2 gene, our research team discovered that HMGA1 is a critical regulator of genes involved in inflammatory signaling and clonal expansion. We therefore hypothesize that HMGA1 contributes to the development of CVD in the setting of CH by activating inflammatory gene networks. To test this, we have developed innovative mouse models and technologies to dissect the underlying gene networks and downstream inflammatory signals. At the completion of this project, we expect to: 1) illuminate the role of HMGA1 in CVD that occurs in individuals with CH, and, 2) identify underlying mechanisms mediated by HMGA1 that could be blocked in therapy. Our proposed studies should lay the groundwork necessary to develop novel therapies to intercept the development of CVD in the setting of CH. Cardiovascular disease (CVD) is the leading cause of death and disability globally. Clonal hematopoiesis (CH), an independent risk factor of CVD, is associated with an increased risk of venous thromboembolism, coronary artery disease, myocardial infarction, and stroke, independent of typical cardiovascular risk factors. Not only is the incidence of CH and CVD rising with our aging populations, but disparities in CVD also put people of color at increased risk of CVD and poor disease outcomes. Our proposed work is significant because: 1) There is an unmet need for treatments to lower the risk of CH-associated CVD. 2) We discovered an epigenetic regulator, HMGA1, that activates inflammatory networks in mouse models of CH. 3) Our preliminary studies also indicate that loss of just a single Hmga1 allele prevents progression and expansion in inflammatory cells in JAK2 mutant CH in mouse models. 4) Importantly, HMGA1 is a key epigenetic regulator in many adult stem cells. We, therefore, propose studies using innovative models and sequencing technologies to elucidate actionable mechanisms to intercept the development of CVD in CH. At the completion of this project, we expect to generate genetic mouse models of CH with varied levels of Hmga1, assess the function of Hmga1 in AS and CVD in a setting of CH, and identify underlying mechanisms modulated by Hmga1 in CH and CVD. Together, these studies will provide the groundwork necessary to develop new therapies to intercept the pathogenesis of atherosclerosis, thrombosis, and CVD in CH.

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Disease Target: Parkinson's Disease

The Efficacy of PFFNB2 in Human Neurons with Synucleinopathies

Lewy body dementia (LBD) is one of the most common dementias, including Dementia with Lewy Body (DLB) and Parkinsons Disease (PD) with Dementia (PDD) [1]. LBD is characterized by the accumulation of aggregated -synuclein (-syn) in the cortex. PD is the second most common age-related neurodegenerative disorder, characterized primarily by progressive loss of motor and non-motor functions and pathologically by the aggregation and accumulation of -syn [1, 2]. Animal models are an tool for studying human disease, but have various critical limitations [1,2]. Human induced pluripotent stem cell (hiPSC)-derived human dopaminergic (hDA) neurons provide a unique cell resource for disease model [3,4]. Here, we will study novel therapies for -synucleinopathies using hDA neurons as a preclinical modeling. Nanobodies are an antibody that target -syn fibrils and could provide an alternative approach to study the pathogenesis of and to treat PD and related -synucleinopathies [5-10]. we have identified a nanobody, PFFNB2, that can specifically recognize -syn preformed fibrils (PFF) over -syn monomers [11]. Advances in adeno-associated virus (AAV)-based gene delivery have provided an attractive approach to continuously express recombinant protein binding to pathogenic targets in disease treatment.

We found that adeno-associated virus (AAV)-encoding EGFP fused to PFFNB2 (AAV-EGFP-PFFNB2) can inhibit PFF-induced -syn serine 129 phosphorylation (p--syn) in mouse primary cortical neurons and prevent -syn pathology spreading to the cortex in the transgenic mice expressing human wild type (WT) -syn by PFF injection [11]. Although PFFNB2 has therapeutic potential in mice, it is also of importance to study the therapeutic efficacy of PFFNB2 in human hiPSC-derived neurons. We hypothesize that, in human neurons modelling -synucleinopathies that PFFNB2 can inhibit pathogenic -syn spreading and decrease toxicity induced by -syn PFF. Our goal is to rigorously study the nanobody PFFNB2 in human neuron models to facilitate its therapeutic development for treating various -synucleinopathies. We have demonstrated that AAV-EGFP-PFFNB2 can inhibit PFF-induced -syn serine 129 phosphorylation (pS129) in mouse primary cortical neurons and prevent -syn pathology spreading. PFFNB2 will have better clinical trial in -syn-related disease if we can prove in human neurons that nanobody PFFNB2 and AAV-EGFP-PFFNB2 can decrease pathogenic -syn spreading and reduce toxicity induced by -syn-PFF. Plan: we will consider clinical trial If this PFFNB2 antibody is effective in human neurons with -synucleinopathies.


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