ACCELERATING *cures*

ANNUAL REPORT

Maryland Stem Cell Research Fund

2021

MSCRF

About Us

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

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.

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Commissioners

Debra Mathews, Ph.D., M.A. (Chair)

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

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

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Appointed by Johns Hopkins University
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David Mosser, Ph.D.

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

Linda Powers, J.D.

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

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Appointed by the Governor
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General Counsel, MD Technology Development Corporation

Curt Van Tassell, Ph.D.

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

Bowen Weisheit, Esq.

Appointed by the Governor
Attorney, President of the Ensign C. Maryland Kelly, Jr. Memorial Foundation

Executive Overview

Amritha Jaishankar, Ph.D.
Executive Director

Celebrating 15 Years

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.

The purpose of the MSCRF is to promote state-funded, scientifically meritorious stem cell research and cures through grants and loans to public and private entities in the state. **Maryland was among very few states that made a visionary investment in regenerative medicine** approaches that were thought to be potentially curative. This field represented a paradigm shift from the traditional focus of the healthcare industry. Today we see the promise of this field being delivered. As we reflect on 15 years of accelerating cures, we are filled with gratitude, pride and hope.

MSCRF Is Making A Difference

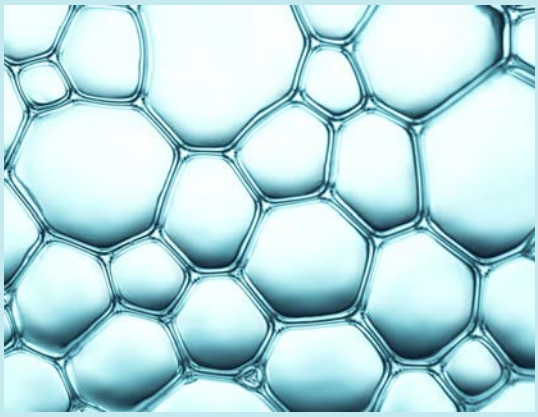
Maryland's investment in MSCRF is a success story with sustainable impact. MSCRF has supported nearly 1,700 jobs in Maryland associated with more than $145 million in compensation and $381 million in economic activity. This economic activity has generated almost $14 million in revenue for the state and for local governments, demonstrating that the State of Maryland has done well while doing good. Moreover, innovative treatments and cures for diseases, and improvements in quality of life and life expectancy are worth billions in social and economic value for Marylanders.

Executive Overview

Since its creation in 2006, **MSCRF has been a leader in growing the stem cell industry in Maryland** and in delivering its mission of advancing stem cell-based research to address the devastating diseases of our time.

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

Our programs have evolved over the years to meet the needs of a rapidly growing industry, but we've stayed true to our mission and continually strive to improve human health by advancing innovative cell-based research, treatments and cures to benefit patients with unmet medical needs.



Executive Overview

Amritha Jaishankar, Ph.D.
Executive Director

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

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

We proactively seek and identify the next promising technology and company, and our priority is to empower our scientists and companies, to enable their success so we can accelerate cures to patients with no viable treatment options.

Moreover, MSCRF has built a vibrant stem cell research and development ecosystem that continues to grow and thrive.

The commitment from the State of Maryland, MSCRF's leadership, and the dedication of our team, our community of scientists, companies, and patients amongst others, have been key to our success. **MSCRF is well-positioned to grow and broaden our impact.** The future of this industry is bright, and we invite you to continue on this path forward with us.

The Maryland Impact

Debra Mathews, Ph.D., M.A.
Chair, Maryland Stem Cell Research Commission

"It has been both exciting and gratifying to watch the MSCRF and the stem cell community in Maryland grow, diversify, and mature over the past 15 years. I have been astounded by some of the research we have been able to fund, starting first in the lab and then on to products and clinical trials. In a very short time, we have seen the promise of stem cell research proven in patients."

Executive Overview

Amritha Jaishankar, Ph.D.
Executive Director

2021 has been a banner year for science and despite another year with COVID-19-related challenges, it was one of tremendous growth and achievements for the regenerative medicine sector, as well as for MSCRF. In 2021, we've worked safe but have continued to innovate, persevere, and collaborate. 2020 was a record-breaking year for the industry on some fronts, but 2021 surpassed it, with ease. **It was the year of record-breaking investments, regulatory approvals** and despite the pandemic, an increase in ongoing clinical trials for a wide range of medical applications. We saw crucial clinical milestones being achieved and strong commercial growth. Amongst many highlights were the promising early outcomes from landmark clinical trials for first in human gene-editing treatments and for stem cell-based therapies for Type 1 diabetes.

At MSCRF, in 2021, we funded 25 exciting research, validation and clinical trial grants through our 6 active programs **(see pages 13 - 14)**. **Our companies have grown,** raised follow-on funds, advanced clinical trials and are working diligently to bring us one step closer to delivering treatments and cures **(see page 16)**. We've supported and stayed connected with our community through another challenging year and have much to celebrate **(see pages 33 -35)**.

Executive Overview

Beyond funding, MSCRF serves as the connective tissue for the regenerative medicine industry in Maryland. Our ongoing outreach, engagement and thought leadership drives scientific collaboration, accelerates innovation, and helps provide valuable resources to the scientists and companies we support. In 2021, we've gathered in person, virtually, and in hybrid formats to share knowledge, to address emerging challenges, to celebrate our accomplishments, and to advance science and cures to patients with even more speed, flexibility and urgency.

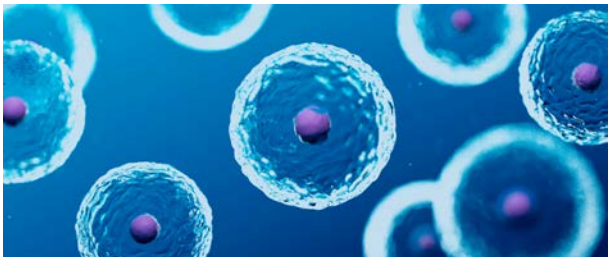
MSCRF has a global presence, and our key industry engagements and collaborations allow our researchers and companies to participate in various national and international stem cell conferences and courses. **This scientific collaboration generates and advances the next medical breakthroughs.** We strive to serve as a regenerative medicine resource hub, to provide information, expertise and connections to our scientists and companies to further advance ideas into therapies.

Great science drives investment and the cell therapy industry that MSCRF has helped build in Maryland serves as an **economic catalyst.**

MSCRF supports the health and economic well-being of the state by supporting cutting-edge stem cell research that may lead to transformative therapies for our patients, as well as by attracting private investment and recruiting and supporting a talented workforce in the region. The economic impact section of this report highlights the impact of MSCRF's programs based on an independent study by the Sage Policy Group **(see pages 17-19).**

MSCRF's mission delivers a human and societal impact beyond the economic impact. Read about some of the research and cures we've supported on **pages 24-32, 36-56**. Our scientists and companies collectively advance the field each day and bring us one step closer to a cure. **Resulting therapies improve the health of many Marylanders and also reduce health care costs for the state** **(see pages 20-22).** Investments in stem cell research can potentially return great value by reducing disease burden, which will pay future health dividends for the State of Maryland.

Executive Overview



2021 was a year of many firsts and one of immense growth for the regenerative medicine field. We have started to see how regenerative medicine approaches have the ability to transform the lives of patients this year, across multiple diseases and modalities. The hope that diseases that shorten life spans, cause life-long suffering and poor quality of life could be mitigated or cured altogether is alive. **This is the future of regenerative medicine and we're on the cusp of making that promise a reality for more patients than ever.** Longer clinical outcome measures in more patients will guide how we move towards the next frontier in 2022.

Good medicine, treatments and cures start with basic science. There is simply no shortcut to good science. Investing in basic and translational biomedical research is important if we want to realize the full potential of regenerative medicine and reduce the burden of diseases for individuals, their families and society.

Executive Overview

In addition, as the field progresses, we simultaneously need to address emerging challenges in commercialization, cost-effective manufacturing, distribution/delivery and access to treatments, as well as payment models to advance this class of therapies.

This past year has shown us how important public funding for research and manufacturing is.

With more investment, as we've seen with COVID-19 research and vaccine manufacturing, the timeline to create medicine is significantly accelerated.

At MSCRF, we know without a doubt, that **the cell and gene therapy industry in Maryland and across the globe will revolutionize healthcare** as we know it - the future is now. We're grateful for the opportunity to build and lead this community and will continue to work tirelessly to advance research and cures to Marylanders and patients around the world. We are looking forward to 2022, it will be an exciting year for MSCRF and for the regenerative medicine community. **Success for Maryland is in our own hands.**

Amritha Jaishankar

Amritha Jaishankar, Ph.D.
Executive Director, MSCRF

2021

AT-A-GLANCE

MSCRF Funding Opportunities

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

During calendar year 2021, we had six active programs and we funded 25 new awards with $7.3 Million. We supported projects addressing a wide range of medical conditions including Parkinson's disease, heart diseases, stroke, autism spectrum disorders, diabetic retinopathy, bone defects, bone marrow failures, sepsis complicated by acute kidney injury and ARDS amongst others.

We identify and support the most promising and innovative stem cell technologies that may provide much needed treatments and cures for patients with no other viable options. While many of the research projects typically study stem cells in the context of a specific disease, some address more than one condition and often these technologies then serve as a platform to expand therapies to a broader set of indications.

MSCRF Six Funding Programs

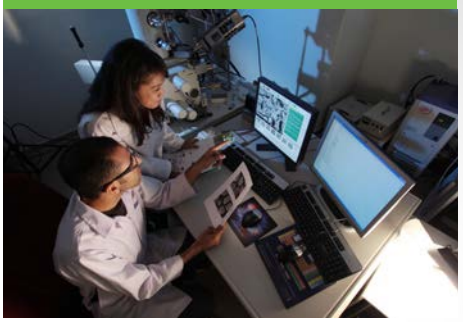
Discovery

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

Validation

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

Launch



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

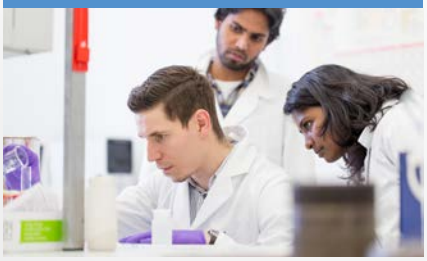
Clinical

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

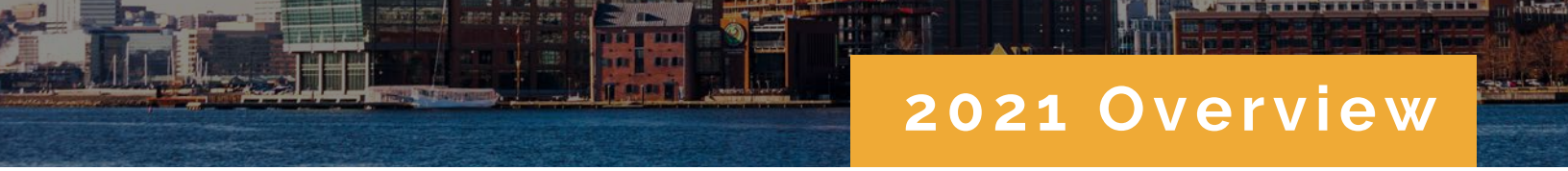
Commercialization

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

Post Doctoral Fellowship



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



2021 Overview

25
GRANTS AWARDED

Johns Hopkins University
University of Maryland
University of Maryland Baltimore
Cartesian Therapeutics
sentien biotechnologies

$7.3M In Committed Funding to Awardees

27
DISEASE INDICATIONS

Achievements

Portfolio Highlights

Regenerative medicine is thriving in Maryland, our research continues to accelerate at a rapid pace, and our companies continue to grow. In 2021, we had much to celebrate with our portfolio. Below are some highlights. For more information on all our portfolio companies, visit https://www.mscrf.org/portfolio-companies.

Vita Therapeutics Inc. raised $32 million in Series A financing to advance development of their lead therapies into clinical trials to treat muscular dystrophies. This will help advance the company's mission to deliver genetically engineered cell therapies to patients with urgent medical needs. Vita was originally founded out of labs of MSCRF-supported faculty Dr. Gabsang Lee and Dr. Kathryn Wagner at Johns Hopkins University and the Kennedy Krieger Institute.

Theradaptive Inc. closed a Series A investment of $6.2 million which will help launch their lead product into clinical trials for treatment of lifelong debilitating conditions such as orthopedic injuries and spinal fusion. The company also received the FDA's breakthrough device designation for its Osteo-Adapt SP spinal fusion implant aimed at treating degenerative disc disease or spondylolisthesis. Through advanced protein engineering, Theradaptive, Inc. develops regenerative therapeutics that are safer and more effective.

RoosterBio Inc. increased it's worldwide presence by collaborating with Sartorius, a leading international partner of the biopharmaceutical industry, to advance their mission and accelerate the development and commercial manufacturing of regenerative medicine products. To further support research efforts, RoosterBio has also launched a new product to aid cell engineering applications.

Seraxis Inc. closed $40 million in Series C financing to advance their cell replacement therapy to treat insulin-dependent diabetes, into clinical trials. This novel regenerative medicine strategy has the potential to impact millions of diabetic patients worldwide.

Longeveron Inc. reported promising results from MSCRF-supported clinical trial using their medicinal signaling cell (MSC)-based therapy to treat infants with a rare and life-threatening congenital heart defect. The company was also granted orphan drug designation by FDA for this treatment.

NeoProgen Inc. received FDA approval to begin Phase 1 clinical trial of their cell therapy for the treatment of heart failure, a leading cause of death in the United States. This approval tails NeoProgen's newly acquired patent for their unique methodology to use neonatal heart-derived medicinal signaling cells (nMSCs) for tissue repair and regeneration. Neoprogen was originally founded from the MSCRF-funded research in Dr. Sunjay Kaushal's lab at the University of Maryland, Baltimore.

MSCRF has generated significant economic and fiscal returns for Maryland. This section presents data and excerpts from a study conducted by Sage Policy Group Inc. in December 2021. The study evaluated the economic contributions of the Maryland Stem Cell Research Fund.

ECONOMIC IMPACT

A Measurable Difference

MSCRF's programs have supported the continuous growth of our economy, created jobs, and generated revenue for our State. MSCRF grants support researchers, physicians, students, and lab technicians as well as scientists and executives at stem cell companies. This creates the next generation of the workforce needed to advance the industry and apply regenerative medicine discoveries to clinical care.

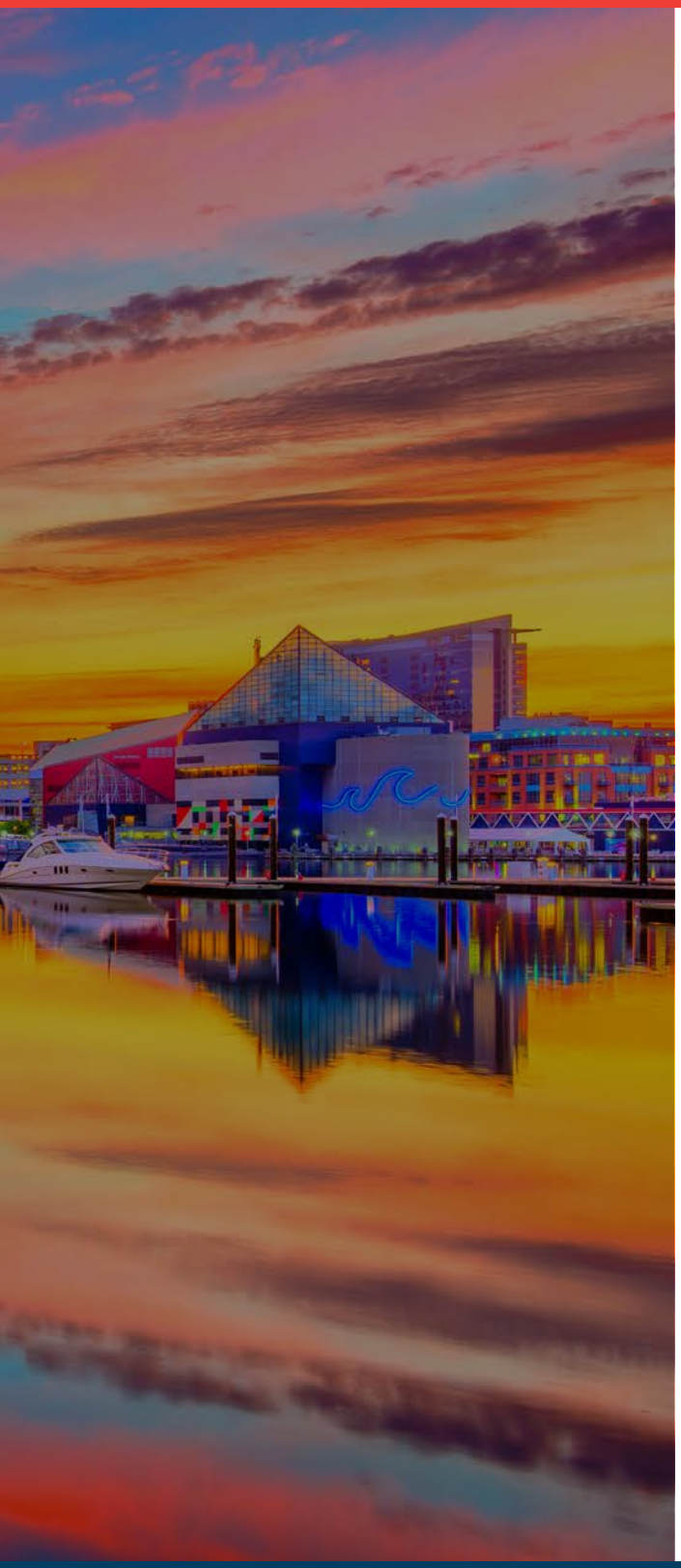
Because the Fund covers the full spectrum of stem cell scientists — from young postdoctoral fellows to experienced researchers in large companies — it contributes to the growth of the life science cluster in Maryland. Some companies operating in Maryland would not exist without MSCRF's support. Many companies in the Fund's portfolio, like RoosterBio and MaxCyte, have large employee bases

The impacts that can be measured are significant.

MSCRF has supported nearly **1,700 jobs** statewide. These jobs are associated with more than **$145 million** in compensation. MSCRF has supported sales of goods and services exceeding **$380 million.** This economic activity has generated almost **$14 million** in revenues for the state and local governments during this time, demonstrating that the State of Maryland has simultaneously "done well while doing good."

The Economic Contributions of the Maryland Stem Cell Research Fund 2021 Update, PREPARED BY SAGE POLICY GROUP, INC. December 2021.

A Measurable Difference



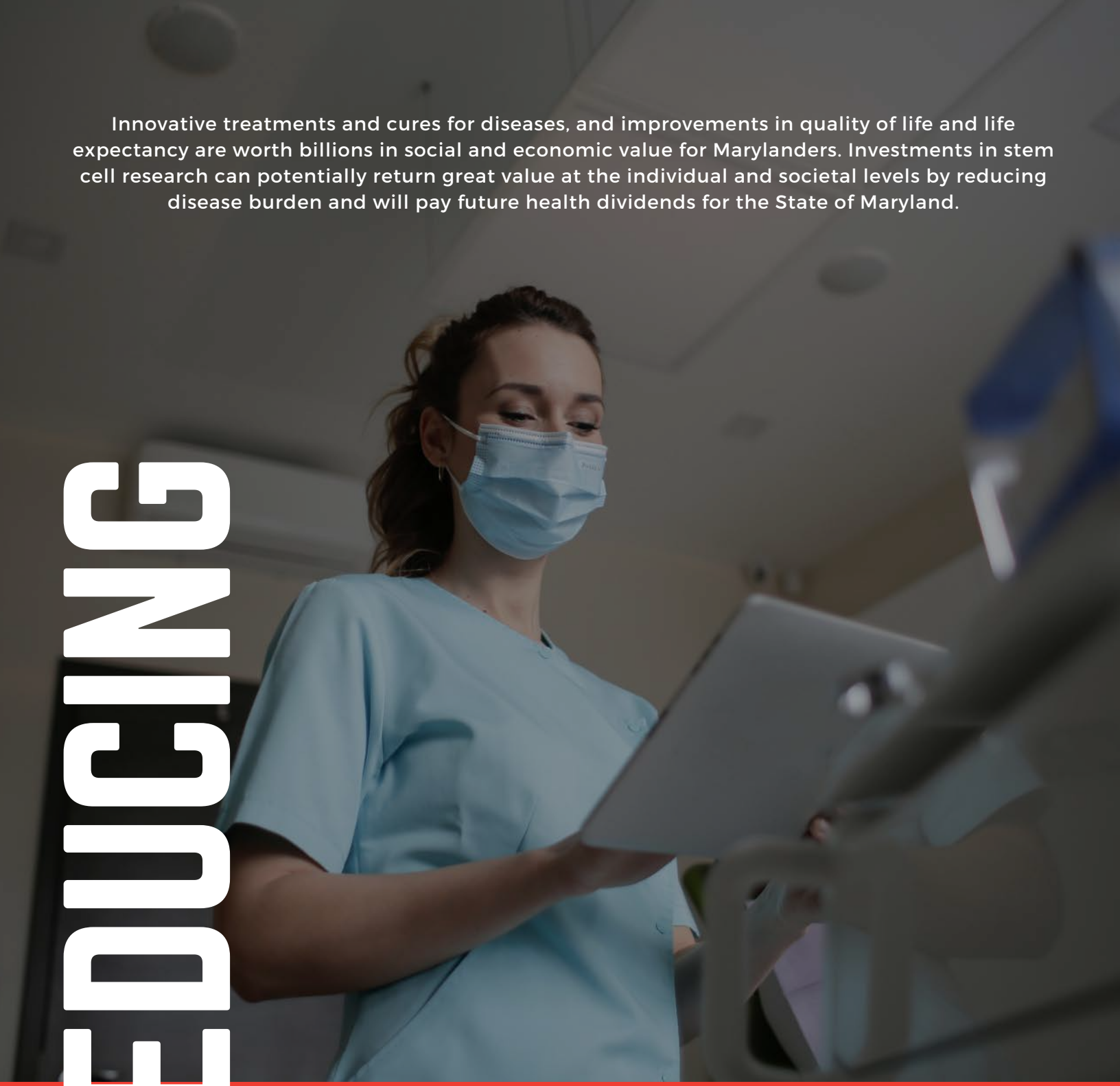
It must be stressed that MSCRF creates impacts that extend far beyond economic and fiscal, as the Fund's goal is to improve human health through the development of new medical strategies that utilize human stem cells for the prevention, diagnosis, and treatment of human diseases and conditions.

The Sage Policy Group report evaluating the economic contributions of MSCRF does not seek to assign dollar values to impacts related to the development of new cures and treatments. The reason for this is simple; it's effectively impossible to determine how many lives have been saved and extended via MSCRF-supported research.

"Economic research estimates the value of a statistical life at $7 million, with a 90 percent confidence interval of $2.4 million to $11.2 million. Therefore, saving a few hundred lives would translate into billions of dollars in social contribution."

The Economic Contributions of the Maryland Stem Cell Research Fund 2021 Update, PREPARED BY SAGE POLICY GROUP, INC. December 2021.

Innovative treatments and cures for diseases, and improvements in quality of life and life expectancy are worth billions in social and economic value for Marylanders. Investments in stem cell research can potentially return great value at the individual and societal levels by reducing disease burden and will pay future health dividends for the State of Maryland.



REDUCING DISEASE BURDEN

DIABETES

The American Diabetes Association estimates that diabetes cost the U.S. $237 billion in direct medical costs and $90 billion in reduced productivity in 2017 alone. Assuming Maryland incurs a proportional share of those costs, diabetes costs the state's economy approximately $6.2 billion per annum.

MSCRF supports research that could accelerate treatments for those with diabetes and related complications. Development of treatments or cures would help Maryland experience billions of dollars in economic benefit.

ARDS

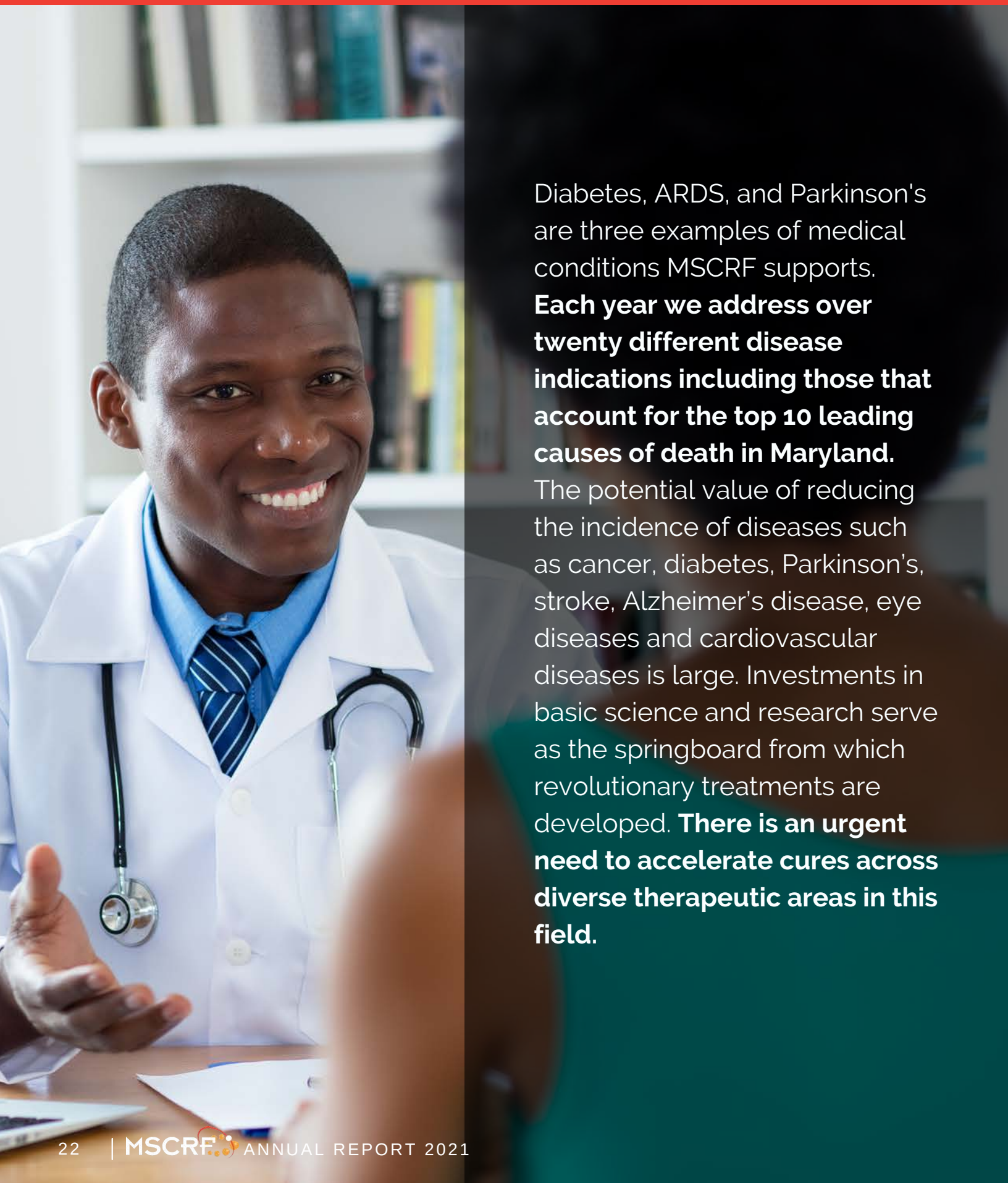
Acute Respiratory Distress Syndrome (ARDS) is a dangerous, potentially fatal respiratory condition and a prevalent complication associated with COVID-19 and other conditions. **ARDS** survivors face a high incidence of long-lasting impairments like cognitive dysfunction and physical impairment. Average lost earnings for a survivor of ARDS are estimated to be **$27,000 during the 12-month period** following discharge, and among jobless survivors there was a 14-percentage point decline in private health insurance coverage. Identifying an effective treatment for ARDS that reduces the incidence of hospitalization and economic dislocation would produce substantial socioeconomic benefits. MSCRF supports stem cell based pre-clinical research and clinical trials addressing **ARDS.**

PARKINSON'S

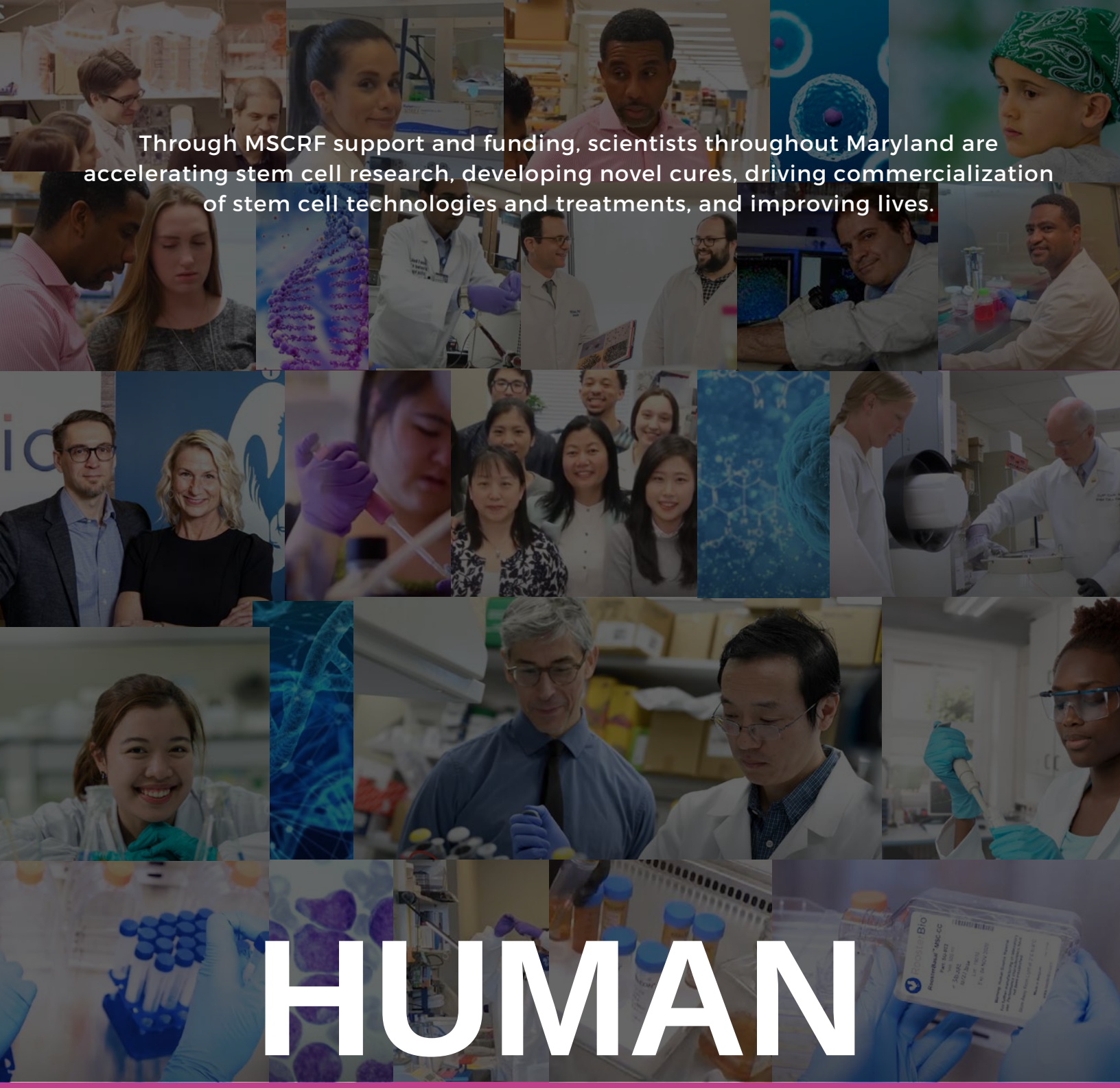
The **Parkinson's** Foundation reports that the direct and indirect effect of Parkinson's, including medical costs and lost income, is $52 billion per year nationwide. If Maryland incurs a proportional share of those costs, Parkinson's disease costs Maryland's economy approximately **$950 million each year.** MSCRF supports research towards prevention and treatment of Parkinson's disease.

The Economic Contributions of the Maryland Stem Cell Research Fund 2021 Update, PREPARED BY SAGE POLICY GROUP, INC. December 2021.

Reducing Disease Burden



Diabetes, ARDS, and Parkinson's are three examples of medical conditions MSCRF supports. **Each year we address over twenty different disease indications including those that account for the top 10 leading causes of death in Maryland.** The potential value of reducing the incidence of diseases such as cancer, diabetes, Parkinson's, stroke, Alzheimer's disease, eye diseases and cardiovascular diseases is large. Investments in basic science and research serve as the springboard from which revolutionary treatments are developed. **There is an urgent need to accelerate cures across diverse therapeutic areas in this field.**



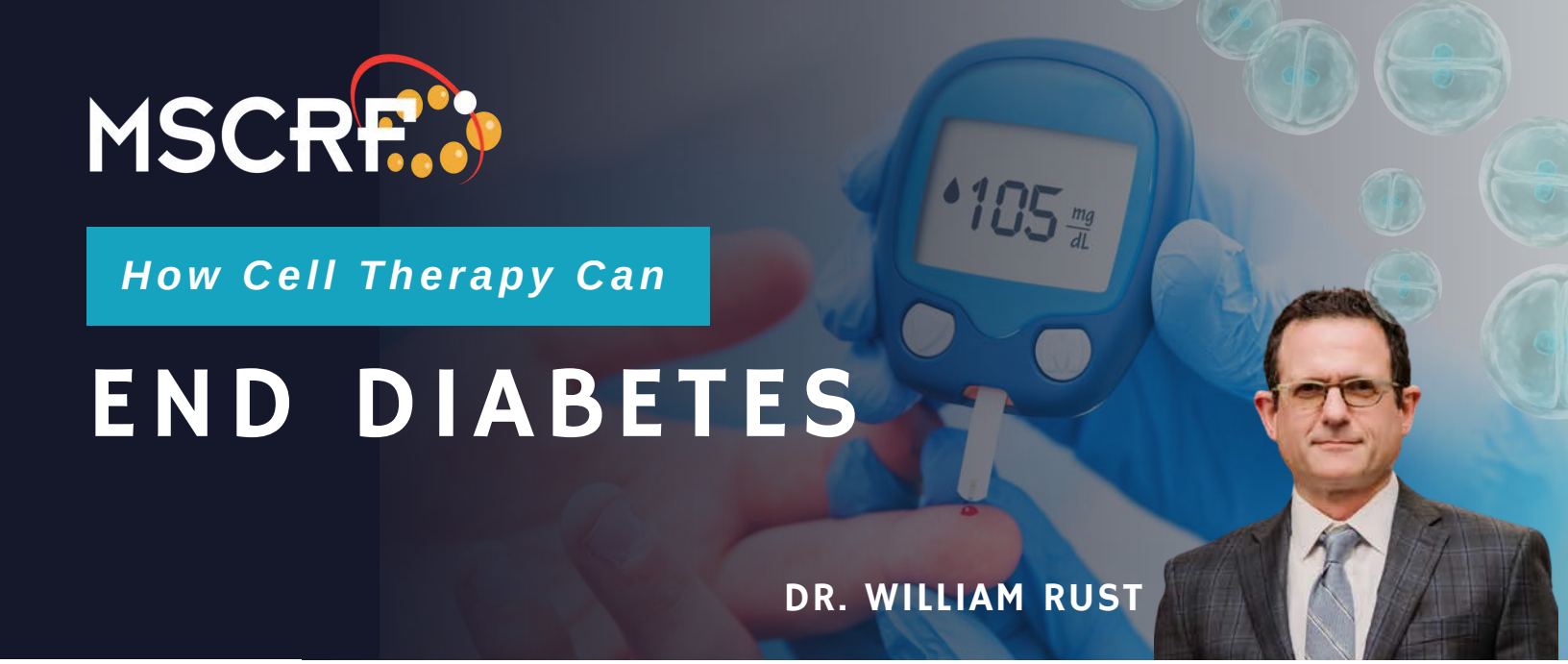
Through MSCRF support and funding, scientists throughout Maryland are accelerating stem cell research, developing novel cures, driving commercialization of stem cell technologies and treatments, and improving lives.

HUMAN IMPACT

MSCRF

How Cell Therapy Can

END DIABETES



DR. WILLIAM RUST

When there's a disease that affects 1 in every 10 Americans and takes 10 years off of someone's life expectancy and can lead to blindness, cardiovascular disease, limb amputation, and kidney failure, we desperately need a cure.

An inability to produce viable islet cells in a lab and transplant them into patients in need remains a problem to curing diabetes. **Seraxis** and the **Maryland Stem Cell Research Fund (MSCRF)** are on a mission to solve this.

There's much to gain from developing islet cells in a lab that are functional, curative, and don't require immunosuppressive drugs–which can feel like an impossible task.

Seraxis founder **Dr. William Rust** knows "the race is on to find a supply of islets that does not come from organ donation so that all of the patients who need this therapy can have it."

Seraxis is leading the charge, and with support of MSCRF funding, its preliminary findings showed exciting promise, and it is on the verge of launching its first clinical trial to show its efficacy. If all goes successfully, Seraxis believes it will be able to commercialize its cellular therapy product in the next 5 years.

MSCRF

Improving Lives With

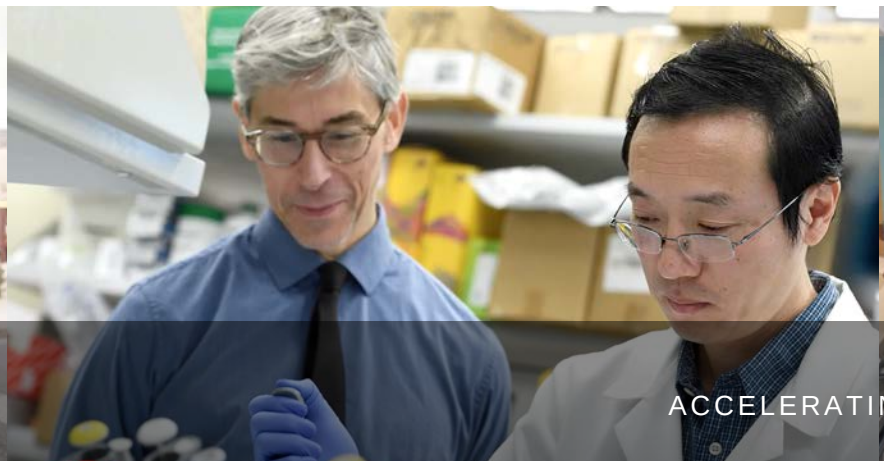
CELL THERAPY

DR. LUIS GARZA HELPS VETERANS

Healing Skin by Regeneration to Help Those with Limb Loss

Using cell therapy, **Dr. Luis Garza**, Associate Professor of Dermatology at the Johns Hopkins School of Medicine, and his lab are conducting clinical trials to help wounded veterans and the 2 million amputees living in the United States. Prosthetics help individuals with limb loss but are limited in use because of pain, discomfort, and skin breakdown from damaged tissue because the skin at the stump site is not meant to bear weight. Dr. Garza's research helps those with limb loss resume their daily activities and/or improve their quality of life. His mission is to give back to the people who have risked their lives for their country and the **Maryland Stem Cell Research Fund (MSCRF)** has made that possible by funding his research.

Scientific breakthroughs in regenerative medicine are the result of taking risks and giving time and investment to promising research. Dr. Garza shares, "we could not have done our work without the Maryland Stem Cell Research Fund." According to him, the research would have died if it were not for the very generous support of the Maryland Stem Cell Research Fund that allowed the Nation's best scientists to come together with careful oversight, advice, and input that created the ecosystem where a promising cell therapy can thrive.



MSCRF

Promising New Research

FIGHTING PARKINSON'S

DR. VALINA DAWSON

At the Johns Hopkins University School of Medicine, **Dr. Valina L. Dawson** leads the Neuroregeneration and Stem Cell programs to battle two global diseases simultaneously: Parkinson's and COVID-19.

Their breakthrough research initiatives have led to the development of new drugs and advanced our understanding of Parkinson's Disease. **One project produced a new drug that is in Phase 2 clinical trials for Parkinson's treatments.**

"There's going to be a large population that has chronic disability or chronic disease, after being infected [with COVID-19]," says Dr. Dawson. "It's a really terrifying medical problem."

But their research is helping shed light on the COVID-19 symptoms of delirium and "brain fog" which will help shape the long-term care of COVID-19 patients. The **Maryland Stem Cell Research Fund (MSCRF)** is proud to support Dr. Dawson and her lab as they are solving the toughest challenges of neurodegenerative diseases.

MSCRF

Radically Simplifying Production Of

STEM CELLS

DR. JON ROWLEY

RoosterBio's stem cell products radically simplify regenerative medicine drug development. RoosterBio Founder Dr. Jon Rowley seeks to industrialize the regenerative medicine supply chain with a set of unique products that radically simplify the incorporation of living stem cells into tomorrow's regenerative drugs.

Having had close to 20 years of experience in manufacturing process development, Dr. Rowley realized in the late 2000s that almost every company was having the same challenge progressing therapies into clinical trials due to the inability to grow stem cell banks. He realized he could help them accelerate their development process by standardizing and providing them MSC cell banks to begin work.

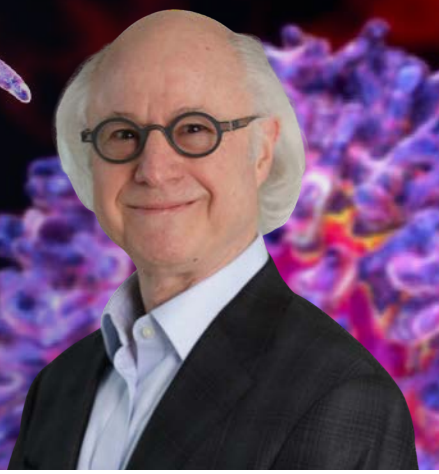
Dr. Rowley accurately understood the field of regenerative medicine was growing and Rooster Bios products took off. **The company started with 5-7 employees in 2013 and is now at 50 employees in 2021.** Dr. Rowley's ability to scale and meet market demand would not have been possible without the investments by the **Maryland Stem Cell Research Fund (MSCRF),** which helped RoosterBio launch two new products in less than three years.

RoosterBio

MSCRF

How Maryland Researchers Are

FIGHTING LEUKEMIA WITH STEM CELLS



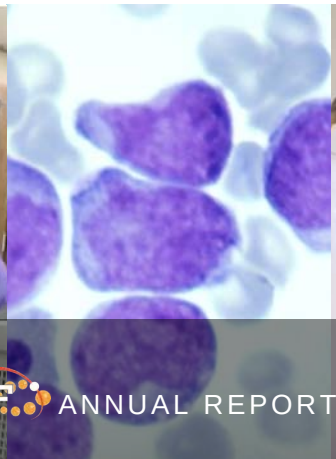
DR. CURT CIVIN

Dr. Curt Civin's, stem cell transplant work has extended the lives of leukemia patients --- one of his patients, "Macy," couldn't be cured of her resistant leukemia without a stem cell transplant, which was the result of Dr. Civin's research.

With funding from the **Maryland Stem Cell Research Fund (MSCRF),** Dr. Civin's team was also able to make an important discovery that identified a small set of snippets of microRNAs that regulate large molecules in stem cells. This discovery allowed his team to discover new drugs that are being tested as treatments for leukemia.

Cancer kills approximately 600,000 patients every year. Leukemias are a portion of this total, but Dr. Civin has a strong reason to believe the drugs they are developing will work for other types of cancers, beyond leukemias.

He noted the MSCRF "is the catalyst to get the fire going that will build into the productive engine to take us all the way to create cures that will help people...that is the most rewarding thing for all of us and it would be the greatest thing for Maryland."



MSCRF

Reseachers Use Stem Cells To

STOP ADVANCED AGING DISEASE

DR. KAN CAO

Someone born with progeria, a premature aging disease, lives just 14 years. But with funding from the **Maryland Stem Cell Research Fund (MSCRF)**, **Dr. Kan Cao**, Professor of the Department of Cell Biology and Molecular Genetics at the University of Maryland, College Park may be on the verge of curing it in the next few years.

As a rare disease affecting only one in every four million births, very little sample material exists to study the disease. Stem cells and the novel approaches to generate different tissues and different cell type lineages from them, offer a path forward.

"The Maryland Stem Cell Research Fund has provided my research lab, the initial essential funding for me to get started," Dr. Cao remarked. **That funding provided the runway to examine both the disease mechanism and investigate multiple, possible treatment options.**

Dr. Cao lab's novel technique, released in a paper she co-authored with colleagues Dr. Francis Collins at NIH and Dr. David Liu at Harvard University, proposed a genetic approach to correct the mutation. A potential single shot cure to the disease. **Dr. Cao's promising research indicates a cure could be possible within the next few years.**

MSCRF

Inspirational Story Of How

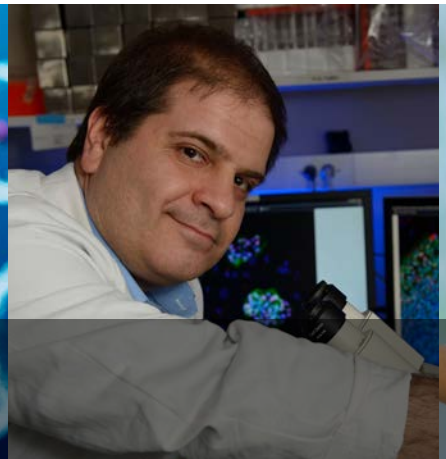
STEM CELL TRANSPLANTS SAVE LIVES

DR. ELIAS ZAMBIDIS

Dr. Elias Zambidis, MD, Ph.D. is a physician-scientist and bone marrow transplant doctor that cares for children who suffers life-threatening disorders of the blood immune system. **He's an expert at conducting stem cell transplants in sick children.**

Dr. Zambidis has helped children like "Jackson," who is alive today thanks to the stem cell transplant Dr. Zambidis performed on him as a infant after being diagnosed with severe combined immunodeficiency.

Dr. Zambidis says, "if I didn't have patients like Jackson to inspire me and make me think how to solve problems that are currently unsolvable I wouldn't be as effective. I've been very lucky to be funded by the **Maryland Stem Cell Research Fund (MSCRF)** since I came onto the faculty of Johns Hopkins."



DR. SHARON GERECHT

RESTORING BLOOD VESSELS

Maryland Stem Cell Research Fund (MSCRF) Supported Researchers Use Stem Cell Approaches to Induce Blood Vessel Repair

Every month, the equivalent of 10% of the population of Baltimore dies from vascular diseases. But a lab that calls that city home leverages funding from the **MSCRF** to develop novel technologies against them.

Dr. Sharon Gerecht, Director of the Johns Hopkins University's Institute for NanoBio Technology, has developed a novel research method to study better how stem cells can induce blood vessel repair and restoration towards normal function.

Because of this novel method, funded by the MSCRF, they've developed new approaches to control the repair mechanism and engineer biodevices leading to rapid wound healing–with several technologies in preclinical and clinical testing stages.

MSCRF

Researchers Discover New Method of

FACIAL RECONSTRUCTION

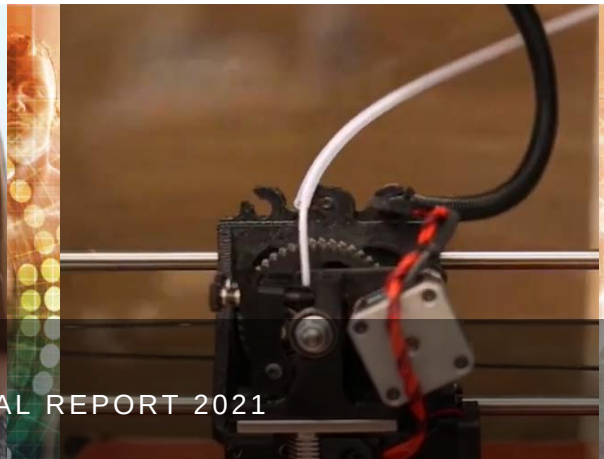
DR. WARREN GRAYSON

Over 200,000 surgeries per year are required to treat patients who have had facial bone loss, and yet, the standard of care involves creating a second injury to remove bone from somewhere in the body to construct another part.

As a professor of biomedical engineering at Johns Hopkins, **Dr. Warren Grayson** and his lab developed a biomaterial-based method to restore the shape and composition of bone that is lost or damaged. They do this by providing a degradable scaffold that will allow the patient's own cells to grow, restructure, and re-form the new bone where it was missing. The patient is left with a native reconstruction of bone in the area they needed it.

Dr. Grayson and his lab have progressed from their initial research phases to standardization and validation of the technology. They believe, thanks to funding from the **Maryland Stem Cell Research Fund (MSCRF)**, they will begin treating patients in the next few years.

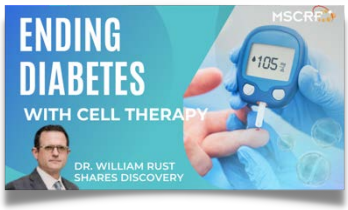
If successful, this dramatically improves the quality of life for those patients and will give them the confidence they need to go out in public regularly. Looking back over their progress, Dr. Grayson says it "all started with that support from the Maryland Stem Cell Research Fund."



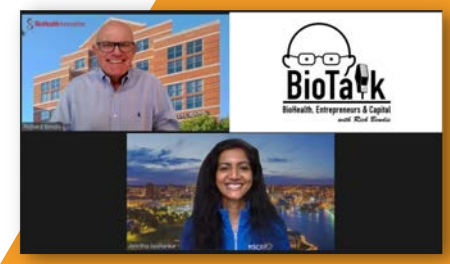
PUBLIC ENGAGEMENT

Scientific Thought Leadership

The scientists we fund are internationally recognized thought leaders and their research is accessible to the public through our channels.



Advancing therapies in a fast-moving field like regenerative medicine requires a global perspective to identify and support the right science at the right time, in keeping with the industry trends. MSCRF's Executive Director and the prominent scientists we work with are regularly invited speakers at numerous stem cell conferences, summits, panels and committees that address and promote regenerative medicine across the globe. **MSCRF leads the way in creating collaborations to advance science and has put Maryland's growing stem cell industry on a global stage.** We thank all our collaborators worldwide for helping us accelerate cures in 2021.



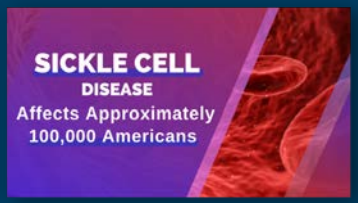
Industry News

We share the latest scientific advances in the stem cell field through our daily regenerative medicine industry news and updates. With over 1,000 articles shared this year, we keep our community connected to cutting-edge research.

Health Education

There is an urgent need to bring awareness to and address a wide range of life-threatening diseases for which no treatments or cures exist.

We thank our patients and the many disease foundations and organizations that share our mission, for their support of our efforts. We look forward to our continued collaboration in accelerating cures together.



In 2021, MSCRF funded 25 research projects addressing a wide range of medical conditions. Read about this research here.

FUNDED ABSTRACTS

25 AWARDEES

Clinical:
Metin Kurtoglu, Ph.D. | Cartesian
Heather Symons, M.D | Johns Hopkins University
Brian Miller, M.S., MBA | Sentien Biotechnologies

Validation:
Warren Grayson, Ph.D. | Johns Hopkins University
Sheikh Amer Riazuddin, Ph.D | Johns Hopkins University

Discovery:
Ivy Dick, Ph.D. | University of Maryland, Baltimore
Ricardo Feldman, Ph.D. | University of Maryland, Baltimore
Xiaoming (Shawn)He, Ph.D. | University of Maryland, College Park
Aaron James, M.D., Ph.D. | Johns Hopkins University
Shaun Kunisaki, M.D., M.Sc.| Johns Hopkins University
Linda Resar, M.D. | Johns Hopkins University
Matthew Trudeau, Ph.D | University of Maryland, Baltimore

Launch:
William "Brian" Dalton, M.D., Ph.D. | Johns Hopkins University
Jie Jiang, Ph.D. | University of Maryland, Baltimore
Younggeon Jin, Ph.D. | University of Maryland, College Park
Deqiang Li, Ph.D. | University of Maryland, Baltimore
Pan Li, Ph.D. | Johns Hopkins University

Post-Doctoral Fellowship:
Michael Barbato, M.D. | Johns Hopkins University
Chelsey Dunham, Ph.D. | Johns Hopkins University
Casey Keuthan, Ph.D. | Johns Hopkins University
Ramesh Marasini, M.S., Ph.D. | Johns Hopkins University
Xiaoli Rong, Ph.D. | Johns Hopkins University
Bo Am Seo, Ph.D. | Johns Hopkins University
Kunyu Zhang, Ph.D. | Johns Hopkins University
Yuxiao Zhou, Ph.D. | Johns Hopkins University

ACCELERATING CURES

CLINICAL

Program

Metin Kurtoglu, M.D., Ph.D.

Cartesian Therapeutics, Inc.
Awardee Amount: $650,000
Disease Target: Acute Respiratory Distress Syndrome

Heather Symons, M.D.

Johns Hopkins University (JHU)
Awardee Amount: $529,938
Disease Target: Primary Immunodeficiencies & Bone Marrow Failures
(2022 1st Funding Cycle)

Phase I/IIA Study of Descartes-30 in Acute Respiratory Distress Syndrome

Acute Respiratory Distress Syndrome (ARDS), including ARDS due to COVID-19, is a severe inflammatory respiratory failure that kills an estimated 80,000 Americans each year; 80% of ARDS patients require mechanical ventilation and no FDA-approved therapies for ARDS are available. ARDS and COVID-19 are thought to be caused or exacerbated by sticky webs of DNA and inflammatory proteins called Neutrophil Extracellular Traps (NETs). Work in this SBIR supports clinical development of Descartes-30, the first clinical-stage therapy specifically designed to eliminate NETs and the first engineered cell therapy specifically targeting ARDS and COVID-19. Descartes-30 cells are composed of Mesenchymal Stem Cells (MSCs), which are engineered using mRNA to secrete enzymes that can dissolve NETs. Mesenchymal Stem Cells are purposefully chosen because: a.MSCs can be collected from healthy donors and can be administered safely to patients with ARDS; b.MSCs can be engineered by a platform developed by Cartesian Therapeutics (RNA ArmorySM) to introduce mRNA which programs the cells to secrete two enzymes that can work together to effectively degrade NETs; and c.When MSCs are administered intravenously, they become trapped within pulmonary microvasculature, which will allow these cells to secrete the enzymes directly into where NETs reside. A multi-center Phase 1/2a clinical study of Descartes-30 in patients with moderate to severe ARDS is now enrolling. If this application is funded, University of Maryland (UM) will be an additional site for the trial. This will allow a unique collaboration opportunity between Cartesian UM: research specimens will be processed at Cartesian same-day without a freeze-thaw procedure, ensuring the highest quality sample for each assay. In addition, a parallel-control group will be enrolled at UM to collect additional biospecimens, which will serve as a comparative control to Descartes-30 treated patients. Comparative biomarker analysis in these two patient groups will significantly increase the validity of biomarker research planned in this clinical study. The Specific Aims of the application are: Aim 1: Determine safety and preliminary efficacy of Descartes-30 study (Kurtoglu, Verceles - 8 months). Milestones: Determine MTD or complete Dose Level 3 without observed dose-limiting toxicity. 50% of treated patients will show 2 point reduction in SOFA score 7 days after first infusion. Aim 2: Define PK/PD and biologic correlates of disease (Kurtoglu, Verceles, Stewart - 8 months). Milestones: establish preliminary pharma-cokinetics, and evidence of lung trafficking and DNase activity, for Descartes-30 in patients with ARDS. Data generated from this Phase 1/2a clinical study will establish the safety and preliminary efficacy of the first engineered cell therapy for ARDS and COVID-19, the first RNA-engineered MSC therapy, and the first therapy in clinical studies specifically designed to degrade NETs. These data will also inform the design of a controlled registration study in patients with moderate-to-severe ARDS. As a fully-integrated cell therapy company with 3 active clinical-stage cell therapies and in-house R&D, cGMP manufacturing and clinical operations, Cartesian is fully prepared and uniquely positioned to rapidly advance development of and commercialize Descartes-30.

Regenerative Medicine to Restore Hematopoiesis and Immune Function in Immunodeficiencies and Inherited Bone Marrow Failures

This is a high impact proposal using adult hematopoietic stem cells in an attempt to cure highly underfunded diseases. PID/IDS and IBMFS are genetic disorders affecting at least 1 in 1,200 persons in the United States and are associated with reduced quality of life and early death. Even with proper diagnosis and treatment, affected patients experience an average of 12 visits to the emergency room and 5 infection related hospitalizations per year at an extraordinary cost of nearly $200,000 per patient per year. PID/IDS and IBMFS fall in the category of rare diseases and historically have received little research funding to improve outcomes. Genetic diseases are prime targets for regenerative medicine and stem cell therapy. Embryonic stem cells and induced pluripotent stem cells have promise, but safety concerns and the fact that HSCs derived from ES and iPSC are not yet capable of long term engraftment limits their potential to cure these disorders, outside of certain types of severe combined immunodeficiencies. HSCs derived from blood or marrow may also be used for regenerative medicine. Their major limitation for PID/IDS and IBMFS patients has been the inability for most patients to find an HLA-matched related donor and the development of complications such as graft-versus-host disease and life-limiting infections after standard myeloablative hematopoietic stem cell transplant regimens. We have shown that post-transplant cyclophosphamide selectively targets alloreactive lymphocytes and spares HSCs after a reduced intensity conditioning regimen. This is important because only 30% of patients in need of an HSCT have an HLA-matched sibling donor, and at most 50% have an HLA matched unrelated donor, with this number dropping to 20% for ethnic minorities. Haploidentical donors are easy to find and virtually guarantee that any PID/IDS and IBMFS patient in need of a transplant will have a rapidly available, unaffected donor. We have developed a novel HSCT regimen that uses a safer, less intense conditioning regimen and HLA-haplo-donors. Johns Hopkins is the national leader in haploHSCT with PTCy for malignant and non-malignant disorders. We have performed over 1500 haploHSCTs with PTCy at our Institution alone for hematologic malignancies and non-hematologic disorders, and are a national and international referral center. Our preliminary data demonstrate that using reduced intensity conditioning allogeneic HSCT therapy with PTCy for PID/IDS and IBMFS minimizes GVHD and treatment-related mortality, maximizes engraftment and cure, and ensures that every patient has an available donor. This innovative preliminary work was first published in 2016 and most recently in 2021. The central goal of this project is to harness the regenerative capacity of adult HSCs to cure patients with PID/IDS and IBMFS. Funding from the MSCRF will enable us to conduct a formal Phase II clinical trial using our novel regimen, perform crucial mechanistic studies, and attempt to establish a new gold standard HSCT platform that maximizes efficacy, minimizes toxicity, and offers a donor for every patient. We will study the recovery of the immune system after HSCT to understand how this platform cures our patients and decreases toxicities. Mechanistic studies will help us better understand how PTCy induces tolerance and will guide us in optimizing the time to discontinue post-transplant immunosuppression on our next generation of therapeutic stem cell trials. The innovation of this trial includes taking a reliable platform and expanding its use to cure PID/IDS and IBMFS patients. Success of this proposal could change paradigms for treating and understanding these disorders and pave the way to cure other nonmalignant disorders with therapeutic stem cells.

Brian Miller, M.S., MBA

Sentien Biotechnologies, Inc.
Awardee Amount: $535,342
Disease Target: Sepsis Complicated by Acute Kidney Injury
(2022 1st Funding Cycle)

Extracorporeal Mesenchymal Stem Cell Therapy (SBI-101) in Severe Sepsis Complicated by Acute Kidney Injury

The clinical trial that is proposed in this grant application aims to evaluate the potential of this cell therapy for the treatment of a sepsis population, namely those that are experiencing multiple organ failure, e.g. in their lungs and their kidneys. These patients have very high mortality rates, in large part due to the over-reactive inflammatory response to infection by the immune system.The overwhelming inflammatory response, sometimes referred to a cytokine storm, is thought to be a very important factor for why sepsis patients become severely ill and don't survive. Sentiens product, SBI-101, is designed to address this inflammatory response by reprogramming the immune system into a healing state. SBI-101 combines a cell type referred to as mesenchymal stromal cells (MSCs) and a medical device. This combination cellular device is administered in combination with blood circuits commonly used in an intensive care unit, such as a dialysis machine.By exposing patients blood, which contains many molecules associated with inflammation, to SBI-101, the MSCs inside of SBI-101 produce a broad variety of their own molecules that join with the patients blood. These MSC secreted factors have been shown in laboratory tests and in animal studies to have this desired effect on the immune system, namely that inflammation goes down and the body starts to heal. In human testing with MSCs as a direct injection, however, these results have not consistently been observed. The scientific community agrees that most, if not the vast majority, of MSCs that are administered intravenously rapidly become trapped in the lungs and therefore become unviable.

This may be part of the reason why clinical testing with MSCs has not yet shown the same potential as has been observed in laboratory testing. SBI-101 was designed to overcome this problem. By keeping the MSCs in the device, cells dont get trapped in the lungs but are still able to interact with the patients blood. SBI-101 retains the benefits of the MSCs namely their ability to sense inflammation and produce their secreted factors while overcoming the limitations of intravenous delivery. SBI-101 has been tested at the low dose in a small number of patients with Acute Kidney Injury, which provided preliminary evidence that SBI-101 is enabling the MSCs to remain viable and produce their secreted factors. This study also showed that these secreted factors may be having their intended effect in reducing systemic inflammation. The next step in clinical development for this product are to determine if a higher dose is safe and elicits a more pronounced biological response, and then expand the study by enrolling more patients to deter-mine if SBI-101 can have meaningful impact on patient outcomes, which is the most important goal of clinical development. It is funding this expansion cohort that is being requested in this proposal. If successful, the concept of SBI-101 can be applied to more patients in an ICU setting with this type of systemic inflammatory response to injury, including trauma, burns, acute lung injury, and acute liver failure.

ACCELERATING CURES

VALIDATION

Program

Warren Grayson, Ph.D.

Johns Hopkins University (JHU)
Awardee Amount: $230,000
Disease Target: Bone Defects
(2022 1st Funding Cycle)

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

Johns Hopkins University (JHU)
Awardee Amount: $229,545
Disease Target: Eye Diseases

Mechanism of Action of Adipose-Derived Stem Cells in Oxygen-Enhanced Bone Regeneration

Each year there are 200,000 surgeries involving the use of bone grafts and implants for facial bone reconstruction of injuries caused by cancer, trauma, or congenital disorders. Of these, there remains a subset of geometrically complex defects for which there is no satisfactory solution. Treatment of these large facial bone defects has additional, unique challenges due to the complex, three-dimensional geometry of the bone. To address this limitation, we propose the use of BiO2-Bone scaffolds: 3D-printed, porous, biodegradable, patient-specific scaffolds, which provide immediate mechanical integrity for calvarial or facial defects and facilitate tissue regeneration. They comprise clinical-grade poly-caprolactone (PCL) combined with Bio-Ossclinical-grade bovine decellularized bone matrix, and oxygen-delivering microtanks, which are hollow microspheres that can provide controlled delivery of oxygen to cells seeded into the scaffold upon transplantation into the defect. My lab has demonstrated (i) the capacity to 3D-print anatomically shaped scaffolds and do quality control analysis, (ii) desirable osteoconductivity and osteoinductivity of scaffolds and bone regeneration in murine models of critical-sized calvarial (skull) defects and porcine models of bone healing in zygoma (cheek bones) when BiO2-Bone is used in conjunction with adipose-derived stem cells. These technologies are uniquely suited to facilitate tailored reconstruction of craniofacial bone defects, enable prolonged cell survival and tissue assembly following transplantation, and promote vascular and bone regeneration at the defect sites. In this study, we propose to investigate the mechanism of action of the transplanted cells and oxygen-delivering biomaterial scaffold using an advanced imaging platform capable of quantitatively assessing cell survival, distribution, differentiation, and interaction with endogenous cellpheno-types.

Validating Efficacy of Cryopreserved, hESC-Derived Corneal Endothelial Cells to Form Corneal Endothelium on Denuded Stroma

Cornea is the outermost, transparent tissue of the eye composed of five layers. Corneal endothelium (CE) is the innermost layer composed of hexagonal cells that are critical for maintaining corneal clarity by mediating hydration through barrier and pump functions. The corneal endothelial cell (CEC) density is approximately 2500 cells/mm2 in adult cornea and the physiological functioning is substantially compromised below a CEC density of 500 cells/mm2 resulting in corneal edema. Corneal endothelial dystrophies are the leading cause of corneal transplantation performed in the US each year, and although keratoplasty, has been successful in treating corneal edema, the worldwide shortage of transplantable-grade donor CE remains an insurmountable obstacle in reducing corneal blindness. We previously generated CECs from human embryonic stem cells (hESCs) and now we have demonstrated that cryopreserved hESC-derived CECs can form a functional monolayer of CE on denuded Descemets membrane (DM) in both rabbits and monkeys when injected in the anterior chamber. Importantly, in many corneal endothelial dystrophies the pathology does not remain localized to the CE but rather also affects the DM, the basement membrane the lies underneath the CE. These include Fuchs Endothelial Corneal Dystrophy (FECD), Posterior Polymorphous Corneal Dystrophy (PPCD), etc. These dystrophies contribute significantly to the overall count of corneal transplantation performed in the US each year, especially FECD having a prevalence of 4% of US population above the age of 40 years. Here, we propose to validate the efficacy of cryopreserved hESC-derived CECs to form a functional CE on denuded stroma in three preclinical models: 1) mammals (rabbits); 2) non-human primates (monkeys), and 3) non-human patients (dogs). Validating the efficacy of cryopreserved hESC-derived CECs to form a functional monolayer on denuded stroma will broaden the commercial utility of hESC-derived CECs to include corneal endothelial dystrophies where the pathology extends beyond CE to the DM.

ACCELERATING CURES

DISCOVERY

Program

Ivy Dick, Ph.D.

University of Maryland, Baltimore (UMB)
Awardee Amount: $345,000
Disease Target: Autism Spectrum Disorder (ASD)

Ricardo Feldman, Ph.D.

University of Maryland, Baltimore (UMB)
Awardee Amount: $345,000
Disease Target: GBA1-Associated PD

Evaluating Therapeutic Targets in Autism Spectrum Disorder

Autism Spectrum Disorder (ASD) is neurological disorder characterized by alterations in social skills, communication and behavior. In many cases, ASD arises from the combined effect of multiple genetic variants. As a result, it is often difficult to generate a robust model system with which to study the underlying mechanisms of ASD. However, a small fraction of patients harbor mutations known to be causative of ASD. Such is the case for Timothy Syndrome (TS), a multisystem disorder which includes ASD as a major feature. The cause of TS is a single point mutation within CaV1.2, an L-type Ca2+ channel (LTCC) subtype which controls the entry of Ca2+ into excitable cells. Several CaV1.2 mutations have been linked to ASD, and our preliminary results indicate that each imparts significant changes to channel gating. While the results argue strongly for increased overall Ca2+ entry in affected neurons, each mutation disrupts Ca2+ in a distinct manner. This large, but variable effect on channel gating represents an untapped opportunity for overcoming some of the obstacles to studying ASD. First, due to the mono-genic nature of TS, the generation of transgenic models is significantly more attainable as compared to idiopathic forms of ASD. Second, the large changes in channel gating due to each mutation enables clear resolution of in-vitro phenotypes. Finally, there is significant evidence that LTCCs and their downstream targets play a role in the pathogenesis of many patients with idiopathic ASD, generalizing the TS results and making the LTCC a promising potential therapeutic target. We therefore aim to leverage the large effects of TS mutations to create a model system with a clear and recognizable cellular phenotype indicative of ASD. To do this, we will employ human induced pluripotent stem cells (iPSCs), which have a unique advantage over other model systems. In particular, iPSCs can either be engineered to contain monogenic mutations such as those in TS, or they can be derived from affected patients, providing a platform which recapitulates even idiopathic ASD. We will therefore derive iPSCs into both individual neurons, and 3D organoids replete with system level neuronal coupling. By identifying the changes in Ca2+ and electrical signaling in ASD linked iPSC derived neurons and organoids, we will not only be able to correlate the specific biophysical changes in CaV1.2 to the relevant ASD phenotype in-vitro, but we will gain new insight into the pathogenic mechanisms of ASD, identify potential therapeutic targets for ASD, and generate a platform with which we will evaluate the efficacy of therapeutic compounds for the treatment of ASD. Moreover, by utilizing a model system capable of recapitulating both monogenic and idiopathic forms of ASD, we lay the foundation for application of our results to idiopathic ASD.

New Therapeutic Targets for Prevention and Treatment of GBA1-Associated Parkinson's Disease

Bi-allelic mutations in GBA1 cause Gaucher disease (GD), which can lead to fatal neurodegeneration. Mono-allelic GBA1 mutations do not cause overt neuronopathy but they are the highest risk factor for Parkinsons disease (PD), and 7% of patients with PD are carriers of GBA1 mutations. GBA1 encodes glucocerebrosidase (GCase), a lysosomal enzyme that catalyzes the hydrolysis of glucosylceramide (GluCer). GBA1 mutations result in elevated levels of GluCer and its deacylated metabolite glucosylsphingosine (GluSph). GluSph is a neurotoxic lipid that is elevated up to 1000-fold in brains from patients with neuronopathic GD (nGD), and to a lower extent in brains from patients with GBA1-associated PD. Mutations in enzymes of sphingolipid metabolism are the cause of >60 lysosomal storage disorders often associated with neurodegeneration, indicating that sphingolipid balance is essential for neuronal survival. Using iPSC-derived dopaminergic (DA) neurons from PD patients harboring heterozygous GBA1 mutations (GBA1/PD-DA) and isogenic controls, we identified a pathogenic cascade that links elevation of GluSph with deregulation of the autophagy/lysosomal pathway (ALP) through mTOR hyperactivation. mTOR hyperactivity in GBA1/PD-DA neurons caused the accumulation and aggregation of -synuclein. Both, mTOR hyperactivation and -synuclein aggregation were prevented by inhibitors of mTOR and acid ceramidase, the lysosomal enzyme that converts GluCer to GluSph. In addition, direct treatment of WT/WT isogenic DA neurons with GluSph recapitulated the mTOR-dependent lysosomal/-synuclein abnormalities seen in the mutant neurons. Further analysis showed that mTOR kinase hyperactivation by GluSph impaired lysosomal function through inactivation of specific regulators of the ALP. We hypothesize that slow but persistent accumulation of GluSph in carriers of GBA1 mutations increases their risk of PD through suppression of normal lysosomal functions required for DA neuronal survival. We propose that GluSph activates an aberrant mTORC1 cascade that destabilizes critical ALP regulators, leading to ALP dysfunction, -synuclein aggregation, and increased neuronal vulnerability. In this application we will examine the role of GluSph-dependent mTOR hyperactivation in deregulation of the ALP, and test the therapeutic efficacy of acid ceramidase inhibitors to prevent the abnormalities caused by GCase deficiency. In Aim 1, we will use GBA1/PD-DA neurons and gene-edited isogenic controls to identify the mechanisms by which GluSph activates this pathogenic sphingolipid/mTOR/ALP cascade. We will determine if Rag GTPases, which are amino acid and nutrient sensors and are specific regulators of mTORC1, are also involved in sensing of elevated GluSph. We will then determine how aberrant mTORC1 activation by GluSph deregulates the ALP. In Aim 2, we will use sensitive assays developed in our laboratory to evaluate the therapeutic efficacy of acid ceramidase inhibitors to restore ALP homeostasis and prevent the functional abnormalities of mutant DA neurons. The work proposed will lead to clinical trials based on a new SRT strategy focused on preventing the abnormal accumulation of GluSph, a more targeted approach than the current substrate reduction therapy with GluCer synthase inhibitors.

Xiaoming (Shawn) He, Ph.D.

University of Maryland, College Park (UMCP)
Awardee Amount: $345,000
Disease Target: Heart/Cardiovascular Diseases (CVDs)

Aaron James, M.D., Ph.D.

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

3D Culture and Differentiation of Human iPSCs for Cardiac Tissue Engineering and Regeneration

Cardiovascular diseases (CVDs) are the leading cause of death globally. A major reason for this is that the human heart has limited capacity of regenerating cardiomyocytes. Therefore, transplantation of stem cell-derived cardiomyocytes has been explored for treating CVDs associated with the loss of cardiomyocytes. Although human embryonic stem cells can be differentiated into cardiomyocytes, it is associated with significant ethical concerns. In contrast, human induced pluripotent stem cells (iPSCs) can be differentiated into cardiomyocytes with no ethical concerns. Besides therapy of CVDs, human iPSC-derived cardiomyocytes are valuable for elucidating the etiology/pathogenesis of CVDs and for evaluating drug cardiotoxicity. Contemporary efforts on human iPSC cardiac differentiation have been focused on cells from 2D culture with low yield. Although a few studies on 3D cardiac differentiation have been reported recently, the outset beating time (OBT) ranges over >7 days for all contemporary 2D and 3D cardiac differentiation approaches, resulting in cardiomyocytes with high heterogeneity in terms of maturity. Moreover, the iPSC-derived cardiomyocytes have to be detached from the 2D substrate, which may induce apoptosis. Lastly, the human iPSCs used for nearly all contemporary 2D and 3D cardiac differentiation studies are made by viral transfection, incurring concerns on their clinical use. We recently developed an improved suspension culture approach for large-scale production of human iPSCs in homogeneous 3D spheroids. Furthermore, we can differentiate the 3D iPSC spheroids into beating cardiac spheroids within ~7 days, compared to >14 days needed by all existing protocols. This is because we can synchronize the beating and shorten the OBT to be within ~1 day for all iPSC-derived cardiac spheroids that beat at ~1 Hz at 37 C, similarly to a normal human heart. These cardiac spheroids can be used directly for tissue engineering and regenerative medicine applications with no need of detachment. Lastly and importantly, we have used episomal induced pluripotent stem cells (eiPSCs) free of viral vector and transgene sequences, ensuring the translational value of this project. However, the mechanism of the aforementioned synchronized 3D cardiac differentiation is not well understood yet, and maturation of the eiPSC-derived cardiac spheroids has not been explored. We hypothesize the synchronized beating is due to the homogeneous size of the eiPSC spheroids used for our 3D differentiation, and maturation of the cardiac spheroids can be improved by encapsulating them in hydrogel (similar to the epicardium encapsulation of human heart). We will test these hypotheses with two specific aims: (1) to understand the mechanism of the synchronized cardiac differentiation in 3D by using human eiPSC spheroids of various sizes for differentiation, and (2) to mature the eiPSC-derived cardiac spheroids by encapsulating them either individually in microscale 3D microcapsules or collectively in bio-printed left ventricle-like 3D constructs. We have extensive experience in working with pluripotent stem cells, microfluidics for encapsulation of cell aggregates, and bio-printing for creating macroscale tissue constructs. These studies may greatly facilitate the use of eiPSCs and the development of 3D cell-based cardiac models or constructs for the prevention, diagnosis, and treatment of heart diseases due to the loss of cardiomyocytes.

Targeting Osteogenic Inhibitors Within Human Pericytes

Autologous stem cell therapies hold great promise for the treatment of defects of bone tissue. However, recent clinical trials using MSC (mesenchymal stem cell) based tissue engineering approaches have demonstrated suboptimal or inconsistent results. This has led our research group to the overarching tenant that development of a translatable cell-based therapy for bone tissue regeneration hinges on the identification of a more well-defined progenitor cell subpopulation. Our team has a long-standing interest in human pericytes for tissue engineering. In fact, the pericytic / perivascular identity of MSCs across human organs was first described by our group (Cell Stem Cell, 2008). However, the tissue-specific attributes of human pericytes has also been increasingly recognized. Funded by the MSCRF, we recently examined differences in FACS purified human CD146+ pericytes from either skeletal or soft tissue sources (Bone Research, 2020). We observed that despite multipotency across all pericyte cell populations, skeletal pericytes had a natural predisposition to mineralize in cell culture and ossify after xenotransplantation. An opposing predisposition to form mature adipocytes was identified among adipose tissue (AT) resident pericytes. In effect, CD146+ human pericytes have a tendency to replicate the microenvironment from which they are derived.A puzzling phenomenon is that human adipose tissue resident pericytes have the ability for transdifferentiate to an osteoblastogenic cell fate, yet this rarely occurs in vivo. We reasoned that basal signaling pathways actively inhibit osteogenic pathways are present among AT pericytes in vivo. To this end, the transcriptome of skeletal versus AT-derived pericytes was analyzed for novel differentially expressed genes (DEGs) that may play this homeostatic role in vivo and could be leveraged for a tissue engineering purpose. Among 26,884 genes expressed across populations, 440 DEGs showed greater than 3 SD increase among adipose tissue-derived pericytes. Of these, 10 genes played some described role in stem cell maintenance or differentiation decisions, of which ZIC1 (Zinc finger protein of the cerebellum 1) was further pursued. ZIC1 acts as a transcriptional activator in organogenesis, particularly in the central nervous system. Indeed, missense and nonsense mutations in Zic1 in mice have recently been found to produce skeletal developmental defects. In preliminary data, gene misexpression studies of ZIC1 demonstrated strong effects on osteogenic versus adipogenic cell differentiation decisions among human pericytes, potentially via regulation of Hedgehog signaling activity. To our knowledge, the transcription factor ZIC1 has never been studied in any context of mesenchymal stem cell fate, the perivascular niche, nor changes in osteo- versus adipogenic differentiation. The current Discovery award seeks to further our thinking in the field, with the central hypothesis that the osteogenic inhibitor ZIC1 maintains the adipose tissue identity of human pericytes, and that gene deletion will drive bone anabolism among implanted human pericytes.

Shaun Kunisaki, M.D., M.Sc.

Johns Hopkins University (JHU)
Awardee Amount: $345,000
Disease Target: Congenital Diaphragmatic Hernia

Linda Resar, M.D.

Johns Hopkins University (JHU)
Awardee Amount: $345,000
Disease Target: Aging, Clonal Hematopoiesis, MDS

Modeling Congenital Diaphragmatic Hernia Lung Development with Stem Cell-Derived Organoids

Congenital diaphragmatic hernia (CDH), a polygenic condition associated with severe pulmonary hypoplasia and pulmonary hypertension at birth, is one of the most common, costly, and severe birth defects managed by neonatologists and pediatric surgeons worldwide. The underlying genetic origins of CDH remain unknown, and more recently, the classical paradigm of mechanical compression by the abdominal organs as the main cause of the underlying lung hypoplasia has been challenged. There is now increasing evidence that there may be primary cell/genetic-based defects in CDH lung morphogenesis that are merely exacerbated by exposure to mechanical compression forces. Given these fundamental questions regarding the embryologic origins of CDH pulmonary hypoplasia, our laboratory seeks to develop human stem cell-based strategies for modeling and treating the CDH fetal lung. Accordingly, we have recently created a consistent CDH lung organotypic model based on human induced pluripotent stem cells (iPSCs) generated from CDH infants. In recently published work (Kunisaki et al., Stem Cells Translational Medicine, 2021), we can reliably differentiate CDH iPSCs into 3D lung-like tissue, termed lung organoids, containing epithelial lumen structures with a robust mesenchyme. The central hypothesis of this proposal resubmission is that periostin (POSTN), a mechanical stress response molecule critical for lung repair, is aberrantly expressed in the CDH fetal lung and is associated with abnormal lung progenitor cell differentiation under mechanical forces. This hypothesis is informed by Supporting Data as well as by published experience suggesting the importance of POSTN in pediatric lung disease. In Specific Aim 1, we will utilize our novel human stem cell-based model to investigate the impact of aberrant POSTN upregulation on mesenchymal and epithelial lung development in CDH. A pathway for therapeutic translation is also proposed in a mouse model of human lung organoid delivery within hydrogel scaffolds. In Specific Aim 2, we will determine the extent to which mechanical compression impairs POSTN-mediated mesenchymal and epithelial lung development in CDH lung organoids. The cornerstone of this proposal is a multidisciplinary team led by an NIH-funded Johns Hopkins pediatric surgeon-scientist (Dr. Kunisaki). This proposal builds on innovative stem cell-based and micro-mechanical technologies. The expected results from these Aims will have advanced the establishment of a unique, human stem cell-based in vitro model to evaluate cell intrinsic properties of CDH lung progenitors and the role of mechanical forces and POSTN during fetal lung development. Further work applying this CDH lung disease-in-a-dish technology will also result in new pathways to clinical translation in CDH, including pharmacologic blockade of POSTN or other novel matricellular targets and organoid-based tissue engineering therapies.

Developing CRISPR Technology to Rejuvenate Blood Stem Cells

We propose to develop innovative CRISPR activator technology to rejuvenate hematopoietic stem cells (HSCs). We focus on the High Mobility Group A1 (HMGA1) chromatin regulator in hematopoiesis. Our scientific premise that HMGA1 is a critical regulator of youthful regenerative function and platelet production from HSC is based on our compelling preliminary data: 1) HMGA1 maintains a de-differentiated, pluripotent state in embryonic stem cells by inducing specific stemness transcriptional networks. 2) Hmga1 fosters self-renewal in adult stem cells of the intestinal epithelium by amplifying Wnt signals (Xian, et al Nature Commun 2017; Resar, et al Cancer Res 2018). 3) Surprisingly, Hmga1 also builds an intestinal stem cell niche comprised of Paneth cells by inducing Sox9; Paneth cells, in turn, secrete Wnt to nurture the stem cells. 4) Mice with global loss of Hmga1 have decreased embryonic viability with loss of about ~40% of knock-out (KO) embryos late in gestation. 5) Those that survive exhibit premature aging phenotypes (early kyphosis, osteopenia, high frequency hearing loss, poor grip strength, decreased gait velocity, premature greying, and poor skin regeneration). 6) In the hematopoietic system, mice with Hmga1 deficiency also develop aging phenotypes, including: a) early thymic atrophy, b) neutophilia, c) thrombocytopenia, and, d) marked defects in competitive repopulating ability in bone marrow transplantation assays with HSC from young adult (6-16 weeks) and aged (18-24 months) mice with further impairment in regenerative function after serial transplantation. 7) In murine models of JAK2 mutant myeloproliferative neoplasms, loss of just a single allele of Hmga1 prevents erythrocytosis, thrombocytosis, splenomegaly, bone marrow fibrosis, and expansion in the megakaryocyte-erythroid progenitors (MEP) and megakaryocytes (Mk). Together, these exciting data led us to the following hypotheses: 1) HMGA1 is key epigentic regulator of regenerative function in HSC and megakaryopoiesis, 2) HMGA1 is required for MEP/Mk expansion, thrombocytosis, and myelofibrosis in myeloproliferative disease. 3) Increasing HMGA1 function in human HSC will enhance regenerative capacity and differentiation to Mk and platelets, while inhibiting HMGA1 will impair regeneration and decrease the Mk and platelet pool. To test this, we propose to harness innovative CRISPR activator (CRISPRa) technology in human CD34+ hematopoetic stem and progenitor cells (hHSPC) to modulate HMGA1 expression and enhance function with the following Specific Aims: 1) To dissect HMGA1 function in HSC and megakaryopoieis, including: A) HMGA1 phenotypes using cell-based and murine preclinical models, and, B) To identify underlying mechanisms by integrating RNAsequencing (RNAseq) with ChIPseq and ATACseq in hHSPC. 2) To develop novel CRISPRa technology: A) To rejuvenate HSC and MEP by activating HMGA1 expression, and, B) To test the function of HSC and MEP engineered with CRISPRa using in vitro colony forming assays and in vivo transplantation in humanized mice. At the completion of this project, we expect to engineer human CD34+ stem and progenitor cells with varied levels of HMGA1, assess their function, and identify underlying mechanisms. Together, these studies will provide the ground work necessary to develop technology to rejuvenate HSCs for aging-related blood diseases and possibly other hematologic disorders.

Matthew Trudeau, Ph.D.

University of Maryland, Baltimore (UMB)
Awardee Amount: $345,000
Disease Target: Long QT Syndrome

Using Non-Canonical Amino Acids to Repair hERG LQT2 Mutants in hiPSC-CMs

Cardiomyocytes derived from induced pluripotent stem cells (iPSC-CMs) are a powerful cellular model system for the study of native cardiac ion channels, cellular electrophysiology properties and cellular mechanisms of cardiac arrhythmias. Here we examine hERG (human Eag-Related Gene) potassium channels and their native correlate, cardiac IKr current, in hiPSC-CMs. We take advantage of action potentials in hiPSC-CMs to examine the cellular electrophysiological impact of dysfunctions in the IKr current. We focus on an understudied class of hERG nonsense mutations that cause Long QT syndrome, a serious inherited condition that causes cardiac arrhythmias, syncope and sudden cardiac death. The proposal is designed to screen the deleterious effects of nonsense amber (TAG) stop codons in hERG that cause LQT in heterologous cells and translate the findings to hiPSC-CMs using CRISPR/Cas9 gene editing. We established amber codon suppression and non-canonical amino acid technology, in which a non-canonical amino acid is inserted at the TAG codon, as a method to ameliorate the dysfunction in hERG TAG LQT mutants. It is not clear how hERG TAG LQT mutants will function under control of the native gene promoters in hiPSC-CMs.

This makes our proposed work vital for understanding the role of nonsense LQT mutants in forming IKr and perturbing cellular electrical properties in gene-edited hiPSC-CMs. The outcomes of our proposed experiments would reveal the impact of autosomal-dominant LQT mutations and establish hiPSC-CMs as a sensitive model for subtle, heterozygous mutations. Our proposed work is also a model for testing precision medicine approaches, such as non-canonical amino acids with amber codon suppression, as tools for critically examining molecular and cellular mechanisms underlying the precursors of arrhythmias in hiPSC-CMs. Our proposed work could be expanded to TAG mutants in other genes linked to other diseases. All our work proposed here directly supports the mission and stated goals of the MSCRF.

ACCELERATING CURES

LAUNCH

Program

William "Brian" Dalton, M.D., Ph.D.

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

Jie Jiang, Ph.D.

University of Maryland, Baltimore (UMB).
Awardee Amount: $338,486,
Disease Target: Rotator Cuff Tears

Leveraging an iPSC-Derived Model of Acquired Sideroblastic Anemia for Novel Therapies

This proposal to the Launch Program of the Maryland Stem Cell Research Fund seeks to leverage a new iPSC-derived model of acquired sideroblastic anemia to increase our understanding of disease mechanisms and to develop novel therapies. Acquired sideroblastic anemia is a devastating complication of the Myelodysplastic Syndromes (MDS), bone marrow failure disorders that are progressive, lethal, and lacking in effective treatments. Targeted therapy in other realms such as cardiovascular disease, diabetes, and areas of cancer have brought great benefit to patients through more effective and less toxic treatments, but patients with MDS and severe, transfusion- dependent anemia in particular have been left behind in this revolution. However, the molecular abnormalities driving MDS have become clearer in recent years, and one gene mutation that is a common, and sometimes the only, driver of MDS is in a spliceosome gene called SF3B1. Mutations in this gene cause abnormal RNA splicing, and they also occur in the vast majority of cases where MDS patients have acquired sideroblastic anemia. However, how SF3B1 mutation leads to abnormal differentiation of hematopoietic stem cells resulting in sideroblastic anemia is unknown, and targeted therapies are lacking. A key barrier to progress is a lack of disease-relevant human cell models, as mice do not recapitulate the human disease. We therefore propose here to combine our developed expertise in SF3B1 mutations and gene targeting with our colleagues at Johns Hopkins to develop an isogenic iPSC-derived model of erythropoiesis to study the role of SF3B1 mutations in stem cell differentiation and to test novel therapies. From previous work, we have identified specific RNA splicing events and metabolic abnormalities suggesting new therapeutic approaches. Thus, we propose to use the Launch Program to move these initial discoveries into the field of stem cell biology and therapy. Consistent with the mission of stem cell research in the State of Maryland, our project promises to develop new treatments through use of human stem cell research for a deadly condition that is itself a disease of stem cells.

Identification and Application of Distinct Perivascular Stem Cell Sub-Populations to Rotator Cuff Repair

Tendon injuries are common orthopedic conditions that significantly impact patients' quality of life. While poorly-vascularized tendons such as the rotator cuff do not heal on their own, well-vascularized tendons such as the Achilles do heal. The exact mechanisms behind this observation are not well-understood. We hypothesize that a significant difference between tendon healing and non-healing is access to vascular supply, specifically, vascular-associated mesenchymal stem cells. Perivascular stem cells (PSCs) are adult mesenchymal stem cells associated with blood vessels throughout the body. PSCs are the combination of two distinct perivascular populations: pericytes and adventitial progenitor cells. Human PSCs (hPSCs) are an attractive mesenchymal stem cell source in terms of clinical potential. They can be isolated from lipoaspirate for same-day use and have the ability to differentiate into all mesenchymal lineages. Recent lineage-tracing studies have linked the cellular identity of fibro-progenitors responsible for fibrous tissue formation after an injury to cells within the perivascular niche. Still, the exact identity of these cells remains unresolved. Some studies have suggested that PSCs or a subset of PSCs are responsible for the native tendon and ligament healing in vivo8,9. Our preliminary scRNA-seq data shows two distinct progenitor cell subpopulations within the adventitial progenitor cells isolated from human adipose tissue, which correspond to previously identified perivascular fibroblasts in mice. We were also able to identify several cell surface antigens for isolating these subpopulations. This proposal will further determine the sub-populations of PSCs responsible for tendon healing and apply them therapeutically to a surgical model of rotator cuff repair. Aim 1: Identify, isolate, and characterize fibro-progenitor sub-populations of human PSCs that are best suited for tendon repair. Hypothesis: Analysis of hPSC with single-cell RNA sequencing (scRNA-seq) will facilitate the isolation and subsequent characterization of specific sub-populations of PSCs that directly contribute to tendon healing. In this Aim, we will perform scRNA-seq on freshly isolated hPSCs from additional donors to expand our preliminary results. We will isolate two PSC subpopulations using fluorescence-activated cell sorting (FACS) and examine their phenotypes using comprehensive MSC characterization assays. We will repeat these experiments on cells isolated from human biceps tendon as a comparison. Aim 2: Determine the ability of hPSC sub-populations to directly contribute to in vivo rotator cuff healing following surgical repair. Hypothesis: Human PSC sub-populations injected into rotator cuff injuries will differentiate into collagen producing fibroblasts that will directly contribute to improved cellularity, healing rates, and mechanical properties of the healing tendon as compared to those without hPSC augmentation. We will evaluate the therapeutic potential of hPSC subpopulations in a rat rotator cuff repair model. Cells will be injected directly into the injury site following surgical repair of a rotator cuff injury. The therapeutic potential of freshly isolated hPSCs, pericytes, and the two sub-populations of adventitial progenitor cells already identified in our preliminary results, will be compared. Human tendon fibroblasts (hTF), non-sorted adipose cells, and injury without cell treatment will be used as controls. Rotator cuff healing will be examined via gate analysis, histology, CT, and mechanical testing.

Younggeon Jin, Ph.D.

University of Maryland, College Park (UMCP)
Awardee Amount: $344,963
Disease Target: Inflammatory Bowel Disease (IBD)

Deqiang Li, Ph.D.

University of Maryland, Baltimore (UMB)
Awardee Amount: $344,907
Disease Target: Dilated Cardiomyopathy

Role of E-cadherin in the Intestinal Stem Cell Homeostasis and Regeneration

Treatment of patients with inflammatory bowel disease (IBD) has been challenging due to disease heterogeneity and the lack of therapeutic targets. In recent years, mucosal healing has emerged as a key therapeutic goal in the clinical management of patients with IBD, as it has been associated with improved long-term clinical outcomes. Intestinal stem cells (ISCs) offer a promising therapeutic target due to their capacity to regenerate the mucosal epithelial barrier which may improve symptoms of IBD. Evidence supports the existence of two functionally different ISC populations: actively prolif-erating ISCs (aISCs, Lgr5 enriched) and slowly proliferating reserve ISCs (rISC, Hopx enriched) are believed to support homeostatic epithelial renewal and injury-induced regeneration, respectively. Adherens Junctions (AJs) are cell-cell adhesion complexes and have critical functions in the epithelium, including stem cells. One of AJs components, [B]-catenin, also plays an essential role in Wnt pathways, which regulates stem cell homeostasis and regeneration. Transmembrane protein E-cadherin is a buffer to sequester [B]-catenin to regulate the Wnt pathway. Specifically, disrupted E-cadherin induces internalization of [B]-catenin to the cytosol, and, subsequently, the nucleus, which increases the transcriptional activity of Wnt/[B]-catenin target genes (Fig. 1A). Despite the critical functions of AJs on the intestinal epithelium, we still have a surprisingly poor understanding of their function in ISCs. Therefore, we will test the central hypothesis that the inactivation of E-cadherin will interrupt the ISCs homeostasis, but will enhance ISCs regen-eration during IBD pathogenesis. Specifically, downregulated E-cadherin will disrupt the ISCs homeostasis with reduced cell-cell adhesion and increased the proliferation of the ISCs. The inactivation of E-cadherin during regener-ation after injury will also enhance aISCs proliferation with increased Wnt target genes and result in improvement of recovery from injury (Fig. 1B). Understanding the impact of AJs on ISCs homeostasis and their contributions to epithelial regeneration is critical to therapeutic targeting for IBD. Aim1. Define the role of E-cadherin during the homeostasis and regeneration in colonoids. Working hypothesis: Downregulation of E-cadherin alters colonoid development and regeneration from injury with interfered ISCs. 1A. Deter-mine the role of E-cadherin during the development of human colonoids. 1B. Identify the role of E-cadherin during regeneration after in vitro IBD-like cytokine/anoxia-induced injury in human colonoids. Aim2. Determine the role of E-cadherin in distinct ISC populations, aISCs and rISCs, during homeo-stasis and regeneration from colitis. Working hypothesis: Conditional knock-out of E-cadherin in aISCs or rISCs will show different responses in ISC homeostasis and regeneration after colitis. 2A. Identify the role of E-cadherin in Lgr5+ specific (Cdh1fl/fl; Lgr5-Cre) and Hopx+ specific (Cdh1fl/fl; Hopx-Cre) conditional E-cadherin knockout mice. 2B. Determine the role of E-cadherin in aISCs and rISCs during regeneration from dextran sodium sulfate-induced colitis.

Mechanisms of Fibrosis in Dilated Cardiomyopathy

Dilated Cardiomyopathy (DCM) is characterized by progressive left ventricle chamber enlargement and reduced contractility, which eventually leads to heart failure. DCM is the most common type of cardiomyopathy and the leading cause of cardiac transplantation. DCM is considered primarily a disease in cardiomyocytes: mutations in genes encoding sarcomeric proteins such as cardiac troponin T (TNNT2) and Titin (TTN) may account for 30% of familial DCM patients. However, the nonmyocyte compartments in the heart including fibroblasts gradually become dysfunctional and profibrotic, which significantly contributes to DCM symptoms and disease progression, although these cells do not express the mutated sarcomeric genes. In fact, DCM prognosis is also directly associated with the extent of cardiac fibrosis. However, it is unclear how fibroblasts in hearts with DCM become profibrotic. A better understanding of this mechanism would likely result in additional therapeutic targets with potentially improved outcomes. Exosomes, small particles that can be secreted and taken up by many types of cells in the body, may play a role in this mechanism. Exosomes contain biologically active cargos such as micro RNAs (miRs). Emerging evidence suggests that cardiac exosomes can serve as biomarkers and play critical roles in a wide range of cardiovascular diseases such as myocardial infarction, heart failure and high blood pressure. In our preliminary studies, we isolated exosomes from induced pluripotent stem cell (iPSCs)-differentiated cardiomyocytes of DCM patients and healthy control individuals. We then cultured human cardiac fibroblasts (HCFs) in the presence of these isolated exosomes. When cultured in the presence of DCM exosomes (DCM-Exos), the expression of fibrotic genes in HCFs was significantly upregulated when compared to control exosomes (CTL-Exos). Consistently, injection of DCM-Exos in mouse hearts resulted in increased fibrosis. Employing miR sequencing analyses, we identified that miR-218 was significantly upregulated in the DCM-Exos. More interestingly, the application of miR-218 mimics to HCFs significantly increased the expression of fibrotic genes, while knockdown of miR-218 in DCM-Exos mitigated their profibrogenic effects. Based on these strong preliminary data, we hypothesize that exosomes released by cardiomyocytes from DCM patients induce fibrosis and contribute to DCM disease progression through miR signaling. To test this hypothesis, we propose two aims. Aim 1: to determine whether DCM-Exos induce cardiac fibrosis and contribute to DCM disease progression. Aim 2: To identify the mechanisms of cellular crosstalk in DCM cardiac fibrosis. Our findings will provide new mechanistic insights into the pathogenesis of DCM, which will serve as the building blocks for developing new treatments. This line of research is truly translational: concurrently studying the pathogenesis of DCM and the resulting potential therapeutics that may lead to improved outcomes for these patients. The findings from this project will surely contribute to the goals of the MSCRF: a better understanding of debilitating human diseases, the development of new medical strategies for the prevention, diagnosis, treatment and cure of human diseases.

Pan Li, Ph.D.

John Hopkins University (JHU)

Awardee Amount: $345,000

Disease Target: Huntington's Disease

Exosomes Derived from Engineered Human iPSCs for the Therapy of Huntington Disease

Huntington's Disease (HD) is a neurodegenerative disorder caused by a CAG repeat expansion (encoding polyglutamine) in the gene huntingtin (HTT). The HD phenotype results primarily from toxic properties of the mutant HTT protein with an expanded polyglutamine tract. There is currently no cure for HD. Our group have previously reported that intracerebroventricular (ICV) injection of a CAG repeat-targeting phosphorodiamidate morpholino oligomer (PMO), a steric-blocking antisense oligonucleotide (ASO), specifically suppressed mutant HTT expression and rescued behavioral deficits in the HdhQ7/Q150 HD knock-in mouse model, with no detectable off-target effects. However, ASOs (including PMOs) do not cross the blood-brain barrier (BBB). Therefore, for targets in the CNS, ASOs must be delivered by repeated lumbar puncture in human patients, a procedure that is invasive in nature, and thus hindering its clinical translation. Exosomes (Exo) are cell-secreted extracellular vehicles with BBB penetration potential, capable of delivering exogeneous therapeutic molecules. iPSCs have been considered as one of the best sources for Exo manufacture, due to high yield, availability of clinical-grade manufacture platform, feasibility to engineer iPSC lines that produce modified Exo for more efficient brain targeting etc. We hypothesize that human iPSCs can be engineered to produce brain-targeting Exo that can deliver PMOs to suppress the toxic mutant HTT protein in neurons and ameliorate the behavioral deficits in the HD mouse model. To test the hypothesis, we have developed the following two specific aims.

Aim 1: To generate engineered iPSCs to produce RFP-labelled and brain-targeting Exo (b-Exo). Aim 2) To determine the efficacy of iPSC-derived b-Exo loaded with PMO for suppressing mutant HTT protein and neurotoxicity in neuron and mouse models of HD. It is innovative to use systemic administration of Exo to deliver PMOs. It is also an innovation to use genome editing to engineer iPSCs that produce modified Exo for brain targeting. The engineered iPSCs will be used as factories for manufacturing Exo that are broadly applicable to deliver therapeutic molecules for the treatment of HD and other brain diseases. The PI, Dr. Pan Li, new to the field of human stem cell research, is currently on her third year as an Assistant Professor at Johns Hopkins School of Medicine. She has established expertise in disease pathogeneses of HD, SCA2 and SCA12, as well as cell and animal modeling of these diseases using CRISPR/Cas9 genome editing approach. She is looking forward to beginning her new journey exploring the use of engineered human iPSC-derived brain-targeting Exo for the delivery of mutant HTT lowering PMOs as a potential therapy for Huntington's disease. If successful, we will establish an effective strategy for HD therapy with potential of clinical translation. Success of this project will also set the stage for future larger scale investigations aimed at using huma iPSC-derived Exo for non-invasive and targeted delivery of therapeutic molecules (such as PMOs) for various neurodegenerative disorders, consistent with the goals of Maryland Stem Cell Research Fund (MSCRF) to promote stem cell based translational research.

ACCELERATING CURES

POST-DOCTORAL FELLOWSHIP

Program

Michael Barbato, Ph.D.

Johns Hopkins University (JHU)
Mentor: Elias Zambidis, M.D., Ph.D.
Awardee Amount: $130,000
Disease Target: Diabetic Retinopathy

Chelsey Dunham, Ph.D.

Johns Hopkins University (JHU)
Mentor: Sharon Gerecht, Ph.D.
Awardee Amount: $130,000
Disease Target: Atherosclerosis

Epigenetic Regulation of Vascular Differentiation from Human Nave Pluripotent Stem Cells

Diabetic retinopathy (DR) results from retinal ischemia and acellular vascular segments. If acellular retinal capillaries could be reconstituted, ischemic death of retinal neurons could be reversed in DR. The mentor's laboratory reported the derivation of hiPSCs with a superior potency of differentiation into vascular progenitor (VP) lineages that could regenerate diabetic blood vessels and is committed to understanding the epigenetic influences which contribute to superior hiPSC differentiation capacity. Prior work showed hiPSCs possessed limited differentiation with lineage skewing. It is suggested that skewing is due to retention of the donor cell epigenetic signature state leftover after classical reprogramming methods. These signatures preferentially tilt the epigenetic landscape resulting in a differentiation potential tethered by donor cell lineages. This problem is evident in chemical nave reversion systems which utilize LIF-2i in murine systems or LIF-5i human culture systems. These small molecule methods have led to epigenetic alterations and imprint region erasure. Despite these clear epigenetic issues, some investigators, explain differentiation skewing by implicating donor differences rather than epigenetic influences. Entering this debate, the mentor's lab reported a novel method for inducing nave reversion with LIF-3i, producing nave human pluripotent stem cells (N-hPSCs) with normal epigenomic configurations. Our group has reported that the functionality of VPs generated from normal and disease-primed conventional hiPSC are significantly improved using LIF-3i. Furthermore, tankyrase/PARP inhibitor-regulated N-hiPSCs represent a new class of human stem cells with high epigenetic plasticity and improved multi-lineage functionality. Our specific aims will define this state to help solidify the dispute about what drives lineage skewing; explain the superior potency of our hematopoietic differentiation; and lay the groundwork for targeted reprogramming, allowing us to generate lineages with improved function-ality like our VPs, efficiently and specifically for individual regenerative medicine.

3D in Vitro and in Vivo Models to Study Vascular Graft Remodeling in Atherosclerosis

We will study vascular graft remodeling with 3D in vitro and in vivo models of atherosclerosis using patient-specific iPSC-derived endothelial cells (iECs). Previously, we successfully developed a small diameter vascular graft (~0.6 mm lumen diameter) that mimics vascular ultrastructure and is made of a natural material, fibrin, shown to interact with cells. iECs are seeded onto the graft luminal surface and cultured in a peristaltic flow-controlled bioreactor, generating a physiological in vitro vascular model. Our system will be the first in vitro atherosclerotic model to use iECs; enabling us to determine differences due to sex and disease, thereby allowing mechanistic evaluation of pathological remodeling. In Aim 1, we will evaluate remodeling and cellular function in this fibrin graft seeded with iECs in pathological (i.e., atherosclerotic) conditions in vitro. The atherosclerotic environment will be induced in vitro via co-culture with monocytes and LDL. Additionally, platelets have been described to exacerbate atherosclerosis and cause thrombosis; they will be added to the co-culture to evaluate their adhesion and activity. In Aim 2, we will mitigate pathological remodeling, endothelial dysfunction, and platelet adhesion in vitro by using a heparinized-fibrin graft. Heparin is an anti-coagulant glycosaminoglycan shown to decrease platelet adhesion and support endothelial cell viability. After establishing and evaluating remodeling in the atherosclerotic platform developed in Aim 1, we will harness the advantage of using a natural material and conjugate heparin to the fibrin graft to determine if it will prevent thrombosis while also directing iECs toward a regenerative phenotype. In Aim 3, we will implant our heparinized-fibrin grafts into atherosclerotic mice (ApoE-/-) to evaluate graft remodeling, endothelial function, and platelet adhesion. This last aim represents an important step toward clinical translation and the need to understand how atherosclerosis modulates graft integration.

Casey Keuthan, Ph.D.

Johns Hopkins University (JHU)
Mentor: Donald Zack, M.D., Ph.D.
Awardee Amount: $130,000
Disease Target: Retinal Degeneration

Long Range Transcriptomic Analysis of Human Retinal Development & Degeneration

Alternative splicing (AS) is a fundamental process to enhance transcrip-tome and proteome diversity and serves as a critical post-transcriptional mechanism for gene regulation of many cell activities. This process is particularly important in neuronal tissues, such as the sensory neurons of the retina, which exhibit some of the highest levels of differentially spliced genes in the body. Retinal development is largely dependent upon finely controlled, cell-type specific patterns of gene expression, and earlier work has suggested that AS may contribute to this complex developmental program. Moreover, a number of splice site mutations and mutations in splicing factors, are associated with retinal degen-eration, yet our understanding of the RNA splicing landscape in the retina is limited. This MSCRF project aims to investigate the AS events that occur during retinal development and the progression of retinal disease using human stem cell-derived retinal organoids. Retinal organoids differentiated from human stem cells recapitulate the in vivo neuron micro-environment and much of the complexities of the human retina. Utilizing cutting-edge long-read sequencing technology, we will comprehensively profile the AS landscape in retinal organoids generated from both wild-type and patient-derived and/or CRISPR-engineered mutant stem cell lines to further understand the role of RNA splicing on a global scale. While computational methods have been developed to extend the RNA splicing information derived from Next Generation Sequencing-based RNA-seq, long-read methods can provide more direct sequencing information on spliced isoforms, as well as modified RNA bases. Overall, the training potential for the proposed research plan and mentorship detailed in this application is high. This project will provide training towards an independent research career using stem cell models while advancing the development of the scientific basis for novel therapeutic strategies, such as the use of retinal stem cells to restore vision in patients suffering from various forms of retinal disease.

Ramesh Marasini, Ph.D.

Johns Hopkins University (JHU)
Mentor: Jeff Bulte, Ph.D., M.S.
Awardee Amount: $130,000
Disease Target: Multiple Sclerosis

In vivo Imaging of Stem Cell-Derived Extracellular Vesicles Using Positive Contrast MRI

The use of experimental stem cell therapy has shown great promise in neurological diseases including multiple sclerosis (MS). However, stem cell therapy is somewhat limited by complex regulatory issues, low cell viability, and high costs. This project proposes to use stem cell-released extracellular vesicles (EVs) and label them with a clinically used gadolinium agent with bright magnetic resonance imaging (MRI) contrast to harness the therapeutic benefit of stem cells as cell-free cell therapy. This technique contrasts with the commonly used inherent dark contrast of iron oxide-labeled magnetic vesicles that makes it difficult to see underlying anatomy and to distinguish them from endogenous voids arising from trauma or hemorrhage, which are commonly encountered in targets of stem cell therapy. The EVs will be isolated from human mesenchymal and glial-restricted precursor cells using differential centrifugation, then labeled with a gadolinium lipid chelate using an insertion technique in the lipid bilayer of the vesicles. EV markers (CD63, CD9, CD81), gadolinium content, and structural integrity of labeled EVs will be tested by examining the markers, gadolinium metal, and size/surface charge analysis, respectively. Labeled EVs will be given via intra-cerebroventricular and intravenous routes and tracked in vivo as bright contrast under MRI for their homing and biodistribution. A mouse experimental autoimmune encephalomyelitis (EAE) model of MS will be employed to evaluate the potential of labeled EVs for therapeutic benefit by reducing host inflammation through cellular communication owing to the molecular function of its parent cells and compared with nave animals.

Xiaoli Rong, Ph.D.

Johns Hopkins University (JHU)
Mentor: Valina Dawson, Ph.D.
Awardee Amount: $130,000
Disease Target: Stroke

Tunable Biomaterial Guided Stroke Repairs by Endogenous and Exogenous Stem Cells

Chronic stroke is a disease of immense public health issue and no promising treatment for the stroke. The institute provides a multidisciplinary expertise in biomedical research environment to conduct chronic ischemic stroke. We propose combinational reparative approaches to promote neural repair following chronic stroke by using the biodegradable hydrogel guided supports (1) endogenous stem cell stimulation, and (2) exogenous stem cell transplantation to promote the functional integration to recovery from the stroke. First, we take advantage of our resident stem cells to restore their neuronal function by developing a tissue-like viscoelastic biomaterials (tissue engineering) there by support internal structures and activate endogenous stem cells to promote repair. We have developed a biocompatible, biopolymer with no neurotoxicity effect to brain tissue. Second, with this neural tissue-like biomaterial, we will achieve safe delivery of human brain cells to the stroke-injured area. Using human ES/iPS, we have developed a high-efficiency rosette-type neural stem cell-derived aggregates (RONA)-method for generating human cortical neural cells that contain excitatory and inhibitory neuronal precursors as well as oligodendrocytes cells. Advantage of these technique is that we can transplant any CNS cell type for the recovery after the post-stroke. Using this strategy, we will employ dynamic observation of survival, proliferation, migration, differentiation, and functional integration of grafted cells to optimize cell delivery interventions to provide behaviorally measurable recovery after in MCAO stroke model. The long-term goal is to responsibly translate cell-based therapies into novel therapies for stroke. In this study, the combinational of neural stem cell biology and regenerative medicine, bioengineering, and neurosurgery and stroke. We will develop a novel stem cell strategy for the treatment of chronic stroke which will pave a bench to bedside approach for clinical trials and strengthen our knowledge in understanding the regenerative stem cell field.

Bo Am Seo, Ph.D.

Johns Hopkins University (JHU)
Mentor: Hanseok Ko, Ph.D., M.S.
Awardee Amount: $130,000
Disease Target: Parkinson's Disease

Neurotoxic Astrocyte Secreted-ExoDrp1 Causes Excessive Mitochondrial Fragmentation and Neurodegeneration of Human Dopaminergic Neurons

Emerging evidence suggests that neurotoxic astrocytes play a role in the onset of neurodegenerative disorders including Parkinson's disease (PD). However, it is still unclear how neurotoxic astrocytes contribute to neurodegeneration in PD. It speculates that toxic factors produced by the neurotoxic astrocytes may play a role in neurotoxicity, yet the toxic factors that mediate neurotoxicity are unknown. As such identification of toxic factors secreted by neurotoxic astrocytes in PD may provide a new insight into the pathogenesis in PD and new strategy for the treatment of PD. In order to identify neurotoxic factors secreted by neurotoxic astrocytes, we conducted mass spectrometry (MS) analysis combined with SILAC (Stable Isotope Labeling with Amino acids in Cell culture) using conditional medium collected from neurotoxic astrocytes (ACM) induced by conditional medium (MCM) collected from -synuclein preformed fibrils (-syn PFF)-activated microglia. Intriguingly, dynamin related protein 1 (Drp1) was identified from the MS analysis. Our preliminary studies indicate that Drp1 is secreted from neurotoxic astrocytes via exosomes (exoDrp1) and exoDrp1 is uptaken by neurons. We also found transported exoDrp1 leads to mitochondrial fragmentation and neurotoxicity in neurons. More importantly, exoDrp1 aggravates human -syn PFF-induced Lewy bodies (LBs) pathology. Based on our preliminary results, we have investigated the contribution of exoDrp1 in human dopaminergic neurodegeneration using both Hg-human embryonic stem cell (hESC)-derived midbrain dopaminergic neurons and PD patients induced pluripotent stem cells (hiPSCs)-derived midbrain dopaminergic neurons. The investigation will provide a new insight into the pathogenesis of PD and new strategy for the treatment of PD.

Kunyu Zhang, Ph.D.

Johns Hopkins University (JHU)
Mentor: Luo Gu, Ph.D.
Awardee Amount: $130,000
Disease Target: Autoimmune Diseases

Yuxiao Zhou, Ph.D.

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

Dynamic Hydrogel Matrix to Prime Mesenchymal Stem Cells for Enhanced Autoimmune Disease Treatments

Autoimmune diseases, such as systemic lupus erythematosus (SLE), are a group of heterogeneous conditions characterized by aberrant immune activation with failure of the regulation to maintain adapted tolerance. Autoimmune diseases are usually chronic and often debilitating, with an enormous medical and socioeconomic burden. Mesenchymal stem cells (MSCs) were initially found to suppress T cell activation and proliferation and evade immune surveillance. Although researchers are bridging the translational gap between scientific observations on MSC function and clinical applications, the understanding of MSC biology is still limited and there is unmet need to optimize their therapeutic effects. Herein, we will investigate the effect and mechanisms of material microenvironment, especially matrix viscoelastic properties, on MSC immunomodulation and application for SLE treatment. Hydrogels are ideal biomaterials for emulating the niche or ECM for 3D cell culture. Recent works have showed the stiffness of hydrogels regulated the TGF- secretion from human MSCs, which could modulate T cell behaviors and activities in SLE. Besides static stiffness, the viscoelastic properties of hydrogel matrix also play critical roles in regulating the behaviors of encapsulated cells. We have recently discovered that the viscoelastic properties of hydrogel induce broad transcriptomic changes of encapsulated MSCs. We will first investigate the transcriptional regulation and cytokine secretion of hMSCs encapsulated in hydrogels with different viscoelastic properties and explore the crosstalk of hydrogel-encapsulated hMSCs and regulatory T cells. We will also evaluate the capability of our hydrogels as cellular regulator for the SLE treatment in animal models. The success of this project will advance our knowledge of microenvironmental factors on human stem cell behaviors and its applications in immuno-modulation. Meanwhile, we will also evaluate our new viscoelastic biomaterials for the potential use of human stem cell-based therapy in autoimmune diseases, and this could benefit healthcare in Maryland and our society.

Building A Predictive Computational Bone Regeneration Model to Optimize the Application of Stem Cells

Critical-sized calvarial bone defects arising from trauma and cancer resection impacts hundreds of thousands of patients each and are challenging to treat. Application of stem cells during scaffold-based treatments hold significant promise for replacing the current autograft gold standard. However, current stem cell-mediated therapy is limited by our empirical understanding of the methods by which stem cells enhance healing. We hypothesize that the ability to predict bone healing patterns as a function of (i) stem cell dosing and differentiation state and (ii) the mechanical microenvironment in the defect would significantly enhance the efficacy of treatment and facilitate their clinical translation. Yet, predicting bone growth after transplantation remains very challenging due to the number of biological and biomechanical factors influencing bone deposition simultaneously and the complex interactions among these factors. Therefore, in this study, we propose to establish a computational model capable of predicting bone regeneration in a rodent segmental mandibular defect in response to the delivery of human induced pluripotent stem cells (hiPSCs) in 3D-printed osteo-inductive and osteo-conductive scaffolds. We will accomplish this in two Specific Aims. In Aim 1, we will establish the computational framework for stem cell-mediated bone regeneration. We will train the model in Aim 2, using existing computed tomography (CT) data from porcine segmental defect model. We will validate the model during bone healing in rat mandibular segmental defects under two different mechanical environments based on the diets provided for the rats. This model will be applied to improve patient-specific treatment plan by optimizing biological and biomechanical factors during healing.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