Robert Abramovitch, Ph.D. – 2022 Awardee

Associate Professor of Microbiology and Molecular Genetics
Michigan State University

"Development of New MmpL3 Inhibitors to Treat Mycobacterial Infections"

Tuberculosis (TB) remains a global health crisis causing ~1.5 million deaths per year. To cure TB, treatment requires daily, multidrug therapy for 6 months. However, with the evolution of drug resistant Mycobacterium tuberculosis (Mtb), current therapies are inadequate to control multi-drug resistant TB.

MmpL3 inhibitors are attractive new agents functioning against drug susceptible and resistant Mtb. We have discovered a new class of MmpL3 inhibitors called HC2099. We optimized and found an orally bioavailable analog that is efficacious in vivo in an acute murine Mtb infection model. However, we encountered a barrier to development caused by in vivo metabolism of HC2099, leading to a short half-life. We have identified the metabolic liability presenting a clear path forward to overcome this barrier to development.

The goal of this proposal is to further optimize the HC2099 series to generate proof-of-concept in vivo efficacy data showing its utility for once daily dosing.
To fulfill these goals, we will complete:

Specific Aim 1. Define structure activity relationships and optimize activity of HC2099 series.
Specific Aim 2. Conduct integrated SAR and pharmacokinetic studies to prioritize compounds for in vivo efficacy studies.
Specific Aim 3. Demonstrate proof-of-concept in vivo efficacy of HC2099 series against Mtb infection.

Drew Adams, Ph.D. – 2022 Awardee

Associate Professor, Dept. of Genetics; Thomas F. Peterson Jr Professor of Novel Therapeutics
Case Western Reserve University

"Targeting Exportin-1 to Block T Cell Activation in Autoimmune Diseases"

My laboratory seeks to validate new targets for drug discovery, and our past work has led to high-impact publications and commercialization. Recently we identified Exportin-1 (XPO1) as the target of multiple small molecules that suppress T cell activation, an important immune process that goes awry in autoimmune diseases. XPO1 is a validated drug target, but safety considerations limit the existing XPO1-targeting drug's use to late-stage cancer patients. In contrast, molecules we have identified show a unique 'low cytotoxicity' profile that suggests XPO1 may have potential as a drug target in autoimmune diseases where cytotoxicity is undesirable. Our goal is now to demonstrate that this novel class of XPO1-targeting molecules is effective in animal models of autoimmune disease and demonstrates diminished toxicity relative to the established XPO1-targeting drug. Our Aims entail 1) using medicinal chemistry to generate an optimized in vivo-active tool molecule, and 2) evaluating both the efficacy of this tool molecule in mouse models of autoimmune disease and its cytopenic effects relative to the approved XPO1 drug. Demonstrating that our novel class of XPO1 modulators is efficacious but also shows a distinct safety profile in vivo will inspire expanded drug discovery efforts toward a new class of immunomodulatory drugs.

Nita Ahuja, M.D. – 2022 Awardee

Chair of Surgery
Yale School of Medicine

"Bedside Liquid Biopsy for Pancreatic Cancer"

Pancreas cancer (PC) is lethal and detected in advanced stages. Early-stage PC detection is the only potentially curative modality. Screening has been recommended in high-risk individuals (HRIs) with significant familial and/or genetic risk. There is no approved screening test, and surveillance relies on imaging modalities (CT scans) or invasive procedures (endoscopic ultrasound). These approaches are available only in selected centers, are expensive, invasive, and prone to interval cancer detection. DNA methylation biomarkers have recently been approved for cancers (Cologuard, SEPT9 for colon cancers) and show promise as cost-effective and safe approaches for PC surveillance

Our blood-based DNA methylation biomarkers (ADAMTS1/BNC1/LRFN5/PXDN) have diagnostic accuracy of 94% for early-stage PC detection. Optimizing the panel with pancreas-specific "tissue of origin" marks may increase diagnostic accuracy from 94% to 99%. We also propose using our DNA methylation panel to monitor HRIs in a pilot study.

An affordable bedside test with excellent diagnostic accuracy will support early detection efforts and identify early curable stage PC, thereby extending patient longevity. If this project succeeds, it can be applied in routine health care. Our proficient multidisciplinary team is led by Dr. Ahuja, a surgeon-scientist with a two-decade history of blood-based biomarkers development.

Ethan Anderson, Ph.D. – 2022 Awardee

Associate Professor of Experimental Therapeutics
University of Iowa

"Development and validation of a piezoelectric immunosensor to rapidly detect a novel biomarker of morbidity and mortality risk in sepsis patients."

Approximately one million people in the U.S. develop sepsis each year and nearly 30% from it. Recent sepsis care guidelines emphasize that a major barrier to improving patient outcomes is early identification of those at greatest risk of organ failure and mortality, so that they can be aggressively treated and closely monitored. We have exciting preliminary evidence that a mitochondrial membrane protein measured with our validated ELISA in blood samples obtained from patients within 24 hours of sepsis diagnosis, is associated with organ failure and mortality. The objective of this project is to validate this biomarker in sepsis mouse models and patients, and then develop a rapid test for this protein using a piezoelectric immunosensor. Preliminary studies suggest that this approach is feasible. Work in Aim 1 will establish the range of this protein's serum concentration in sepsis mouse models and patients to model organ failure and mortality risk. In Aim 2 we will determine the technical specifications of the immunosensor required for rapid detection and quantification of the protein in serum. This project will be the first steps toward development of a point-of-care biosensor which could rapidly identify high-risk patients early in the progression of a very deadly condition.

Fariba Behbod, PharmD, Ph.D. – 2022 Awardee

Professor
University of Kansas Medical Center

"Elucidating the Role of Enhancer Reprogramming in DCIS Malignancy"

Having no reliable biomarkers for risk stratification of ductal carcinoma in situ (DCIS) has resulted in overtreatment of indolent disease as well as undertreatment for a small subpopulation at high risk for metastasis. We hypothesize that the evolution of DCIS epithelial cells into invasive and metastatic cancer cells is associated with enhancer reprogramming and subsequent acquisition of an epithelial stem-like signature.

Aim 1. Study the epigenome and transcriptome associated with evolving breast epithelial cells during the transition of DCIS to invasive breast cancer using simultaneous scATAC/scRNA-sequencing in patient derived DCIS models that evolve into invasive and metastatic cancers versus those which remain indolent. Established algorithms (CytoTRACE) will be used for predicting cellular stemness.

Aim 2. Investigate the role of BCL9 and its binding partners (pS-STAT3 and CREBBP) in enhancer reprogramming, epithelial stemness and DCIS invasive and metastatic progression using RNA- and ChIP-sequencing.

Impact: The development of biomarkers for risk stratification in DCIS. Absence of biomarkers of epigenome reprogramming and cellular stemness may identify women at low risk who do not require further treatment avoiding overtreatment. Additionally, the presence of DCIS cells which have acquired epigenome reprogramming and cellular stemness may justify early systemic therapy including drugs that target the epigenome.

Luisa Escobar-Hoyos, Ph.D. – 2022 Awardee

Assistant Professor of Therapeutic Radiology
Yale School of Medicine

"Making cancer cells look like bacteria: Developing antigen-mimicry cancer vaccines."

Our objective is to create a vaccine that can induce the clearance of tumors in pancreatic cancer. The most lethal malignancies uniquely express the neoantigen K17, and we have found that patients with prior exposure to the pathogenic bacterium, Streptococcus pyogenes, lack K17-expressing tumors. This led us to identify bacterial antigens that mimic K17, and, using cell cultures, we showed that T cells from donors or patients with previous exposure to S. pyogenes can eliminate K17 cancer cells. Thus, we will test the hypothesis that anti-S. pyogenes immunity or anti-K17 immunity can cause clearance of K17-expressing cancer cells. We will carry out critical proof-of-concept vaccination studies in animal models of pancreatic cancer using mRNA-based expression of bacterial and K17-derived antigens, and a drug delivery system based on GMAB immunoglobulin, which we have shown can deliver mRNA efficiently to tumors in vivo, a condition that is required to elicit immunoclearance at the tumor site. If successful, we will demonstrate elimination of K17-expressing tumors in mice, paving the way for development of a vaccine to treat the large proportion of patients who have the most aggressive form of pancreatic cancer.

Amanda Garner, Ph.D. – 2022 Awardee

Assistant Professor of Medicinal Chemistry
University of Michigan

"Decoding the Druggable Transcriptome"

Following completion of the Human Genome Project, it was revealed that only ~2% of our genome encodes for proteins, and the overwhelming majority of our transcribed genome is comprised of often highly conserved non-coding RNAs. Through expanded exploration of RNA biology, a diversity of structure and function has been revealed putting RNA at the forefront of medicine, making the targeting of RNAs with small molecules attractive as a novel therapeutic strategy. Yet, despite significant efforts in the field, our ability to directly drug RNA molecules has been wrought with challenges. We believe that this lack of success is due to the use of reductionist-based approaches that are in contrast to what we know about RNA biology: that it is a complex and underexplored area of science. To match this complexity of cellular RNA structure-function, through the aims proposed, we seek to develop an integrated chemotranscriptomic pipeline to facilitate the target agnostic discovery of RNA-binding small molecules with disease-relevant cellular phenotypes allowing us to finally decode the druggable transcriptome and enable the systematic discovery of RNA-targeted small molecule therapeutics.

Justin Lathia, Ph.D. – 2022 Awardee

Associate Professor of Cellular and Molecular Medicine
Cleveland Clinic Lerner Research Institute

"Development of an anti-cancer stem cell therapy for glioblastoma by targeting the epigenetic state via WDR5"

Our long-term objective is to synthesize a brain penetrant, WDR5 inhibitor with sufficient potency to decrease cancer stem cell (CSC) proliferation, self-renewal, and viability. We plan to accomplish this goal through (1) structure informed compound design and (2) rigorous experiments designed to evaluate drug efficacy in vitro and in vivo. In Aim 1, we will develop new compounds altering motifs known to enhance brain permeation, increase potency, and decrease off-target effects based on a WDR5 tool compound. We will evaluate drug properties central to brain penetrant compounds such as plasma protein/tissue binding, target affinity, predicted clearance, solubility, and brain specific pharmacokinetics. In Aim 2, we will evaluate our top compounds for inhibition of cancer cell growth, induction of cell-death, disruption of the WRAD complex, reduction of the methylation that promotes CSC growth, and increasing survival in preclinical glioblastoma models. We also evaluate predicted mechanism of action through a variety of in vitro assessments and tumors isolated from in vivo studies. These studies will facilitate the development and translation of novel brain penetrant WRD5 inhibitors to attenuate CSCs in glioblastoma and can be expanded to other cancers in which the WRAD complex drives CSCs and tumor growth and brain metastasis.

Craig Levin, Ph.D. – 2022 Awardee

Professor of Radiology, Physics, Electrical Engineering, and Bioengineering
Stanford University

"Multiplexed Positron Emission Tomography Imaging of Promising Biomarkers for Immune Checkpoint Inhibitor Therapy"

Immunotherapy has revolutionized medical oncology. The use of immune checkpoint inhibitors (ICIs) has demonstrated revolutionary results in cancer treatment, but only a portion of patients can achieve complete disease remission. Current diagnostic imaging based on the tumor size measurement alone is not adequate to assess initial response to immunotherapy and disease evolution. Therefore, there is an urgent need for the development of new non-invasive imaging assays that can tell whether a patient's cancer responds to the immune treatment at an earlier timepoint than that allowed by the current methods. A commonly explored approach to imaging immune response uses radiolabeled antibodies targeting membrane associated markers, but this single biomarker imaging strategy has not yet demonstrated reliable prediction of treatment response. In this project, a team of investigators will bring expertise in chemistry, physics, engineering, molecular imaging, and cancer immunology to develop a novel non-invasive multiparametric imaging assay that can sensitively detect two (or even three) biomarkers in the tumor in response to the immune therapy. This new imaging assay will be evaluated in a preclinical mouse model of colorectal cancer and the results will pave the way to future clinical translation for more efficacious selection and accurate monitoring of cancer immunotherapy.

Karen Liby, Ph.D. – 2022 Awardee

Professor of Pharmacology and Toxicology
Michigan State University

"Novel RXR Agonist for Neurofibromatosis"

Neurofibromatosis type 1 (NF1) is a complex disease driven by inactivation of the NF1 gene, leading to constitutive RAS activation, uncontrolled cell growth and immune cell infiltration. Our novel compound, MSU42011, suppresses tumor growth, drives immune cells toward an anti-tumor phenotype, and decreases p-ERK expression in a RAS-driven lung cancer model, but has never been tested for NF1. We hypothesize that MSU42011 will inhibit tumor growth in NF1 mouse models and target immune cells that drive disease progression. Macrophages co-cultured with conditioned media or NF1 cells will be treated with MSU42011. Macrophage activation and polarization will be analyzed by measuring cytokine expression and cell surface markers via qPCR, ELISAs and flow cytometry. To explore complex interactions in the tumor microenvironment, effects of drug treatment on immune phenotypes will be evaluated using tri-cultures of macrophages, T cells and tumor cells. In vivo efficacy of MSU42011 will be tested in mice with established neurofibromas or malignant peripheral nerve sheath tumors. Tumor growth and toxicity will be monitored and immune cell populations in tumors quantified by flow cytometry and immunohistochemistry. The pharmacokinetic profile and selectivity of MSU42011 will be evaluated. These studies are designed to advance MSU42011 into the clinic.

Feng Lin, B.Sc., Ph.D. – 2022 Awardee

Professor of Molecular Medicine
Cleveland Clinic Lerner Research Institute

"Development of a New Drug for Patients With T Cell Lymphoma"

T-cell lymphomas (TCL) are complex clusters of aggressive blood cancers with unsatisfactory management options. In pilot studies, we identified CD6 as a novel therapeutic target for TCL. We developed an antibody-drug conjugate (ADC) specific for CD6 (CD6-ADC) using a conventional anti-CD6 monoclonal antibody (mAb) and found that this ADC is highly effective in selectively killing TCL cells both in vitro and in vivo. Nanobodies, heavy-chain only antibodies produced in camelids, are the next generation of mAb-based therapeutics. Given the many advantages that nanobodies have over the conventional mAbs, especially their excellent tumor penetrating capacity, we are developing the next generation of CD6-ADC using nanobody technology. We have immunized an alpaca and identified more than 120 anti-CD6 nanobody candidates. In this project, we will rigorously characterize these candidates to identify the best anti-CD6 nanobodies, use them to develop the next generation of CD6-ADC, and evaluate their treatment efficacy and potential adverse effects both in vitro and in vivo in a preclinical model of TCL. These high-risk studies, if successful, will generate the next generation of CD6-ADC and provide the required proof of concept to translate it into a much-needed new drug for patients with TCL.

Jonathan Marchant, Ph.D., M.A. – 2022 Awardee

Professor
Medical College of Wisconsin

"IDENTIFYING NEW DRUGS TO TREAT AN INFECTIOUS DISEASE OF POVERTY"

Diseases caused by parasitic flatworms impact millions of people. These infections cruelly target some of the most disadvantaged, with an especial burden on children worldwide. Many infections are treated with a drug called praziquantel (PZQ), the sole clinical agent available, but not an ideal treatment: for 40 years we have not understood how PZQ works, and it is not effective against every parasitic disease. Therefore, discovery of new anti-parasitics is a priority.

Our team recently identified the target of PZQ, overcoming a long-standing roadblock. The target is an ion channel, named TRPMPZQ, responsible for PZQ-dependent worm paralysis and elimination. Our discovery explains why PZQ is effective against some diseases and not others, as the TRPMPZQ binding site differs between parasites. With this knowledge in hand, we are optimally placed to discover novel drugs.

Therefore, in this 'Catalyst' proposal, we will take two independent, but parallel approaches to identifying novel chemotypes active at TRPMPZQ. We will take a rational drug engineering approach (Aim 1) and an unbiased drug screen (Aim 2). By adhering to 10 quantitative metrics that benchmark success of our assays, we aim within 18 months to discover novel agents that can be further progressed in subsequent work.

Tay Netoff, Ph.D. – 2022 Awardee

Professor of Biomedical Engineering
University of Minnesota

"Rational, Targeted Electrical Brain Circuit Intervention for Mental Illness"

We will demonstrate a new approach to treating mental illnesses, based on directly changing the underlying biology (activity in defined brain circuits) through targeted electrical stimulation. Brain stimulation is a rapidly growing approach to treating a range of illnesses, but has a fundamental limitation: it is difficult to determine the "dose" that will produce a desired circuit change. Patients' self reports are often inaccurate and change too slowly to be useful for tuning stimulation.

We have developed a new approach, based on a core insight: many mental disorders are characterized by "stuck", inflexible thought/behavior patterns. We have developed a way to measure that behavioral inflexibility in seconds, using computer-based testing. We have further developed electrical stimulation methods that reverse the deficit and make patients more flexible– and patients feel less anxious or depressed when we do. To bring these methods into trials, we need to turn them from academic lab code into a robust and tested application that can meet FDA Investigational Device Exemption requirements. In our Catalyst project, we will perform that development and testing, engage with FDA to verify that our tests meet their criteria, and formalize the design of a clinical trial in major depression.

Mark Paterno, P.T., Ph.D. – 2022 Awardee

Professor of Sports Medicine
Children's Hospital Medical Center dba Cincinnati Children's Hospital Medical

"Identifying Early Indicators of Outcome to Inform Novel Rehabilitation Paths"

The healthcare system is failing young patients who suffer musculoskeletal (MSK) injuries and seek to return to prior levels of function as many suffer subsequent injuries and few are able to return to pre-injury levels of activity. Our long-term objective is to improve outcomes for youth who sustain MSK injury by reducing the incidence of subsequent injury and enabling patients to return to and maintain pre-injury levels of activity through an innovative and personalized rehabilitative care pathway. To achieve this, the focus of this observational cohort study is to validate current discharge criteria and identify key modifiable biomarkers, present early in the rehabilitation process, as indicators of poor outcome. This important step towards our long-term goal is foundational to the development of a paradigm shift in standard care, specifically a shift towards a personalized, adaptable, algorithmic rehabilitation process. This work has the potential to create accessible and objective clinical metrics that personalize return to sport clearance and improve long-term health of millions of young patients who sustain acute MSK injury annually.

Arjun Raman, M.D., Ph.D. – 2022 Awardee

Assistant Professor
University of Chicago

"The 'statistical' design of synthetic microbiomes"

Complex microbial communities ('microbiomes') are ecosystems that critically contribute to human physiology. A major goal is to design such communities to unlock their immense potential in addressing challenges affecting human health. However, it is remarkably difficult to rationally engineer microbiomes due to their immense complexity—the number of possible interactions between the thousands of component microbes is astronomical. Thus, what strategy can be used to design functional microbiomes? We have developed a 'top-down' approach that combines the ability to print thousands of microbiomes in parallel and emerging mathematical inference methods with the goal of designing microbial communities that perform specific functions. As a first instance of using our approach, we created two designed microbial consortias (DMCs) comprised of 24 and 46 bacterial strains that suppress Klebsiella pneumoniae—a pathogen that is resistant to many antibiotics and causes severe disease. Our goals are (i) to mechanistically understand why our DMCs work, (ii) investigate whether we can deduce a 'core' set of bacterial members to reduce the size of our DMCs, and (iii) apply the general approach of creating DMCs to suppress other antibiotic resistant pathogens. Ultimately, we hope to define DMCs as novel therapeutic modalities for a range of health-relevant conditions.

Jeffrey Schneider, Ph.D. – 2022 Awardee

Assistant Professor
Rush University Medical Center

"Glycoengineering Anti-HIV antibodies to increase delivery to the brain and block viral egress from the CNS"

There is no cure for HIV despite great strides in combination anti-retroviral therapy(cART) to control HIV/AIDS. Once cART is interrupted, HIV re-emerges from sanctuary sites to reseed the body. One such site is the central nervous system (CNS). Our recent chimeric human brain mouse model demonstrates that HIV egresses from the brain to peripheral organs following cART cessation. Therefore, it is critical to develop therapies targeted to the brain to block this egress if a viable cure strategy is going to be achieved. We propose to target anti-HIV neutralizing antibodies to the brain to block this egress. CNS antibodies have a distinct glycosylation profile to support their retention in the CNS in comparison to bulk IgG. We propose a novel methodology to target antibodies to the brain, involving engineering antibodies to be more "CNS like" through glycosylation and subclass manipulation (Aim 1). We will assess these "CNS like" anti-HIV antibodies for CNS penetration and potential to block HIV viral egress following cART cessation in our chimeric human brain mouse model (Aim 2). Collectively, these studies will explore the validity of engineering CNS-like antibodies to increase penetration to the brain and ability to block viral egress following cART cessation.

Jennifer Woyach, M.D. – 2022 Awardee

Assistant Professor of Internal Medicine
The Ohio State University Wexner Medical Center

"Optimizing BTK Inhibitor Therapy in CLL"

Chronic lymphocytic leukemia (CLL) is the most prevalent adult leukemia. Covalent Bruton's Tyrosine Kinase inhibitors (BTKi) ibrutinib and acalabrutinib have been paradigm-shifting in CLL, however, patients do relapse. Our previous work has identified that the vast majority of patients with ibrutinib-refractory CLL develop mutations in BTK or its immediate downstream target PLCG2. New approaches to therapy in patients who relapse on these agents are of critical importance.

In this proposal, we evaluate a novel selective inhibitor of BTK which can bind the protein both covalently and noncovalently. This allows for differential binding with wild type (covalent) vs mutant (noncovalent) BTK. A dual inhibitor may represent a strategy which prevents resistance, thereby significantly extending remissions. In this project, we will perform preclinical work on this BTKi, NW775 to evaluate efficacy in CLL cells as well as mouse models. We will also use CRISPR screens to evaluate potential combination partners, which we will extensively validate.

At completion, we will have preclinically evaluated a promising new agent for CLL and performed validation studies for potential combinations. We expect that this project will lead to clinical studies with the potential of this mechanism to change the standard approach for patients with CLL.

Xiaoyu Zhang, Ph.D. – 2022 Awardee

Assistant Professor of Chemistry
Northwestern University

"Discovering and developing neoantigen inducers as new and effective cancer immunotherapeutics"

Cancer immunotherapy relies on cytotoxic T lymphocytes (CTLs) to recognize proteins or mutation-derived peptides displayed on cancer cell surfaces in order to eliminate cancer cells. Mutation-derived peptides, or neoantigens, that display on major histocompatibility complex I (MHC-I) on cancer cell surfaces are particularly relevant to effective cancer immunotherapy. Although exome sequencing and in silico algorithms are used to predict MHC-I-associated neoantigens, only a handful of neoantigens have been identified experimentally. Given that protein turnover rate is correlated with MHC-I antigen presentation, I hypothesize that the neoantigens presented under physiological conditions represent only a small fraction of all potential neoantigens, most of which may exist in an unprocessed state. Inducing the presentation of those otherwise 'invisible' or insufficiently presented neoantigens by small molecules, or neoantigen inducers, has the potential to convert immunologically silent cancer into an immunosensitive state. In Aim 1, we will demonstrate the therapeutic potential of inducing the presentation of a CDK4_R24C neoantigen to elicit CTL responses. In Aim 2, we aim to discover a new knowledge of cancer on a genome-wide scale – the presentability of cancer neoantigens. In Aim 3, We will develop neoantigen inducers to induce the presentation of 'invisible' cancer neoantigens.

Franziska Bleichert, Ph.D. - 2021 Awardee

Molecular Biophysics and Biochemistry
Yale School of Medicine

"Targeting DNA Replication Initiation for Cancer Therapy"

Harnessing DNA replication stress to induce cell death has been a successful approach in cancer therapy. One attractive but underexplored strategy to selectively enhance replication stress in cancer cells is to inhibit the licensing of replication origins, i.e., the loading of replicative helicases onto DNA. We hypothesize that origin licensing inhibitors would serve as powerful cancer drugs, either in stand-alone or combination therapy, as they sensitize cancer cells to replication stress through a mechanism distinct from established chemotherapeutics, eventually causing cell death. Here, we propose to use high-throughput biochemical screening to identify small-molecule origin licensing inhibitors. Positive hits will be tested in biochemical and cellular assays to establish dose-responses and identify compounds that are most effective at inhibiting DNA replication initiation. These studies will be combined with X-ray crystallography and cryo-electron microscopy to understand the structural mechanisms by which identified compounds selectively inhibit replicative helicase loading. The outcomes of these efforts will pave the way for the development of the first origin licensing inhibitors as anticancer therapeutics and, in the long term, have the potential to yield a novel class of cancer drugs.

Vladimir Bogdanov, Ph.D. - 2021 Awardee

Internal Medicine
University of Cincinnati

"Preclinical Development of a First-In-Class Humanized Antibody Targeting Alternatively Spliced Tissue Factor"

This proposal is focused on pancreatic ductal adenocarcinoma (PDAC) – a highly lethal cancer with poor treatment options. We study alternatively spliced Tissue Factor (asTF), a protein whose expression is high in tumor tissue and blood of patients with PDAC. asTF activates integrins, which promotes proliferation, migration, and metastasis of cancer cells. We developed 1) a humanized, inhibitory monoclonal anti-asTF antibody termed hRabMab1 with a favorable pharmacokinetic profile and tumor-suppressing properties in vivo; and 2) asTF-specific ELISA. The Specific Aims we propose are: 1. Test the hypothesis that hRabMab1, a first-in-class biologic, may help treat PDAC. Our preliminary data shows that hRabMab1 suppresses the growth of pre-formed, orthotopically grown PDAC tumors when administered intravenously as a single agent. We will assess hRabMab1's ability to suppress the growth of several PDAC cell lines and patient-derived xenografts in combination with standard-of-care regimens; and hRabMab1's ability to impede metastases. 2. Test the hypothesis that measuring circulating asTF may help evaluate response to therapy. We found that asTF levels in plasma drop post-treatment; this extends our earlier findings that plasma asTF levels are high in PDAC patients compared to healthy subjects, and positively correlate with non-resectability. In this aim, we will determine if asTF levels rise with recurrence post-surgery. In partnership with investigators conducting a Phase II trial examining an adaptive approach to neoadjuvant therapy, we will determine if plasma asTF rises in those subjects who recur, and whether said rise precedes the emergence of conventional signs of recurrence. Our studies will help us validate asTF as a new target and a biomarker in PDAC, bringing this technology closer to the clinic.

Karlene Cimprich, Ph.D. - 2021 Awardee

Chemical and Systems Biology
Stanford University

"A Novel Biomarker for the Detection of Genomically Unstable Cancers"

Early cancer detection is a critical factor in patient survival. However, sensitive and specific diagnostic tools are limited. Many cancers exhibit genome instability, a hallmark of cancer. Among the causes of genome instability are mutations in DNA repair genes, including the hereditary breast cancer susceptibility genes, BRCA1 and BRCA2. Mutations in BRCA1/2 lead to elevated levels of R-loops, nucleic acid structures that form when nascent RNA hybridizes to the template DNA. Although R-loops have regulatory roles in the cell, their deregulation causes DNA damage and genome instability. We have identified RNA-DNA hybrids as a product of R-loop processing, which accumulate in the cytoplasm of cells lacking BRCA1/2. We hypothesize that these hybrids could be a circulating biomarker suitable for liquid biopsy and thus, early cancer detection. Our approach has three aims with the overarching goal of evaluating hybrids in the serum of BRCA1/2-deficient high-risk individuals. We plan to detect and sequence extracellular hybrids from BRCA1/2-deficient cell lines (aim 1) and serum samples (aim 2), and test a quantitative qPCR-based assay for serum hybrid analysis (aim 3). A highly sensitive, non-invasive, and inexpensive method for early cancer detection in individuals of elevated genetic risk would transform current management and surveillance schemes.

Swetha Gowrishankar, Ph.D. - 2021 Awardee

Anatomy and Cell Biology
University of Illinois College of Medicine at Chicago

"Evaluation of the In Vivo Efficacy of Novel Autophagy Activators for Amelioration of Alzheimer's Disease Pathology in the 5xFAD Mouse Model"

Alzheimer's disease (AD) is a progressive neurodegenerative disease that affects over 5 million people in the United States and is characterized by amyloid plaques, tau tangles, loss of synapses and ultimately, neuronal death. FDA-approved drugs for AD treat the symptoms of the disease but do not improve the underlying cell damage that leads to disease progression, highlighting the need for novel neuroprotective therapeutic options. Dysfunction in the autophagic and lysosomal pathways, which are critical for protein and organelle homeostasis in neurons, is associated with different stages of the disease, and thus modulation of autophagy has emerged as a new strategy for developing AD-targeted drugs. To this end, we have identified novel, small-molecule autophagy activators in a high-throughput screen that rescued key pathological features in human neuronal culture models of the disease. We hypothesize that these novel activators will restore optimal autophagy, clear protein aggregates, prevent disease progression, and ameliorate AD symptoms in the 5xFAD mouse model. We will test this hypothesis through determination of the drug metabolism and pharmacokinetic parameters of these two autophagy activators to identify a lead compound and evaluation of the in vivo efficacy of this compound in 5xFAD mice.

Nikki Johnston, Ph.D. - 2021 Awardee

Otolaryngology
Medical College of Wisconsin

"Aerosolized HIV Inhibitors for the Treatment of Laryngopharyngeal Reflux (LPR)"

More than 20% of the US population suffer from LPR with no effective medical therapy. We have identified two, well tolerated, drugs which hold promise for the treatment of LPR. While the FDA has approved a proof-of-concept clinical trial using the high dose oral formulation (Transformational Application), the long-term objective is to develop a metered inhaler for local delivery. Local administration of a low dose to the laryngopharynx can achieve the same therapeutically effective drug level as the high dose, oral formulation. It follows that the use of a lower dose will be associated with fewer and/or less severe side effects. We therefore propose to:

Specific Aim 1: Develop a water-based formulation of fosamprenavir and darunavir that is amenable for inhalation administration in humans, and

Specific Aim 2: Compare the dose response of oral and inhalation administration of fosamprenavir and darunavir for pepsin-mediated laryngeal epithelial damage in our established LPR in vivo mouse model.

Aerosol formulation offers the potential for generating Intellectual Property and thereby the means to connect with an industrial partner to carry out the detailed drug product development needed for an effective treatment for the millions of people in the US that suffer from LPR.

Mark Kaplan, Ph.D. - 2021 Awardee

Microbiology and Immunology
Indiana University

"Inhibitors Targeting Peanut-Specific Allergic Reactions"

Allergies are a result of allergen proteins cross-linking allergen-specific IgE (sIgE) on the surface of mast cells and basophils. The diversity and complexity of allergen epitopes, and high-affinity of the sIgE–allergen interaction have impaired the development of allergen-specific inhibitors of allergic responses. This study presents the design of food allergen-specific sIgE inhibitors termed covalent heterobivalent inhibitors (cHBIs) that selectively form covalent bonds to only sIgEs, thereby permanently inhibiting them. We have developed peanut-specific inhibitors that have demonstrated efficacy and specificity in blocking mast cell or basophil degranulation and anaphylaxis using in vitro assays, ex vivo samples from peanut-allergic patients, and humanized mouse models. The next critical step in moving these inhibitors to testing in humans is more formal toxicology analysis and establishing efficacy of the inhibitors for cutaneous anaphylaxis as a prelude to testing inhibition of skin prick testing. Thus, the specific aims of this application are to 1. Validate the inhibitory potential of cHBIs for cutaneous reactivity in humanized mouse models; and 2. Assess the cHBI toxicity and pharmacokinetics in non-human primate models. Together, these Aims will provide the critical data required to move forward with IND approval and preparation for phase I clinical trials.

Jonathan Kurtis, M.D., Ph.D. - 2021 Awardee

Pathology and Laboratory Medicine
Brown University

"Apoptosis-inducing Anti-Malaria Drugs Targeting PFGARP"

The overall aim of this application is to discover novel therapeutics for Plasmodium falciparum malaria. P. falciparum is a leading cause of morbidity and mortality in developing countries, infecting hundreds of millions of individuals and killing over 300,000 children each year 1. The spread of parasites resistant to the artemisinin family of compounds 2 threatens recent progress achieved by antimalarial campaigns and underscores the urgent need to identify new anti-malarial drugs.

In previous work, we discovered PfGARP, a previously unrecognized vaccine candidate found only in P. falciparum. Antibodies to the highly invariant carboxyl-terminal of PfGARP (PfGARP-A, aa 411-673) inhibit parasite growth in vitro by 99% compared to controls ($P < 0.001$) by killing trophozoite stage parasites. In confocal and transmission electron microscopy studies, PfGARP localized to the exofacial surface of the RBC membrane in trophozoite and early schizont infected RBCs, but not to other parasite stages or uninfected RBCs. Importantly, the growth inhibition assays are performed in the absence of any immune effector molecules (complement) or cells- thus the remarkable anti-parasite effect of anti-PfGARP results from antibody binding alone. This is further supported by the killing effect of recombinant mAb (KD 2.9 nM, (95% CI = 1.3 – 5.9 nM)) and its rec monovalent Fab that target aa 443-459 (VKNVIEDEDKDGVEIIN) of PfGARP.

The Scientific Premise of this application is that PfGARP is a high-value druggable target based on: 1) its surface expression on infected RBCs, 2) the absence of any significant amino acid homology with human host proteins, and 3) the ability of antibody binding to PfGARP to kill essentially all parasites within 12-24 hours.

In the current proposal, we will: 1) conduct a targeted, high-throughput drug screen to discover drugs which mimic the lethal activity of antibodies recognizing PfGARP, 2) optimize and down select these candidates, and 3) validate these new drug candidates in a humanized mouse model of P. falciparum.

Kelvin Lee, M.D. - 2021 Awardee

Medicine
Indiana University

"Targeting PIM2 and its Regulation of the c-Myc Oncogene in Multiple Myeloma and Other Cancers"

Multiple myeloma (MM) is a malignancy of plasma cells that is the second most common hematologic malignancy (20% of all cases), and remains incurable for almost all patients. The primary cause of treatment failure is upregulation of pro-survival resistance mechanisms, and identifying these mechanisms remains key for new therapeutic development. We have recently reported that the serine-threonine kinase PIM2 has a major pro-survival role in MM, and that inhibition of PIM2 with a first-in-class PIM2-selective non-ATP-competitive kinase inhibitor JP11646 (JP) caused significant MM cell death in vitro and in vivo in preclinical MM models. Unexpectedly, JP was much more effective than the ATP-competitive PIM kinase inhibitors due to JP's unique ability to downregulate of PIM2 gene and protein expression. This has led to our findings that PIM2 has critical but previously unrecognized kinase-independent (KI) functions, which would be a change in paradigm. One such PIM2 KI function that is inhibited by JP is induction of the expression (mRNA and protein) of the c-Myc oncogene, which is dysregulated in ~50% of all human cancers, including MM. Our additional findings suggest a completely novel mechanism where JP disrupts PIM2 interaction with a partner protein (possibly c-Myc itself) that results in downregulation of c-Myc expression and loss of c-Myc driven PIM2 gene expression, collapsing a self-reinforcing loop that sustains both PIM2 and c-Myc expression. Targeting c-Myc expression through PIM2 inhibition represents an entirely unexplored therapeutic approach. The overall goal of this proposal is to develop JP11646 for the treatment of refractory/relapsed MM, and potentially for other cancers. The Specific Aims of the proposal are:

Aim 1. Define the kinase-independent mechanisms by which PIM2 supports MM survival, and how JP11646 inhibits these.

Aim 2. Conduct preclinical and IND enabling studies for a phase I clinical trial of JP11646 in refractory/relapsed multiple myeloma.

Tristan Maerz, Ph.D. - 2021 Awardee

Orthopaedic Surgery
University of Michigan

"Targeting Pathological Rspo2-mediated Wnt/β-Catenin Signaling as a Novel Treatment for Osteoarthritis"

Recent evidence has associated overactivation of canonical Wnt signaling through β-catenin(cWnt) with multiple pathological processes in osteoarthritis (OA). The cWnt agonist, R-spondin 2 (Rspo2), is a soluble ligand that our preliminary data demonstrates is sufficient to induce joint degeneration in vivo, making it a promising therapeutic target. The antidepressant drug Mianserin was recently identified as an Rspo2 inhibitor by blocking Lgr5 binding.
Our overall objective is to elucidate the role of Rspo2 in OA pathogenesis and test the efficacy of sustained intra-articular Rspo2 inhibition using Mianserin-loaded polymeric microspheres. We will:
Aim 1: Demonstrate the joint-protective effect of Rspo2 ablation. We hypothesize that joint injury-induced Rspo2 potentiates OA by activating cWnt signaling in multiple intra-articular cell types, promoting pathology. Using a global, inducible Rosa26-CreERT2; Rspo2flox mouse, we will globally ablate Rspo2 at the time of injury (Rspo2cKO) and comprehensively evaluate OA severity.
Aim 2: Test the efficacy of sustained intra-articular Rspo2 inhibitor therapy as a novel OA treatment. We will formulate PLGA microspheres to deliver the Rspo2 inhibitor Mianserin to the joint, assessing dose-dependent pharmacokinetics and pharmacodynamics of Mianserin release in vivo. Then, we will test the OA disease-modifying effects of Mianserin-loaded PLGA-MS in mice, benchmarking against repeated direct Mianserin injections.

Reshmi Parameswaran, Ph.D. - 2021 Awardee

Medicine
Case Western Reserve University

"BAFF CAR-NK Cells: An Efficacious and Safe Immunotherapy for B Cell Cancers"

CAR-T immunotherapies have produced remarkable clinical responses, but several challenges remain including disease relapse due to antigen escape/decreased CAR-T persistence, fatal side effects (cytokine release syndrome, neurotoxicity), the cost and time to produce CAR-T cells. We have developed novel B cell activating factor (BAFF) ligand-based BAFF CAR with multi-receptor specificity to target BAFF receptors (BCMA, TACI, BAFF-R) expressed by B cell cancers. We deliver BAFF-CAR to human NK cells using a non-viral TcBuster transposon platform, enabling safer, simpler, and more cost-effective CAR-NK production. Our preliminary data demonstrates in vitro and in vivo cytotoxicity of BAFF CAR-T cells against human malignant B cell lines. The overall objective of this proposal is to develop, optimize and evaluate BAFF-CAR NK cell therapy for the treatment of B cell malignancies. Aim 1: Achieve stable, non-viral integration of various BAFF CAR constructs in human NK cells. Aim 2: In vitro functional validation and characterization of BAFF CAR-NK. Aim 3: In vivo evaluation of dosing and efficacy of BAFF-CAR-NK cells using mantle cell lymphoma (MCL) xenograft NSG mouse model. Persistence of engineered NK cells will be evaluated in parallel with efficacy, as well as examination of preliminary safety endpoints and disease relapse.

Richard Pomerantz, Ph.D. - 2021 Awardee

Biochemistry
Jefferson Medical College of Thomas Jefferson University

"PROTACs for Targeting BRCA-Deficient Cancers"

DNA polymerase (Polq) is a unique polymerase-helicase DNA repair protein that has recently been validated as a synthetic lethal drug target in cancer cells harboring mutations in BRCA1 or BRCA2 (BRCA) tumor suppressor proteins. Polq promotes DNA repair via the microhomology-mediated end-joining (MMEJ) pathway (also referred to as alternative end-joining). Polq is essential for BRCA-deficient cells, but is dispensable for normal cells and Polq null mice show no phenotypes. Thus, Polq is synthetic lethal with BRCA1/2. Consistent with this, recently published Polq inhibitors (Polqi) selectively kill BRCA-deficient cells, while showing no effects in BRCA-proficient cells. Yet, whether these early stage Polqi will lead to clinically effective drugs is unknown.

Towards the goal of developing a clinically effective Polqi, our laboratory and collaborators have taken a different approach for targeting Polq: to develop a Proteolysis Targeted Chimera (PROTAC) Polq degrader to completely abolish all Polq activities in cancer cells. Because Polq null mice are healthy with no phenotypes, simultaneous inactivation of Polq polymerase (Polq-pol) and helicase (Polq-hel) enzymatic domains is expected to be a safe and a highly effective therapeutic method. Based on this rationale, we plan to develop a Polq PROTAC targeting the Polq-pol domain as a unique therapeutic for treating BRCA-mutant cancers.

Ying Sun, Ph.D. - 2021 Awardee

Human Genetics
Children's Hospital Medical Center dba Cincinnati Children's Hospital Medical

"Novel Brain-penetrable and Orally-available Small Molecule Pharmacological Chaperones to Treat Gaucher Disease and Parkinson's Disease"

Our drug discovery effort seeks to develop brain-penetrable and orally available small molecule pharmacological chaperones to restore defective acid β-glucosidase (GCase) as a new disease-modifying treatment for neuronopathic Gaucher disease and Parkinson's disease. We have identified novel small molecules that are non-inhibitory chaperones capable of improving multiple important endpoints: a) GCase activity, b) GCase substrate reduction and c) penetration through the blood brain barrier. We are presently at the critical optimization stage and will select lead compounds to advance as drug candidates for preclinical studies. In this proposal, we will optimize our lead compounds to improve activity and bioavailability. Newly designed compounds will be screened to increase GCase activity using cell-based multi-well screening platform. The chaperone property of selected compounds will be evaluated in fibroblasts and neurons carrying common GBA1 mutations found in Gaucher disease and Parkinson's disease patients. Selected compounds will be assessed for CNS drug properties, biodistribution, and preclinical evaluation in our diseased mouse models. Upon completion of the proposed milestones, we will have identified promising drug candidates of non-inhibitory chaperones that will be implemented for IND-enabling studies and advance a drug candidate into clinical trials to test the compound's benefit for patients with Gaucher and Parkinson's diseases.

Sherry Thornton, Ph.D. - 2021 Awardee

Rheumatology
Children's Hospital Medical Center dba Cincinnati Children's Hospital Medical

"Biomarkers for the Advancement of Targeted Therapies for Children with Juvenile Arthritis"

Juvenile idiopathic arthritis (JIA) is the most common chronic inflammatory rheumatic disease of childhood, affecting 1:1,000 children worldwide. This prevalence is about the same as juvenile diabetes. Currently, physicians lack laboratory tests to support them in the initial choice and sequence of medications prescribed to a child with JIA. This deficiency increases not only health care cost but also adversely impacts the prognosis of children with JIA. What is needed is a JIA-Rx Biomarker Panel, i.e., a comprehensive, yet concise group of biomarkers that helps anticipate the response of a child with JIA to a given immunosuppressive treatment. Over the next 1 year, we will (1) delineate serum biomarkers that individually, or in combination, forecast response of JIA therapy; (2) develop a mathematical algorithm to provide a numeric estimate for the probability of experiencing response to therapy, and (3) test the performance of these candidate biomarkers and algorithm in children with JIA who are newly started on TNF-blocking medications. We will use serum samples, gene expression RNAseq data, and clinical information from 187 children with JIA who participated in a 44-week, double-blinded international clinical trial (NCT02592434) of tofacitinib. Data and samples have been donated by Pfizer to CCHMC Rheumatology. We will enroll 60 children with JIA who are newly prescribed TNF inhibiting medication and study them prospectively. We expect to delineate an actionable JIA-Rx Biomarker Panel and interpretation rules to immediately support treatment decisions for children with JIA.

Wei Xu, Ph.D. - 2021 Awardee

Oncology
University of Wisconsin-Madison

"CARM1 Inhibition Enhances Immunotherapy Response in Triple-Negative Breast Cancer"

Triple-negative breast cancer (TNBC) is the most aggressive breast cancer. Treatment options for this subtype are limited, as it is lacks expression of ER, PR, and HER2. Immune checkpoint inhibitors (ICI) have shown promising effects, but response rates are low, underscoring the need for combination therapy. However, there are many critical roadblocks for developing combination regiments, including identifying which combinations will be effective, and identifying biomarkers to select patients that are most likely to benefit from treatment.

This project is based on our groundbreaking discovery of the role of CARM1-mediated BAF155 methylation in modulating the efficacy of immunotherapy. We found that pharmacological inhibition of CARM1 boosted host immune responses by enhancing cytotoxic T cell activity and tumor infiltration, leading to tumor regression. Moreover, a combination of CARM1 inhibitor with ICI synergistically inhibited tumor growth. CARM1 is overexpressed in TNBC and is a druggable enzyme with several pharmaceutical inhibitors. Our studies suggest that inhibiting CARM1 methylation of BAF155 primes tumors to become immunologically 'hot', and sensitizes BC to ICIs.

Furthermore, methylated BAF155 (me-BAF155) is readily detected in circulating tumor cells of metastatic BC patients, making me-BAF155 a potential biomarker for selecting patients for the combined CARM1i and immunotherapy.

Hongwei Yu, M.D. - 2021 Awardee

Anesthesiology
Medical College of Wisconsin

"A Novel Analgesic Approach for Chronic Pain: Small Peptide Inhibition of Nav1.7 in Anatomically Targeted Sensory Neurons"

The peripheral nervous system is an appealing site for devising new analgesic treatments since primary sensory neurons (PSN) play a central role in the development and maintenance of chronic pain, and can be safely medicated in a highly targeted fashion. The Nav1.7 sodium channel plays fundamental roles in mediating pain, so development of PSN-targeted NaV1.7 inhibitors could provide highly effective analgesia. However, success in creating small molecule NaV1.7 inhibitors remains elusive. For a new approach, we propose a strategy in which small peptides derived from Nav1.7 ion channels are developed as interfering peptide aptamers (iPAs) for highly effective and selective blockade of pain signaling. These will be delivered selectively to anatomically targeted sensory neurons by recombinant adeno-associated viral (AAV) vectors to achieve analgesia without side effects or addiction potential. Candidate peptides will be identified by combined computational and experimental strategies and tested for selective and effective NaV1.7 channel blockade. Analgesic efficacy will be tested in established animal pain models representing neuropathic and arthritis pain, following AAV-NaV1.7iPA injection into peripheral segmental nerves. Preliminary observations with a prototypic NaV1.7iPA candidate predict highly safe and effective analgesia. Achievement of these goals will provide candidate agents suitable for development in the subsequent transformational phase.

Jennifer Yu, M.D., Ph.D. - 2021 Awardee

Cancer Biology, Radiation Oncology
Cleveland Clinic Lerner Research Institute

"Targeting Hypoxic Cancer Stem Cells to Improve Glioblastoma Treatment"

Glioblastoma is an incurable primary brain tumor that is characterized by regions of hypoxia and marked resistance to radiation. A major roadblock in the treatment of glioblastoma is the inability to kill the cells that give rise to disease recurrence, the glioma stem-like cells (GSCs). GSCs are highly resistant to standard cytotoxic treatments, have a high capacity for self-renewal, and are frequently located in hypoxic areas, which renders them even more difficult to kill with radiation and chemotherapy. Here, we propose to develop a new therapeutic strategy to kill these GSCs, with the long-term goal of extending glioblastoma patient survival. We have found that the long non-coding RNA (lncRNA) Lucat1 is an important regulator of GSC response to hypoxia. Lucat1 is frequently overexpressed in glioblastoma and is associated with poor prognosis. Our data support that Lucat1 is induced by hypoxia and forms a positive regulatory loop to promote HIF1alpha-mediated signaling. Lucat1 helps to maintain GSCs in hypoxia and promote tumor growth. By targeting Lucat1, GSCs fail to adapt to hypoxia, resulting in cell death. Silencing Lucat1 extends animal survival in mouse models of glioblastoma. Importantly, our data suggest that targeting Lucat1 has fewer side effects than direct inhibition of hypoxia regulator HIF1alpha. In this study, we propose to develop and optimize novel anti-sense oligonucleotide (ASO) therapeutics that inhibit Lucat1-HIF1alpha signaling and test the efficacy of these ASOs in mouse models of glioblastoma. Our findings will provide a new therapeutic approach for targeting GSCs in hypoxia to improve glioblastoma control.

Hermine Brunner, M.D., M.B.A., M.Sc. - 2020 Awardee

Pediatrics, Division of Rheumatology
Children's Hospital Medical Center dba Cincinnati Children's Hospital Medical

"Lupus Nephritis Biomarkers for the Advancement of Therapies for Lupus Nephritis"

Lupus, incurable autoimmune disease affects round 1.5 million persons living in the United States. Among the main risk factors for poor disease outcome is the presence of lupus nephritis (LN). The applicants have discovered and initially validated a panel of LN-biomarkers [NGAL,MCP-1,Kim-1,ceruloplasmin,adiponectin,hemopexin]. Considering the urine levels of these LN-biomarker we delineated an algorithm, the Renal Activity Lupus Index (RAIL), where higher scores reflect more kidney inflammation as seen on kidney biopsy. Changes in RAIL scores allow us to predict LN flares and LN response to therapy at least 3 months earlier than when using current clinical tools. Further, our pilot studies suggest that the RAIL score can be used to refine the dosing of current LN therapies; we observed that high-dose intravenous steroids lead to a dramatic reduction of the RAIL score that does not occur when only daily oral steroids are used for LN therapy.

Critical Scientific Roadblock to be addressed: To enable the application of these highly promising LN-biomarkers and the RAIL algorithm in clinical care and for research, following work needs to be conducted: (1) development of a high-quality multiplex assay (RAIL-MPLA) to rapidly measure all of the LN-biomarkers concurrently; (2) additional validation of the RAIL in an independent cohort of adults and children.

Approach: Working together with Ethos Research & Development and pharmaceutical companies, we will: (1) develop a RAIL-MPLA; (2) define changes of RAIL scores that reflect clinically relevant improvement of LN and reference values for absent, controlled and active LN across age-ranges; and (3) test the hypothesis that high-dose intravenous steroids result in a significantly higher reduction of the RAIL score compared to standard doses of oral steroids alone.

Expected Results/Deliverables:

- Develop a novel clinically actionable multiplex assay that accurately quantifies the biomarkers used in the RAIL within 4 hours of sample receipt.
- Increase the usability of RAIL by refining the interpretation of RAIL scores
- Immediately advance the treatment of LN by providing the scientific underpinning for the preferred use of intravenous rather than oral steroids alone.

Christina Curtis, Ph.D. - 2020 Awardee

Medicine
Stanford University

"Identifying and Targeting the Drivers of Metastasis and Resistance in High-Risk of Relapse Estrogen-Receptor Positive Breast Cancer"

Women with early-stage estrogen receptor-positive (ER+) breast cancer face a persistent risk of distant recurrence and breast cancer death up to 20 years post diagnosis. We have recently reported the 20 year follow-up of nearly 2000 early-stage breast cancer patients with accompanying tumor molecular profiling. This work identified 11 integrative clusters (ICs) of breast cancer, defined based on integration of genomic copy number alterations and transcriptional profiles, with markedly variable risk of recurrence over time. Four of these ICs had exceedingly high risk of recurrence – over 40 percent 20 years post diagnosis – and together comprised nearly one-quarter of ER+/HER2- patients. Importantly, each of these ICs harbored a characteristic and putatively targetable gene amplifications. We hypothesize that therapy targeted at the driver genes present in each of the high-risk ER+/HER2- IC groups will reduce tumor cell proliferation, providing a path forward to design targeted therapeutic strategies aimed at preventing recurrence and reducing mortality associated with lethal metastatic breast cancer. Having identified and validated biomarkers of breast cancer relapse, several of which themselves represent potential new therapeutic targets, we propose to functionally characterize the drivers of the high-risk Integrative Clusters (ICs), to uncover their oncogenic dependencies and to elucidate mechanisms of resistance to endocrine and targeted therapies. Accordingly, we will leverage advances in CRISPR technology to perform pooled multiplexed CRISPR/dCas9-interference (CRISPRi) screens in representative 2D cell lines and 3D spheroid cultures to delineate 3D-specific vulnerabilities (Aim 1). In tandem we propose reciprocal experiments in which we oncogene engineer non-malignant mammary cells via CRISPR/dCas9-activation (CRISPRa) pooled screens to define the genes that promote malignant growth (Aim 2). Further, we will exploit a powerful cellular barcoding techniques to trace clonal dynamics during treatment and delineate the functional determinants of response to targeted and endocrine therapies in representative patient-derived organoid models (Aim 3). These complementary approaches will systematically define novel therapeutic targets and mediators of drug sensitivity in aggressive ER+ breast cancer, thus informing subsequent clinical trials.

Richard Harvey, M.D. - 2020 Awardee

Physical Medicine and Rehabilitation and Physical therapy and Human Movement Sciences
Rehabilitation Institute of Chicago

"HUMMINGBIRD: Advancing Technology for Motor Recovery in Hand and Fingers for Stroke and Spinal Cord Injury"

The purpose of this project is to develop a usable bedside hand therapy device that that takes advantage of early neuroplasticity following stroke and cervical spinal cord injury (SCI) to retrain functionally relevant movement in individual fingers of the neurologically impaired hand. It is well established in neuroscience that neural recovery and neuroplasticity at the level of cortex in animals and humans is dependent on active motor practice. In this Catalyst project we will determine and assure that the second-generation device we have develop, which has not yet been used in humans, is usable in the clinical setting and that patients with stroke and cervical SCI find it a meaningful tool for relearning hand movement. We will also assess whether use of the device daily for 2 weeks results in improved functional hand use, improve finger strength and improved ability to control individual finger movements. Although these abilities are foundational to functional manipulation of objects with the hand, there is presently no specific therapeutic interventions, nor time available in conventional early (acute) rehabilitation to address these goals. Thus, early intensive hand recovery is an obvious but unmet need in neurorehabilitation. Our goals for this one-year project are determine if the device:

1. can facilitate improve hand function.
2. can improve finger strength and individuation.
3. is usable in a clinical setting and serves as a meaningful therapy tool to patients with stroke and cervical SCI.

If successful, this project will lead to a refined therapy protocol in preparation for a larger clinical trial to establish efficacy of this training approach for both stroke and cervical spinal cord injury. We chose to study both patient populations as we believe this tool can have benefit for both groups and to study both provides generalizability to a wider group of patients needing rehabilitation. If we achieve our long-term goal, this device will evaluated in a large multi-center pivotal trial in preparation for transition to the clinical marketplace as new rehabilitation technology that has proven scientific evidence supporting its efficacy in early hand rehabilitation.

Hannelore Heemers, Ph.D. - 2020 Awardee

Cancer Biology
The Cleveland Clinic

"Developing Citron Kinase Inhibition As A Mechanistically Novel Approach To Overcome Cancer Treatment Resistance"

Treatment resistance causes nearly all cancer deaths. Novel treatments that bypass this resistance and inhibit the biology that drives cancer progression are needed to improve patient survival. Using prostate cancer (CaP) as model, we isolated citron kinase (CIT) as a target for such a therapy. CIT is a previously unrecognized novel druggable target that is functionally distinct from current targets yet is a critical determinant of cancer cell proliferation and induces cancer growth and treatment resistance. CIT silencing inhibited growth of cell lines and xenografts representing different stages of CaP progression and diverse forms of treatment resistance but not of benign epithelial prostate cells. CIT's stimulation of cancer progression relied entirely on its kinase activity, isolating CIT's kinase activity as a completely new target. A specific CIT inhibitor has not yet been developed. By analyzing the target spectrum of kinase inhibitors and CIT kinase assays, we identified 3 multikinase inhibitors that inhibit CIT, decrease CaP cell proliferation and inhibit CIT substrate phosphorylation. Because these drugs are not selective for CIT and thus not likely to succeed as cancer therapies, we developed a lead compound that inhibits CIT at low doses, has improved CIT selectivity and promising DMPK. Our objective is to develop our lead compound into first-in-class selective CIT inhibitor as a novel cancer treatment. We hypothesize that novel CIT inhibitors will overcome acquired resistance to conventional cancer therapies, which will be reflected in the phosphorylation status of CIT substrates. We will test 2 Specific Aims:
1. To determine the therapeutic efficacy of novel CIT inhibitors during cancer progression using multiple chemistry and crystallography approaches and kinome screens to improve CIT specificity and DMPK and verifying growth inhibition of clinical relevant treatment-resistant cancer models.
2. To determine the substrates by which CIT kinase action conveys aggressive cancer behavior using integrated state-of-the-art biotin-based proximity ligation assays, kinase substrate arrays, and mass spectrometry approaches.
We expect these studies to have a significant positive impact because they will provide CIT inhibition as an entirely new and functionally diverse cancer treatment and the phosphorylation status of CIT substrates as a treatment-specific biomarker of response.

David Hildeman, Ph.D. - 2020 Awardee

Pediatrics, Division of Immunobiology
Children's Hospital Medical Center dba Cincinnati Children's Hospital Medical

"IL-10 Blockade to Boost Influenza Vaccination in Non-human Primates"

Declining immune function is well described in the elderly, and leads to increased risk and severity of infection, poorer control of cancer, and impaired responses to vaccination, all major public health concerns. Further, the elderly population is at high risk of mortality if infected with new viral pathogens, such as SARS-CoV2. So, even if vaccines are quickly developed against emerging pathogens, they are likely to have reduced efficacy in the elderly. Thus, there is an urgent need to identify strategies to increase vaccine responses in elderly humans.
Our exciting and newly published data show that levels of IL-10, an immune suppressive cytokine, are increased dramatically with age. Further, we found that the majority of this IL-10 is produced by a population of cells called T follicular helper (Tfh) cells, which are normally critical for productive antibody responses. Notably, these aged, IL-10-producing Tfh cells are found in both mice and humans. Strikingly, neutralization of IL-10R signaling in aged mice substantially increases antibody responses, nearly to the levels observed in young mice. In monkeys, we defined the pharmacokinetics of an IL-10 neutralizing antibody as well as a soluble receptor. Given these compelling data, we hypothesize that Tfh10 cells play an important role in impaired age-related vaccine responsiveness that is conserved between mice, monkeys, and humans. Here, we propose to determine if short-term IL-10 blockade will restore Flu vaccine responsiveness in aged macaques. We propose an iterative approach, assessing whether concomitant blockade of IL-10 alongside flu vaccination will: (i) increase anti-influenza neutralizing antibody responses in response vaccination without increasing systemic inflammation (ii) promote resistance to influenza challenge. These studies will likely have important translational implications, as a short-term blockade of IL-10 signaling could be envisioned to improve vaccine responsiveness in elderly humans. Our long-term goal is to develop therapeutic strategies to enhance protective immune responses in the elderly.

Evangelos Kiskinis, Ph.D. - 2020 Awardee

Neurology
Northwestern University Feinberg School of Medicine

"Development of Spherical Nucleic Acid-Based Anti-Sense Oligos for the Therapeutic Treatment of Pediatric Epilepsy Disorders"

Heterozygous mutations in KCNQ2, which encodes a pore-forming K+ channel subunit responsible for neuronal M-current, cause neonatal epileptic encephalopathy (EE), a complex disorder presenting with severe early-onset seizures and impaired neurodevelopment. The condition is exceptionally difficult to treat, partially because the effects of KCNQ2 mutations on the development and function of human neurons are unknown. Using induced pluripotent stem cells (iPSCs) and gene editing we have established KCNQ2-EE disease model systems, and measured the functional properties of patient-derived neurons using electrophysiological and optical approaches at single-cell resolution. We find that patient-derived excitatory neurons develop intrinsic and network hyperexcitability that mimics epileptic electroencephalogram (EEG) activity. Using heterologous expression systems, we have also found that some disease-causing variants exhibit a dominant-negative effect, reducing the channel activity by more than 50%. We hypothesize that deleting the mutant transcript will restore channel activity and alleviate the associated neuronal firing defects. Here, we propose to collaborate with Exicure Inc, to design, screen and test allele-specific antisense oligonucleotides (ASOs), which will target and degrade the mutant KCNQ2 allele. Exicure, is a clinical-stage biotechnology company developing spherical nucleic acid (SNA) based ASOs for genetic disorders. We will assess the specificity of ASOs using established digital droplet PCR assays and examine the efficacy of ASOs by measuring their ability to restore the firing activity of patient-derived neurons. If successful, our work will provide a rational therapeutic approach for this devastating disease.

Eric Morrow, M.D., Ph.D. - 2020 Awardee

Molecular Biology, Cell Biology and Biochemistry
Brown University

"Development of a Preventative Treatment for a Novel Neurometabolic Disorder in Childhood"

Intellectual disabilities are common and carry high lifetime costs to families. We have identified a new neurodevelopmental disorder caused by loss-of-function mutations in the mitochondrial enzyme glutamate pyruvate transaminase 2 (GPT2). GPT2 disease involves postnatal microcephaly, cognitive disability, epilepsy, and progressive spastic paraplegia. GPT2 localizes to mitochondria and catalyzes the reversible addition of an amino group from glutamate to pyruvate, yielding alanine and alpha-ketoglutarate, a metabolite in the tricarboxylic acid (TCA) cycle. Metabolic diseases in children, such as GPT2 disease, may be amenable to treatments, via dietary restrictions or supplements, that can prevent intellectual disabilities when treated early. With the prospect of newborn screening supported by genome-wide sequencing on the horizon, there will be new opportunities to intervene in childhood brain disease. Therefore, we rapidly need to determine which new neurometabolic diseases may be amenable to interventions in early childhood. Our preliminary data provide support for disease mechanisms wherein GPT2 plays a critical role in alanine synthesis, as well as in neuronal anaplerosis. Anaplerosis (filling-up) is the metabolic process whereby TCA cycle intermediates are replenished. Anaplerosis is important during high biosynthetic demand, such as during brain development, when TCA cycle intermediates are consumed for synthesis of macromolecules for cell growth. We have established potential treatment strategies for GPT2 disease that may be implemented in the near term. To guide these interventions, we propose to complete needed pre-clinical studies. Our overriding hypothesis is that GPT2 is required for metabolic mechanisms central to neuronal and axonal growth during brain development, particularly in long projection neurons of the motor system. In Aim 1, we will test mechanism-based treatments in our mouse model of GPT2 disease. Our Gpt2-null mouse recapitulates key aspects of disease, such as motor abnormalities, akin to spastic paraplegia seen in patients. In Aim 2, to translate our basic findings to the clinic, we will establish the infrastructure and collaborations, including with industry, to develop a protocol for biomarker studies and a clinical trial in patients. The impact of this research is that we will establish the basis for therapeutics in a childhood disease for which there is no current treatment.

Lonnie Shea, Ph.D. - 2020 Awardee

Biomedical Engineering
University of Michigan

"A Cell Capture Implant to Predict Acute Cardiac Allograft Rejection"

Over 36,000 solid organ transplants are conducted annually in the US, costing $30 billion. Immunosuppressive drugs protect these donor grafts from acute rejection but increase the risk of opportunistic infections and cancer, especially in pediatric transplant recipients, who require immune suppression for decades. As there is no method for determining which grafts will be rejected, immunosuppression is aggressively applied in a one-size-fits-all approach.

We have developed a novel implantable scaffold device to monitor recipient immune response to the transplant that would enable the use of personalized immunosuppression while maintaining graft acceptance. We employed these scaffold devices in a murine skin allograft model to develop a highly sensitive and specific scoring system from a 19 gene biomarker signature to distinguish between recipients with healthy grafts and recipients with rejecting grafts.

In heart transplantation, organ scarcity requires that clinicians remain vigilant in preventing acute cardiac allograft rejection (ACAR) through frequent graft biopsy and aggressive immunosuppression, at the expense of toxicities due to over-suppression. A novel surveillance method is urgently needed to calculate the early risk of transplant rejection to allow personalized immunosuppression regimes. Here, we present a novel subcutaneous scaffold implant that collects biomarkers of graft health that will predict ACAR onset to preserve graft function while reducing the need for frequent graft biopsy and overly aggressive immunosuppression. In this proposed work, we will employ these minimally-invasive cell capture scaffolds in four cohorts of heterotopic heart transplants in mice in which we will identify a signature gene panel of graft rejection, validate this scaffold biomarker signature, assess the predictive power of the signature, and employ the biomarker signature to monitor the immunosuppression response. We hypothesize that the dynamic changes captured in the scaffold device as the allograft is recognized as non-self by the recipient immune system will enable the development of a novel predictive biomarker panel for ACAR. This minimally-invasive immune cell-capturing scaffold could prove transformative in directing and personalizing immunosuppression in solid organ transplant recipients.

David Spiegel, M.D., Ph.D. - 2020 Awardee

Chemistry
Yale School of Medicine

"Development of Bifunctional Molecules that Cross the Blood-Brain Barrier and Degrade Pathogenic Neurological Proteins"

Unique challenges are presented when treating neurological disease due to the physical obstacle formed by the Blood-Brain Barrier (BBB). Many neurodegenerative diseases result from the accumulation of pathogenic proteins and could potentially be avoided with the early detection and removal of these species. The Spiegel lab is developing a novel class of bioactive molecules that can bind proteins non-covalently and chaperone them across the BBB. These small molecules will be used for the removal of pathogenic proteins from the brain as a novel therapeutic approach to the treatment of neurological diseases. The bifunctional small molecules consist of a protein-targeting motif and a motif for the transport of the target protein across the blood-brain barrier, and subsequent degradation.

Stephen Strittmatter, M.D., Ph.D. - 2020 Awardee

Neurology
Yale School of Medicine

"Neural Repair for Spinal Cord Injury by Axon Regeneration"

Devastating and persistent functional deficits occur after Spinal Cord Injury (SCI), despite survival of nearly all neurons. Partial recovery would be adequate for patients to gain a level of independence in wheelchair transfers, bowel and bladder management, and locomotion. Today, there is no approved medical therapy for the over 300,000 individuals in the USA with SCI, rendering therapy a totally unmet medical need. Because the primary cause of disability is disconnection of networks by axon transection, axon regrowth has the potential to provide recovery by restoring connectivity, without requiring "new" cells. Unfortunately, without therapeutic intervention, the adult brain and spinal cord produce extremely limited reparative axon growth after damage.

We surveyed the mouse genome in an unbiased approach for loci with undiscovered functions in axon regeneration. This screen identified 400 genes whose suppression yielded greater axon regeneration in vitro. In Preliminary Studies, we leveraged the in vitro results to identify pathways that might be targeted to improve CNS neural repair in vivo using a simple optic nerve crush model. In control eyes, very few axons regenerate, but knockdown of 40 of the in vitro hit genes produced significantly increased regeneration. These genes were validated gene editing using CRISPR/Cas9 in vitro and in vivo.

In the current proposal, we will test two of these novel regeneration genes as attractive therapeutic candidates for their ability to promote axon growth and behavioral recovery after spinal cord injury in mice. We hypothesize that their demonstrated axon regeneration activity will translate into improved neural repair. Validation of this hypothesis will support development of their potential in translational research.

Ross Summer, M.D. - 2020 Awardee

Medicine
Thomas Jefferson University

"Preventing De-condensation of Nascent Chromatin in the Treatment of Pulmonary Fibrosis"

The goal of this project is to develop an effective therapy for Idiopathic pulmonary fibrosis (IPF), a devastating lung disease that progressively scars the lung and has a prognosis worse than many aggressive cancers. A defining feature of IPF is the differentiation of fibroblasts into myofibroblasts, followed by the production of massive amounts of extracellular matrix (ECM) and the formation of collagen-rich scars, which restrict movement of the lung and impede respiratory gas exchange. While two anti-fibrotic drugs are approved to treat IPF, neither therapy is highly effective. Our long-term objective, and the focus of this high-risk, high-yield application is to develop a new, effective therapy for IPF. Previous work has shown that differentiation of embryonic and hematopoietic stem cells requires the transient decondensation of chromatin at sites of repressed genes and that failure to do so prevents the binding of critical differentiation transcription factors (TF). The hallmark of condensed arrays of nucleosomes in chromatin is H3K27me3, and transient global removal of this mark is required for activating repressed genes during differentiation of stem and progenitor cells. The same mechanism was also found in more differentiated naïve T cells during their differentiation into Th1 and Th2 helper cells, leading us to hypothesize that removal of H3K27me3 chromatin marks might also dictate the differentiation of fibroblasts into myofibroblasts. Because transient de-condensation of the H3K27me3-marked chromatin is achieved by the activities of the H3K27me3 de-methylases (KDM) UTX and JMJD3, we hypothesized that inhibiting these enzymes via a small molecule 'epigenetic' inhibitor GSKJ4 would arrest pulmonary fibrosis. In preliminary studies presented here, we show that GSKJ4 effectively blocks the formation of myofibroblasts in vitro and limits the production of extracellular matrix genes in experimentally induced IPF mouse models. We now wish to extend these observations in hopes of moving this concept to the clinic. To achieve this goal, we propose 3 rationally designed Specific Aims that are mechanistically link but fully independent. Studies in this application aim to challenge current approaches to the treatment of IPF and provide support for targeting nascent chromatin in the treatment of a wide assortment of fibrotic diseases.

Allan Brasier, M.D. - 2019 Awardee

Medicine
University of Wisconsin-Madison

"Targeting the Myofibroblast Epigenome for First-In-Class Treatment of Chronic Obstructive Pulmonary Disease, COPD"

Chronic Obstructive Pulmonary Disease (COPD) affects 13.5 million (M) people in the US with a regional age-standardized death rate that will be the 4th leading cause of death by 2020. This obstructive lung disease is characterized by acute episodic decompensations, "exacerbations" associated with increased symptoms that result in substantial morbidity and costs through unscheduled hospital visits. Importantly, exacerbations are associated with more rapid declines in pulmonary function for which no effective therapies exist. These patients suffier substantially reduced quality of life and over half will die of acute-on-chronic respiratory failure.

COPD is initially triggered by oxidative airway injury that results in the activation of a pathogenic mesenchymal myofibroblast population in the small bronchioles of the airway. Myofibroblasts produce fibronectin and collagen that reduce the normal elastic properties of the lung and impair gas exchange.

The broad goal of this Catalyst Award is to advance first in-class therapeutics that target myofibroblast transdifferentiation. This advance will come in two stages, each a focus in this application. First, there are no high resolution, non-invasive methods for detection of airway remodeling or determination of therapeutic response. We will validate an integrated proteomic and imaging diagnostic for airway remodeling based on our unbiased pharmacoproteomics study of inhibiting BRD4 in airway remodeling. Quantitative selective reaction monitoring assays of a panel of airway remodeling proteins will be paired with minimally invasive optical imaging of mucosal collagen deposition using optical coherence tomography (OCT). Second, we will advance a lead highly selective BRD4 inhibitor to an aerosol formulation encapsulated in proprietary Dendron Micelles. Understanding that foci of myofibroblasts are initially formed in the small bronchioles, we will develop aerosolized therapeutics that target fibronectin-rich foci. Nanoparticle formulation have additional advantages that they will enhance duration of effect, and reduce potential systemic toxicity. We will demonstrate enrichment using advance mass spectrometry based imaging, and demonstrate efficacy in an established model of COPD. By advancing, in parallel, minimally invasive biomarkers and targeted therapeutics, we will be uniquely poised to translate these into first-in human studies

John Bushweller, Ph.D. - 2019 Awardee

Molecular Physiology and Biological Physics
University of Virginia

"RUNX1-ETO Targeted Small Molecule Therapy for t(8;21) Acute Myeloid Leukemia"

The gene encoding RUNX1 (AML1) is disrupted by the t(8;21) that is associated with 4-12% of adult acute myeloid leukemia (AML) patients and ~13% of pediatric AML patients. The t(8;21) results in a fusion protein containing the N-terminus of RUNX1, including the Runt domain, fused to almost all of ETO. The RUNX1-ETO (also called AML1-ETO) fusion protein acts as a dominant repressor of RUNX1 function, dysregulating the expression of multiple genes required for normal hematopoiesis and, in cooperation with secondary mutations, leads to the development of leukemia. The RUNX1-ETO fusion protein has clearly been established as the primary driver of t(8;21) AML. About 60-70% of t(8;21) patients are alive at 5 years, however disease recurrence is the major treatment failure with 30-40% of these patients relapsing after standard intensive chemotherapy, highlighting the need for new approaches to treatment. The standard chemotherapy used to treat these patients has serious long-term side effects, which is particularly problematic for pediatric patients who will deal with these effects throughout their lives as well as for older patients who can't tolerate standard chemotherapy as well. In order to overcome this, it is essential to develop drugs which directly and selectively target the RUNX1-ETO fusion protein driver to treat the disease.

We propose to develop small molecule inhibitors of RUNX1-ETO that block its ability to bind to DNA and which selectively inhibit RUNX1-ETO while having minimal if any effect on wildtype RUNX function. Such a high level of selectivity of action has rarely been achieved, but we have previously done so in targeting the CBFβ-SMMHC fusion protein driver in inv(16) AML. We are proposing to develop a hetero-bivalent compound which targets both the Runt domain of RUNX1 (DNA binding domain) and the TAF domain of ETO (the nearest ETO domain to the Runt domain in the fusion protein). We have screened and optimized a compound which binds to the TAF domain displacing HEB with a low μM IC50. We have identified compounds which covalently react with Cys residues on the Runt domain. For this grant, we will focus on covalently linking the TAF domain inhibitor to optimized Cys reactive compounds. Linker length and chemical structure will be varied to identify optimal linkers. Compounds will be evaluated in t(8;21) cell lines for their ability to inhibit growth, alter the expression of RUNX1-ETO target genes (qPCR), and ability to inhibit binding of RUNX1-ETO to its genomic sites (ChIP).

Lin Guo, Ph.D. - 2019 Awardee

Biochemistry and Molecular Biology
Thomas Jefferson University

"Developing Therapeutic Agents to Rescue Neurotoxicity of FUS Aberrant Phase Transition"

It is now universally appreciated that accumulation of misfolded proteins, which can acquire alternative proteotoxic states, causes a series of deleterious molecular events resulting in numerous lethal neurodegenerative diseases. Among these, amyotrophic lateral sclerosis (ALS) is the most common motor neuron disease. There are no effective therapies for ALS and very few treatment options. Therefore, new therapeutic target and strategies are greatly needed. ALS-linked mutations have been discovered in several prion-like domain (PrLD) containing nuclear RNA-binding proteins (RBPs) including FUS, which is a stress granule (SG) component. Cytoplasmic mislocalization and inclusion formation, which are common pathological features of FUS proteinopathies, have been connected to persistent SGs. Upon stress, ALS disease proteins are recruited to SGs, which are reversible cytoplasmic membraneless organelles that form through Liquid-liquid phase separation (LLPS) and behave like liquid-droplets. Because SGs condensate ALS disease proteins such as FUS that are intrinsically aggregation-prone, if SGs are not cleared in time, FUS can go through aberrant phase transition to form solid fibrils that can induce toxicity and neurodegeneration. The long-term goal of this project includes understanding the molecular mechanisms underlying the aberrant phase transitions of SGs and leveraging our understanding of aberrant phase transition to develop therapeutic agents to mitigate the neurotoxicity of these pathological events. This proposal focuses on developing two types of therapeutic agents to rescue FUS neurotoxicity caused by aberrant phase transition. We have discovered a novel function of nuclear import receptor-Kapbeta2 in reversing FUS aberrant phase transition and aggregation. However, ALS-causing mutations in FUS PY-NLS such as P525L reduces Kapbeta2's activity as protein disaggregase. Therefore, the first goal of the proposal is to discover small molecules that can enhance Kapbeta2's activity to reverse FUS P525L aberrant phase transition. Kapbeta2 reverses FUS aberrant phase transition by binding to the nuclear localization signal PY-NLS in the C-terminus of FUS. Thus, other FUS-binding biomolecules might also prevent and reverse FUS LLPS and aggregation. Indeed, our preliminary data show FUS-binding RNA can prevent and reverse FUS aggregation. Therefore, the second aim of the proposal focuses on developing RNA oligonucleotides to reverse FUS aberrant phase transition and defining their therapeutic potential in mitigating FUS neurotoxicity. The in vitro activities of the agents developed in this proposal will be characterized using pure protein biochemical and biophysical assays. Top ranking agents will then be validated in iPSC-derived motor neurons for their ability to mitigate FUS aberrant phase transition and the resulted neurotoxicity.

Yogendra Kanthi, M.D. - 2019 Awardee

Internal Medicine, Division of Cardiovascular Medicine
University of Michigan

"Combating Venous Thrombo-Inflammation with Precision Bio-functional Therapies"

Venous thromboembolism (VTE), comprised of deep venous thrombosis and secondary pulmonary embolism, affects 900,000 people and is the third leading cause of cardiovascular death. Current therapies are limited to anticoagulation, which carries significant bleeding risks and don't address the inflammatory processes that initiate and propagate VTE. Therapies that directly target inflammatory processes would address the underlying catalyst of disease and have the potential to fundamentally transform the treatment of VTE.

A distinguishing feature of VTE is the recruitment of leukocytes to the thrombus. We discovered that this is driven by marked neutrophil activation, the expulsion of DNA as neutrophil extracellular traps (NETs), and inflammasome activation to release a potent inflammatory cytokine, interleukin 1-beta(IL-1beta). This phenomenon further polarizes the vascular environment in a self-amplifying loop culminating in VTE. We showed that hyperactive neutrophils are more prone to form NET 'scaffolds' for thrombus expansion, and that systemic delivery of anti-IL-1beta antibodies (Ab) can markedly reduce neutrophil activation during thrombogenesis. There is currently no therapeutic approach to specifically target neutrophils, and systemic IL-1beta inhibition is limited by suppression of other innate immune functions essential in host-defense. This represents a critical roadblock in the translation of our findings into the clinic for patients with VTE.

Our goal in this Falk Catalyst proposal is to overcome this current roadblock by developing a synthetic bio-functional molecule to precisely target neutrophils for inhibition during thrombogenesis. This proposal builds on an existing collaboration between experts in venous thrombo-inflammation and vascular-targeting drug carrier design to develop a neutrophil-tropic particle that directs to the inflamed neutrophil and arrests thrombosis expansion. The Aims outlined here will optimize our novel nanoparticles to exclusively target to neutrophils (Aim 1, Particle optimization); and determine whether this approach effectively reduces in vivo and in vitro neutrophil activation and venous thrombogenesis (Aim 2, Efficacy). Our proposal is driven by our innovative discoveries, expert cross-disciplinary collaboration, and strong preliminary data, with the long-term objective of developing a neutrophil-inhibiting 'precision' therapy for patients who suffer from venous thromboembolism. The results of these studies will provide a solid rationale for us to translate our discoveries during the Transformational phase of this Award, and form the basis of an innovative and more complete strategy to treat one of the leading cardiovascular diseases.

The University of Michigan has a unique history of organizing around research questions across disciplines, providing a robust foundation for the Catalyst and Transformational phases of this program.

Chaitan Khosla, Ph.D. - 2019 Awardee

Chemistry
Stanford University

"A Novel Therapeutic Approach for Major Depressive Disorder"

Major depressive disorder (MDD) is among the leading causes of illness and disability worldwide. It is a severe and life-threatening disease. Childhood trauma and insulin resistance are known risk factors for MDD. The pathophysiology of MDD remains poorly understood, and there is a serious dearth of new druggable biological targets to guide the development of improved therapeutics. This proposal outlines a research plan to identify a fundamentally new drug therapy for MDD.

In rodent models of depression, the biomolecule acetyl-carnitine promotes a rapid anti-depressant response. Endogenous acetyl-carnitine levels are also lower in the plasma of patients with MDD compared with age- and sex-matched healthy controls. Several older studies involving human subjects have also reported neurophysiological and neuropharmacological effects of oral acetyl-carnitine, although these studies were either uncontrolled or under-powered. While not a typical drug-like substance, acetyl-carnitine has properties that make it a promising lead for our purposes. It is classified as a Generally Regarded as Safe (GRAS) substance by the FDA, and is orally bioavailable, while also being transported across the blood-brain barrier.

Our goals for this Catalyst Research Award are to assemble a multidisciplinary team of researchers with relevant backgrounds in chemistry, neuropharmacology, and psychiatry to launch a comprehensive drug discovery effort aimed at optimizing the activity of acetyl-carnitine in MDD. Our technical aims are to: (1) Implement robust in vitro and in vivo assays based on our model for the mode of action of acetyl-carnitine in MDD; (2) Design and synthesize acetyl-carnitine analogs, and test them in the above assays; and (3) Compare the activity of the most promising analog vesus acetyl-carnitine in a rodent model of depression. If successful, these team-building and technical achievements will pave the way for a more intensive pursuit of a safe and effective drug candidate for MDD therapy under the Transformational Research Award Program.

Jeannette Messer, D.V.M., Ph.D. - 2019 Awardee

Inflammation and Immunity
The Cleveland Clinic

"Crafting New Weapons for the Fight Against Infectious Diseases"

Infectious diseases caused by bacteria, viruses, fungi, and single-cell parasites kill millions of people worldwide, each year. Although these microorganisms are very different in many ways, the first step of infection with each of them is microbial adherence to human or host tissues. Regardless of the microorganism, adherence essentially always involves binding between a protein on the surface of the microbe and a carbohydrate ligand on the host. Without this binding, microbes cannot infect host tissues and cause disease. It is clear that blocking microbial adherence mechanisms shared among microbes would be a powerful and nearly universal way to treat or prevent infectious diseases. However, no conserved aspects of microbial adherence have ever been identified and there are no proposed strategies to target this type of dangerous microbial behavior.

We have identified a conserved amino acid motif (CAMo-1) with an associated molecular feature in a large number of adherence proteins from bacteria, viruses, fungi, and protozoa. In our preliminary work, we identified this novel drug target and validated it experimentally by showing that a host protein binding to this structure blocks microbial adherence to human cells. The goal of this study is now to identify and develop small molecules that bind to this structural feature and block microbial adherence to host cells for treatment and prevention of these infectious diseases. We hypothesize that we can identify specific small molecules that bind to microbial adherence proteins at CAMo-1 to block the attachment of pathogenic microbes to human tissues. We will test this hypothesis using three different screening approaches as outlined in our specific aims: Aim #1) Use purified recombinant proteins to identify small molecules that bind to microbial adherence proteins at CAMo-1. Aim #2) Use bead-based microbial mimics to identify small molecules that prevent microbial adherence protein binding to host cells. Aim #3) Use live microbes to identify small molecules that prevent microbe binding to host cells. In each of these aims we will be performing primary and secondary screens using diversity and fragment small molecule libraries. Successful completion of this study would provide small molecule leads for development of a completely new type of antimicrobial drug designed to target dangerous microbial behaviors, regardless of the microorganism. This would be a paradigm shift in how these diseases are conceptualized and treated and a major advance towards our long-term objective to provide cures for infectious diseases in which no cures currently exist.

Jon Parquette, Ph.D. - 2019 Awardee

Chemistry and Biochemistry
The Ohio State University

"Antioxidant Nanoscaffold Technology for Combinatorial Treatment of Diabetes"

Diabetic patients often develop neuropathies and have a greater risk for vascular or Alzheimer's dementia compared to those without this disease. The epidemic of these diseases indicates a need to improve the treatment of diabetes and reduce neurodegeneration. Patients with diabetes must use increasingly higher doses of therapeutics over time, which increases the risk for side effects. These side-effects are partly a consequence of the fact that insulin is a growth factor that recruits glucose for anabolic processes. Consequently, diabetic patients treated with insulin are prone to weight gain and dyslipidemia that increases the risk of cardiovascular mortality. In this work, a series of short peptides are being designed and synthesized, which undergo self-assembly into nanofibers upon exposure to physiological conditions. The positive charge on the nanofibers electrostatically binds negatively charged molecules, including insulin, and can concomitantly bind to critical receptors, i.e. LepR, to stimulate glucose uptake as a cytokine. One peptide, AAC2, alone and bound to insulin, rescued mice from T1D and T2D in preliminary studies. These preliminary data provide the basis for a central hypothesis/question to be tested in this work: Can these peptides improve glucose tolerance and reduce neurodegeneration related to diabetes, based on their interaction with LepR? The overall goal is to develop and optimize this class of therapeutics with dual properties: (1) as a scaffold to bind and stabilize hormones, such as insulin, and (2) as a concomitant activator of LepR to improve glucose uptake, energy balance, and to reduce associated neurological complications. The specific aims of this work are: (1) to optimize the structure of AAC, (2) to modulate the length and stability of the AAC nanofibers, (3) to examine the glycemic and antioxidant effects of AAC variants in vitro, and a future aim will be to (4) to determine the pharmacokinetic and pharmacodynamic profile of the candidate AAC molecule in Akita mouse models of T1D and high-fat diet induced model of T2D.

Anne Rowley, M.D. - 2019 Awardee

Pediatrics
Ann & Robert H. Lurie Children's Hospital of Chicago

"A New Human Hepacivirus as an Etiologic Agent of Kawasaki Disease"

Kawasaki Disease (KD) is the leading cause of acquired heart disease in children in developed nations. KD can result in coronary artery aneurysms that can lead to lifelong heart disease, myocardial infarction, and death. The clinical and epidemiologic features support an infectious etiology in genetically susceptible children, but the cause has eluded more than 50 years of study. Delayed and missed diagnoses increase the risk of coronary artery aneurysms. The development of urgently needed diagnostic tests and improved therapies are dependent upon identifying the etiology. In this study, we propose to determine if a new human hepacivirus is an etiologic agent of KD. Recent studies have shown that 1-2 weeks after infection with any specific pathogen, 75% of peripheral blood plasmablasts target that specific infection. In preliminary studies, we analyzed the peripheral blood plasmablast response at 1-3 weeks after KD fever onset using single cell RT-PCR and made 61 monoclonal antibodies (Mab) from these plasmablasts. We used these Mab to determine their target antigens. We found that 33/61 Mab, derived from 9/11 KD patients, identify intracytoplasmic virus-like inclusion bodies (ICI) in ciliated bronchial epithelium of KD children but not infant controls. Using a viral peptide discovery array and/or ELISA, we found that 6 of the 33 (18%) Mab, derived from 3 KD patients with coronary artery aneurysms, recognize multiple similar peptides of hepacivirus non-structural protein 4A (NS4A). An optimized NS4A peptide completely blocks binding of these Mab to KD ICI, indicating the presence of a hepacivirus-like protein in the ICI. We hypothesize that at least a subset of KD cases are due to a previously unidentified hepacivirus. To test this hypothesis, we will identify the KD-associated hepacivirus using a specifically designed pathway for bioinformatics analysis of our KD tissue RNAseq dataset containing >4 billion reads. In addition, we will obtain additional viral proteome data by screening viral peptide arrays/phage display libraries with KD Mab that bind to KD ICI but do not recognize NS4A. We will test KD patients and childhood controls for serologic response to and presence of KD-associated hepacivirus. These studies will lead to improved diagnosis and treatment of KD, enable prevention, and reduce healthcare costs from the long-term consequences of coronary artery aneurysms arising in young childhood.

Sarah Slavoff, Ph.D. - 2019 Awardee

Chemistry
Yale School of Medicine

"Targeted Degradation of a Melanoma Transcription Factor"

Melanoma, the deadliest skin cancer, was diagnosed in approximately 90,000 Americans and led to over 9,000 deaths in 2019. While BRAF inhibitors and checkpoint inhibitors have revolutionized treatment of metastatic melanoma, these therapies are limited by rapid development of resistance and low response rates, respectively. New therapies, and entirely novel drug targets, are therefore critically needed, ideally targeting not only melanomas but also congenital giant nevi that are precursors to melanomas and are currently removed by repeated surgery. An innovative approach originally developed by Prof. Craig Crews at Yale University, leverages molecules called proteolysis targeting chimeras, or PROTACs, to degrade – rather than inhibit – previously "undruggable" classes of proteins, including transcription factors. PROTACs also have the potential to enhance immunotherapy because the proteolytic peptides arising from target protein degradation are immunogenic. In this work, we propose development of PROTACs targeting a melanocyte-specific transcription factor, SOX10, that is required for melanoma cell, normal melanocyte and nevus cell proliferation. PROTACs consist of a ligand to the protein of interest, a flexible linker, and an E3 ubiquitin ligase ligand. We will therefore first develop a ligand to SOX10 using two high-throughput screening approaches for which preliminary feasibility has been established (Specific Aim 1). While ligand development is in progress, we will utilize a previously reported biotechnology-based strategy to demonstrate that PROTAC-mediated degradation of an engineered SOX10 fusion protein kills melanoma cells (Aim 2). Finally, we will create a small PROTAC library based on our novel SOX10 ligands, from which optimal members will be selected based on their ability to induce degradation of the transcription factor, inhibit melanoma cell, normal melanocytes and nevus cell proliferation, and eliminate melanocytes from human skin tissue in organotypic culture (Aim 3). In the future, we envision that the best PROTACs identified in this study will be further optimized and elaborated into drug candidates for testing in animal models prior to translation to the clinic. More broadly, this work will advance current cutting-edge efforts to drug transcription factors that have been refractory to traditional inhibitor development.

Fei Song, M.D., Ph.D. - 2019 Awardee

Neurology and Rehabilitation
University of Illinois College of Medicine at Chicago

"Therapeutic Targeting of Disease Progression in ALS"

ALS patients desperately need new approaches and directions to combat this disorder. While motor neuron loss in the spinal cord has been a central area of research focus for this disease, there is extensive pathology in ALS that spans from the neuromuscular synapses to the spinal cord, to the lateral corticospinal tract, and to the cerebral cortex. It is still not known how and where this disease begins nor the molecular mechanisms of how the disease progresses once it starts.

This application represents an innovative and uniquely humanized, collaborative approach for human ALS. It will combine clinical and basic research on rapidly-acquired human postmortem tissues to identify and validate new targets for drug development using a novel injury based model of disease progression. While gene mutations in animal models that rely on rarer forms of the disease with distinct genetic abnormalities have been used extensively, these animal models have not yielded effective therapeutics. The unique aspect here is that we instead focus on targeting disease progression once the disease has already begun, as it does when our patients first present with their disease. Our central hypothesis to be tested here is that the degree of pathological change will correlate with a distinct group of genes, proteins, and biological pathways that will lead us to novel drug targets of disease progression. Currently, we have leads to suggest that inflammation is key to this as well as a custom designed, patented fusion protein that targets this. Targeting disease progression offers a 'final common pathway' approach to treat ALL ALS patients, not just those with rare genetic abnormalities.

The catalyst phase will bring together a diverse team for genomic analysis and mining of our novel tissue repository. Our group has pioneered human tissue functional genomics, cellular predictions, biomarker identification, and drug target identification from human tissues. In parallel, it will build a new animal model of ALS disease progression by bringing together a multidisciplinary team to test targets generated from the human tissue data. These parallel teams will come together for the transformational phase where our top drug targets will be translated into small molecular and/or biologic therapeutics to test on the new animal model of ALS disease progression. In this later phase, our goal will be to select our top three drugs for preclinical testing, toxicology, and filing of IND applications.

Takanori Takebe, M.D., Ph.D. - 2019 Awardee

Gastroenterology, Hepatology and Nutrition
Cincinnati Children's Hospital Medical Center

"Novel Human Organoid Transplant Therapy Against Pediatric Liver Disease"

Disorders affecting urea metabolism are among the most common inborn errors of metabolism in the liver. Defects in metabolism of waste nitrogen from the breakdown of protein and other nitrogen-containing molecules lead to elevated blood ammonium level that causes neurotoxicity and can be fatal. Currently, low protein diets and liver transplantation are the only available therapies for disorders of urea metabolism. Procurement of cadaveric livers or use of hepatocytes/stem cell-based replacement therapies, has remained a significant challenge, driving intense research towards developing alternative liver regeneration strategies for human application. Our own current focus leverages a "self-condensation" culturing methodology wherein human induced pluripotent stem cells (hiPSC) are developmentally specified and together with vascular progenitors, self-organized into 3-D vascularized miniature livers ("organoids"). Our success with this approach is highlighted by rescue of a mouse model of liver disease using these cultured hiPSC-derived human liver organoid (hiPSC-HLO) transplants.

More recently we showed that hiPSC-HLO can be produced at a scale and purity for detailed testing in animal models. Building on this recent success, we now propose to conduct a preclinical study for the treatment of newborns who have congenital rare urea cycle disorders, more specifically, ornithine transcarbamylase deficiency (OTCD). The initial goal is to provide bridge therapies during the waiting period for liver transplants when surgery is not yet feasible and a liver not available. Our interim goals are (1) To determine if hiPSC-HLO transplants provide pivotal efficacy in alleviating urea cycle disease in our rodent models, and (2) To assure safety without tumorigenic complications by defining critical quality attributes (CQAs) in the hiPSC-HLO manufacturing process. The proposed analyses utilizing urea cycle disorder mice (generated under a severely immunocompromised background) will make it possible to determine the efficacy for correcting hyperammonemia, survival and behavioral deficits. Concurrent monitoring of tumor formation will ensure safety of our proposed organoid based approach.

In addition to providing preclinical assessment of utility of hiPSC-HLO in the OTCD model, our proposal has broader implications for efficacy in multiple hepatic dysfunctions related to protein metabolism, bile acid synthesis and export, and coagulation factor synthesis. This will expand the utility of our methodology for many other clinical indications such as liver cirrhosis, which is a significant cause of global health burden with more than one million deaths per annum.

Hongwei Yao, Ph.D. - 2019 Awardee

Molecular Biology, Cell Biology & Biochemistry
Brown University

"Identification of Carnitine Palmitoyltransferase 1A as a Novel Target for Bronchopulmonary Dysplasia"

Ventilatory support, including oxygen supplementation, has saved countless premature infants, yet these therapies have also led to bronchopulmonary dysplasia (BPD) in premature babies, which can persist into adolescence and adulthood. The pathology of this disease is characterized by alveolar and vascular simplification in the lungs. Although current therapies, including antennal steroids and surfactant, have greatly improved survival of premature infants, the prevalence of BPD and its consequences on the lung have not been reduced. My long-term objective is to develop effective drugs to prevent or treat BPD.

Carnitine palmitoyltransferase 1 (Cpt1), a rate-limiting enzyme of the carnitine shuttle system for beta-oxidation during fatty acid oxidation (FAO), may be key for developing these drugs. Cpt1 has three isoforms: Cpt1a, Cpt1b and Cpt1c, of which Cpt1a has acyltransferase activity and a high affinity to carnitine to generate acylcarnitine for mitochondrial transport. My previous studies showed for the first time that hyperoxic exposure reduces levels of Cpt1a and FAO in neonatal lung endothelial cells (ECs), leading to apoptosis. Pharmacological inhibition or genetic deletion of Cpt1a aggravates hyperoxia-induced alveolar and vascular simplification, characteristics of BPD, in neonatal mouse model of BPD. This proof-of-concept study suggests that Cpt1a reduction causes hyperoxic lung injury in neonates. Nevertheless, whether enhancing Cpt1a attenuates neonatal hyperoxic lung injury remains unclear.

We hypothesize that enhancing Cpt1a level and activity in neonatal mice ameliorates hyperoxia-induced persisting lung injury into adulthood. To test this hypothesis, we propose two Specific Aims using pharmacological activators (i.e., C89b and L-carnitine) and genetic approaches (i.e., Cpt1a gene knockout and overexpression) to determine their impact on lung injury and repair.

Aim 1: Determine whether overexpression and activation of Cpt1a reduces hyperoxia-induced lung EC dysfunction in vitro. We will genetically overexpress and pharmacologically activate Cpt1 to determine whether this attenuates hyperoxia-induced lung EC dysfunction, including apoptosis, reduced proliferation, migration, and angiogenesis.

Aim 2: Validate whether targeting Cpt1a protects against hyperoxia-induced persisting lung injury in mice. We will expose neonatal mice (<12 h old) to different concentrations of hyperoxia for 3 days, and allow them to recover in air until adulthood. These mice will be treated with L-carnitine or C89b, or Cpt1a plasmids will be transfected into lungs to determine whether this ameliorates hyperoxia-induced lung function decline as well as alveolar and vascular simplification.

These proposed studies will set the groundwork for Cpt1a as a novel therapeutic target for preventing BPD, potentially impacting tens of thousands of lives.

Shirit Einav, M.D. - 2018 Awardee

Medicine (Infectious Diseases) and Microbiology and Immunology
Stanford University

"Towards Predicting and Preventing the Development of Severe Dengue"

Dengue virus (DENV) is a global health threat for which no effective vaccines or approved antivirals exist. 5-20% of symptomatic patients progress to severe dengue (SD), manifested by complications and sometimes death. Early administration of supportive care reduces mortality, however, there are no accurate means to predict which patients will progress to SD. Our goal is to better understand the virus-host interplay involved in SD pathogenesis in order to develop prognostic tools for early identification of patients at risk for progression to SD and host-targeted antivirals.

We established a unique cohort in Colombia--dengue patients who present prior to progressing to SD. Moreover, we developed a novel virus-inclusive, single-cell transcriptomic (viscRNA-seq) platform, which transforms our ability to monitor host gene expression dynamics with viral abundance in thousands of individual cells. Additionally, we used a novel multi-cohort analysis of the publicly available gene expression data sets to identify and validate a 20-gene set predictive of SD. This discovery is groundbreaking since this gene set is generalizable across a variety of countries and ages. Lastly, we demonstrated a proof-of-concept for the utility of host-targeted approaches to combat DENV. We hypothesize that combining viscRNA-seq profiling in our dengue cohort samples and the multi-cohort analysis will identify biomarkers of SD whose mechanistic validation can also reveal host targets for antiviral therapy.

In Aim 1, we will conduct viscRNA-seq analysis in longitudinal PBMC samples from the Colombia cohort to map an atlas of DENV cellular targets and identify candidate biomarkers predictive of SD. To functionally validate these biomarkers and identify novel druggable host functions for antiviral therapy, we will decipher the roles of prioritized top hits identified in our viscRNA-seq studies in SD pathogenesis and the DENV life cycle.

Aim 2 will determine the feasibility and biological rationale for predicting SD by the promising prognostic 20-gene set. We will validate this gene set in silico and prospectively, monitor its dynamics during the disease course, and define its specificity. Lastly, we will decipher the roles of some of the 20-gene products in SD pathogenesis and the DENV life cycle.

This bold, interdisciplinary proposal will further transformative single-cell and bioinformatics technologies, provide insights into SD pathogenesis with an unprecedented resolution, and yield generalizable gene sets predictive of SD and host functions as candidate antiviral targets. By advancing the development of dengue prognostic assays and antiviral strategies (years 2-3), this work has the potential to improve human health.

Christopher Hadad, Ph.D. - 2018 Awardee

Chemistry and Biochemistry
The Ohio State University

"Development of a Novel Treatment for Organophosphorus Chemical Nerve Agent Poisoning"

Many organophosphorus (OP) compounds are chemical warfare agents or pesticides and are significant threats to human society as over 200,000 people die every year from OP exposure. These chemical agents inhibit the enzyme acetylcholinesterase (AChE) in the human body, and upon exposure to OP pesticides, AChE undergoes an aging process, rendering current oxime treatment to be ineffective. Our team has recently demonstrated the first small drug-like molecules that can reverse the effects of aging for AChE in vitro, thereby fully reversing the effects of OP exposure. The objective for this proposal is to expand on these alkylating compounds to improve selectivity, binding and reactivity with the aged forms of AChE in order to form stable adducts which can then be reactivated, and while doing so, enhance the drug-like properties for improvements in dosages and time for effective treatment. Thus, this proposal will improve the efficacy of these compounds in order to develop new therapeutics against the toxicological effect for the global use of OP pesticides.

Oliver Jonas, Ph.D. - 2018 Awardee

Radiology Department
Brigham and Women's Hospital/Dana Farber Cancer Institute in collaboration with The Leukemia & Lymphoma Society, Ilinois Chapter

"Use of an Implantable Microdevice for in Situ Drug Sensitivity Testing for Cutaneous Lymphoma"

T- and NK-cell lymphomas comprise 10% of non-Hodgkin's lymphomas in Western countries. Specifically, advanced cutaneous T-cell lymphoma (CTCL) and peripheral T-cell lymphoma (PTCL) have a dismal survival. To date their treatment has been largely derivative or empiric, with few biomarkers to guide therapeutic selection. Three fundamental barriers have prevented successful translation in TCL: 1) the low incidence of each TCL subtype, which limits sample availability and clinical trial enrollment; 2) heterogeneity across subtypes, which further complicates biologic interrogation; and 3) a lack of faithful model systems for in vitro and in vivo studies.

To directly address the need for patient-specific biomarkers that guide therapeutic selection, we have developed a rapid parallel in vivo assay that consists of implantable microdevices (MDs) placed inside the native tumor microenvironment. Each MD contains up to 20 reservoirs, which are individually loaded with a unique agent or drug combination. It is implanted directly into tumor and remains in situ for ~24-72h. Following implantation, drugs from each reservoir are released into distinct regions of tumor tissue. A coring needle is used to retrieve the MD with surrounding tissue. Native tumor tissue surrounding MDs can be analyzed by multi-parameter immunohistochemistry or immunofluorescence, RNAseq, or other approaches to determine individual drug effects. This completely new paradigm for discerning targeted tumor-specific drug effects allows immediate readouts of drug activity directly from the affected tissue. The key advantage of this approach is the high throughput of targeted perturbations induced by released drugs within the native tumor architecture, which, through precise spatial overlay, provides a combined measurement of drug release and phenotypic readout of cell/tissue response, across a large set of drugs relevant to the treatment of TCL.

Application of this microdevice, to determine the optimal treatment for each patient, could be transformative for the care of patients with TCLs. We will test the MD in situ within tumors, a process that has never been undertaken in lymphoma models. The results of this study will serve as the basis for registering the MD with the FDA to allow investigation in patients with TCL and as preclinical data to define mechanisms of response and resistance for multiple novel agents. We propose the following Specific Aims.

Aim 1. Define the activity of FDA-approved and investigational drugs across TCL models in vivo.
Aim 2. Validate methods for multiparametric assessment of TCL biopsies with embedded MDs.
Aim 3. Finalize planning for clinical trial sample analysis.

Anthony Koleske, Ph.D. - 2018 Awardee

Molecular Biophysics and Biochemistry
Yale School of Medicine

"Modulators of TRIO Guanine Nucleotide Exchange Factor Activities as New Therapeutics for Schizophrenia, Autism, and Related Disorders"

Schizophrenia, autism spectrum disorder, bipolar disease, and epilepsy are genetically related and highly debilitating neurodevelopmental disorders that affect >3% of the world population. Current therapies for these diseases do not ameliorate all symptoms and have not improved in efficacy in decades. Our goal is to develop better drugs to treat these disorders.

TRIO is among the genes most frequently impacted by heterozygous rare damaging genetic variants in individuals with schizophrenia, autism, and related disorders. TRIO contains two guanine nucleotide exchange factor (GEF1 and GEF2) domains which catalyze GTP exchange on the Rac1 and RhoA GTPases, respectively, to regulate synapse development and function. Most of the disorder-associated mutations in TRIO are heterozygous and disrupt the GEF1 and/or GEF2 activities. Deletion of one TRIO allele in mice compromises normal brain development and function. Together, these data strongly implicate reduced TRIO function as a causative factor in neurodevelopmental disorders.

We propose to test the hypothesis that boosting TRIO's enzymatic activities might lead to therapeutic benefits in individuals suffering from SCZ, ASD, and related disorders. Specifically, we propose to identify small molecules that can modulate TRIO's GEF1 and GEF2 activities and to test their ability to rescue phenotypes arising from TRIO haploinsufficiency.

Aim 1. To screen for small molecule modulators of TRIO GEF1 and GEF2 activities. We will use a fluorescence-based, 384-well plate assay that measures the TRIO GEF1-mediated exchange of GTP for GDP on Rac1 to implement a pilot high throughput screen of an approximately 5,000 compound library enriched with CNS-drug like properties. We will perform a similar pilot screen on TRIO GEF2 activity on RhoA. These screens will identify both positive and negative regulators, and, because both GEF domains will be screened against the same library, hit compounds with initial selectivity will be identified and prioritized. We will validate lead compounds in cells using genetically-encoded Rac1 or RhoA FRET biosensors.

Aim 2. To test the ability of TRIO GEF modulators to rescue defects in TRIO+/- neurons. Cortical neurons and slices from TRIO+/- mice exhibit defects in dendrite structure and synaptic structure, transmission, and plasticity. We will apply our battery of quantitative cellular, anatomical, and electrophysiological assays to probe whether validated activators from the initial screen can remediate structural and functional defects in TRIO+/- neurons. Active hits from Aim 2 will ultimately be advanced into a drug discovery cascade for advancement toward a clinical candidate in future studies.

Nicholas Leeper, M.D. - 2018 Awardee

Surgery
Stanford University

"Precision Nanotherapies for Cardiovascular Disease"

Atherosclerosis is the process underlying heart attack and stroke. Despite recent advances, atherosclerotic cardiovascular disease (CVD) remains the leading cause of death in the United States. Most current therapies are directed against cardiovascular risk factors (such as hypertension and elevated cholesterol levels). However, much of the population's risk of developing disease occurs independently of traditional risk factors. Therapies that directly target the plaque would instead address the root cause of disease and have the potential to fundamentally transform how CVD is treated.

A characteristic feature of the atherosclerotic plaque is the pathological accumulation of diseased and dying cells in the necrotic core. We discovered that this phenomenon is driven by the marked upregulation of a key 'don't eat me' molecule known as CD47. This renders vascular cells 'inedible' and resistant to 'efferocytosis' (programmed cell removal). We showed that systemic delivery of anti-CD47 antibodies (Ab) could reactivate efferocytosis within the lesion, thus dramatically reducing plaque development and vascular inflammation. However, systemic antibody-based therapy also caused off-target clearance of red blood cells. This induces an anemia which represents a critical roadblock in the translation of our findings into the clinic for CVD patients.

The broad, long-term objective of our Falk Catalyst proposal is to overcome this current roadblock by developing a 'precision' nanotherapy that delivers anti-CD47 therapy specifically to the plaque. We are collaborating with experts in nanomedicine, bioengineering, and immune cell biology to develop a vascular-tropic nanoparticle that homes to the inflamed macrophage and reactivates phagocytosis in the plaque, thereby preventing atherosclerosis without off-target toxicity. The aims outlined in this Catalyst Award proposal will determine whether our pro-efferocytic nanoparticles specifically accumulate in the macrophage-rich plaque and prevent atherosclerosis (Aim 1, Efficacy), and validate that this approach effectively avoids the off-target clearance of healthy tissue seen with systemic anti-CD47 therapy (Aim 2, Safety). Our proposal stems from our major innovative discovery and promising preliminary data, with the long-term goal of developing an efferocytosis-activating 'precision' therapy for patients who suffer from atherosclerotic CVD. The results of these studies will allow us to move towards translating our discovery into humans during the Transformational Award phase, and form the basis of a completely new strategy to prevent or even cure the leading cause of death in the United States.

Gye Young Park, M.D. - 2018 Awardee

Medicine
University of Illinois College of Medicine at Chicago

"A New Strategy of Asthma Treatment Using a Dendritic Cell-Centered Approach to Prevent Allergen Sensitization"

Despite current medical treatment, a large number of patients with asthma develop chronic refractory asthma with long-term disability. Innovative approaches for treatment of asthma are needed to reduce the economic and social burden. People become asthmatic through the process known as 'sensitization' against specific allergens such as house dust mites. Dendritic cells (DCs) play a key role in this sensitization process. DCs are able to recognize allergens, and present the immune determinants of an allergen to T and B cells to generate immunoglobulin E (IgE). Thus, the allergen-reactive IgE is a hallmark for systemic sensitization of the allergen. Upon re-exposure to the already sensitized allergens, DCs capture the re-entered allergen and present the allergen to memory T and B cells, which induce a robust allergic inflammation resulting in the clinical symptoms of asthma. This indicates that DCs play an important role not only in the initial sensitization, but also exacerbation of asthma in already sensitized individuals. Intervention of DC function in this process could create a novel therapeutic opportunity for treating asthma at very early steps of allergic inflammation by blocking allergen sensitization. We found that the allergen-exposed airway epithelial cells secrete Colony Stimulating Factor-1 (CSF1) into airways where they bind to its receptor (CSF1R) on recruited dendritic cells in airways. The binding between CSF1 (ligand) and CSF1R (receptor) enhances the expression of CCR7 on DCs. CCR7 is the key molecule for DC migration and subsequent antigen presentation. We also confirmed that the small molecule inhibitor of CSF1R successfully inhibited CCR7 expression on DCs and subsequent specific IgE production. Based on this novel immunologic mechanism of allergen sensitization, we hypothesized that the inhibition of the CSF1-CSF1R pathway has a therapeutic benefit for asthma treatment. To examine the effectiveness of CSF1R inhibitor in the treatment of asthma, we have synthesized the encapsulated nanoparticle carrying CSF1R inhibitors. This particle can be delivered to the lung via inhalation or intranasal delivery. Before moving forward with a clinical trial, we will examine the effectiveness of the nanoparticle of CSF1R inhibitor and look for possible adverse reactions in an animal model of asthma. This proposal is based on our strong scientific premise that the CSF1-CSF1R pathway plays a critical role in allergen sensitization and development of subsequent allergic inflammation. The proposed experiment will set the groundwork for the potential use of CSF1R inhibitor in clinical practice.

Aaron Ring, M.D., Ph.D. - 2018 Awardee

Immunobiology
Yale University

"Breaking the Vicious Cycle of Necroptosis and Inflammation with a Therapeutic "Molecular Shroud""

Sepsis is a syndrome characterized by a maladaptive immune response to infection that leads to hypotension, organ dysfunction, and death. It is a common disease with an exceedingly high fatality rate and was deemed a global health priority by the World Health Organization in 2017. Despite decades of clinical and basic science research, there are no effective treatment options for sepsis other than early administration of antibiotics and supportive care. A growing body of research has illuminated the critical role of the inflammation-induced programmed cell death pathway necroptosis in the pathogenesis of sepsis. Necroptotic cell corpses are inflammatory, particularly towards myeloid cells, which can drive a vicious cycle of increased myeloid cell activation, cytokine production, and further necroptosis that sustains sepsis pathophysiology. However, the mechanism by which necroptotic corpses promote and sustain inflammation remains unresolved. To address this question, we recently identified a family of cell surface receptors that specifically bind to the surface of necroptotic cells, but not viable or apoptotic cells. To interrogate the function of these receptors and their potential contribution to the pathophysiology of sepsis, we generated recombinant decoy proteins as pharmacologic antagonists of the receptors. In vitro, these antagonists attenuated the effect of necroptotic cells to activate inflammatory signaling in macrophages. Furthermore, these antagonists dramatically improved survival in an in vivo experimental endotoxemia sepsis model. This effect was evident even when the drugs were administered well after the acute inflammatory phase; by contrast, most other experimental agents are ineffective at this time point and work only when given before or immediately after the initiation of sepsis. Thus, we have uncovered a new therapeutic strategy to break the "vicious cycle" of inflammation in sepsis by "shrouding" necroptotic corpses from immune recognition with novel therapeutic candidate drugs. The goal of our proposal is to understand the biology of these molecular sensors of necroptotic corpses, to identify their ligand(s), to understand how our agents protect animals from sepsis, and to conduct key proof-of-concept studies of the efficacy of the proteins in preparation for an Investigational New Drug (IND) application to enable clinical trials. Our efforts will therefore shed new light on the biological mechanisms of sepsis and will lay the foundation for transformative therapeutic approaches for this deadly disease.

Arun Sharma, Ph.D. - 2018 Awardee

Surgery
Ann & Robert H. Lurie Children's Hospital of Chicago

"Supramolecular Nanomolecules Abrogate Inflammation In A Mouse Model Of Ulcerative Colitis"

Approximately 900,000 people in the United States are affected by ulcerative colitis (UC) and patient numbers increase yearly. While its etiology is unknown, symptoms of UC typically include chronic inflammation of the large intestines and are accompanied by numerous complications. Medical costs range from $4-14 billion annually within the United States and treatments range from oral medications to injectable agents that circulate widely throughout the body. As these treatments eventually fail, surgery is performed to remove parts of the diseased tissue. One-third of those with UC will require surgery during their lifetime, drastically affecting quality of life. The current regimen of injectable drugs to treat UC has multiple side-effects, including increased risk of certain cancers. Hence, there is still an important unmet clinical need for safe and effective treatments. Our proposed innovation is to engineer potent anti-inflammatory agents in the form of supramolecular anti-inflammatory peptide amphiphiles (AIF-PAs). The AIF-PAs will target inflamed areas in the lower gastrointestinal tract so that a high proportion of the treatment goes directly to the affected area. Hence, we propose that the non-invasive delivery of AIF-PAs will attenuate inflammation in a pre-clinical mouse model of UC that closely mimics the human condition while simultaneously promoting would healing. In order to validate our hypothesis, we will first synthesize, characterize, and optimize supramolecular AIF-PAs that can be targeted to the site of lower gastrointestinal tract inflammation and deliver effective anti-inflammatory agents (Specific Aim 1, months 1-3). We will then evaluate the efficacy of AIF-PAs to inhibit lower gastrointestinal tract inflammation using a targeted delivery platform in an established mouse model of UC (Specific Aim 2, months 4-12). Initial data gleaned from these studies will set the foundation to initiate clinical studies that will meet our long-term objectives. The ultimate goal of this study is to create a product that can be successfully utilized in the clinical arena to treat those afflicted with varying degrees of UC. In order to meet this criteria, our AIF-PAs, in-part, need to be: 1) able to reduce lower gastrointestinal inflammation with minimal dosing, 2) synthetically reproducible on a large scale at clinical grade levels for patient delivery, 3) non-immunogenic and non-toxic-, 4) biodegradable-, and 5) biocompatible-in humans. These issues need to be addressed in subsequent years of funding.

Anita Shukla, Ph.D. - 2018 Awardee

School of Engineering
Brown University

"Combating the Global Threat of Antibiotic Resistance with Bacteria-Triggered Biomaterials"

Antibiotic resistance is a growing health threat worldwide. Although microbes evolve resistance mechanisms inherently, resistance can be exacerbated by prophylactic and systemic antibiotic administration and lengthy exposures to antibiotics at sub-inhibitory concentrations. Localized drug delivery has the potential to provide rapid antibacterial therapy at the site of an infection, while preventing offsite toxicity and reducing susceptibility to resistance. However, most currently existing local antibiotic delivery devices are plagued by low drug loadings and release below inhibitory concentrations. We propose to develop a library of bacteria-responsive polymeric building blocks that can be used to formulate biomaterials for triggered antibiotic delivery at the site of an infection. Responsive biomaterials undergo a change in properties under specific stimuli, which can be coupled with the release of encapsulated therapeutics. Existing infection responsive materials rely on non-specific triggers, such as pH, to trigger drug release. In the proposed work, we will utilize beta-lactamases, which are bacteria produced enzymes, to serve as the bacteria-specific drug release trigger. Beta-lactamases hydrolyze the beta-lactam ring in beta-lactam antibiotics (among the most commonly prescribed drugs in the world) and are the most prevalent cause of resistance to these antibiotics. They are produced by many bacteria including the ESKAPE pathogens (Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa, and Enterobacter species) that cause nosocomial infections and commonly develop resistance. In the first aim of this project, we will synthesize polymer-beta-lactam conjugates using previously developed beta-lactam molecules. Synthetic and naturally-derived biocompatible polymers including poly(ethylene glycol), alginate, hyaluronic acid, and gellan will be explored. This polymer-beta-lactam library will be thoroughly characterized for beta-lactamase triggered degradation. In the second aim, we will develop a range of bacteria-responsive biomaterials using these building blocks. The materials will vary in size from nanoparticles for injectable therapies to micro-scale self-assembled films for device coatings to macro-scale hydrogels for antibacterial wound dressings. Antibiotics effective against ESKAPE pathogens will be incorporated into these materials. Biomaterial physical properties and beta-lactamase responsiveness will be assessed. In the final aim, we will investigate the in vitro antibacterial efficacy of these responsive materials against the ESKAPE pathogens. Results from this study will provide a strong basis for application to the Falk Transformational Awards program, in which we will optimize these materials for use in preclinical infection models, examine in vivo efficacy and biocompatibility, and evaluate antibiotic resistance development in the presence of these materials.

John Tilton, M.D. - 2018 Awardee

Department of Nutrition
Case Western Reserve University

"Treatments and Cures for Genetic Diseases Caused by Premature Termination Codons"

Premature termination codons (PTCs) are genetic mutations that result in truncated and inactive proteins. The World Health Organization estimates that over 10,000 human diseases are caused by mutations within a single gene, and 10-25% of these are believed to be the caused by PTCs. While individually rare, collectively genetic diseases caused by PTCs affect many patients and their families and represent a significant burden to the health care system. Many of these diseases result in severe developmental or neurological defects or are lethal in infancy and early childhood, and almost none have effective treatments or cures. Several treatment and curative modalities for PTCs have been proposed. Suppressor tRNAs are engineered tRNAs that recognize the termination codon but add the appropriate amino acid instead of terminating the chain, enabling read-through of the PTC and the producing the functional protein. CRISPR-Cas9 guided single base editors can change individual base pairs within DNA, correcting the underlying mutations and potentially curing these diseases. Both approaches have been effective in vitro but are limited by the lack of an in vivo-compatible platform to deliver these therapies to tissues within patients. The objective of this study is to determine whether our novel, rationally engineered nanoscale PrOtein Delivery (nanoPOD) platform can deliver suppressor tRNAs (Aim 1) or CRISPR-Cas9 guided single base editors (Aim 2) in vivo and enable read-through or correction of PTCs. We will employ a powerful mouse model that contains a DNA sequence – including a PTC – from patients suffering from glycogen storage disease type 1a (GSD1a) upstream of red-shifted luciferase (akaLuc) and fluorescent reporter (Venus) proteins. If read-through or correction of the patient-derived PTC occurs, these enzymes are produced and are detectable using whole-animal bioluminescence or cryofluorescence imaging, respectively, allowing precise determination of on- and off-target delivery of our therapeutics. We will also characterize the kinetics and duration of treatment as well as monitor toxicity and immunogenicity of the nanoPOD platform. Finally, in Aim 3, we will develop a transgenic mouse model of GSD1a that incorporates the patient-derived PTC that will be used to evaluate the efficacy of our interventions in vivo to prepare for a potential Falk Transformational Award. These animals will be shared with the research community to stimulate research into GSD1a treatments and cures. The successful completion of this will lay the foundation for transformative treatments and cures for GSD1a and other devastating genetic diseases caused by PTCs.

Wei Xu, Ph.D. - 2018 Awardee

Oncology
University of Wisconsin-Madison

"Development of Novel Agents for Treatment of Endocrine Resistant Breast Cancer"

Approximately 70% of breast cancers express estrogen receptor alpha (ERα) and are, therefore, treated with endocrine therapies. Although many patients benefit from Tamoxifen or aromatase inhibitors (AIs) in adjuvant and metastatic settings, approximately 50% of responsive tumors eventually relapse due to the development of resistance. One emerging mechanism of resistance is the clonal evolution of mutations in a "hotspot" within the ligand-binding domain (LBD) of ESR1, the gene encoding ERα. ESR1 mutations occur in 10-20% of patients with metastatic ERα-positive disease who received endocrine therapies. The mutations led to ligand-independent ERα activity that promotes tumor growth and metastasis, and reduced efficacy of ERα antagonists resulting in endocrine resistance. The mutant ERα proteins are also more resistant to selective estrogen receptor degraders (SERDs) such as faslodex. AZD9496 and GDC-0810, two newly developed, orally bioavailable selective estrogen receptor degraders (SERDs) are in clinical trials. However, both displayed mild estrogen activity in endometrial cells and increased uteri weight in rat models, raising the concern that the mixed agonist/antagonist activity may increase risk of endometrial cancer. Thus, our goal is to identify a new class of ERα blocker to treat metastatic, ERα-expressing breast cancer.

This study builds on our recent discovery of a natural plant product, Diptoindonesin G (Dip G) that significantly decreases ERα protein levels and, importantly, is insensitive to ESR1 mutations. We showed that Dip G acts via a mechanism distinct from all known endocrine-therapy agents. Instead of binding to ERα, the direct target of Dip G is CHIP/STUB1, an E3 ubiquitin ligase that controls ERα stability. Consequently, Dip G can degrade mutant ERα more effectively than faslodex within the therapeutic window. We hypothesize that Dip G and its analogues will be effective for treating mutant ERα expressing, endocrine-resistant tumors. This application will focus on the synthesis of novel Dip G analogues to determine the basic pharmacophore of Dip G and improve the potency and pharmacological properties of Dip G (Aim 1), which will be performed by co-I Dr. Weiping Tang (School of Pharmacy, UW-Madison). The mechanism of action of Dip G will be studied (Aim 2) and the anti-cancer effects of Dip G and its analogues will be evaluated in endocrine-resistant models and compared with other SERDs (Aim 3). By targeting mutant ERα for degradation, these novel therapeutic agents will help reversing endocrine-resistance and provide a cure to metastatic patients.

Emad Alnemri, Ph.D. - 2017 Awardee

Biochemistry and Molecular Biology
Thomas Jefferson University

"Role of DFNA5 in the Anti-Tumor Immune Response"

Tumor immunogenicity, defined as the ability of the tumor itself to trigger an anti-tumor adaptive immune response, is one of the most important determinants of successful anti-cancer therapy. The immunogenicity of a tumor depends on its antigenicity, conferred by neo-antigens generated in the course of oncogenesis, and adjuvanticity, the latter provided by damage-associated molecular patterns (DAMPs) released from stressed or dying tumor cells by a process called immunogenic cell death (ICD). Corroborating the importance of ICD in the treatment of cancer, traditional chemo- or radiotherapies that induce ICD in tumors yield a far superior and durable response to those therapies that don't. In addition, combinatorial strategies that boost the release of DAMPs from dying tumor cells can efficiently convert non-immunogenic cell death into a bona fide ICD. Nevertheless, despite these important insights into the key role of ICD in tumor immunogenicity, there is still much to learn about the signaling and molecular pathways that induce or modulate ICD. Hence, a better understanding of the mechanistic aspects of ICD and the signaling pathways that regulate it can help us better predict the responsiveness of certain tumors to different therapies as well as reveal candidate molecular targets to enhance ICD of tumor cells and aid in cancer treatment. We have recently discovered a signaling pathway that switches apoptotic cell death into a necrotic form of cell death, allowing efficient release of DAMPs from apoptotic cells. We discovered that activation of caspase-3 during apoptosis in transformed and non-transformed cells leads to caspase-3-mediated cleavage of the gasdermin protein DFNA5 after Asp270 generating a necrotic pore-forming DFNA5-N fragment that targets the plasma membrane to induce its permeabilization. Creation of these plasma membrane pores by the necrotic DFNA5-N fragment allows the release of intracellular DAMPs such as HMGB1, DNA, and ATP. The ability of these events to switch apoptosis into a potentially immunogenic form of cell death demonstrates a novel mechanism that could explain how immunogenicity of tumor cells arise. Therefore, the experimental plan outlined in this Catalyst Award proposal will test the novel concept that the caspase-3/DFNA5 pathway constitutes one of the major checkpoints determining the immunogenicity of cancer cells. Elucidation of the role of this pathway in the regulation of tumor immunogenicity will provide a solid rationale for a Falk Transformational Award that will allow us to exploit DFNA5 as a biomarker and a target to enhance anti-cancer therapies.

Ernesto Bongarzone, Ph.D. - 2017 Awardee

Anatomy and Cell Biology
University of Illinois College of Medicine

"Promoting Remyelination in Multiple Scerosis by Delivering Therapeutic MicroRNAs Via Oligodendrocyte-Targeting Microvesicles"

Current therapies reduce the frequency and magnitude of immune attacks in multiple sclerosis (MS) but do not stimulate remyelination. Lack of remyelinating therapies is one of the major roadblocks to treat MS patients. Although the CNS contains abundant oligodendrocyte progenitors (OPCs) capable to differentiate in myelinating oligodendrocytes, most OPCs fail to remyelinate during MS. At least two factors contribute to this failure: inadequate stimuli to promote differentiation and abnormal expression of myelinating-inhibitory signals in demyelinated axons. Although numerous mechanisms regulating OPC differentiation are known, their translation into remyelinating therapies has not been achieved yet.
Our project to the Falk Foundation will address the current roadblock in remyelination therapies by taking advantage of our successful experience with promyelinating microRNAs and our experience with extracellular microvesicles (EVs). Various micro-RNAs (i.e. miR-219) have potent proremyelinating properties, constituting excellent candidates for therapy. The main difficulty is their delivery to OPCs in the CNS during disease, with minimal off-target. EVs are ideal vehicles for CNS therapy because they can be loaded with different therapeutic biologicals, elicit limited or none toxicity and immunogenicity, and are taken up by most cells.

To test our hypothesis that receptor-mediated endocytosis of EVs loaded with proremyelinating miRs can enhance remyelination in MS, our catalyst project will design and optimize a cell-free delivery system to selectively target OPCs to improve the delivery of miRs. We will produce bone-marrow derived mesenchymal stem cells engineered to produce EVs which express surface recognition proteins with high affinity binding to PDGFrα receptors in OPCs. We have chosen PDGFrα receptors as our target receptor for endocytosis of EVs because PDGFrα is highly expressed in OPCs, the target cells for remyelination and because we have identified a set of small peptides that bind to this receptor in OPCs. We show that one of these peptides (OL-2) have the highest selectivity for OPC binding, with minimal binding to other glial cells such as astrocytes and microglia. Our catalytic proposal stems from strong preliminary data, with a defined target for surface recognition, and consolidated methods to deliver a measurable product: EVs targeting OPCs.

Upon generation of effective OPC-targeting EVs and optimization of conditions for proremyelinating miR loading, the subsequent transformative phase will determine the therapeutic effects of single vs combined miRs to promote remyelination in EAE via intrathecal injections of OPC-targeting EVs. Achievement of the transformative award will produce measurable deliverables ready for consideration for IND and clinical trials.

Mark Kay, M.D., Ph.D. - 2017 Awardee

Pediatrics
Stanford University

"Enhancement of Therapeutic AAV-Mediated Gene Targeting Without Nucleases"

The overarching goal of this project is to develop a small molecule inhibitor that can transiently increase recombinant adeno-associated virus (rAAV)-mediated homologous recombination-(HR) levels such that a single-dose administration in neonates or adults can treat individuals suffering from life-threatening metabolic disorders.

Even though classical rAAV vectors show early promise in the clinic, there are still limitations: (1) Inability to get life-long persistence in neonates/infants/children from any growing tissue such as the liver. (2) rAAV-vector administered into neonatal mice results in high rates (>50%) of hepatocellular carcinoma because rare integration events activated a proto-oncogenic because of the strong promoter used to drive the transgene. Additionally, nuclease-mediated rAAV approaches are being considered for in vivo genome editing. This presents several problems: (1) Multiple rAAVs are required to transduce the same cell. (2) No means to limit expression or integration of the vector containing the promoter driven nuclease. (3) Off-target cutting, immunogenicity and/or insertional mutagenic risks remain.

We developed a new technology for HR that overcome the problems cited. Our nuclease-free, promoterless AAV-mediated HR technology, named GeneRideR, uses a vector containing a ribosome skipping sequence and therapeutic protein coding sequence flanked by homology arms to an endogenous gene. After HR, transcription from the endogenous gene locus produces a chimeric mRNA producing both the endogenous and therapeutic protein. This technique has been used to treat three mouse models of human genetic liver diseases. However for many diseases, the threshold for correction will require higher rates of HR. We have recently identified 20 genes that when knocked out enhance rAAV-mediated HR. Independent validation for one of these genes confirmed that in its absence, AAV-HR is 10-fold higher. We propose to: (1)continue to validate the other genes identified in cells and mice, and (2)plan a small molecule screening strategy to identify small molecules that can be used to temporarily increase AAV-mediated HR. With as little as a 5-fold increase in HR, this technology becomes widely applicable to hundreds of diseases for which there is no current treatment.

Our ultimate goal is to make the GeneRideR technology a universal plug-play therapeutic that can be used to treat a myriad of genetic diseases by simply changing the therapeutic protein coding sequence in the vector. The major advantages of this tactic compared to current gene therapy/editing approaches is that it has the potential for single administration lifelong cure in neonates, or adults, and mitigates current concerns for vector-induced cancers.

Ari Melnick, M.D. - 2017 Awardee

Medicine/Hematology & Medical Oncology
Weill Medical College of Cornell University

"SIRT3 as a Therapeutic Target for Therapy Eesistant B-cell Lymphomas"

Therapy resistance remains one of the most important challenges in treating patients with aggressive B-cell lymphomas. Yet little is known about the specific mechanisms that cause chemo-immunotherapy resistance in patients. Through a rationally designed screening strategy our research identified SIRT3 as a critical driver of these resistant forms of DLBCL. SIRT3 mRNA and protein expression is highly significantly and reproducibly linked to inferior outcome in DLBCL, independent of all other known risk factors including IPI, cell of origin, double hit, etc. We showed that whereas SIRT3 does not play a role in normal B-cell development, loss of SIRT3 in mice is required for B-cells to undergo malignant transformation. Our preliminary data show that SIRT3 loss of function causes proliferation arrest and cell death in almost all DLBCL cell lines. SIRT3 is also required for human DLBCL cells to engraft and form tumors in mice. Investigation into mechanism of action show that a crucial function of SIRT3 in DLBCL cells is to maintain a constant flow of Acetyl CoA to the cytoplasm, to maintain various anabolic synthetic pathways. Another key function appears to be control of oxidative stress response. Loss of SIRT3 in DLBCL cells causes them to literally "die of starvation", manifested by massively increased autophagy, depletion of metabolic precursors, and other features. Using a rational approach we designed small molecules that selectively inhibit mitochondrial SIRT3. These molecules exert identical effects to the genetic loss of function of SIRT3. Collectively, these data lead us to hypothesize that i) SIRT3 is a critical mediator of chemotherapy resistant DLBCL, ii) that SIRT3 mediates these effects by maintaining unique aspects of DLBCL metabolism and enabling these cells to tolerate extreme metabolic and oxidative stress characteristic of these highly proliferative tumors, iii) by the same token these features enable DLBCLs to tolerate exposure to chemo-immunotherapy, iv) SIRT3 targeted therapy will potently suppress the most aggressive and resistant forms of DLBCL and v) SIRT3 expression and metabolic profiles will serve as a useful biomarker to guide SIRT3 inhibitor deployment to the clinic. The aims of our TRP will address these various points and lead to the clinical translation of an entirely novel therapeutic concept specifically geared to those patients that most urgently need improved therapies.

Mark Mitton-Fry, Ph.D. - 2017 Awardee

Medicinal Chemistry and Pharmacognosy
The Ohio State University

"Novel Therapies For MRSA and Innovative Methods For Tackling Bacterial Resistance"

Our long-term goal is to deliver Novel Bacterial Type II Topoisomerase Inhibitors (NBTIs) into clinical trials for multidrug-resistant pathogens. This application proposes the design and synthesis of NBTIs targeting MRSA with a diminished resistance potential. Our central hypothesis is that potent dual inhibition of gyrase and TopoIV will reduce the spontaneous resistance rate and that a novel linker moiety will afford improved cardiovascular safety. Our synthetic route delivers new analogs in six steps, with structural diversification in the final step. Our rationale is that the synthetic efficiency and superior physicochemical properties will enable optimal dual target inhibition while preserving cardiovascular safety. These efforts will be guided by innovative x-ray crystallographic and computational homology models.

1) Design and synthesize potent dual inhibitors with reduced cardiovascular safety liability
Our working hypothesis is that optimization of the enzyme-binding moiety will improve TopoIV inhibition. Twelve analogs will be synthesized initially. Subsequent cycles of design and synthesis, guided by results from the Aims below, seek ten-fold improvements in the TopoIV/gyrase ratio of our lead compound and in the spontaneous resistance frequency. We hypothesize that cardiovascular safety issues are primarily driven through hERG inhibition and that the lower basicity and lipophilicity of the linker will minimize hERG inhibition.
2) Quantify anti-MRSA potency, propensity for resistance, and safety
Previous research supports our working hypothesis that dual inhibition will reduce spontaneous resistance and improve activity against MRSA with mutated DNA gyrase. Gyrase and TopoIV inhibition will be measured for each compound alongside Staphylococcus aureus minimum inhibitory concentrations (MICs). Spontaneous mutation frequencies will be determined for our lead compound and for an optimized analog with superior dual target inhibition. MICs against a lab-generated mutant S. aureus with resistance to our lead will be used to assess whether superior dual inhibition translates to improved whole cell mutant activity. hERG inhibition and mammalian cytotoxicity (K562 cells) will be determined for prioritized compounds.
3) Optimize TopoIV inhibition guided by computational and structural methods
Our working hypothesis is that molecular-level understanding of target binding will enable optimized target inhibition. We will determine x-ray crystal structures of NBTIs in ternary complex with DNA and gyrase as proof of feasibility, and we will express TopoIV enzyme in preparation for future crystallography efforts. We will build a homology model of the NBTI, TopoIV, and DNA ternary complex as a complementary approach and use this model to inform the rational design of analogs with improved TopoIV inhibition.

Harikrishna Nakshatri, Ph.D., B.V.Sc. - 2017 Awardee

Surgery
Indiana University Purdue

"The Effect of Extra Physiologic Oxygen Shock/stress (EPHOSS) on Cancer Stem Cell and Drug Sensitivity Measurements"

Preclinical studies of primary cancer cells are done after cells are removed from patients or animals at ambient atmospheric oxygen (~21%) yet, oxygen concentrations in organs are in the ~3-10% range, with most tumors in an hypoxic environment in vivo. While effects of oxygen tension on tumor cell characteristics in vitro have been studied, it is only after the cells were first collected in ambient air. Dr. Broxmeyer's lab recently showed that hematopoietic stem cells exposed to ambient air within minutes undergo irreversible differentiation through a phenomenon termed extra physiologic oxygen shock/stress (EPHOSS). Therefore, results of many stem cell-related studies likely need to be interpreted with caution, and re-evaluated because the cells were first collected and processed/propagated under ambient air. With growing interest in cancer stem cells (CSC), it is crucial to determine whether current methods of collecting/studying tumor cells in ambient air influence numbers, gene expression profiles, and drug sensitivity of CSCs in tumors due to EPHOSS during tissue collection. We will address this important question using breast cancer animal models and patient-derived samples, particularly focusing on metastatic cancer cells. Studies using properly collected and processed metastatic cells are needed. Recent studies showed that metastases depend on signaling networks distinct from those of primary tumors, due to independent evolution. Drugs effective against primary tumors may be ineffective against metastases. We observed that mammary tumors from MMTV-PyMT mice collected and propagated under 3% oxygen manifest more CD61+ tumor cells compared to tumors of the same mice propagated under ambient air. CD61+ mammary tumor cells have 50-fold higher CSC activity due to enhanced CD61-KRAS-RalB-NF-κB signaling, and are resistant to receptor tyrosine kinase inhibitors (RTKis). Aim 1 will functionally characterize tumor cells collected and propagated at 3% oxygen vs. ambient air for CSC activity, gene expression profiles, and sensitivity to RTKis and chemotherapy. Aim 2 will determine whether metastatic tumor cells from patient pleural effusions and ascites fluid with and without EPHOSS show distinct CSC properties and drug sensitivity using our recently modified primary cell culturing system. Our results could provide paradigm-shifting information in the CSC field for drug-screening efforts, and provide a more rational basis to change tissue collection procedures for a truer understanding of in vivo functional characteristics of CSC/metastatic tumor cells.

Xin Qi, Ph.D. - 2017 Awardee

Physiology & Biophysics
Case Western Reserve University

"Identification of Enhancers of Mitochondrial Function as Candidate Therapeutics of Huntington's Disease"

Mitochondrial dysfunction is an early prominent feature in patients with neurodegenerative diseases such as Alzheimer's (AD), Parkinson's (PD) and Huntington's disease (HD). Significantly, we recently reported in vitro and in vivo proof of concept that suppression of mitochondrial impairment is a useful strategy for treatment in models of these diseases. We demonstrated that improving either impaired mitochondrial dynamics or aberrant mitophagy with rationally designed peptides was protective in both neurons derived from patient induced pluripotent stem cells (iPSCs) and mouse models of these diseases. Because peptides often face challenges during drug development, we sought small molecules that increase mitochondrial function as a new therapeutic approach. The objective of this study is to optimize an existing lead molecule to enable deeper in vivo evaluation of the hypothesis that enhancing mitochondrial efficacy is a novel therapeutic strategy for neurodegenerative disease. We have recently developed and validated a series of cell-based assays in 384-well format to identify small molecules that reduce mitochondrial depolarization and bioenergetic failure as well as cell death. We focus on HD that is a fatal and inherited neurodegenerative disease with no treatment available, as a disease model. High-throughput screening identified a number of molecules, including the GSK3alpha/beta inhibitor CHIR99021, as enhancing mitochondrial function and cell viability in an HD culture model. Preliminary in vivo studies found that CHIR99021 reduces neuronal loss, behavioral deficits and animal lethality in an HD R6/2 transgenic mouse line. Notably, past reports cast doubt on GSK3 as a drug target in HD, and knockdown of GSK3alpha and GSK3beta is insufficient to enhance mitochondrial function or block cell death. Among 12 GSK3 inhibitors evaluated in dose in our in vitro assays, only one other scaffold (AZD1080) is effective. These results suggest that targets beyond GSK3 likely contribute to the phenotypes seen in vivo and have led us to formulate the hypothesis that CHIR99021 that stimulates mitochondrial activity without targeting GSK3 could have therapeutic benefit for HD. In Aim 1, we will identify molecular targets beyond GSK3 that mediate the effects of AZD1080 and CHIR99021 in HD models. In Aim 2, we will assess efficacy of CHIR99021 in both HD patient neurons and a chronic HD mouse model. The successful completion of our studies will validate our approach of enhancing mitochondrial efficacy and provide a chemical lead for further drug development toward novel therapeutics for HD and a wider range of neurological disorders marked by dysfunctional mitochondria.

Eric Shusta, Ph.D. - 2017 Awardee

Chemical and Biological Engineering and Department of Neurological Surgery
University of Wisconsin-Madison

"A New Strategy for Brain Cancer Immunotherapy"

The healthy blood-brain barrier (BBB) physically separates the central nervous system from the bloodstream and prevents effective delivery of many therapies. However, under certain pathological conditions such as brain cancer, the BBB is disrupted such that brain cells and surrounding extracellular matrix (ECM) are exposed to the systemic circulation. For the incurable brain cancer, glioblastoma (GBM), core tumor regions often enhance on magnetic resonance imaging (MRI) due to contrast permeability across disrupted BBB, but the invasive tumor margin remains behind an intact BBB. Such BBB heterogeneity has made GBM very difficult to treat, especially to eradicate therapeutically resistant GBM cells in the invasive margin likely responsible for recurrence. To address this issue, we propose an immunotherapy that first targets BBB disruption and accumulates at tumor sites of exposed brain ECM. Then, an immunogenic peptide capable of spreading to the invasive margins is released to generate a cytotoxic T cell response throughout the tumor volume.

To generate this therapeutic platform, we use a family of antibody-like targeting molecules called Variable Lymphocyte Receptors (VLRs). VLRs are lamprey antigen receptors that are particularly adept at binding glycosylated structures and therefore well-suited for targeting glycosylation-rich brain ECM. Using state-of-the-art VLR screening paradigms, we have identified multiple VLRs that preferentially bind brain ECM. After intravenous injection into tumor-bearing mice, the VLRs home to BBB permeability sites, indicating a capacity to specifically deliver GBM therapy. In this proposal, the VLRs will be fused with immunogenic peptides (VLR-IPs) that can be activated proteolytically by the GBM microenvironment, enabling IP spread throughout the tumor volume to mediate a cytotoxic T cell response. To validate the VLR-IP platform, we will first test the capability for the VLR-IPs to activate T cells in vitro, using both human and murine lymphocyte cultures. Next, the VLR-IPs will be administered to mice bearing an orthotopic, syngeneic murine GBM and therapeutic efficacy determined. The proposed work will therefore evaluate the potential of the VLR-IP platform as an innovative GBM treatment and motivate further detailed translational studies. If successful, ECM targeting of therapeutic payloads would also become a viable approach in many other neurological diseases that exhibit BBB disruption including stroke, traumatic brain injury and multiple sclerosis.

Stephen Strittmatter, M.D., Ph.D. - 2017 Awardee

CNNR, BCMM 436295 Congress Avenue
Yale School of Medicine

"Fyn Kinase Inhibition for the Tauopathy in Fronto-Temporal Dementia and Glaucoma"

Neurodegenerative diseases produce diverse symptoms, but share some molecular mechanisms. Pathology related to the misfolding, phosphorylation and accumulation of microtubule-associated protein Tau, has been observed in several diseases, including Alzheimer's, Fronto-Temporal Dementia and glaucoma. Our previous work has mapped a signaling pathway in Alzheimer's from Amyloid-ß oligomers to synapse loss through Fyn kinase, and Fyn inhibitors are being tested in a Phase 2 trial.

It is well known that Fyn associates with Tau, and our data show that Fyn inhibition reduces Tauopathy in Alzheimer's mice. Therefore, we propose that Fyn kinase inhibition may provide effective treatment for other Tauopathies. Here, we will investigate Tau-dependent Fronto-Temporal Lobar Degeneration (FTLD-Tau) and glaucoma.

We will treat mice modeling FTLD-Tau with the Fyn inhibitor, AZD0530 (Saracatinib), at doses effective in Alzheimer's models. We will utilize two models, a transgenic strain over-expressing human mutant P301S Tau, and intracerebral injection of pathological Tau extracted from human autopsy brain. We will assess the ability of Fyn inhibition to reduce Tau pathology, synpase loss, cell degeneration and memory impairment, each of which is well documented in this strain. Critically, our Preliminary Data show that the drug eliminates memory dysfunction in this transgenic model. In addition, we seek to validate a role for Fyn by treating mice injected with human pathological Tau, and monitoring the extent of induced mouse Tau misfolding and the spreading of pathology. Together these data will determine whether Fyn inhibition generally, and AZD0530 particularly, is a therapeutic candidate for FTLD-Tau.

Vision loss in glaucoma is driven by increased intraocular pressure (IOP), and reducing IOP is the mainstay of therapy, though benefit is partial. There is a recognized unmet need for neuroprotective therapy to prevent progressive vision loss. It has been shown that glaucomatous retina exhibits Tauopathy, and one of our team members showed that silencing Tau expression in the eye eliminates ganglion cell loss. We propose that Fyn inhibition will reduce Tauopathy in the glaucomatous eye, thereby preserving function and cell number separately from IOP lowering by a neuroprotective mechanism. Importantly, our Preliminary Data reveal that retinal pattern ERG signals, which are reduced with elevated IOP, are maintained by Fyn inhibition in a glaucoma model. We propose to assess the ability of AZD0530 treatment to preserve retinal ganglion cell numbers and function in two glaucoma models. The outcomes will provide an assessment of whether Fyn inhibition might provide the first neuroprotective therapy for glaucoma.

Frits van Rhee, M.D., Ph.D. - 2017 Awardee

The Myeloma Institute
University of Arkansas College of Medicine

"Expanded Natural Killer Cell Therapy for High-Risk Multiple Myeloma"

Despite major advancements in progression-free and overall survival through the introduction of novel agents, relapse remains a major problem in high-risk multiple myeloma (HRMM). Our group is focused on the development of novel immunotherapeutic strategies for HRMM. In this innovative study, we will combine three highly-active agents: autologous expanded natural killer cells (auto-ENKs) that avidly kill myeloma targets, the anti-SLAMF7 antibody (ab) elotuzumab (Elo) which stimulates and redirects ENKs to myeloma, and the interleukin-15 superagonist ALT-803, a cytokine uniquely able to potently stimulate the expansion, activity, and persistence of natural killer cells. This regimen will be administered after auto-stem cell transplant (ASCT) to patients with previously-treated gene expression profile-defined HRMM in a FDA- and IRB- approved clinical trial. We safely treated HRMM patients in frank relapse with ENKs in a previous clinical trial, but found that the activation state of the infused ENKs was rapidly lost, likely due to suboptimal support provided by low dose IL2 and the suppressive effects of the bone marrow microenvironment (BM-ME). We have performed preliminary studies examining ways to enhance this approach. We found that Elo substantially enhances the activity of auto-ENKs, and ALT-803 extends the activation state and promotes the proliferative capacity of ENKs in vitro. We hypothesize that our combinatorial approach will maximize the efficacy of ENKs by 1) targeting the auto-ENK to myeloma using the antibody elotuzumab; 2) delivering therapy after ASCT, when the disease burden has been significantly reduced rendering the BM-ME more conducive to immune effectors; and 3) supporting transferred ENKs with ALT-803 rather than IL2. The principal endpoint will be response. The activation and persistence, anti-MM cytolytic ability, and homing of ENK will be assessed post-infusion by studying the peripheral blood and BM compartments. Effects of ALT-803 on the post-ASCT immune reconstitution of NK and T cells will be studied. We will also explore the potential adverse impact of the tumor ME on the ENKs to identify therapeutically actionable targets and evaluate the presence of immunosuppressive T regulatory cells. We hope that this combinatorial approach will transform NK immunotherapy and can be extended to other malignancies.