

**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”

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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 suffer 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”

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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 CBF $\beta$ -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  $\mu$ M IC<sub>50</sub>. 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”

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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”

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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”

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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 antidepressant 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 versus 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.

“Crafting New Weapons for the Fight Against Infectious Diseases”

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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”

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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 glyceemic 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”

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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”

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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”

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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”

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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”

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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 antenatal 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”

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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”

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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, Illinois Chapter*

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

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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”

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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<sup>+/-</sup> neurons. Cortical neurons and slices from TRIO<sup>+/-</sup> 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<sup>+/-</sup> 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”

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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”

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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””

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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”

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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 wound 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.

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

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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”

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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 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”

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Approximately 70% of breast cancers express estrogen receptor alpha (ER $\alpha$ ) 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 ER $\alpha$ . ESR1 mutations occur in 10-20% of patients with metastatic ER $\alpha$ -positive disease who received endocrine therapies. The mutations led to ligand-independent ER $\alpha$  activity that promotes tumor growth and metastasis, and reduced efficacy of ER $\alpha$  antagonists resulting in endocrine resistance. The mutant ER $\alpha$  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 ER $\alpha$  blocker to treat metastatic, ER $\alpha$ -expressing breast cancer.

This study builds on our recent discovery of a natural plant product, Diptoindonesin G (Dip G) that significantly decreases ER $\alpha$  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 ER $\alpha$ , the direct target of Dip G is CHIP/STUB1, an E3 ubiquitin ligase that controls ER $\alpha$  stability. Consequently, Dip G can degrade mutant ER $\alpha$  more effectively than faslodex within the therapeutic window. We hypothesize that Dip G and its analogues will be effective for treating mutant ER $\alpha$  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 ER $\alpha$  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”

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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 Sclerosis by Delivering Therapeutic MicroRNAs Via Oligodendrocyte-Targeting Microvesicles”

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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 PDGFR $\alpha$  receptors in OPCs. We have chosen PDGFR $\alpha$  receptors as our target receptor for endocytosis of EVs because PDGFR $\alpha$  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.

“Enhancement of Therapeutic AAV-Mediated Gene Targeting Without Nucleases”

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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 Resistant B-cell Lymphomas”**

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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”

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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.

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

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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- $\kappa$ B 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”

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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”

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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”

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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- $\beta$  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, synapse 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”

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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.

“Leveraging the microRNA Interactome for Cancer Drug Discovery”

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MicroRNAs (miRs) comprise a large family of small, ~21–23 nucleotide noncoding RNAs that have emerged as key post-transcriptional regulators of gene expression and act by silencing the translation of target mRNAs. To date, there are ~1,000 predicted human miRs believed to control the activity of >60% of all protein-coding genes. Not surprisingly, these small RNAs have been shown to play crucial roles in nearly all aspects of human biology from development to disease. In cancer, global down-regulation of tumor suppressor miRs (TS-miRs) is commonly observed and has been demonstrated to be a causative feature in tumorigenesis. Loss can stem from genetic mutation or deletion, promoter methylation or dysregulation of miR biogenesis. Of these mechanisms, alteration of global miR biogenesis is receiving increased attention due to recent findings demonstrating the critical role that miR-binding proteins (miR-BPs) play in the inhibition of this process, ultimately stimulating TS-miR degradation and cancer development. Current strategies for restoring TS-miR function rely on replacement therapy, whereby chemically-modified and formulated mature miR mimics are delivered to cells and tissues. Despite the promise of these agents in xenograft mouse models, similar to RNAi-based therapeutics, many liabilities exist. These include the requirement for chemical modifications to enhance *in vivo* pharmacokinetic/pharmacodynamic properties, hybridization-based off-target effects, toxicity, drug delivery problems and high cost. As such, no miR-targeted drugs have yet been approved for use. To overcome these challenges and provide a druggable and transformative solution for restoring TS-miR levels and conquering cancer, we propose to target miR–miR-BP interactions. Our overall goal is to discover and develop small molecule-based inhibitors of these interactions to prohibit the degradation of specific TS-miRs, thus providing the basis for next-generation miR-targeted therapeutics for cancer treatment. These proposed investigations are based on the hypothesis that regulatory miR-BPs specific for select miRs have been evolved as a quality control mechanism for controlling mature TS-miR levels, and this network is hijacked in cancer. If successful, these studies will catalyze the development of new therapeutics for the treatment of cancer, in addition to the identification of new miR–miR-BP targets for future drug discovery efforts. Our Specific Aims are as follows: (1) To develop a high-throughput screening (HTS) assay of the let-7–Lin28 miR–miR-BP interaction for small molecule inhibitor discovery, and (2) To develop a strategy for the discovery of miR-BPs using chemically-modified pre-miR probes.

Jeffrey Glenn, M.D., Ph.D. - 2016 Awardee

*Medicine*  
*Stanford University*

“Targeting RNA Secondary Structure--Towards a New Class of High Barrier to Resistance Antiviral Agents.”

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Our broad, long-term objectives are to develop a new class of antiviral therapeutics that target essential RNA secondary structures present in viral genomes, and that can be developed for all viruses for which no effective therapies exist.

As proof-of-concept, we will seek to target the influenza virus RNA secondary structure that we have recently discovered by SHAPE (selective 2'-hydroxyl acylation analyzed by primer extension) and demonstrated to be a universal influenza virus packaging signal--absolutely conserved in all viral isolates from 1918 epidemic flu to H1N1 and H5N1 bird flu--that mediates both packaging of the influenza virus genome as well as in vivo disease. In particular, we will test our hypothesis that potent pharmacological inhibitors of the virus' packaging and life cycle can be identified using SHAPE, which offers a dramatic means of overcoming the critical scientific roadblock of conveniently determining viral RNA secondary structure targets, and identifying molecules capable of disrupting those targets. Our specific aims are to: 1) Perform the first SHAPE-based small molecule screen, and identify small molecules capable of disrupting the RNA secondary structure of the influenza virus packaging signal; 2) Identify a candidate lead molecule by determining the anti-influenza virus activity of the most potent hits from the SHAPE screen; 3) Optimize the lead molecule by determining its structure-activity relationships (SAR) using medicinal chemistry to synthesize analogs and assessing their SHAPE and antiviral activity; 4) Nominate a pan-genotypic anti-influenza virus development candidate with the best therapeutic index.

Successful accomplishment of our specific aims will not only result in a novel universal pan-genotypic inhibitor of influenza virus with a high barrier to the development of resistance that can be developed for clinical use; the exact same approach can then also be undertaken to develop an exciting new class of drugs targeting virtually any virus of interest--thereby opening up new avenues for treating and curing a wide range of diseases.

**Zhiwei Hu, M.D., Ph.D.** - 2016 Awardee

*370 West 9th Avenue*

*The Ohio State University College of Medicine*

**“Targeting Tissue Factor as a Novel Oncotarget for Immunotherapy of Triple Negative Breast Cancer Using a Second Generation Icon”**

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Triple-negative breast cancer (TNBC), representing ~15% of breast cancer diagnosed across the globe, is considered as incurable malignancy. Due to the lack of surface markers for current targeted therapy, there are currently no molecularly targeted therapies approved for TNBC.

To eliminate the mortality associated with TNBC, there is an urgent need to discover novel surface markers for TNBC and develop corresponding targeted therapy for TNBC. We hypothesize that tissue factor (TF) is a novel surface marker and oncotarget in TNBC and that TF-targeted immunotherapy is effective for the treatment of TNBC. The rationale behind the hypothesis is that (i) our preliminary studies demonstrate that TF is over-expressed on TNBC cancer cells as well as the tumor neovasculature in over 85% of TNBC patients (n=14) when using standard paraffin-embedded tumor tissues or in nearly 60% of TNBC patients (n=157) when employing tissue microarray slides. Importantly, TF is not expressed on normal breast gland and quiescent endothelial cells of normal vasculature. Thus, TF is a novel surface marker in TNBC and targeting TF may constitute a novel targeted therapy for a majority of TNBC patients. (ii) Dr. Hu's prior work demonstrated that TF-targeted therapies are effective and safe in preclinical animal studies and clinical studies. He has co-invented and patented a TF-targeting Immuno-Conjugate agent named ICON that consists of factor VII (fVII, the natural ligand to TF) fused to the Fc region of human IgG1. As a neovascular-targeting agent, ICON has shown therapeutic efficacy and safety in preclinical animal models against solid cancers, macular degeneration (AMD) and endometriosis and in a Phase I trial for AMD patients. (iii) Dr. Hu has improved ICON by reducing 50% of molecular weight and completely eliminating its pro-coagulant effects yet with intact binding to TNBC, as a second-generation TF-targeting immunotherapy agent, called L-ICON. The major goal and specific aims of this proposal are to evaluate the novel L-ICON as single agent therapy and combination therapy with current immune modulatory agents (such as immune checkpoint inhibitors) and natural killer (NK) cells for TNBC. To ultimately translate the findings into clinic for TNBC patients, Dr. Hu is collaborating with breast cancer expert and clinical trialist, GMP manufacturing NK cell expert, pathologist and biostatistician on this proposal. If successful, the significance and impact of this project is that it will provide novel targeted approaches for the treatment of TNBC and hopefully to eliminate the mortality associated with this disease.

**Joseph Leventhal, M.D., Ph.D. - 2016 Awardee**

*Comprehensive Transplant Center  
Northwestern University*

**“Clinical Transplant Tolerance Induction by the Use of Ex Vivo Expanded Antigen Specific Regulatory T Cells”**

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Organ transplantation currently relies on nonspecific immunosuppressive agents (IS) given life-long to prevent graft rejection. These drugs have significant and cumulative toxicities, including nephrotoxicity and increased rate of infections, malignancy, diabetes, and hypertension. The elusive goal since organs were first transplanted in humans is to establish donor-specific immunologic tolerance, a state where a donated organ is accepted as "self," eliminating the need for IS. Regulatory CD4+CD25+FOXP3+ T cells (Tregs) have been shown to induce tolerance in transplantation models and to be elevated in tolerant human recipients. However, there have been limited efforts to harness their therapeutic potential in the clinic.

Our long-term goal is to develop tolerogenic protocols that will lead to controlled IS minimization or withdrawal after organ transplantation. As a step in that direction, the overarching goal of this proposal is to develop a recipient derived, DONOR-specific expanded regulatory T cell "product" for use in kidney transplant recipients, to be pursued under two specific aims.

Specific Aim 1: To further define and optimize the conditions for laboratory-based expansion of allospecific Tregs

(A) Our approach will be to verify if greater expansion without the loss of efficacy can be obtained by a non-specific restimulation with anti-CD3/CD28-beads on day 14, following the initial donor-specific stimulation with "donor" irradiated B cells on Days 0 and 7. (B) After this optimization, we will make two more small-scale Tregs expansions in the laboratory and then test these for their in vivo safety and efficacy (below).

Specific Aim 2: To optimize the conditions for large scale (i.e., clinically applicable) expansion of allospecific Tregs. The technology optimized in the laboratory will be transferred to the GMP Facility where two large-scale expansions will be carried out.

The Tregs expanded in both the laboratory (n=2) and the GMP Facility (n=2) will be tested for their (a) in vivo safety, testing whether they would lose their suppressive properties following infusion and instead cause rejection of human skin grafts on NSG mice, and (b) in vivo efficacy, determining if they protect human skin grafts on humanized NSG mice from rejection. Completion of these studies will enable us to file for an IND from the FDA to perform a future phase I clinical safety trial during the Transformational Award Phase, and then to a future phase II efficacy trial. The significance of this proposal lies in its broad applicability for induction of tolerance in organ and tissue transplantation.

**Richard Lieber, Ph.D.** - 2016 Awardee

355 E. Erie St.

*Rehabilitation Institute of Chicago*

“Breakthrough Technology to Measure Human Muscle Function”

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Here at the Rehabilitation Institute of Chicago, we treat over 55,000 patients per year with a variety of diagnoses ranging from traumatic brain injury, spinal cord injury and stroke to Parkinson's disease and cerebral palsy. Virtually all of these patients suffer from movement disorders and weakness. Currently, the gold standard for diagnosis of neuromuscular problems involves painful and sometimes impossible muscle biopsies or very qualitative clinical exams. We believe the device described in this proposal to measure muscle properties will revolutionize treatment of these problems. Using this device, patient muscle function can be diagnosed and objectively characterized in a matter of seconds. This would have dramatic impact on the course of patient care and clinical decisions since muscle structure and function are so central to patient well being. While a few tools are available to collect in vivo muscle data, none can measure sarcomere properties noninvasively or with this degree of resolution. Necessary attributes are: simultaneous sampling across millimeters of muscle tissue, real-time sampling, compatibility with gross movement, inducing no or minimal tissue damage, and resolving nanometer-scale sarcomere length changes.

To solve this challenge, we are developing a new method termed resonant reflection spectroscopy (RRS). RRS is an optical method that relates reflected spectra to the muscle sarcomere structure. Unlike current methods, RRS operates through a minimally invasive optical probe, collects large sample sizes of sarcomere lengths, and is compatible with muscle movement and activity. In the catalyst stage, our goal is to develop RRS so that it can be routinely used in clinical settings. Specific aim 1 will develop a new optical source so RRS can be applied across general muscle architectures and geometries. Specific aim 2 will result in a robust optical probe designed for use in the clinical setting. Specific aim 3 will result in a packaged RRS system that can be used in a future transformational award across clinical settings. Our ultimate goal is to revolutionize the way that our patients with neuromuscular disorders are diagnosed and treated.

**David Masopust, Ph.D.** - 2016 Awardee

*Microbiology and Immunology, Medical School  
University of Minnesota*

“Ablating Tumors with Resident Memory T Cells”

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This proposal will address major limitations in the clinical effectiveness of emerging cancer immunotherapy regimens. Adoptive cell therapies and administration of checkpoint blockade antibodies, which depend on CD8 T cells of the patient's immune system to specifically kill tumor cells, are predicted to revolutionize how cancer is treated. This proposal will exploit tissue resident memory CD8+ T cells (TRM) to overcome two major limitations of cancer immunotherapy efficacy: 1) tumors represent a strongly immuno-suppressive environment, which antagonizes immune-based therapies, and 2) therapies need to localize to tumors but current approaches are systemic and not anatomically targeted. TRM are a recently defined lineage of T cells that comprise the most abundant immunological surveyors of non-lymphoid tissues in mice and humans. TRM are established throughout the body as a consequence of natural infections and vaccines. TRM reactivation, achieved via topical application of cognate peptide, rapidly licenses these cells to direct the activation of all arms of the immune system, triggering a highly immune-stimulatory environment. This response, referred to as the 'sensing and alarm' function, occurs locally and recruits adaptive immune cells and serum antibody to the site of TRM reactivation. Reactivated TRM also potently activate local innate responses, dendritic cell priming, and natural killer cell activation. We hypothesize that TRM reactivation can reverse the immunosuppressive tumor microenvironment and can be exploited to enhance existing adoptive cell transfer and checkpoint blockade therapies. This proposal will answer two major questions. 1) Can TRM reactivation be exploited to promote an immune-stimulatory environment within solid tumors and mediate tumor regression? 2) Does TRM reactivation recruit engineered anti-cancer chimeric antigen receptor (CAR) T cells and checkpoint blockade antibodies to solid tumors? These questions will be addressed in autochthonous inducible tumors of the female reproductive tract (uterine and/or ovarian cancer) and skin (melanoma) in mice. Successful outcome will provide proof-of-principle demonstration of a human translatable strategy for improving upon cancer immunotherapies by modifying tumor microenvironments and focusing therapies to tumor sites. Future pursuits would interrogate TRM biology, specificity, and function within human tumors and will define regimens for therapeutic application.

**Steven McMahon, Ph.D.** - 2016 Awardee

233 S. 10th Street, Suite 609  
Thomas Jefferson University

“Modulation of the Anti-tumor Immune Response by the Myc Oncoprotein, Implications for Cancer Therapy”

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Perhaps the most common hallmark of cancer is activation of the MYC oncogene. Nearly all tumor cells are strictly dependent on high MYC levels in order to drive malignant phenotypes like rapid proliferation, elevated invasion and migration, reprogrammed metabolism, and survival in the presence of cellular stresses like hypoxia and genetic damage. Despite this central role in the biology of cancer, strategies for directly targeting MYC have not been successful. We report here evidence suggesting that MYC may regulate an additional hallmark of cancer, i.e. immune evasion. At the molecular level, MYC drives expression of the TNF receptor family member CD30/TNFRSF8. While CD30 expression is known to be important in rare forms of lymphoma (e.g. Cutaneous T Cell Lymphoma/CTCL and Anaplastic Large Cell Lymphoma/ALCL), we demonstrate that more common lymphoid malignancies, and also common epithelial tumors, express CD30 as a surface antigen. Remarkably, CD30 levels are controlled by MYC in all tumor types we examined. Confirming the functional relevance of these observations, we demonstrate that blocking CD30 expression in either lymphoid or epithelial tumor cells causes rapid cell death. Thus, CD30 may serve as a biomarker and potential therapeutic target in cancers with elevated MYC. Furthermore, based on published findings on CD30 in Hodgkin's Lymphoma, it likely serves as an immune checkpoint molecule to provide immune evasion properties to MYC-driven tumors. The experimental plan outlined in this Catalyst Award proposal will formally test these highly impactful predictions. Successful completion of this proposed research will provide a solid rationale for a Falk Transformational Award that explores methods for exploiting these biomarker, targeted therapy and immune checkpoint blockade strategies.

**Markus Müschen, M.D., Ph.D.** - 2016 Awardee

*Department of Systems Biology  
Beckman Research Institute of City of Hope*

“CD25 As Therapeutic Target in Refractory B Cell Malignancies”

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B cells critically depend on continuous survival and proliferation signals from a functional B cell receptor (BCR). In >80% of B cell malignancies, the tumor clone is driven by an oncogenic BCR-mimic. Oncogenic BCR-mimics include BCR-ABL1, EBV-encoded oncoproteins, RAS-pathway and NF- $\kappa$ B-pathway activating lesions. While CD25 mediates IL2 signaling on T cells, we recently revealed that CD25 is a critical feedback regulator of B cell receptor (BCR) and oncogenic BCR-mimics in human B cell malignancies. Indeed, we found that CD25 is a reliable biomarker of tumor clones driven by oncogenic BCR-mimics (e.g. BCR-ABL1, LMP2A, BRAF, NF- $\kappa$ B) and genetic experiments demonstrated that CD25 is critical for the initiation of B cell leukemia and lymphoma in transplant recipients. Surface expression is rapidly induced by activity of PKC- $\delta$  and NF- $\kappa$ B downstream of the BCR. CD25 then recruits an inhibitory complex to the surface to reduce kinase signaling downstream of the BCR or its oncogenic mimics. Analysis of three clinical cohorts revealed that high expression levels of CD25 are associated with poor clinical outcome in various B cell malignancies. While CD25 expression is associated with drug-resistance, inhibition of CD25 sensitizes multiple B cell malignancies to conventional drug-treatment.

Based on these and other findings, we propose three Aims to (1) elucidate mechanisms of CD25 regulation, (2) explore usefulness of pharmacological suppression of CD25 and (3) CD25 as target for immunotherapy in B cell malignancies.

**Aim 1:** Mechanisms of CD25 regulation in B cell malignancies: Which mechanisms control transcriptional regulation (e.g. Rel-A/NF- $\kappa$ B) and surface expression (phosphorylation of cytoplasmic tail by PKC- $\delta$ ) of CD25 and how are these events related to oncogenic mimicry of BCR signaling?

**Aim 2:** Pharmacological suppression of CD25 in human B cell malignancies. For suppression of CD25 at the transcriptional level, we will test the Rel-A small molecule inhibitor IT-901 (genetic validation based on RelAfl/fl, Nfkb1fl/fl leukemia and lymphoma cells). To inhibit translocation to the cell membrane, we will inhibit PKC- $\delta$  function by Enzastaurin and CID2858522 (genetic validation based on leukemia and lymphoma cells from Prdkcdf/fl and analog-sensitive Prkcd mice).

**Aim 3:** CD25 as a target for immunotherapy of B cell malignancies. We will test the efficacy and specificity of antibody-based targeting of CD25+ B cell malignancies in humanized NSG-KitW41 mice. In two experimental series, antibody-drug conjugate (ADC) against CD25 (ADCT-301) will be studied.

Tommi Raij, M.D., Ph.D. - 2016 Awardee

*Center for Brain Stimulation  
Rehabilitation Institute of Chicago*

“Re-Wiring the Human Brain”

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Re-wiring the human brain represents a critical frontier in clinical neuroscience. Behaviors and mental function are emergent properties of large-scale neuronal networks where the connectivity strengths between nodes define the network. Correspondingly, many neurological and psychiatric symptoms arise from network-level derangements where the structural and/or functional connectivities between separate brain areas have been altered by the disease process (e.g., sub-cortical stroke, traumatic brain injury) and/or secondary maladaptive network changes. While the adult brain has little capacity to re-grow damaged long-range axons, it might be possible to restore functional connectivity by training the brain to use an alternate route connecting the areas, with the ultimate goal of improving motor/behavioral/cognitive function. However, currently there are no clinical treatments available for restoration of functional connectivity between specific brain areas.

Here, we propose to develop a non-invasive brain stimulation/recording technique to functionally re-wire the human brain. Inspired by our previous animal studies and existing literature in healthy humans using paired associative stimulation (PAS), the instrument system utilizes two transcranial magnetic stimulator (TMS) channels to activate two cortical regions in precisely orchestrated temporal patterns to selectively up- or down-regulate functional connectivity between them. To this aim, we will develop new stimulation sequences and monitor the ensuing interregional connectivity changes with source-resolved electroencephalography (EEG). To improve the navigation and targeting accuracy of brain stimulation, we will use the newest-generation small but powerful cooled TMS coils, combined with our published TMS E-field computations with individual magnetic resonance images (MRIs) and a TMS navigator device. The TMS-EEG-MRI data will be correlated with connectivity estimates from high-angular resolution diffusion imaging (HARDI) diffusion MRI tractography and resting-state functional MRI (rs-fMRI), along with behavioral measures.

In summary, this proposal develops non-invasive techniques to transform our capability to manipulate brain interregional functional connectivity in humans, therefore laying the foundation for a new class of network-level therapies in disorders that involve white matter lesions or aberrant functional connectivity due to other reasons. We build the techniques within studies in healthy human subjects and stroke/TBI patients, but the findings will be applicable to a broad range of neurological and psychiatric disorders. If the Catalyst phase of the award is successful, the techniques may be applied in a controlled clinical trial during the Transformational phase. The TMS, EEG, and MRI devices are noninvasive, safe, and already FDA-approved, and the experiments can be conducted in their allotted time frames.

**Stephen Waggoner, Ph.D. - 2016 Awardee**

*Center for Autoimmune Genomics and Etiology (CAGE)  
Cincinnati Children's Hospital Medical Center*

*"Harnessing Natural Killer Cells to Treat Autoimmune Disease"*

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Systemic lupus erythematosus (SLE) is a devastating autoimmune disease in which autoantibodies drive potentially fatal tissue damage in hundreds of thousands of individuals in the U.S. Tragically, the disease frequently affects ethnic minorities, children, and women of childbearing age. There is no cure for lupus and the current standard of care for lupus relies heavily upon the symptom-suppressing use of immunosuppressive drugs that are associated with harmful side effects. To achieve remission in lupus, new treatments must be developed to restrain the pathogenic production of autoantibodies by B cells.

The laboratory of Dr. Stephen Waggoner at Cincinnati Children's has discovered a novel and critical role for natural killer (NK) cells in suppression of antibody production by B cells. His lab is currently funded to pursue means of inhibiting this regulatory function of NK cells to improve immune responses during vaccination and chronic infection. In this proposal, an innovative alternative strategy aimed at harnessing NK cells to eliminate follicular helper T cells (TFH) and suppress B-cell production of autoantibodies is proposed as a novel means to improve treatment and potentially cure lupus. There is strong precedence for the clinical use of NK cells in therapy of cancer. Investigators at our institution are developing chimeric antigen receptors (CARs) that can be expressed in NK cells to increase their ability to eliminate patient tumors. The premise of the current proposal, supported by Dr. Waggoner's preliminary studies, is that CAR-expressing NK cells can also be used to kill normal, non-cancerous cells that are central to the pathogenesis of autoimmune diseases like lupus.

This Catalyst Award will enable development of a novel CAR construct and transduction of primary human NK cells isolated from the blood of lupus patients. The subsequent experiments supported by this award will test the hypothesis that this CAR construct endows patient NK cells with the ability to specifically recognize and kill TFH cells. Furthermore, the ability of these CAR-expressing NK cells to suppress antibody production by B cells from lupus patients in vitro and in vivo will be evaluated.

This proposal represents an important paradigm shift in NK-cell biology and highlights a transformative use of NK cells in clinical therapy of lupus. Our broad, long-term objective is to introduce CAR-expressing NK cells into patients in order to reduce autoantibody secretion and promote remission of lupus and other TFH-mediated autoimmune diseases (e.g. rheumatoid arthritis) for which we have no cure.

**Andrew Aplin, Ph.D.** - 2015 Awardee

*Sidney Kimmel Cancer Center  
Thomas Jefferson University*

“Targeted Inhibitor Strategies in Uveal Melanoma”

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Uveal melanoma (UM) is the most common intraocular malignant tumor in adults. Even after treatment of the primary tumor, 20-50% of patients succumb to metastatic disease. There are no FDA-approved therapies for metastatic UM and patient survival is poor. Our long-term objective is to inform effective treatment options for advanced stage UM. UM frequently (90%) harbors mutations within alpha subunits of heterotrimeric G proteins, either GNAQ or GNA11. Mutant GNAQ/11 activate the MEK-ERK1/2 signaling pathway. Additionally, mutations in BAP1 are associated with aggressive disease and higher likelihood of metastasis. In many patients, macro-metastases do not develop for years, even decades, indicating early tumor dissemination and subsequent dormancy of disseminated tumor cells. When macro-metastases develop, the response to targeted therapies is low. In phase II and III trials, the MEK inhibitor, selumetinib, had a <15% partial response rate and marginally improved median progression-free survival compared to standard chemotherapy. How mutant forms of GNAQ, GNA11 and BAP1 promote tumor initiation and progression in UM is poorly characterized and mechanisms of dormancy and therapeutic resistance are not known. We propose to create novel cell lines expressing fluorescently tagged forms of mutant G alpha q and 11 to test for altered subcellular localization and trafficking of mutant versus wild type forms of G alpha q and 11. We will also characterize the ability of novel inhibitors to block activated GNAQ and growth of UM cells. To characterize dormancy, we will generate inducible UM models to modulate BAP1 and test effects on response to dormancy inducing agents. Finally, we will develop novel reporter models to monitor signaling pathways and response to targeted inhibitors in UM cells in vivo. This multi-PI application brings together expertise in signal transduction, mechanistic basis of dormancy, novel UM models and clinically relevant targeted inhibitors. The synergy between team members is expected to advance the understanding of the late development of overt metastatic disease in UM, shift the paradigm of metastasis treatment and address unmet clinical needs.

**Samira Azarin, Ph.D.** - 2015 Awardee

*Chemical Engineering and Materials Science  
University of Minnesota*

“Scaffold-Based Vaccination Strategy To Target Metastatic Disease”

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Novel treatments targeting metastatic cancer are urgently needed, as cancer mortality typically occurs upon the disrupted function of vital organs due to the growth of metastatic cancer cells. While focal ablation technologies such as application of heat, cold or electrical pulses to destroy tumor cells have shown promise in treatment of some solid tumors, they have not been successfully applied to disseminated disease. It has recently been shown that polymer-based biomaterial scaffolds can capture metastasizing cells *in vivo*, which provides an opportunity to apply focal therapy to disseminating cells by concentrating them within a scaffold. The long-term goal of this proposal is to therapeutically target metastatic disease using a novel vaccination strategy that combines biomaterials, focal therapy, and immunotherapy to lyse captured metastatic cells and release tumor antigens that will promote robust CD8<sup>+</sup> cytotoxic T-cell (CTL)-mediated destruction of disseminated cancer cells.

This proposal utilizes a clinically-relevant, immunologically robust mouse melanoma model system to demonstrate proof-of-concept of this novel therapeutic approach through completion of the following Specific Aims: (1) Utilizing polymeric scaffolds to attract metastatic melanoma cells, (2) Determining the optimal focal therapy strategy for release of tumor-specific antigens, and (3) Evaluating the immune response following focal therapy applied within implanted polymeric scaffolds *in vivo*. Poly(caprolactone) scaffolds will be engineered to recruit B16 melanoma cells. In parallel, heat, cryotherapy, and irreversible electroporation (IRE) will be applied to B16 cells *in vitro* to determine which focal therapy releases the most tumor-specific antigens upon cell lysis, and these lysates will then be loaded onto scaffolds containing immunostimulatory signals and implanted in mice to determine if the lysates can activate CTLs *in vivo* and promote an anti-tumor response following secondary challenge by tumor inoculation in the groin. The addition of immune checkpoint blockade (i.e. anti-CTLA-4 treatment) will also be evaluated to enhance the CTL response to the lysates. Finally, the focal therapy will be applied to disseminated cells captured by immunostimulatory biomaterials scaffolds *in vivo* in combination with immune checkpoint blockade, and the ability of the CTL-mediated anti-tumor response to inhibit growth of lung metastases will be evaluated. This approach is patient-specific yet does not require large-scale *ex vivo* expansion of cells, and thus it has the potential to be broadly applicable to many types of cancer.

**Paul Bollyky, M.D., Ph.D.** - 2015 Awardee

*Medicine, School of Medicine  
Stanford University School of Medicine*

“Targeting Bacteriophage to Treat Biofilm Infections”

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The bacteria *Pseudomonas aeruginosa* is a major human pathogen responsible for much of the morbidity and mortality associated with diabetic wounds, cystic fibrosis, and nosocomial infections. This massive disease burden is caused by biofilms - slimy coats of bacteria and polymers that allow *P.aeruginosa* to stick to catheters and other surfaces and resist both antibiotics and the host immune response.

We have discovered a surprising and unprecedented role for bacteriophage (viruses produced by bacteria) in biofilm formation. We have learned that the extracellular matrix produced by *P.aeruginosa* self-assembles into a liquid crystal through entropic interactions between polymers and Pf phage, a bacteriophage that is ubiquitous among clinical isolates of *P.aeruginosa*. This liquid crystalline structure enhances biofilm function and bacterial fitness by increasing adhesion, resistance to desiccation, and antibiotic tolerance.

These findings are groundbreaking in several ways. First, they indicate that Pf phage have a unique, symbiotic relationship with their bacterial hosts. Second, they implicate Pf phage in human biofilm infections. Third, the identification of a critical role for Pf phage in biofilm formation creates exciting opportunities for novel classes of anti-microbial agents to treat *P.aeruginosa* infections.

In light of these findings, we propose to generate innovative therapeutics that will treat biofilm infections by targeting Pf phage. We will focus on two therapeutic strategies in year 1 of this program with a plan to make the agent that shows the greatest potential our sole focus in years 2-3.

In Aim 1, we will develop anti-viral compounds effective against *P.aeruginosa* biofilms. Our hypothesis is that we can prevent biofilm formation by suppressing bacterial production of Pf phage. We will first develop a high-throughput reporter system to quantify Pf phage production by *P.aeruginosa*. Then, we will screen a 300,000 molecule compound library for candidates that inhibit Pf phage production. Finally, we will test the efficacy of these anti-viral molecules against *P.aeruginosa* biofilms and clinical isolates.

In Aim 2, we will develop antibodies that prevent and treat *P.aeruginosa* infections by targeting Pf phage. Our preliminary data suggest such antibodies indeed inhibit biofilm formation. First, we will screen a panel of monoclonal antibodies for efficacy against biofilm formation in vitro. Then we will develop a *P.aeruginosa* wound infection model and use this to test the efficacy of anti-phage antibody treatments in vivo.

Together, these aims represent a bold and radically unconventional approach to treating *P.aeruginosa* Biofilm infections and improving human health.

**Steven Dowdy, Ph.D.** - 2015 Awardee

*Cellular and Molecular Medicine*  
*University of California San Diego*

“Development of Next-Generation Antibody-RNAi Conjugates (ARC) to Treat AML”

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Due to chromosomal translocations, Acute Myeloid Leukemia (AML) cells that express the Mixed Lineage Leukemia (MLL) gene fused to the AF9 gene (MLL-AF9) are devastatingly aggressive, difficult to treat malignancies. Unfortunately, the current standard of care remains at 40% disease-free survival (DFS) at five years for children and <10% for adults over 60. Consequently, there is a great need to develop precision medicines that can selectively kill AML cancer cells and their clonogenic cancer stem cells, without harming normal cells.

siRNA-induced RNAi responses can selectively target each and every MLL fusion oncogene, including MLL-AF9. Unfortunately, despite RNAi's promising therapeutic features, siRNA drugs are unstable in serum and due to 40 negatively charged phosphates, siRNAs cannot enter cells. To address these issues, we spent 8 years synthesizing a next-generation RNAi trigger, called RiboNucleic Neutral (siRNN) prodrugs. siRNNs are synthesized with neutral bioreversible phosphotriester groups that mask the negative charge and result in many drug-like properties of extreme stability and deliverability. Once inside cells, ubiquitous cytoplasmic thioesterase enzymes unmask siRNNs and convert them into negatively charged, active siRNAs that induce robust RNAi responses. Importantly, unlike all other anti-cancer therapeutics, RNAi therapeutics can evolve at pace with the AML cancer cell's mutation rate to treat recurrent disease. In contrast, all current anti-cancer therapeutics are static molecules where unfortunately, the cancer cells often become resistant after a relatively short period of time.

The goal of this transformative high risk/high gain Catalyst Award is to develop anti-CD33 Antibody-RNAi Conjugates (ARC) using our next-generation siRNN RNAi triggers to target MLL-AF9 fusion oncogenes and selectively kill AML cancer cells.

**Aim 1. Develop anti-CD33 MLL-AF9 ARC and Optimize Delivery into AML Cells.**  
siRNNs bridging the MLL-AF9 t(9;11) fusion site will be conjugated to the C-terminus of anti-CD33 humanized monoclonal antibodies. We will optimize ARC delivery and induction of RNAi responses in AML cells.

**Aim 2. In Vivo Optimization of Anti-CD33 MLL-AF9 ARC Delivery into AML Tumors.**  
We will optimize anti-CD33 MLL-AF9 ARC delivery and induction of RNAi responses in preclinical mouse models of AML.

Successful development of MLL-AF9 ARC here will be a springboard for extensive preclinical optimization using patient-derived (PDX) AML samples in a Transformational Award application with the ultimate goal to declare a MLL-AF9 ARC clinical candidate for IND enabling studies and eventual clinical trials. siRNN ARC technology has the potential to fundamentally transform how we treat human disease, from cancer to pandemic flu to heart disease.

**Nickolai Dulin, Ph.D.** - 2015 Awardee

*Medicine*

*University of Chicago Pritzker School of Medicine*

“Development of a Novel Small Molecule for Treatment of Fibrotic Diseases”

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Fibrosis represents a critical mechanism in the pathogenesis of a wide range of important diseases that remain poorly treated; salient examples include: congestive heart failure, secondary or idiopathic pulmonary fibrosis, advanced kidney disease, scleroderma, and liver cirrhosis. There is a huge clinical need for new treatments that prevent fibrosis. Across these various fibrotic diseases, there is a unifying cellular mechanism that leads to scar deposition: the transformation of tissue fibroblasts into an activated phenotype called "myofibroblasts". Myofibroblasts are the principal cells that deposit extracellular matrix proteins and are responsible for scar formation during fibrosis. Therefore, disrupting cellular mechanisms which induce and/or maintain myofibroblast differentiation may be a potential strategy to attenuate fibrosis of various organs.

We have discovered a small molecule ("C085") that inhibits myofibroblast differentiation in vitro and inhibits experimental pulmonary fibrosis in living mice, without obvious immediate toxicity during 2 weeks of daily administration. Our collaborators have synthesized a new (and patentable) synthetic analog ("C308") that also inhibits myofibroblast differentiation in vitro. Now, we propose to confirm the anti-fibrotic efficacy of C308 in vivo in three models of organ fibrosis -- heart, lung, and kidney -- and to define the molecular mechanisms by which C308 inhibits myofibroblast differentiation. To achieve our goal, we propose two specific aims: (i) determine whether C308 inhibits organ fibrosis in heart, lung, and kidney, using two models of fibrosis for each organ; and (ii) determine the mechanisms by which C308 inhibits myofibroblast differentiation and function.

This research matches perfectly the high risk, high reward intent of the Falk Catalyst Award, for if successful it could reveal a new strategy for treating fibrotic diseases and would position us to apply for Transformational Research Award in order to achieve our final goal -- move C308 to clinical trials as antifibrotic medicine.

**John Imig, Ph.D.** - 2015 Awardee

*Pharmacology & Toxicology*  
*Medical College of Wisconsin*

“Novel Therapy for Kidney Disease”

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Renal disease in cancer patients and those being treated for cancer is an emerging and rapidly growing healthcare issue. Newer cancer therapies have increased cure rate and survival time, but therapy-associated fluid and electrolyte abnormalities and acute and chronic kidney injury are common. This healthcare issue has led to the development, evolution, and emergence of a subspecialty, Onco-Nephrology. This project will tackle this emerging cancer arena and focus on novel therapeutics to protect the kidney from cancer therapy-associated toxicity. Kidney disease afflicts 33 million in the United States and chronic kidney disease (CKD) accounted for over \$60 billion in Medicare costs. Cancer treatments and radiation exposure represent a lesser-known cause of CKD. Thus there is a significant clinical need for novel therapies to effectively treat and stop CKD in cancer patients. We have identified EET analogs/mimetics with anti-hypertensive, anti-inflammatory, and anti-oxidative activities that will be particularly beneficial for hypertensive patients and patients undergoing chemotherapy and radiation exposure. Our central hypothesis is that EET analogs are a novel therapeutic for CKD caused by hypertension, chemotherapy, and radiotherapy. The rationale that underlies the research proposed is that EET analogs are a novel chemical entity (NCE) with multi-faceted pharmacological activities well suited for the treatment of CKD. Our long-term goal is to complete preclinical requirements for an Investigational New Drug (IND) application for EET analogs for the treatment of CKD in cancer patients and as a consequence of chemotherapy or radiotherapy. The overall objective of this application is to identify a lead candidate EET analog that prevents or arrests the progression CKD in cancer patients. We will also optimize the EET analog chemical scaffold and develop kidney-targeted EET analogs. Our preliminary studies strongly suggest that the experimental studies and outcomes will have an important positive impact and these will make a significant advance towards fulfilling the preclinical IND filing requirements for an EET analog as a CKD therapy. Guided by strong preliminary data, our central hypothesis will be tested by pursuing two specific aims: 1) Test the hypothesis that EET analogs will prevent or mitigate the development of kidney disease associated with cancer therapies; 2) Identify a lead EET surrogate drug candidate and develop EET analogs that target the kidney to enhance their therapeutic potential. The proposed research is original and innovative, because the novel small molecule EET analogs demonstrate great potential as a therapeutic approach improving outcomes in cancer patients.

**Mark Rosenblatt, M.D., Ph.D.** - 2015 Awardee

*Ophthalmology & Visual Sciences*  
*University of Illinois College of Medicine at Chicago*

“Nanoengineered Silk Biomaterials for Ocular Regenerative Medicine”

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Damage to the ocular surface is a common endpoint for an array of corneal conditions including trauma, infection, and inflammation. In many cases the regeneration of the ocular surface epithelial barrier is impeded by the underlying pathology leading to persistent epithelial defects, or encroachment of con-corneal epithelium onto the ocular surface with concomitant risk of corneal ulceration and loss of vision. In these cases, reconstruction of the ocular surface may be necessary, and the use of biomaterials (such as amniotic membrane) may be required. We have characterized the ability of transparent and biocompatible silk films with modified surface topography to modulate the properties of corneal epithelium. We hypothesize that silk film biomaterials with engineered surface topographies modulate corneal epithelial cell behavior and gene expression, and can be optimized for ocular surface reconstruction. Specific aim 1 will investigate the role of surface topography in regulating the expression of genes responsible for cell differentiation in vitro. Specific aim 2 will investigate the role of surface topography in regulating regenerative potential in vitro. Specific aim 3 will investigate the role of surface topography in the differentiation of epithelial cells on silk film biomaterials in vivo.

**Stephen Strittmatter, M.D., Ph.D.** - 2015 Awardee

CNNR, BCMM 436295 Congress Avenue  
Yale School of Medicine

“Validation of Targets to Rescue Synapse Loss in Alzheimer's Disease”

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Central to the pathophysiology of cognitive dysfunction in Alzheimer's disease (AD) is the loss of synapses, with an impairment of plasticity at surviving synapses. Therapeutic efforts to intervene in AD have focused on the Amyloid-beta peptide as an upstream trigger for synaptic disease, but clinical trials have been disappointing so far. Additional validated targets for AD therapy are needed, in particular those focused more directly on synaptic deficits.

One approach to target identification for AD is to study the biochemical basis for Amyloid-beta oligomer (A-beta-o) toxicity in neurons. We defined an A-beta-o --PrP-C--mGluR5--Fyn cascade that damages synapses in AD models. In this cascade, mGluR5 is a druggable target. Here, we seek to validate the preclinical efficacy of specific mGluR5 agents that preserve physiological function while blocking A $\beta$ o pathophysiology in mouse transgenic models.

A second approach to identify targets for AD therapy with direct clinical relevance is through genetic studies of Late Onset AD (LOAD) risk. The largest GWAS analysis of LOAD identified a short list of genes whose common variants alter risk, providing potential new targets for AD therapy. We considered whether any of these might be directly linked to synaptic dysfunction in AD. Nearly all of the LOAD risk genes are hypothesized to bind A-beta, to alter A-beta metabolism, to regulate endocytosis, or to modulate immune function. Therefore, their action on synaptic dysfunction must be indirect. From the list of AD genetic risk factors, Pyk2 (also PTK2B or FAK2) is the only gene recognized to encode a protein concentrated at post-synaptic densities with direct effects on synaptic plasticity. Of note, the Pyk2 protein physically associates with mGluR5 and Fyn, so its study is supported by our biochemical approach as well. Our second goal in this project is to validate Pyk2 inhibition as a target for disease modification in AD. We will use both genetic and pharmacological tools to assess this potential target.

This Falk Catalyst project seeks to validate two targets with high potential for effective disease modification of Alzheimer's. This is the first step in advancing one or both approaches into a full drug development program during a transformational phase.

**Su-Chun Zhang, M.D., Ph.D.** - 2015 Awardee

*Waisman Center  
University of Wisconsin-Madison*

“Refine Cell Therapy Outcomes by Remote Regulation of Neural Circuitry in Non-Human Primates”

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Stem cell therapy holds promise for some of the most debilitating diseases, such as Parkinson's disease (PD). Its prospect is raised substantially with the development of human induced pluripotent stem cells (iPSCs) and our ability to guide iPSCs to functionally specialized cells. However, transplanted neurons do not always integrate into a correct circuit, which may result in unwanted outcomes. In PD, a degenerative disorder resulting from loss of dopamine (DA) neurons in the midbrain and insufficient DA release in the striatum, transplantation of human fetal ventral midbrain tissue into the striatum can restore DA release and ameliorate symptoms. However, uncontrolled movements, named graft-induced dyskinesia (GID), are present in some patients, partly due to unregulated release of DA from grafted cells. Hence, an ability to tune up or down the activity of grafted cells will not only overcome side effects but also refine therapeutic outcomes.

We intend to tackle this fundamental issue by building a functional "switch" into grafted neurons. For the present (one-year) catalyst project, we propose to engineer monkey iPSCs to express both the active and inhibitory forms of DREADDs (designer receptor exclusively activated by designer drug) that are regulated by respective designer drugs. The iPSCs with the "bidirectional switch" will be differentiated to dopamine neurons, which will be transplanted to the brain of a monkey model of PD. This will mimic potential future clinical application. We will then determine if the activity of and transmitter release from the differentiated neurons are up or down regulated by peripheral (remote) application of the designer drugs after transplantation into the monkey brain as well as in vitro. Successful completion of the study will lay down a solid foundation to evaluate the feasibility of refining the therapeutic outcomes in a preclinical nonhuman primate model of PD.