2020 RESEARCH ANNUAL

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Allergy and Immunology

Principal Investigator: Edward Zoratti, M.D.
ICAC 3 - Inner City Asthma Consortium Infrastructure (UM1AI114271) Subcontract

The objectives of the Inner-City Asthma Consortium are to implement a long-range scientific plan to reduce asthma severity and prevent asthma among inner city children and to identify the mechanisms involved in the immunopathogenesis of asthma in these populations. The specific objectives are to: 1) conduct clinical trials to evaluate the safety and efficacy of promising immune based therapies in reducing asthma severity and preventing disease onset in minority children residing in inner cities in the United States; 2) conduct research to delineate the underlying mechanisms of such therapies as an integral part of the clinical trials undertaken by the Consortium; 3) conduct clinical studies on the immunopathogenesis of asthma onset, progression and severity; and 4) develop and validate surrogate/biomarkers to measure disease stage, progression and therapeutic effect.

Principal Investigator: Edward Zoratti, M.D.
Project 2-Maternal Factors and their Effect on a Child's Gut Microbiota and IgE Production (P01AI089473-06)

This application builds on the findings of our initial P01 designed to examine relationships between environmental factors, especially pets, the infant gut microbiota and pediatric allergic asthma. We have shown that: 1) dogs alter the microbial composition of dust in homes, 2) children born into homes with dogs have different developmental patterns of gut microbiota and of IgE, 3) a distinct pattern of gut microbial composition at 1 month of age is related to heightened risk of sensitization to multiple allergens at 2 years and of asthma at 4 years, and this pattern is influenced by numerous maternal characteristics, 4) sensitization to multiple food and inhalant allergens at 2 years is strongly related to asthma at 10 years, 5) the metabolic profiles of stools are related to later allergic sensitization 6) 12,13-DiHOME, a metabolite in stool, promotes development of Th2lymphocytes and lowers development of Treg lymphocytes in an in vitro assay, and 7) in another study, theme conial microbiota is distinct in neonates born to mothers with asthma. Our complementary mouse studies have shown that: 1) gavaging with dust from homes with dogs reduces lung inflammation from allergen sensitization and from respiratory syncytial virus (RSV) infection, 2) dog dust gavaged mice have increases *Lactobacillus johnsonii* in their ceca 3) oral administration of live *L. johnsonii* confers protection against pulmonary inflammation induced by allergen and RSV, 4) *L. johnsonii* alters the function of bone marrow derived dendritic cells, 5) mice orally supplemented with *L. johnsoni*i have altered serum metabolic profiles, and 6) mouse pups born to *L. johnsonii*-supplemented mothers are protected against allergen challenge and RSV infection. Collectively

these findings showing the influence of maternal factors provide the basis for this application's focus on the maternal gut and vaginal microbiotas during pregnancy, and how these relate to infant gut microbial development and risk of allergic asthma. Project 1 focuses on the relationship of maternal environmental and dietary factors, including maternal and infant gut microbiotas, to the child's developing a high-risk for asthma phenotype by age 2 years. Project 2 proposes a detailed examination of relationships between maternal and child microbiota, breast milk composition and IgE development amongst a cohort of pregnancies in which the mother has current allergic asthma. Project 3 synergistically interacts with Projects 1 & 2 and also uses specimens from 10-year-old allergic asthma cases and controls in the initial P01 birth cohort to examine gut microbes producing metabolites associated with a lowered risk of allergic inflammation and how they are transferred from mother and established in offspring. Project 4 will use mouse models to examine the relationships between manipulation of maternal microbiota and immune development in offspring. We anticipate that together these studies will show that interventions directed at the gut microbiota of mothers during pregnancy and of high-risk neonates after birth could reduce the risk of allergic asthma in childhood. Such findings would provide the foundations of a rational strategy to prevent allergic asthma.

Cardiology/Cardiovascular Research

Principal Investigator: Dennis Kerrigan, Ph.D.
The Effect of High Intensity Interval Training and Surgical Weight Loss on Distal Symmetric Polyneuropathy Outcomes (R01DK115687-02) Subcontract

Subrecipient will collaborate with the PTE to recruit and train subjects for a bariatric surgery high intensity interval training (HIIT) study. As the Sub recipient PI, Dr. Kerrigan has experience in HIIT studies for both patients with cardiovascular disease and cancer. Additionally, he oversees a weekly exercise class of 10-15 new patients prior to bariatric surgery who could be eligible for study. Dr. Kerrigan would oversee day-to-day operations of the project, be responsible for the protocol adherence, and supervise the research exercise physiologist. The research exercise physiologist would recruit potential subjects, communicate with the PTE project manager, and perform exercise training to those randomized into the HIIT group. Training will be conducted at one of Henry Ford Health System exercise facilities located in Detroit, West Bloomfield and Livonia.

Aims Include:

1. Identify and recruit subjects who will undergo bariatric surgery.
2. Identify and recruit subjects who are candidates for bariatric surgery but do not undergo surgery.
3. Perform the supervised HIIT training protocol.

Principal Investigator: Steven Keteyian, Ph.D.
The Improving ATTENDance to Cardiac Rehabilitation (iATTEND) Trial (R33HL143099)

The numbers of U.S. adults with either diabetes or pre-diabetes is staggering with nearly 40% of the population affected. In U.S. minority populations, such as African Americans, the diabetes rates are nearly twice as high as those of non-Hispanic white Americans. Skeletal muscle insulin resistance appears to be a nearly universal precursor to overt type 2 diabetes (T2D), and both insulin resistance and T2DM are often accompanied by mitochondrial dysfunction. With 16,569 base pairs and 13 protein-encoding genes, the mitochondrial genome is diminutive when compared with the ~6 billion base pairs in the diploid nuclear genome. To date, genomewide association studies of the nuclear variants have failed to explain a large proportion of the heritability of T2D, but rare mitochondrial mutations have been clearly implicated in T2D syndromes. Mitochondrial genetics has a number of complexities that haven't been collectively considered in existing studies. First, cells possess hundreds to thousands of mitochondria, so the effect of a given variant may depend on mitochondrial number, as reflected in the DNA copy number. Second, there can be subpopulations of mitochondria within cells such that only portion of mitochondria carry a particular variant – a situation known as heteroplasmy. Moreover, copy number and heteroplasmy can differ between tissue types. Third, a number of genes encoding mitochondrial proteins are located in the nuclear genome. This implies that crosstalk between genomes is required to coordinate gene expression and the efficient production of essential mitochondrial

complexes, such as those involved in electron transport. Therefore, epistatic interactions between genes on both genomes (i.e., mitonuclear interactions) may influence risk of T2D. We have two large study populations that will help us devolve these complexities and their role in T2D – the Study of Asthma Phenotypes and Pharmacogenomic Interactions by Race-ethnicity (SAPPHIRE) and the Diabetes Multi-omic Investigation of Drug Response (DIAMOND). Through the TOPMed program, we will have whole genome sequence data on 3,596 African American adults in SAPPHIRE. African Americans are a particularly interesting population to study because limited numbers of mitochondrial haplogroups and large-sized chromosomal ancestral blocks minimize the numbers of comparisons needed to identify potentially important mitochondrial variants and mitonuclear interactions associated with T2D (Aim 1). The ongoing DIAMOND study will provide a population of T2D patients and controls in which to replicate the findings from Aim 1 using DNA isolated from skeletal muscle. Given the primacy of skeletal muscle in the pathogenesis of T2D, it is important to replicate mitochondrial findings from Aim 1 in this tissue (Aim 2a), as well as use it to identify unique variants associated with T2D and insulin resistance (Aim 2b). Lastly, given the described effect of exercise training on reversing skeletal muscle insulin resistance, we will investigate how exercise affects the heteroplasmy and copy number of variants identified in the earlier aims and how these changes relate to changes in insulin resistance (Aim 3).

Principal Investigator: David Lanfear, M.D., and Hani Sabbah, Ph.D.
Plasma Metabolomics and Myocardial Energetics in Heart Failure (R01HL132154)

Heart failure (HF) remains an enormous public health problem despite advances in treatment. Disease progression and response to therapy in HF varies widely between individuals, but breakthrough technologies such as genomics and metabolomics are helping to unravel the disease heterogeneity that confounds patient management. Perturbed energy metabolism may be a key contributor to cardiac dysfunction and the development of clinical HF. Evidence from a variety of sources indicates that impaired structure and function of the energetic apparatus in the myocardium contributes to disease severity, progression, and may influence response to treatment. However, in order to advance these observations toward meaningful interventions for HF patients, several key steps are still needed: 1) confirming the importance of metabolic variation in human HF, 2) developing noninvasive markers of myocardial energetic status, and 3) identifying promising targets for intervention. Our proposed project is a series of interwoven translational investigations in humans and dogs with HF to define the association of plasma metabolite levels with disease severity, myocardial energetics, and disease progression. This project leverages substantial infrastructure already in place, a large existing genetic cohort study, available plasma samples suitable for metabolomic profiling, comprehensive translational laboratory capabilities, and a cohesive multidisciplinary research group focused on HF. Together the planned studies will address the overarching hypothesis that the peripheral metabolomic signature can indicate disease progression/treatment responsiveness in HF patients and that this is driven by altered myocardial energy metabolism. If true, then these data will help advance personalized therapy and identify novel targets for HF intervention, leading to improved outcomes for HF patients.

Endocrinology and Metabolism

Principal Investigator, Arti Bhan, M.D.
Epidemiology of Diabetes Interventions and Complications (U01DK094157) Subcontract

The Diabetes Control and Complications Trial (DCCT, 1983-1993) compared intensive therapy aimed at near normal glycemia versus conventional therapy with no specific glucose targets in 1441 subjects with type 1 diabetes (T1DM). In 1993, after a mean follow-up of 6.5 yrs, the study showed conclusively that intensive therapy reduced the risks of retinopathy, nephropathy, and neuropathy by 35-76%, and that hyperglycemia was a primary determinant of complications. We also described potential adverse effects of intensive therapy; assessed its effects on cardiovascular disease (CVD) risk factors, neurocognition and quality of life; and projected the lifetime health-economic impact. DCCT intensive therapy was then adopted world- wide as standard-of-care for T1DM. The Epidemiology of Diabetes Interventions and its Complications (EDIC, 1994-present) is the observational follow-up study of the DCCT cohort, with 95% of those surviving actively participating. Most outcomes are evaluated annually. CVD events and deaths are carefully documented and adjudicated. EDIC has notably discovered that the early beneficial effects of intensive treatment on complications have persisted for over 10 years despite the similar HbA1c levels during EDIC in the two groups, termed metabolic memory. Remarkably, former intensive therapy also

greatly reduced the risk of CVD events. DCCT/EDIC collaborators have also conducted numerous ancillary studies, with separate funding, most recently including measurement of cardiac function on cardiac MRI and measurement of biomarkers of oxidative stress and inflammation as determinants of complications. The overarching goals for the next 5 years are to follow at least 90% of the surviving cohort; to describe accurately the study-long effects of glycemia (HbA1c) and other established and putative risk factors on diabetes complications and the metabolic memory effects of prior DCCT intensive therapy; and to expand knowledge regarding T1DM and its complications by supporting collaborations for new research funding applications to maximally utilize the cohort, phenotypic data set, and collected biologic and genetic samples. The specific scientific aims are to 1) evaluate effects of risk factors, biomarkers and glycemia on risk of clinical CVD; 2) assess the long-term changes in CVD risk factors; 3) describe effects of DCCT intensive versus conventional therapy on mortality; 4) evaluate risk factors for severe retinopathy/nephropathy; 5) assess effects of diurnal glycemic variation on complications; and 6) conduct eight new research projects involving new measurements and analyses.

Principal Investigator: D. Sudhaker Rao, M.D.
Clinical Assessment of Vertebral Bone Quality Using Direct Biomechanical and Textural Analysis via Digital Tomosynthesis (W81XWH1910374)

This project relates to the Topic Area “Musculoskeletal Disorders”, and specifically to the encouragement area of research on measures to improve diagnosis, prediction and optimization of health outcomes. This is because the proposed project ultimately aims to improve the accuracy of assessment for spinal bone fragility and fracture risk. The bones of the spine (vertebrae) are the most frequently fractured ones due to osteoporosis. These fractures are economically costly and burden the patients with many downstream problems including back pain. Military personnel are known to be at greater risk for these fractures and complications. An accurate assessment of vertebral fracture risk is essential for appropriate and timely intervention for the prevention of fracture. This research also relates to the Topic Area “Diabetes”, because a diabetic cohort will be included in the study. Current standard techniques for fracture risk assessment rely on radiographic bone density scans. Additional information regarding the patient’s demographic status and medical history is also incorporated in tools predicting fracture risk. However, these techniques are not very sensitive in identifying who will have a fracture and who will not. This is not too surprising, considering the fact that the information used in the assessment is a crude indirect measure of bone strength not based on biomechanics. To address this concern, we developed a new method, in which two images of a patient’s vertebra are taken in the presence and absence of the patient’s body weight by having them stand and lay down for both images respectively. The images are obtained using digital tomosynthesis (DTS), a system that is similar to computed tomography (CT). The advantages of DTS over CT are that DTS allows for standing and lying images to be captured, offers high resolution and exposes patients to less radiation than CT. The two sets of images are compared using an advanced computational method and deformations in the vertebra caused by standing are measured. From the displacement measurements, vertebral stiffness and overall displacement are calculated as metrics of strength and factor of safety (factor safety is a measure of how strong the bone is relative to the loads it normally experiences). Information on bone microstructure, additional to bone density, is known to increase accuracy in predicting fractures. We can also derive properties related to bone microstructure from DTS images without the biomechanical test. These properties are determined by quantifying the texture in the bone image and called textural properties. We developed these methods in the laboratory in detail using cadaveric vertebrae and laboratory-standard imaging and strength testing. We also performed pilot human studies to establish feasibility of the methods in the clinic. What remains to be determined is how successful the methods will be in the clinical environment for identifying individuals who are at risk. Therefore, this study will be a clinical validation of the new biomechanical and textural DTS methods. In order to determine the ability of DTS methods to correctly identify at-risk patients, the approach will be to compare patients who have conditions or diseases that are known to increase their risk of fracture to normal patients. Therefore, a group of osteoporotic patients with an existing vertebral deformity, a group with primary hyperparathyroidism (pHPT) and a third group with diabetes will be compared to normal patients. These diseases are considered service-related and thus represent a greater risk for military families. Importantly, each of these diseases increase the risk of fracture but alter bone in different ways that are not always detectable by bone density scans. For example, osteoporosis primarily results in loss of bone mass, pHPT alters the organization of bone structure and affects the cortical bone (in the case of a vertebra, the dense bony shell surrounding the vertebra), and diabetes alters the quality of the bone material without reducing bone mass. By studying these groups using DTS and comparing the assessment of bone strength to standard bone density

results, we will; i) establish for which types of patients and to what extent the DTS methods might be useful, ii) identify in which way the method must be performed for best results, iii) better understand how differences in bone quality specific to each disease affect the biomechanical outcome, and iv) establish a group of patients that we can follow up for longer term results. In the long term, the method will be useful in other clinically significant issues such as low back pain associated with vertebral fractures, implant stability, degenerative and congenital diseases of the skeletal system resulting in deformities, skeletal response to drug, exercise and disuse.

Principal Investigator: Davida Kruger, N.P.
The Insulin-Only Bionic Pancreas Pivotal Trial: Testing the iLet in Adults and Children with Type 1 Diabetes the "Insulin-Only Bionic Pancreas Screening Protocol" (UC4DK108612)

This multi-center randomized control trial (RCT) will compare efficacy and safety endpoints using the insulin-only configuration of the iLet Bionic Pancreas (BP) System versus a control group using CGM during a 13-week study period. Participants may be enrolled initially into a screening protocol and then transfer into the RCT protocol, or they may enter directly into the RCT protocol. At the completion of use of the BP system (end of RCT for BP Group), participants will enter a 2–4 day Transition Phase and be randomly assigned to either transition back to their usual mode of therapy (MDI or pump therapy) based on therapeutic guidance from the iLet BP System or transition back to their usual mode of therapy based on what their own insulin regimens were prior to enrolling in the RCT.

Gastroenterology

Principal Investigator: Stuart Gordon, M.D.
Chronic Hepatitis Cohort Study II (CHeCS-II) (U18PS005154)

Hepatitis B (HBV) affects over 1.25 million Americans, and hepatitis C (HCV) over 3.2 million Americans. In the decades to come, more than 150,000 Americans are expected to die from these conditions unless steps are taken to increase awareness, diagnosis, and access to necessary care and treatment. Emerging interferon-free, direct-acting all-oral antiviral (DAA) treatments have changed the landscape of HCV treatment and care. These treatments appear to be safer than interferon-based treatments and provide exceptionally high rates of sustained virological response (SVR). Both HBV and HCV treatment guidelines have been updated to reflect evidence regarding initiation of new therapies; however, the evidence for those recommendations is largely based on clinical trials conducted under highly controlled conditions in restricted patient populations with limited data collection. Significant health disparities—across race, sex, age, and co-infection (with HIV or dual hepatitis)—may limit the generalizability of these populations. Data from longitudinal cohorts of “real world” hepatitis patients are needed to assess the population impact of rapidly evolving antiviral therapies, to understand the spectrum of disease and its natural history, and to evaluate the public health impact of chronic viral hepatitis. The Chronic Hepatitis Cohort Study (CHeCS) is the first comprehensive longitudinal cohort study of chronic viral hepatitis in the USC and has served as a model platform for observational data collection in this population. Since 2010, CHeCS has reported valuable information and expanded knowledge on many facets of hepatitis disease and policy. We propose to build upon CHeCS to develop “CHeCS-II,” in order to achieve the long-term goal of applying this rich data and infrastructure resource to inform public health planning, policy decisions, and clinical management of HBV and HCV. To achieve this, we will leverage the established CHeCS infrastructure, which has: (1) a diverse, real-world, non-veteran-based US cohort of >3,000 HBV, >11000 HCV, and >500 HIV co-infected patients receiving care through four U.S. health systems; (2) an experienced multidisciplinary team; (3) an efficient system for patient identification and data collection. We will provide scientific leadership to identify research findings and priorities by: (1) Offering seamless collaboration across study sites and with the Centers for Disease Control (Aim 1); (2) Expanding our HCV cohort to over 14,000 patients with >2 years’ follow-up; (3) Increasing follow-up of HBV patients to >5 years; (4) Collecting additional data regarding social determinants of health, including access to and uptake of care (Aim 2); (5) Applying rigorous analytical approaches to develop an in-depth understanding of health disparities and comorbidities, as well as investigating how these differences impact access to and uptake of antiviral therapy; (6) Advancing translation of this research to inform hepatitis-related policy and practice (Aim 3).

Hypertension and Vascular Research

Principal Investigator: Gustavo Ares-Sarmiento, Ph.D.
Role of Ubiquitin Ligase adaptor FBXL13 on Salt-sensitive hypertension (1K01DK123192)

Gustavo Ares, Ph.D., is a Research-Scientist Instructor training in the integrative renal physiology related to hypertension at Henry Ford Health system. In this revised application, Dr. Ares aims to determine whether a high salt diet stimulates the E3 ubiquitin-ligase FBXL13-NKCC2 interaction, enhancing NKCC2 degradation, favoring NaCl excretion thereby preventing an increase in blood pressure. Dr. Ares' immediate goal is to acquire the research training and professional skills necessary to transition to an independent extramurally funded investigator. His long-term goal is to establish his own research program with a focus on identifying novel targets of loops diuretics and pharmacological interventions to treat hypertension. Dr. Ares' Career Development Plan consists of improving his: 1) research skills; 2) Networking and collaborations; 3) professional development through attendance of presentations at weekly journal clubs, seminars, course and national scientific meetings; 4) mentoring skills; 5) writing manuscripts and grants. Dr. Ares' progress will be assessed thru bi-weekly to monthly meetings with each member of the mentoring team. Environment: Dr. Ares and his mentor, have assembled a strong team of co-mentors and advisors to guide him through the research project. Primary mentor: Dr. Pablo Ortiz a NIH-funded scientist with strong records of successful in renal physiology and hypertension. Secondary mentor: Dr. Jeffery L. Garvin is a professor of physiology at CWRU Cleveland, with extensive experience in regulation of salt and water transport along the renal nephron. Secondary mentor: Dr. Peter Kaiser, professor and Chair of Biological Chemistry at UC Irvine, has extensive experience in the ubiquitin-proteasome system, and the E3-ubiquitin ligases with special interest in the Skp1- Cullin-FBox family complex. The supportive research team includes T. Pavlov, Ph.D. and Mariela Mendez, Ph.D. working in renal physiology; Pamela Harding Ph.D., N-E. Rhaleb, Ph.D., Suresh Palaniyandi, Ph.D. working in cardiovascular physiology/pathophysiology. Research: Hypertension is a highly prevalent condition involving the kidney's inability to excrete excess salt. Abnormally enhanced NaCl reabsorption thru the apical Na+/K+/2Cl- cotransporter (NKCC2) by the thick ascending limb of the loop of Henle (TAL) is associated with salt-sensitive hypertension in humans. NKCC2 inhibitors has many side effects, therefore they are not used. This project study the role of a novel E3 ubiquitin ligase FBXL13 on NaCl reabsorption and blood pressure regulation under normal or high salt diet. In Aim I, we study the effect of high salt on NKCC2 ubiquitination, surface phosphor (Thr96-101 and Ser126) and total NKCC2 expression and activity. In Aim II, we will study the FBXL13-NKCC2 interaction. In Aim III We study the role of FBXL13 on NaCl reabsorption and blood pressure regulation. This application will advance our knowledge which may lead to new strategies for the treatment of hypertension and promote the development of novel and specific loop diuretics.

Principal Investigator: Pamela Harding, Ph.D.
Opposing Effects of Prostaglandin E2 EP3 and EP4 Receptors on Mitochondrial Function in the Failing Heart (1ROHL148090)

Heart disease and heart failure are important causes of morbidity and mortality, affecting approximately 300 million people, at an enormous cost. Although current treatments slow the progression of these diseases, there has been little progress in preventing the compensated heart transitioning to that of a failing one. The Prostaglandin E2 (PGE2) receptor subtypes EP3 and EP4 are most abundant in the heart and activate different signaling pathways (Gαi for EP3, Gαs for EP4). Compelling data from my laboratory shows that EP3 expression is increased in the pathologically diseased heart produced by myocardial infarction (MI) or Angiotensin II- dependent hypertension (Ang II-HTN), that stimulation of the EP3 receptor decreases cardiac contractility whereas EP4 increases it and that overexpression of EP4 in the failing heart improves cardiac function. The failing heart switches from fatty acid (FA) oxidation to reliance on glucose. Coupled with this are alterations in mitochondrial function. Thus, mitochondrial dysfunction is an important player in the pathogenesis of heart failure. Our previous gene array data showed dramatic down regulation of mitochondrial genes in mice in which the EP4 receptor was deleted in cardiomyocytes; allowing PGE2 to act via the EP3 receptor and new data shows that an EP3 agonist reduces Complex I activity and ATP levels in adult mouse cardiomyocytes. mRNAs for proteins that alter both FA oxidation and their transport into mitochondria; specifically, carnitine palmitoyl transferase (CPT) were also down regulated in EP4 KO hearts. In other tissues, CPT activity was reportedly regulated by

the transcription factor/orphan receptor NR4A2 (Nurr1) in a PGE2-dependent process but whether this occurs in the heart is unstudied. Since heart failure is characterized by reduced FA oxidation, we propose the novel overall hypothesis: EP3 is increased during cardiac injury and impairs mitochondrial function due to reduced fatty acid import via diminished CPT activity. This is mediated by decreased activity of the transcription factor, NR4A2. Ultimately these events contribute to heart failure. To test this hypothesis, we propose 3 aims that will use a new conditional and cardiomyocyte-specific EP3 KO mouse model coupled with an EP3 overexpressing transgenic mouse to determine whether upregulation of cardiomyocyte EP3 contributes to impaired contractile function and reduced mitochondrial function in heart failure caused by Ang II-HTN and MI. The study also examines whether PGE2 via its EP3 receptor reduces import of fatty acids into mitochondria via decreased activity of the transcription factor NR4A2 which subsequently reduces CPT activity; and whether these events reduce subsequent ATP levels. The proposal employs a multidisciplinary approach including physiological, biochemical and imaging studies that will impact the treatment of heart failure.

Principal Investigator: Emilio Mottillo, Ph.D.
Direct Analysis of Lipolysis-mediated Signaling Events (5R00DK114471)

Project Summary/Abstract Obesity has reached epidemic proportions and is tied to the greater prevalence of metabolic disorders such as diabetes and cardiovascular disease. While the precise mechanisms by which obesity causes diabetes are not entirely clear, mounting evidence suggests that the body's normal process of sensing lipids is disrupted. This inability to properly detect lipids can lead to lipotoxicity and cause detrimental effects in key insulin sensitive tissues. Thus, an important scientific goal, and that of this NIH Pathway to Independence Award, is to understand the mechanisms by which cells sense lipids and thereby maintain lipid homeostasis. The training component of this application builds upon the candidate's interest and background in imaging metabolism and metabolic signaling/energy sensing, while providing a unique environment to train in career development activities related to a team science approach of doing research. The research training component will utilize a unique set of tools that will allow the candidate to probe the direct effects of lipolysis independent of transmembrane-protein kinase A (PKA) signaling and image fatty acid metabolism. The candidate will gain experience in global analysis techniques of phosphoproteomics and lipidomics, and super-resolution imaging. Utilizing these tools and training, the candidate will determine 1) the signals directly generated by lipolysis, and 2) the dynamics of lipid trafficking and lipolysis-derived signals within a cell. Central to this aim is the hypothesis that signals directly produced by lipolysis function to maintain lipid homeostasis and are highly dynamic. The research component of the award will be accomplished by the following specific aims: Aim 1: Identification of signals that are generated directly by lipolysis. Lipolysis is known to produce signals, but up to this point the direct effects of lipolysis were not distinguishable from transmembrane-PKA signals. Utilizing novel synthetic ligands that activate ABHD5, a lipase co-activator protein, Aim 1 will be accomplished by the following sub-aims: 1a: To identify ABHD5-dependent lipid mediators. Utilizing a lipidomic approach, the candidate will identify the bioactive lipids produced by ABHD5 that regulate downstream metabolism. 1b: To identify ABHD5-dependent kinase activation pathways. Using a phosphoproteomic approach, the candidate will determine the phosphorylation events, kinases and pathways that are a direct consequence of ABHD5 activation. Aim 2: To determine the trafficking dynamics of fatty acids and their metabolites and lipid mediators. This will be accomplished by the use of newly developed genetically encoded fluorescent sensors that allow the monitoring of the temporal and spatial dynamics of fatty acids and fatty acyl-CoAs. The proposed K00/K99 is well aligned with the mission of the NIH and the NIDDK and will train a promising scientist to understand the mechanisms that regulate lipid homeostasis and potentially the pathways that are disrupted during obesity, a significant public health priority.

Principal Investigator: Pablo Ortiz, Ph.D.
Fructose Induced Salt-Sensitive Hypertension: Role of Thick Ascending Limb Transport (R01DK107263)

A high-fructose diet is linked to the epidemic of hypertension, diabetes, and obesity. Up to 25 million Americans consume up to 20% of their calories from added fructose1,2. We found that feeding rats a fructose-enriched diet (20%) *for 4 weeks did not increase blood pressure. However, a fructose-enriched diet combined with high salt (4% Na) caused salt-sensitive hypertension within 1 week* (Figures 1,11); prior to the development of metabolic abnormalities. The initial phase of salt-sensitive hypertension is in part mediated by a renal defect that prevents NaCl excretion during high salt intake. The thick ascending

limb (TAL) reabsorbs 25% of filtered NaCl. Enhanced TAL NaCl absorption is related to salt-sensitive hypertension in humans and rodents3-5. However, the mechanism by which a fructose-enriched diet rapidly (1 week) causes salt-sensitive hypertension is not clear and the role of TAL NaCl absorption in this process is completely unknown. NaCl reabsorption by the TAL depends on the apical Na/K/2Cl cotransporter NKCC2, the target of loop diuretics. Our preliminary data show that a fructose-enriched diet enhanced NKCC2 phosphorylation at Threonine (Thr)96,101. NKCC2 phosphorylation at Thr96,101 activates NKCC26,7. *Our data show that NKCC2-mediated NaCl transport is abnormally elevated in rats fed fructose plus a high salt diet*. However, the effects of fructose and *the signaling induced* in the TAL and the distal nephron have not been studied. Our data show that plasma and urine fructose increase rapidly after fructose intake. Thus, fructose reaching the nephron may be transported in by a fructose channel, activating protein kinase signaling. The only kinases known to phosphorylate Thr96,101 of NKCC2 are SPAK (STE20/SPS1- related proline-alanine-rich kinase) and OSR1 (Oxidative Stress Responsive 1) kinases. *In the TAL, these kinases specifically phosphorylate NKCC2. In the distal convoluted tubule (DCT), these kinases specifically phosphorylate the thiazide sensitive NaCl transporter NCC.* We found that a 20% fructose diet increases SPAK/OSR1 phosphorylation in TALs. In addition, stimulation of β-adrenergic receptors (β-AR) in the TAL activates NKCC213. A fructose-enriched diet may increase sympathetic activity by 2 weeks12, or enhance the sensitivity or signaling of β-AR. Our preliminary data show that β-AR stimulation increases SPAK/OSR1 phosphorylation in TALs. *In the Dahl salt sensitive (SS) rat, NKCC2 and SPAK/OSR1 phosphorylation are abnormally enhanced in a normal salt diet. It is not known whether this increases the effect of fructose on blood pressure and NaCl absorption*. *We hypothesize that a fructose-enriched diet enhances thick ascending limb (TAL) and distal tubule (DCT) NaCl absorption by inducing NKCC2 and NCC phosphorylation via SPAK/OSR1 kinases and enhanced β-AR signaling. These effects occur within 1 week, prior to metabolic alterations, and are maintained chronically (16 weeks), promoting salt- sensitive hypertension in normal rats.* In *Dahl SS rats, abnormally elevated SPAK/OSR1 in the TAL, enhances the effect of fructose on blood pressure in normal- or high-salt diets*.

Principal Investigator: Suresh Palaniyandi, Ph.D.
4-hydoxy-2-nonenal in Mitochondrial DNA Damage and Contractile Dysfunction in Diabetic Heart: A Role for Aldehyde Dehydrogenase 2 (R01HL139877)

Diabetes mellitus (DM) afflicts 26 million people in the US. 40-70% of these diabetics die of cardiovascular complications. We and others found that DM increases reactive oxygen species (ROS)-mediated aldehydes like 4-hydroxy-2-nonenal (4HNE) generation. 4HNE forms covalent bonds with macromolecules known as adducts, which lead to cellular damage and decreased cardiac function. Aldehyde dehydrogenase (ALDH2) is a mitochondrial enzyme that detoxifies 4HNE in the heart. We and others have reported that in streptozotocin- induced hyperglycemic models increase in 4HNE protein adducts and decrease in myocardial ALDH2 activity correlate with cardiomyopathy. Although we think this causes cardiac dysfunction, the exact mechanism is unclear. However, most diabetic patients have type-2 DM. Thus, it is imperative to investigate whether hyperglycemia-induced 4HNE and lower ALDH2 activity contribute to cardiac dysfunction in type-2 DM models. We recently demonstrated that high glucose stress or 4HNE administration decreased mitochondrial respiration with increased mitochondrial DNA (mtDNA) damage in cultured cardiomyocytes. In our preliminary study using type-2 diabetic mouse heart, we found an increase in mitochondrial levels of 8-hydroxyguanine (8OHG), an oxidized mtDNA product, which is primarily repaired by 8-oxoguanine glycosylase (OGG)-1. Next, we found increased 4HNE adduct formation on OGG-1 and reduced cardiac OGG-1 levels. These data suggest that 4HNE adduction on OGG-1 reduces its level and activity thereby raising the unmetabolized 8OHG level. Thus, we postulate that 4HNE-mediated mtDNA damage is part of the mechanism by which lower ALDH2 causes mitochondrial respiratory dysfunction and thus cardiac contractile dysfunction. To test our idea, we will use a high-fat diet induced type-2 DM model in wild type (WT) C57BL/6 and ALDH2*2 mutant mice. This mutation mimics East Asians with the E487K variant (ALDH2*2), which exhibits lower ALDH2 activity. We will overexpress ALDH2 gene in the myocardium *in situ* or treat our diabetic mice with Alda-1, the only specific drug available to improve the catalytic activity of both WT and mutant ALDH2. We propose following three specific aims: **Aim 1- Hyperglycemia in models of type-2 diabetes reduces ALDH2 activity in cardiac myocytes by increasing 4HNE adduction with ALDH2: Aim 2- Increased 4HNE adduct formation on mtDNA and OGG-1 causes mtDNA damage and poor mitochondrial respiration in type-2 DM: Aim 3- Augmenting ALDH2 activity reduces 4HNE-mediated mtDNA damage and thereby cardiomyopathy progression in type2-DM.** This study will identify a novel role of ALDH2 in type-2

DM mediated cardiac dysfunction and establish that ALDH2 could be a therapeutic target for restoring cardiac function in type-2 diabetic patients.

Principal Investigator: Nour-Eddine Rhaleb, Ph.D.
Ac-SKDP in the Treatment of Cardiac Dysfunction in Hypertension or Ischemic Heart (R01HL136456)

Hypertension is a major health care burden in the United States, affecting 1 in 3 adults. Hypertension is associated with concomitant coronary artery disease with myocardial infarction (MI) and heart failure (HF). In this study, we will define how N-acetyl-seryl-aspartyl-lysyl-proline (Ac-SDKP) protects cardiac structure and function in a mouse model of HF that will be induced in two models [angiotensin II (Ang II) hypertension- or permanent left anterior descending coronary ligation (LAD)]. We and others reported that Ang II-induced hypertension or LAD resulted in HF associated with cardiac structural remodeling and impaired function. Ac- SDKP is successively produced from thymosin ¿4 (T¿4) by two enzymes, meprin ¿ and prolyl oligopeptidase (POP). Circulating and tissue Ac-SDKP depends on the angiotensin converting enzyme (ACE) activity, since Ac- SDKP is mainly degraded by the N-terminal active side of ACE (ACE-N). ACEi are first-line drugs to treat HF. ACEi have strong side effects such as hypotension, cough, rash, angioneurotic edema, hyperkalemia, and dysgeusia, whereas Ac-SDKP has none, even at high dosages (up to 48 mg/kg/d). Also, Ac-SDKP is down- regulated in the myocardium of dogs and patients with chronic HF. Whether and how Ac-SDKP therapy could rescue hypertension- or LAD-induced cardiac complications remain to be elucidated. Increasing circulating Ac- SDKP not only inhibited fibrosis and mediators of inflammatory cell infiltration into the injured myocardium, but it also improved cardiac function in mice with LAD or hypertension (preliminary data). We have found that Ac-SDKP inhibits endoplasmic reticulum (ER) stress in cardiac fibroblasts in vitro and in mice with MI and restores phosphor-AKT in hypertensive hearts. Activation of ER stress is detrimental to the endothelium, cardiac fibroblasts, and cardiomyocytes. These findings set the scientific premise of this work, providing foundational work that Ac-SDKP represents a beneficial supplement to the existing cardiac pharmacotherapy. Our central hypothesis is that Ac-SDKP protects and potentiates cardiac protection against heart failure via the inhibition of ER stress. We propose to use the mouse model of heart failure induced by hypertension or LAD to address the following 2 two aims: (1) we will determine whether Ac-SDKP protects the heart and provides additional cardiac protective effects to ARBs, ACEi, or eplerenone in mice with MI or hypertension, (2) and we will demonstrate that Ac-SDKP improves cardiac function in mice with hypertension or LAD by inhibiting the detrimental ER stress via the PI3K/AKT pathway. A number of conditional and tissue-specific knockout female and male mice will be employed. A team with significant expertise is recruited for this project, which will apply a combination of state-of-the-art in vivo, cell and molecular techniques including measurements of cardiac remodeling and function by echocardiography in non-anesthetized mice and radiotelemetry, which can detect the blood pressure, the electrocardiogram, and the heart rate of conscious mice. These studies will help define the cause-effect relationship between Ac-SDKP and HF and its mechanism towards the protection from HF.

Pulmonary

Principal Investigator: Jayna Gardner-Gray, M.D.
Clinical Centers (CC) for the NHLBI Prevention and Early Treatment of Acute Lung: Reevaluation of Systemic Early Neuromuscular Blockade (U01HL123031)
Subcontract

Purpose: This study is evaluating whether giving a neuromuscular blocker (skeletal muscle relaxant) to a patient with acute respiratory distress syndrome will improve survival. Half of the patients will receive a neuromuscular blocker for two days and in the other half the use of neuromuscular blockers will be discouraged. **Trial Summary**: Study Design: This is a multi-center, prospective, 2-arm, unblinded, randomized clinical trial of two management strategies of neuromuscular blockade (also called skeletal muscle relaxant and muscle relaxant). Purpose: To assess the efficacy and safety of early neuromuscular blockade in reducing mortality and morbidity in patients with moderate-severe ARDS in comparison to a control group with no routine early neuromuscular blockade. Sample Size: This trial will enroll approximately 1400 subjects from PETAL network hospital ICUs.

Sleep Medicine

Principal Investigator: Philip Cheng, Ph.D.
Clinical Translation of Phenotypes of Shift Work Disorder (1K23HL138166)

Shift work disorder (SWD) is a significant threat to public health and safety; over 6 million shift workers in the United States experience the debilitating symptoms of excessive sleepiness and insomnia and suffer functional impairments that increases the risk of catastrophic industrial accidents. However, patients with SWD are often inadequately treated because the pathophysiology is not well-characterized, and current diagnostic assessments do not identify specific treatment targets. Consequently, clinicians are unable to deliver precise interventions that target the underlying causes of SWD. The proposed project in this career development award will address these gaps by taking the initial steps of translating two phenotypes of SWD for clinical use. Previous research has indicated that SWD can arise from two independent pathways that can be categorized as pathophysiological phenotypes. The first is the circadian misalignment phenotype, characterized by poor adjustment of the biological clock to the nocturnal work schedule. The second is the sleep reactivity phenotype, characterized by a trait vulnerability to sleep disturbance triggered by environmental stressors. Both phenotypes lead to symptoms of sleepiness and insomnia in SWD and is not currently distinguished in the clinic; however, the requisite treatments for each pathophysiological phenotype are entirely different. As such, the appropriate intervention of SWD requires that these phenotypes be adequately characterized and identified in the clinic. The proposed aims will complete the requisite foundational research to launch the translation of these phenotypes of SWD for clinical use. The first research aim will examine the stability of each phenotype in shift workers to characterize them as either state or trait phenotypes, which will impact both assessments of interventions. The second research aim will identify the specific clinical attributes that can be used to index the phenotypes in a brief, accurate, and cost-effective assessment tool. Finally, the third research aim will identify differences in cognitive and performance deficits between the two phenotypes so that accidents and injuries can be preempted with targeted interventions. To successfully complete the research aims, and to support my long term goal of conducting translational research to improve the health and productivity of shift workers, this career development award will provide further training in the following areas: (1) development of clinical screening tools, (2) advanced methodologies in clinical and translation research, (3) feasibility of real-world behavioral interventions for shift work disorder, and (4) advanced field measurement of circadian phase. In combination, the training activities outlined in this career development award will provide the necessary expertise for a sustained career in translational research and circadian medicine.

Principal Investigator: Christopher Drake, Ph.D.
Sleep to Reduce Incident Depression Effectively (STRIDE) (1R56MH115150)

Abstract Prevention of major depressive disorder (MDD) is a public health priority and is in critical need of innovative strategies that preemptively identify those at-risk in order to enable early intervention. A recent meta-analysis of over 20 longitudinal studies found the risk for incident depression among individuals with insomnia disorder is nearly three times that for normal sleepers, thus making insomnia a potential point of entry for depression prevention. Identification and treatment of insomnia typically occurs in primary care, and is commonly treated with hypnotic medications; however, hypnotics have significant limitations, including increased risk for residual impairment, falls in the elderly, and abuse. Cognitive behavioral treatment of insomnia (CBT-I) has been recommended as a first line approach with demonstrated efficacy that is sustained beyond initial therapeutic intervention. However, effective and widespread implementation of CBT-I is severely limited by the national shortage of trained practitioners in clinical practice. A stepped care approach rooted in primary care holds potential for innovative accessibility and delivery of CBT-I, improving insomnia therapeutics, and reducing rates of MDD by targeting a robust yet modifiable risk factor in insomnia. Our proposed stepped care model uses digital cognitive behavioral therapy (dCBT-I) as an accessible, least-restrictive, first line intervention that reduces specialist time and resources, and adds clinician based face-to-face CBT-I only for refractory patients who need a more personalized, flexible, and durable therapist driven approach. We propose a large-scale clinical trial in the primary care setting that utilizes a stepped care model (SMART design) to determine the effectiveness of dCBT-I alone and in combination with face-to-face CBT-I for insomnia, and the effects of these sleep interventions on the prevention of MDD. An important innovative component of the trial is the 1 and 2-year follow-up assessments to determine the durability of

effectiveness over time and assess the impact on depression incidence and relapse. Early risk-detection and prevention is especially critical in those at elevated risk for depression to reduce health disparities. Thus, individuals with significant vulnerability to MDD, such as high sleep-reactivity, low socioeconomic status, and racial minorities will be included in significant numbers to test for potential moderation of treatment effects stratified by risk. Finally, improving sleep through insomnia treatment may reduce nocturnal rumination, which may mitigate progression toward MDD. As such, we will determine whether changes in nocturnal rumination (i.e., target), a modifiable risk-factor, mediates the effects of CBT-I and dCBT-I on MDD incidence and relapse. This project will test a highly scalable model of sleep care in a large primary care system to determine the potential for wide dissemination to address the high volume of population need for safe and effective insomnia treatment and associated prevention of depression

Principal Investigator: Timothy Roehrs, Ph.D.
Risks for Transition from Therapeutic Hypnotic Use to Abuse (R01DA038177)

The acknowledged drugs of choice for the pharmacological treatment of insomnia are the benzodiazepine receptor ligand hypnotics (BzRL). Our nighttime studies show that with therapeutic doses used either short-term or chronically, the abuse liability of BzRLs ininsomnia is not seen universally and is relatively low. The data from our last grant, a first-ever study, showed the abuse liability of chronic zolpidem use in insomniacs was low. Yet case reports and retrospective studies continue to report BzRL dependence and for the majority of these cases the abuse developed through initial therapeutic use. In our study some subjects showed an increase in dose across time. Understanding the transition from therapeutic use to abuse and identifying risk factors, such as specific patient and drug characteristics, is both mechanistically and clinically important. Our preliminary data have shown that a subset of insomniacs, those insomniacs that have signs of hyperarousal as reflected by elevated Multiple Sleep LatencyTest (MSLT) scores, increased their nightly zolpidem dose across time. BzRLs have differential receptor binding affinities and associated anxiolytic or antidepressant properties. Zolpidem has selective alpha 1 BzRL affinity and little mood activity and thus may show less risk for transition from therapeutic use to abuse than another currently frequently prescribed BzRL with less alpha subtype selectivity such as eszopiclone. We propose to study the abuse liability of a selective (zolpidem) vs nonselective (eszopiclone) hypnotic during chronic use (six months) in an at-risk sub-population (insomniacs with hyperarousal shown by elevated MSLTs). The proposal is highly innovative as it reflects a paradigm shift in understanding the abuse liability of hypnotics. In the end, this proposal will generate a unique set of data addressing a number of previously clinically important unanswered questions regarding hypnotic abuse by insomniacs (i.e., its likelihood as a function of arousal state and specific hypnotic pharmacology, of dose escalation over time and change in mood/drug effect ratings over time). It will provide clinicians with behavioral indicators of abuse risk.

General Internal Medicine

Principal Investigator: David Willens, M.D., MPH, FACP
eASSIST A Post-Visit Patient Portal Tool to Promote Colorectal Cancer Screening (R01CA197205-05) Subcontract

The goal of this study is to develop and test a patient portal tool (e-Assist) for engaging and supporting primary care patients to make decisions about and to obtain colorectal cancer screening. Henry Ford Health System will serve as the performance site for this study. As such, the e-Assist tool will be programmed by HFHS programmers and all day-to-day aspects of the study will be coordinated by staff at Henry Ford's Center for Health Policy and Health Services Research with oversight from the study PI and team. These tasks include identifying eligible participants and coordinating all elements of communication with patients (letter, phone interviews, etch.) In addition, HFHS staff will conduct focus groups and cognitive interviews with patients for preliminary testing of the e-Assist tool.

Hematology/Oncology

Principal Investigator: Ding Wang, M.D., Ph.D.
SWOG Network Group Operations Center of the NCTN (U10CA180888) Subcontract

The impact statement in SWOG's network operations grant application succinctly summarizes our work and our goals: By continuously improving inclusion, engagement, and scientific innovation, SWOG will enhance cancer clinical trial development and conduct, reducing the burden of human neoplasm. The SWOG National Clinical Trials Network Group has established itself as an innovative, collaborative, a n d cost-effective NCTN constituent. SWOG has 60 years of trial experience, and its work has led to the Food and Drug Administration approval of 14 regimens, changing and informing oncologic practice hundreds of time more. In our 2013 grant application, we promised to make unique contributions to the new NCTN enterprise, and we successfully did so over the last five years. We are strongly committed to furthering our efforts over the next six. SWOG designs and directs high-value, pathway- and immune-driven oncology research, with the goal of achieving practice-changing results that are meaningful to both persons affected by cancer and investigators. The group's current network includes more than 1,000 member sites, with 5,000 physicians who practice across the United States, Canada, South Korea, Mexico, Saudi Arabia, and South America. Twenty-three NCI-designated cancer centers number among our members, as do 22 Specialized Programs in Oncology Research Excellence. From early 2014 through mid-2017, SWOG investigators published more than 188 cancer treatment articles and abstracts and enrolled 12,819 patients into NCTN therapeutic trials. SWOG actively collaborates in NCTN direct research and administrative functions and has developed training and education tools used throughout the network. SWOG's mission is to significantly improve lives through cancer clinical trials and translational research. The following guiding principles, ratified in 9/2017, are the foundation upon which we build to achieve that end: — We make patients our absolute highest priority — We ensure that the best science drives our research — We embrace and encourage diversity in leadership and membership, to effectively solve problems in cancer — We demand integrity, accountability, and ethical behavior in SWOG — We foster and mentor young investigators, to ensure excellent clinical research for future generations Over the next grant cycle, we will provide an efficient, innovative, and nimble network capable of developing and conducting abroad framework of clinical and translational trials; we will meaningfully contribute to the NCTN; and we will help patients lead longer and meaningful lives. SWOG will remain an innovative force in the design of the next generation of oncologic therapies.

Part II – All Other Clinical Departments

- **Dermatology**
- **Emergency Medicine**
- **Neurology**
- **Neurosurgery**
- **Orthopaedics/Bone & Joint**
- **Otolaryngology**
- **Pathology**
- **Pediatrics**
- **Psychiatry/Behavioral Health**
- **Radiation Oncology**
- **Women's Health**

Dermatology

Principal Investigator: Aimin Jiang, Ph.D.
b-catenin in Vaccine-induced Anti-tumor CD8 T Cell Immunity (5R01CA198105)

One major obstacle for the success of dendritic cell (DC) vaccines is host DC-mediated immunosuppression. Cross-priming, a process which DCs activate CD8+ T cells through cross-presentation, plays a major role in generating anti-tumor CD8+ T cell immunity. Tumor antigen cross-presentation by host DCs, however, often induces CD8+ T cell tolerance instead of immunity. Thus, there is a critical need to better understand whether and how tumors modulate cross-priming to suppress CD8+ T cell immunity. The long-term goal is to develop strategies to block tumor-induced immunosuppression to augment CD8+ T cell immunity and improve cancer vaccine efficacy. The objectives in this application is to elucidate the underlying mechanisms of how tumors inhibit cross-priming through β-catenin in DCs, and validate blocking β-catenin signaling as a novel strategy to improve cancer vaccine efficacy. The central hypothesis is that tumors differentially regulate DCs' cytokine induction through β-catenin to inhibit cross-priming, and blocking β-catenin's function in cross-priming augments vaccine-induced anti-tumor CD8+ T cell immunity. This hypothesis has been formulated on the basis of preliminary data produced in the applicant's laboratory. The rationale is that once it is known how tumors suppress CD8+ T cell responses through β-catenin, strategies targeting β-catenin signaling can then be developed to improve cancer vaccine efficacy. Three specific aims are proposed: 1) To determine whether activation of β-catenin in DCs suppresses anti- tumor CD8+ T cell immunity under diverse cancer vaccinations. 2) To elucidate the molecular mechanisms of how tumors inhibit cross-priming through β-catenin in DCs. 3) To determine whether blocking β-catenin pharmacologically improves cancer vaccine efficacy. We will carry out adoptive transfer, phenotypic and functional assays including multiple cross-priming assays, real-time PCR, in vivo killing assays and DC-targeted vaccinations with DC-specific knockout mice with either active or inactive β-catenin. The project is innovative because: (1) Based on the findings that β-catenin mediates tumor-induced suppression of CD8+ T cell immunity by inhibiting cross-priming, we will determine whether a novel strategy targeting DCs' function in cross-priming improves vaccine efficacy. (2) Using genetic and pharmacological approaches to alter β-catenin expression, this proposal will elucidate the mechanisms of how tumors inhibit cross-priming through β-catenin in DCs. As β- catenin inhibitors have been tested in pre-clinical studies and clinical trials, this proposal will additionally provide direct evidence to support the application of β-catenin inhibitors in cancer vaccines. The proposed research is significant, because it addresses how cross-priming is modulated by tumors to achieve DC-mediated immunosuppression, a fundamental but unanswered question in cancer immunology and DC biology, and more importantly will validate modulating β-catenin signaling as a novel strategy to improve cancer vaccine efficacy.

Principal Investigator: Qing-Sheng Mi, M.D., Ph.D.
Genetic and Genomic Dissection of Psoriatic Arthritis (R01AR063611)

HFHS will participate in this project by continuing its longitudinal assessment of patients with cutaneous psoriasis (PsC) and psoriatic arthritis (PsA), through our existing collaboration in IPART (the International Psoriatic Arthritis Research Team). It will also continue to collaborate with PI James Elder and Co-PI Dafna Gladman (University Health Network, Toronto, CA) to provide serum samples to identify serum mircor-RNA (miRNA) biomarkers of conversion of PsC to PsA. Under the direction of Co-PI Qing-Sheng Mi, HFH will assay serum mi RNAs for the purpose of biomarker identification and provide guidance for the assessment of biological functions of identified serum miRNA biomarkers. HFH (through IPART) will contribute additional DNA samples from patients with PsC and PsA as well as normal controls for expanded GWAS genotyping of psoriasis, PsC, and PsA. Finally, HFH (through IPART) will contribute additional blood samples for assessment of blood mRNAs and miRNAs as biomarkers for progression of PsC to PsA. Applicant Identifier 19-PAF02365 submitted in response to Opportunity ID PA-18-484, is incorporated herein by reference as applicable.

Principal Investigator: Qing-Sheng Mi, M.D., Ph.D.
Uncover the New Subsets of Epidermal Langerhans Cells (1R61AR076803)

Langerhans cells (LCs) are skin-resident dendritic cells (DCs) expressing the C-type lectin Langerin (CD207) that mediate both adaptive immunity and immune tolerance in skin and are involved in various types of skin diseases. Adult LCs are originated from embryonic yolk-sac-derived macrophages and fetal liver monocytes in the steady state. Interestingly, LCs could also be derived from the bone marrow or peripheral monocytes and repopulate the skin under inflammatory conditions. However, due to the lack of molecular profiles at individual LC level, a significant gap remains in our understanding on how a single CD207+ epidermal LC population can induce both immunity and tolerance. Fortunately, new technologies such as the single-cell RNA-sequencing (scRNA-seq) can evaluate cell-to-cell transcriptomic variation, while the single-cell assay for transposaseaccessible chromatin using sequencing (scATAC-seq) can assess the epigenomic heterogeneity at single-cell resolution in an unbiased manner. Recently, we identified two major LC subsets in mice, ATF3+Bal2a1b- (mLC1) and ATF3-Bal2a1b+(mLC2) subsets, and three major LC subsets in human including ATF3+ (hLC1) subset using scRNA-seq. We also found in ATF3 knockout mice that lack of ATF3 enhances LC maturation and promotes LCs-induced Th1 and Th17 cell differentiation suggesting immune suppressive function induced by ATF3+LC1. Hence, these preliminary data support our hypothesis that LCs are heterogeneous consisting of distinct subset with different immune functions. Our objective is to use single-cell analysis platforms plus the LC fate-mapping and mutation mouse models to further validate this. We will pursue two Specific Aims in the R61 phase: Aim 1) Characterize the gene signatures and regulatory elements of mLC1 and mLC2 by profiling LCs during embryonic, young, and aging development at steady-state and repopulated LCs at inflamed state using scRNA-seq and scATAC-seq; Aim 2) Generate ATF3negEGFP reporter mice to fate-map ATF3+LC1 embryonic development and the dynamic change of ATF3+LC1 and ATF3-LC2 subset at steady state during adult and aging development and at inflammatory state and functionally characterize LC subsets in vitro by sorting ATF3EGFP+ LC1 and ATF3- LC2 cells and rederiving ATF3.loxp mice, which will be crossed with hLangerin-Cre mice to generate LCspecific/time induced ATF3KO for in vivo functional study. In the R33 phase, we will pursue the following Specific Aim: Aim 3) Functionally characterize ATF3+LC1 subset in vivo using LC-specific ATF3 deletion hLCcre.ATF3KO mice to evaluate the potential immune regulation function of ATF3+LC1 subset in the different disease models, including autoimmune vitiligo, melanoma, and fungi infection models. Our work will uncover the mystery of LC subsets with their specific functions, which will provide new insights into the biology of LCs and lead to the development of LC-based intervention strategies for skin diseases.

Principal Investigator: Qing-Sheng Mi, M.D., Ph.D.
microRNAs and NKT Cell Development and Function (R01AI119041)

Natural killer T (NKT) cells are an evolutionarily conserved subset of T cells that are developmentally and functionally distinct from conventional T cells. The ability to quickly secrete large quantities of a variety of cytokines upon activation enables NKT cells to be potent regulators of diverse immune responses. The deficiencies in NKT cell number and function have been linked to the development of many diseases. However, *a significant gap* remains in our understanding of how the development and function of NKT cells are precisely regulated. MicroRNAs (miRNAs), a recently discovered class of evolutionarily conserved small non- coding RNAs, negatively regulate the expression of protein-coding genes and thereby control essential biological functions and contribute to the development of many diseases. We

were the first to report that the deletion of Dicer (a key enzyme for miRNA biogenesis) during hematopoiesis results in a significantly reduced NKT cell number and impaired NKT cell maturation and function, without alternating conventional T cell development in the thymus, suggesting that miRNAs are required for NKT cells. Our *long-term goal* is to understand how miRNAs regulate NKT cell development and function. While more than 1000 experimentally reported miRNAs, very few specific miRNAs are linked to NKT cells so far. Our *objective* here is to define specific miRNAs and their targets that regulate NKT cell development and function. Using miRNA arrays, we recently identified dynamic expression of miRNAs, including miR- 155, and miR-17-92 cluster, during NKT cell development and activation. These findings plus our recent other report lead to our *central hypothesis* that these dynamically expressed miRNAs serve as critical regulators controlling NKT cell development and function through fine-tuning of specific target genes. Here we will further test this hypothesis. We will investigate how dynamic and miR-155 and miR-17-92 expression regulates NKT cell development and function using specific miRNA mutant mice with the gain or loss of miRNA gene. The results from proposed studies may not only illuminate the new immunological and molecular mechanisms underlying NKT cell development but may also facilitate the development of new and more efficient intervention strategies for autoimmune diseases, infection, and cancer based on the NKT cell therapy.

Principal Investigator: Qing-Sheng Mi, M.D., Ph.D.
Roles of HDAC3 in Epidermal Langerhans Cell Ontogeny and Function (R01AR069681)

Langerhans cells (LCs), the skin residing dendritic cells (DCs), form a contiguous immune network in skin and are involved in allergy, infection, cancer, and autoimmune disease development. However, the regulatory mechanisms involved in the development and functions of LCs have not been completely elucidated. Histone deacetylases (HDACs) are enzymes that regulate gene expression by modifying chromatin structure through removal of acetyl groups from target histones or directly deacetylating nonhistone proteins and represent a key epigenetic regulatory mechanism. HDAC inhibitors (HDI) are shown to have anti-tumor and anti- inflammatory effects in a variety of diseases, in which LCs play an important role. However, the mechanisms underlying the clinical effectiveness of HDI remain largely unknown. We recently reported that the inhibition of Class I/II HDACs by Trichostatin A (TSA) regulates the homeostasis and function of LCs in vitro and in vivo and modulates the non-coding miRNA expressions in LCs, while miRNAs also control LC development and function. Our preliminary data indicate that LCs express all Class I/II HDACs. To evaluate the role of individual HDACs in LC development and function, we generated knockout (KO) mice with selective deletion of HDAC3 (Class I) or HDAC4 (Class II) in epidermal LCs. Interestingly, LC number was significantly reduced in LC-HDAC3KO mice, but unaffected in LC-HDAC4KO mice. Furthermore, LC maturation and function were altered in LC-HDAC3KO mice. Thus, we hypothesize that HDAC3 is a key epigenetic component that controls LC development and function. In Aim 1, we will investigate the roles of HDAC3 in LC development and homeostasis, using LC-HDAC3KO mice for homeostasis after birth and using constitutive Csf1r-specific HDAC3-deletion mice (Csf1r-HDAC3) and inducible Csf1rspecific HDAC3-deletion (Csfr1.Mer-HDAC3) mice for early embryonic LC development; Aim 2, we will investigate the roles of HDAC3 in LC function, using inducible LCER. HDAC3KO mice. In Aim 3, we will elucidate the molecular mechanisms and signaling pathways by which HDAC3 regulates LC development and function, by combining cDNA array, miRNA array and ChiP-Seq techniques. The proposed studies will uncover the epigenetic regulatory mechanisms of HDAC3 in LC development and function and may also elucidate new mechanisms for HDI therapy.

Principal Investigator: Qing-Sheng Mi, M.D., Ph.D.
Serum MicroRNA Biomarkers of Islet Autoimmunity (R01AI123258) Subcontract

Under Dr. Mi's leadership, the team at Henry Ford Health System will perform miRseq profiles and quantitative miRNA analysis on serum samples using the Exiqon RT-PCR platform. Based on preliminary data, a custom panel of 188 microRNAs will be used. This strategy will allow greatly reducing the cost of measuring microRNAs by almost 50% and yet allow to study serum microRNA extensively; making it possible to measure a larger number of samples for increased statistical power. Over the course of the four-year program, we anticipate measuring microRNA levels in 600 serum samples from the DPT1 cohort, as described in the experimental plan. In addition to this, the team at Henry Ford Health System will perform miRseq to define potential candidates that may be missed by the Exiqon platform.

Principal Investigator: Li Zhou, M.D.
miRNAs Regulate Skin Langerhans Cell Ontogeny and Function (R01AR072046)

Langerhans cells (LCs), the skin residing dendritic cells (DCs), control both the induction of adaptive immunity, and immune tolerance in skin and are involved in variety of skin disease development. However, the regulatory mechanisms involved in the development and functions of LCs have not been completely elucidated. MicroRNAs (miRNAs), a class of non-coding small RNAs, are recognized as important regulators of protein-coding genes through the inhibition of mRNA translation. Using Cre-loxP Dicer deletion mouse models, our laboratory and others have reported that deletion of miRNAs by CD11c-Cre or hLangerin-Cre significantly reduced the number and interrupted the function of LCs, indicating that miRNAs are required for LC homeostasis and function after birth. While there are more than 1000 experimentally reported miRNAs, very few individual miRNAs are linked to LCs so far. We were the first to report that miR-150 and miR-223 differentially regulated LC-induced T cell proliferation and cytokine production. Most recently, our embryonic lineage-tracing studies showed that miRNAs, including miR-17-92cluster, regulate LC embryonic development. Furthermore, using miRNA arrays, we identified that mature LCs have a unique miRNA gene expression profile compared to immature LCs, and that miRNA expression is dynamically changed during LC embryonic ontogeny. These findings led to our central hypothesis that the dynamically changed miRNAs may serve as critical regulators controlling LC ontogeny, homeostasis and function through fine-tuning specific target genes. In Aim 1, we will investigate the roles of miRNAs in LC ontogeny and homeostasis. Constitutive or inducible Csf1r-specific individual miRNA mutant mice will be used for studying embryonic LC ontogeny and LC repopulation after inflammation, while LC-specific Dicer or individual miRNA mutant mice will be used for LC homeostasis after birth. In Aim 2, we will investigate the roles of miRNAs in LC function, inducible LC-specific Dicer or individual miRNA mutation mouse models will be used. In Aim 3, the direct target gene(s) of miRNAs and related signaling pathways involved in LC development and function will be investigated by the combination of RNA-seq, miRNA bioinformatics and related target functional validation strategies. The proposed studies will uncover the dynamic miRNA-mRNA regulation and related molecular mechanisms and signaling pathways that control LC development and function, which will not only provide new insight into the biology of LCs, but may also facilitate the development of LC-based intervention strategies for diseases.

Emergency Medicine

Principal Investigator: Christopher Lewandowski, M.D.
Longitudinal Assessment of Post-traumatic Syndromes (U01MH110925)

Each year, more than 40 million Americans present to US emergency departments (EDs) for evaluation after trauma exposure (TE). While the majority **of** these individuals recover, an important subset develops adverse posttraumatic neuropsychiatric sequelae (APNS). These APNS include traditionally categorized outcomes such as posttraumatic stress disorder (***PTSD***), depression, minor **Traumatic** brain injury (MTBI), and regional or widespread pain. However, these previous definitions **of** outcome have limited progress, and we now appreciate that the actual trajectories **of** APNS are multidimensional, incorporating a range **of** specific outcomes that may be best understood, and optimally targeted for intervention, by dividing across specific domains **of** functioning. This application, submitted in response to RFA-MH-16-500, proposes to identify and characterize the trajectories **of** the most common trauma-induced APNS within these domains **of** functioning using the RDoC classification system. 5,000 patients presenting to the ED after trauma will be screened, recruited, and will receive initial baseline evaluation in the ED, including blood collection and psychophysical, survey, and neurocognitive evaluation. They will be closely monitored over the next 8 weeks using innovative technologies (a wrist wearable for continuous-time monitoring **of** daytime physiology and sleep; a smart phone app for continuous-time monitoring **of** GPS and daily “flash” surveys; weekly web-based neurocognitive tests; periodic mixed-mode surveys; serial saliva collection; deep phenotyping [blood collection, fMRI, psychophysical evaluation]) and then followed less intensively using similar procedures (including deep phenotyping) over the remainder **of** a 52-week follow-up period. Adaptive sampling and state-of-the-art statistical methods will be used to (1) optimize precision in characterizing RDoC construct trajectories and (2) test theoretically-guided, “high yield” hypotheses evaluating the effects **of** pre-trauma, peritraumatic, and recovery-related factors on these trajectories and on multivariate RDoC construct trajectory profiles. The longitudinal schedule **of**

rich, granular, multidimensional data collection in the study has been specifically designed to evaluate those constructs most important to post-TE outcomes and to test the proposed hypotheses. Ensemble machine learning methods will be used to develop tiered-targeted clinical decision support models to identify individuals at high risk **of** specific, common APNS outcomes. The close-knit ED research network that will undertake the study has a strong track record **of** prospective research on APNS and is ideally suited to carry out this exceedingly complex study. The study has been designed to be a resource for the entire field (for example, it has been designed and budgeted to collect and store a great many more biological samples at the ***NIMH*** Biorespository than we can analyze, for use by other investigators).

Principal Investigator: Christopher Lewandowski, M.D.
ICECAP: Influence of Cooling duration on Efficacy in Cardiac Arrest Patients (ICECAP) Trial (SUBK00012911)

Cardiac **Arrest** is a common and devastating emergency **of** the heart and the ***Brain***. More than 380,000 patients suffer out **of** hospital **Cardiac Arrest** (OHCA) each year **in** the US. Improvements **in** cardiac resuscitation (the early links **in** the "chain **of** survival" for **Patients** with OHCA) are tempered by our limited ability to resuscitate and protect the ***Brain*** from global cerebral ischemia. Neurological ***Death*** and disability are common outcomes **in** survivors **of Cardiac Arrest**. Therapeutic **Cooling of** comatose **Patients** resuscitated from shockable rhythms may markedly increase the rate **of** good neurological outcome, but poor outcomes still occur **in** as many as 50%, and the benefit **of Cooling in** those resuscitated from ***Asystole*** and pulseless electrical activity has not been evaluated **in** a ***Randomized*** study. Even **in Patients** with shockable rhythms, prior trials showing **Efficacy** have been questioned. Therapeutic **Cooling** is already a guideline-recommended and commonly used treatment **in** comatose survivors **of Cardiac Arrest**, but because **of** limited data, the optimal duration and ***Patient Selection*** criteria remain unknown and **Cooling** devices are not FDA approved for this indication. ***preclinical*** data and mechanistic studies strongly suggest that durations **of** hypothermia longer than those typically used may minimize ***Brain Injury***. This study will determine if identifying an optimal **duration** of therapeutic hypothermia can improve outcomes, and if development **of** a **duration *response*** curve can substantiate **Efficacy in** a wider ***patient population*** **of Cardiac Arrest** survivors. We hypothesize that longer durations **of Cooling** may improve either the proportion **of Patients** that attain a good neurological recovery or may result **in** better recovery among the proportion already categorized as having good outcome. The overarching goal **of** this project is to identify ***Clinical*** strategies that will increase the number **of Patients** with good neurological recovery from **Cardiac Arrest**. The results **of** this **Trial** will be immediately significant, impacting both ***Clinical*** practice and regulatory ***Evaluation***. The **Trial** uses ***innovative*** adaptive dose finding methods that allow exploration **of** a wide range **of** potential durations and efficiently allocate subjects where they will be most informative. The study methods also include ***innovative*** approaches to traditional outcome assessment and ***innovative*** outcome assessment tools, including the ***NIH*** Toolbox. The study will be conducted **in** the ***NIH*** SIREN Emergency ***Clinical*** Trials ***Infrastructure***. SIREN leverages existing resources to achieve economies **of** scale, maintain talented rapidly responding teams to screen and enroll subjects **in** the emergency department setting, and to continue ***Clinical*** investigations through the ICU stay and beyond with proven performance.

Principal Investigator: Jacob Manteuffel, M.D.
Clinical Trials Network: New England Node: CTN0099 ED-INNOVATION supplement (SUBK00012911)

Study site principal investigator (PI) will be responsible for oversight of all local scientific and administrative processes and procedures required for implementation of ED-initiated buprenorphine in their ED, including use of both SL and XR BUP formulations, and conducting the randomized clinical trial (RCT). They will develop ED protocols, including site-specific standard operating procedures (SOPs) for study and if needed, will establish referral sites including developing partnerships with community opioid treatment providers and programs. They will engage their IT colleagues to ensure that the necessary reports will be available on time. They will hire the research associates (RAs) and assist with their training and supervision. In addition, they will assist in training the ED providers, physicians, advanced practice practitioners (APPS) and nurses on how to administer and prescribe both formulations of buprenorphine. They will work with the pharmacy to be sure that both formulations are in the ED pyxis or easily accessible to the ED providers. They will ensure that all study forms are completed accurately during the baseline index visit, as well as at the 7- and 30-day assessments. They

will be responsible for all the local CQI and monitoring of the research protocol and ensuring quality, and for securing initial and ongoing Institutional Review Board (IRB) approval utilizing the single IRB.

Neurology

Principal Investigator: Jieli Chen, Ph.D.
Diabetic Stroke Cardiac Dysfunction; Treatment with CD133 + Exosomes (R01HL143432)

Cardiovascular complications are primarily responsible for the high morbidity and mortality in people with stroke and diabetes mellitus (DM). Cardiovascular diseases are roughly three times higher in patients with neurological deficits than in patients without neurological diseases. DM is a prominent risk factor for cardiovascular diseases and cerebral ischemic stroke. Our preliminary data show that ischemic stroke and type two DM (T2DM) each induces cardiac dysfunction, while T2DM animals subjected to ischemic stroke exhibit profound cardiac dysfunction compared to non-stroke T2DM mice or non-T2DM stroke mice. Therefore, there is a compelling need to develop therapeutic approaches specifically designed not only to reduce neurological deficits, but also to decrease cardiac dysfunction after stroke with diabetes. Our preliminary data indicate that treatment of stroke in T2DM mice with exosomes derived from human umbilical cord blood isolated CD133+/KDR+ cells (CD133+Exo) 3 days after stroke not only improves neurological and cognitive outcome, but also significantly improves cardiac function and increases heart microRNA (miR)126 and miR29b expression. In a novel and clinically relevant approach, based on our robust preliminary data, we propose to investigate the underlying cardioprotective therapeutic mechanisms of CD133+Exo treatment of stroke in T2DM mice, and we will test the hypothesis that miR126 and miR29b mediate CD133+Exo-induced cardiac protective effects in male and female mice in vitro and in vivo. Two Aims are proposed. Aim 1: To investigate the effect of cerebral ischemic stroke and stroke-related factors (age, sex and T2DM) on cardiac and neurological function in mice. To test the therapeutic effects of CD133+Exo treatment of T2DM-stroke in male, female and aged mice, time window, dose response, multiple doses and combination with anti-diabetic drug (Metformin) studies will be performed. Aim 2: To investigate the mechanism of CD133+Exo induced cardiac protective effects in male and female T2DM-stroke mice in vitro and in vivo. We will focus on miR126 and miR29b, and will test: 1) whether CD133+Exo treatment of T2DM-stroke increases heart and serum miR126 or miR29b levels; 2) whether increasing miR126 or/and miR29b expression in heart or/and serum mediates the CD133+Exo induced cardiac beneficial effects in male and female T2DM-stroke mice; 3) whether the miR126/Spred-1 and/or the miR29b/DPP4 signaling pathways mediate CD133+Exo treatment induced myocardiocyte protection of cultured cardiomyocytes. A major significance of our investigations is that it opens up important and novel ways to understand how exogenously administered CD133+Exo communicate with and alter heart cells by means of miR delivery to thereby activate endogenous cardiac protective events. This proposal is highly clinically relevant and if successful, it will significantly impact the treatment of stroke, diabetes, and cardiac dysfunction. Importantly, this proposal will elucidate novel mechanisms of action and generate therapeutic targets for CD133+Exo treatment of cardiac dysfunction after stroke with T2DM in male, female and aged mice.

Principal Investigators: James Ewing, Ph.D., Neurology and Stephen Brown, Ph.D., Radiation Oncology MRI Signatures of Response to High-Dose Radiotherapy in Rat Models of Cerebral Tumor (R01CA28596)

In some cases, e.g. small brain tumor metastases, responses to single or multiple fraction high-dose radiation therapy (HD-RT) have been remarkable, suggesting that HD-RT tumor control is at least as effective as biologically equivalent doses of conventional fractionated radiation therapy (CF-RT), even in radioresistant tumors. Although the mechanism for its effectiveness is not well understood, HD-RT is becoming accepted practice for a variety of tumors, including brain tumors.

Our recent preclinical study using MRI measures of short-term changes in tumor physiology after HD-RT in a small-animal model of cerebral tumor suggests a physiological response that includes vascular effects but is multifactorial and temporally variable. Hypothesizing that these short-term changes may both explain the increased effectiveness of HD-RT, and serve as a predictor of long-term response, we propose to investigate the relationship between short-term physiological changes after HD-RT and long-term outcome as a result of that therapy. In counterpoint, we will also study physiological changes during and after CF-RT. Detailed poroelastic modeling is proposed

to generate a map of local solid and fluid parameters (stress, flow) that will help explain short-term changes in physiology. Aim 1 studies short-term changes in measures of tumor physiology as predictors of response. Aim 2 describes the behavior of these same measures over the course of CF-RT. Our long-range goals are to develop noninvasive biomarkers of response that predict tumor control after HD-RT and CF-RT, and to describe physiological changes and related biomarkers that might be used to optimize the order and timing of RT and adjuvant chemotherapies.

Principal Investigator: Shailendra Giri, Ph.D.
Novel Regulation and Targeting of Macrophages Metabolism in Neuroinflammatory Disorders (R01AI144004)

Myeloid cells play a critical role in CNS demyelination and axonal destruction of multiple sclerosis (MS) and experimental autoimmune encephalomyelitis (EAE). The early phase of the disease is characterized by the presence of pathogenic activated macrophages (M1 type), while the recovery phase is associated with alternatively activated macrophages (M2 type) which release anti-inflammatory cytokines that resolve the pathogenic inflammation. Activated M1 macrophages depend on glycolysis to boost biosynthetic pathways to produce inflammatory mediators. However, anti-inflammatory M2 macrophages rely primarily on mitochondrial respiration. Adenosine monophosphate-activated protein kinase (AMPK) regulates energy metabolism, and thus controls the balance between glycolysis and mitochondrial respiration. We reported previously that AMPKα1 knockout (KO) mice develop severe EAE indicating AMPK activation is protective, yet the molecular mechanism by which AMPK regulates EAE disease progression is not known. AMPKα1-KO macrophages exhibit a hyperinflammatory phenotype and have a lower rate of metabolism. AMPKα1-KO macrophages also show glycolysistricarboxylic acid (TCA) cycle remodeling, which results in an imbalance in the levels of the endogenous metabolites, succinate and itaconate, which regulate pro- and anti-inflammatory macrophage functions, respectively. Their levels are tightly controlled by succinate dehydrogenase (SDH) and immune responsive gene 1 (IRG1), respectively. We *hypothesize* that the loss of AMPKα1 remodels the glycolytic-TCA pathway causing an imbalance in the levels of succinate and itaconate, which promotes an M1 phenotype over an M2 phenotype. This, in turn, promotes Th17 cells and suppresses T regulatory cells leading to a hyperinflammatory CNS immune response and CNS tissue damage. To test our hypothesis, we have generated monocyte-specific AMPKα1 KO and macrophage-specific, constitutively active AMPKα1T172D transgenic mice. In **Aim 1**, we will examine how the loss or gain of function of AMPKα1 in macrophages regulates M1 versus M2 macrophage polarization and consequently, Th17 and Tregs differentiation and disease outcomes. Studies under **Aim 2** will elucidate the mechanism by which the loss of AMPKα1 reprograms glycolysis-TCA metabolism leading to an imbalance of succinate and itaconate metabolites in macrophages, which in turn, determine the macrophage phenotype. The proposed study is expected to have a positive impact by elucidating the metabolic regulatory mechanism responsible for macrophage plasticity during disease and investigating AMPKα1 as a potential therapeutic target for MS. Our innovative genetic mouse models and precise metabolomics approach will allow us to identify the apparent rewiring of cellular metabolic pathways specific to AMPKα1 in hyperinflammatory cells. Ultimately, this process could be exploited to tailor novel therapeutic strategies to resolve or limit autoimmune inflammation in the CNS.

Principal Investigator: Shailendra Giri, Ph.D.
Endogenous Metabolite Restricts GM-CSF Signaling Pathway in Pathogenic Macrophages to Ameliorate CNS Autoimmunity (1R01NS112727)

Identifying a therapeutic option that can modulate the innate immune response without generally suppressing the immune system as a whole has been a key barrier to improving treatment for patients with MS. Using metabolic profiling, we have reported that resolvin D1 (RvD1), a pro-resolving lipid metabolite of omega-3 polyunsaturated fatty acids, is significantly decreased in the plasma of patients with MS. Consistent with this finding, MS patients have lower levels of omega-3 metabolites, which are precursors of resolvins, compared to healthy controls, a finding that has been replicated in animal models of the disease, experimental autoimmune encephalomyelitis (EAE). Notably, we found that daily supplementation with RvD1 significantly attenuated clinical symptoms in both chronic and relapsing remitting EAE. These data are provocative for their translational potential, particularly because the immune system is not depressed by RvD1 treatment as it is with steroids and most other MS therapies. The immunomodulatory effect of RvD1 is mediated through its receptor, formyl peptide receptor 2 (FPR2), leading to modulation of AMP-activated protein kinase (AMPK), an important regulator of cell

metabolism. In other human disease models, the RvD1-FPR2 signaling cascade protects by inducing an anti-inflammatory phenotype in macrophages. However, the mechanism affording this protection remains elusive. Granulocyte-macrophage colony-stimulating factor (GM-CSF) is a key player both in the pathology of both MS and EAE, promotes an inflammatory environment and neuronal damage. There is a growing interest in inhibiting the pro-inflammatory effects of GM-CSF signaling as a therapeutic target in MS. In our preliminary work, we found that RvD1 treatment inhibited GM-CSF signaling in macrophages and that this inhibition was AMPK de-pendent. However, how RvD1 affects AMPK activity and GM-CSF signaling to attenuate EAE is unclear. Our **long-term goal** is to identify natural endogenous signaling mechanisms that can be harnessed to treat autoimmune diseases, particularly MS. Our **overall objective** here is to determine the mechanism of action of RvD1 in resolving inflammation and disability in mouse models of MS. Our **central hypothesis** is that RvD1 attenuates EAE disease progression by abrogating GM-CSF signaling resulting in the polarizing of pro-inflammatory macrophages into an anti-inflammatory phenotype. And the underlying mechanisms of macrophage phenotype switch are through FPR2-AMPK-dependent metabolic reprogramming. To test this hypothesis our specific aims are: **1) to identify the effects of RvD1 on the cellular phenotype and function of CNS-infiltrating macrophages in EAE**; and **2) to determine the effects of RvD1 on metabolic reprogramming in macrophages.** We will address these aims with a combination of immunological, biochemical and innovative metabolomic approaches that are already well in hand. The proposed studies will form the foundation for the development of innovative therapeutic strategies to resolve inflammation during MS with no side effects and will likely apply to other diseases involving pathogenic activation of the immune system.

Principal Investigator: Quan Jiang, Ph.D., and Xu Cui, Ph.D.
Investigation of D4-F Effects on Neurovascular Remodeling after Diabetic Stroke (R01NS097747)

Ischemic stroke patients with Diabetes mellitus (DM) exhibit a distinct risk-factor and etiologic profile and a worse neurovascular prognosis than non-DM patients. Therefore, there is a compelling need to investigate neurovascular changes after stroke in the DM and non-DM population and to develop therapeutic approaches specifically designed to reduce neurological deficits after stroke. Type 2 diabetes (T2DM) constitutes 90% of diabetic patients and is associated with low high-density lipoprotein cholesterol (HDL-C), impairment of the anti-oxidative capacity of HDL-C, low phosphorylation of endothelial nitric oxide synthase (p-eNOS), and with reduced ATP-binding cassette transporter A1 (ABCA1) gene expression. D-4F is an economical apolipoprotein A-I (ApoA-I) mimetic peptide, presently employed in clinical trials to reduce coronary atherosclerosis in patients with acute coronary syndrome. However, the therapeutic effects of D-4F in post-ischemic stroke have not been investigated. Our preliminary data show that D-4F treatment of stroke starting 2h or 24h after ischemic stroke improves recovery of neurological function in both T2DM and non-DM mice and also increases p-eNOS and ABCA1 in the ischemic brain. In a novel and clinically relevant approach, based on our robust preliminary data, we propose to use D-4F in the treatment of stroke in the non-DM and T2DM population in mice. We seek to develop D-4F as a novel neurorestorative therapy to reduce white matter (WM) dysfunction and vascular damage, in T2DM and non-DM mice when treatment is initiated at 24h after onset of ischemic stroke. In addition, most development of stroke treatments has focused on young adult animals, but not on old animals, the prevalent population with stroke. Increased age also increases neurological impairment after stroke. We have also developed and implemented multimodality MRI imaging which can dynamically monitor neurovascular remodeling in both the animal and the patient. In the current study, we will measure WM and vascular changes and elucidate the mechanisms of action of D-4F in young adult and aged animals with and without T2DM after stroke. Our hypothesis is that D-4F increases ABCA1 and p-eNOS signaling activity which mediates vascular and WM remodeling and in concert improve functional outcome after stroke. We, therefore, propose two highly integrated and longitudinally designed Specific Aims. Aim 1 will investigate the delayed (24h after stroke) therapeutic effects of D-4F in non-DM and T2DM in young adult and aged mice after stroke. The differences in cerebral WM and vascular changes, and neurological functional outcome after stroke between non-DM and T2DM mice treated with or without D-4F will be analyzed. MRI will be employed to measure the dynamics of neurovascular reorganization underlying therapeutic response and recovery. In Aim 2, using eNOS knockout mice and specific loss of brain ABCA1 mice, we will investigate the mechanisms by which D-4F promotes neurovascular remodeling and hence, neurological recovery. The long-term objective of this RO1 is to develop a neurorestorative treatment for stroke in patients with or without diabetes.

Principal Investigator: Quan Jiang, Ph.D.
Glymphatic and Cognitive Impairment of Aging and Diabetes (RF1AG057494)

The objective of this application is to investigate glymphatic impairment and cognitive deficits during progression of aging with and without diabetes. Emerging data1-5 indicate that the glymphatic system in the brain mediates the cerebrospinal fluid (CSF)-interstitial (ISF) exchange and solute clearance from the brain parenchyma. However, despite the well-described dysfunction of the glymphatic system in the development of neurodegenerative conditions, there is still no reported study that focuses on the role of the glymphatic system in the development of cognitive impairment during aging and aging with type-2 diabetes (DM). Using noninvasive MRI methodologies to investigate cerebral solute waste clearance in middle-age control and type-2 diabetic (DM) rats, we have found increased impairment of the glymphatic system, as indicated by reduced clearance of interstitial Gd-DTPA in brain parenchyma, primarily in the hippocampus and hypothalamus in DM rats (**Fig.2&3**). In parallel, 3D confocal microscopic analysis of the brain-wide distribution of fluorescent tracers revealed increased delayed clearance of ISF in the hippocampus and hypothalamus from DM rats (**Fig.2&3**). Impairment of the glymphatic system in DM rats was shown to be highly correlated with cognitive deficits as measured by an array of cognitive tests including the Morris Water Maze (MWM) for hippocampal related learning and memory. Importantly, histopathological analysis shows that delayed clearance of interstitial solutes is associated with sporadic cerebral microvascular thrombosis in the hippocampus 2 months after hyperglycemia (15 months from birth), while extensive microvascular thrombosis and para-vascular accumulation of beta-amyloid (Aβ) are detected at 4 months after induction of hyperglycemia (17 months from birth), suggesting that the impairment of the glymphatic system leads to Aβ accumulation. Collectively, our preliminary data, for the first time, demonstrate that non-invasive MRI methodologies can detect DM-induced early impairment of the glymphatic system which is highly correlated with hippocampal related dysfunction of learning and memory. Based on our novel preliminary data, we will employ MRI and 3D confocal microscopy to evaluate and quantitatively measure kinetic clearance parameters of the glymphatic system during progression of aging with and without DM (Aim 1). We will then investigate whether impairment of the glymphatic system predicts cognitive dysfunction, the sensitivity and association between impairment of the glymphatic system, the onset of brain vascular dysfunction, and cognitive deficits during aging with and without DM (Aim 2). Data generated from this application will provide new insights into aging and age-matched DM associated impairment of the glymphatic system and the relationship of the glymphatic system with vascular and cognitive dysfunction.

Principal Investigator: Quan Jiang, Ph.D.
Interaction Between Glymphatic and Vascular Systems for Waste Clearance in Brain (R01NS108463)

The objective of this application is to first develop and validate micro vessel measurement for the entire brain to enhance detection sensitivity of micro vessels by ten-fold using superparamagnetic iron oxide (SPIO) enhanced susceptibility weighted imaging (SWI, SPIO-SWI) and then to investigate the interaction between glymphatic and vascular systems for waste clearance in the diabetic brain. Emerging data indicate that the glymphatic system in the brain mediates the cerebrospinal fluid (CSF)-interstitial (ISF) exchange and solute clearance from the brain parenchyma and plays an important role in neurological diseases1-6. Despite many milestone achievements, conclusive findings on the solute efflux pathways are relatively limited. Consequently, the interaction between vascular and glymphatic systems on waste clearance, especially with neurological diseases, is unclear.

The paucity of research into the efflux pathway may be attributed in part to technical difficulties, such as the challenging need to perform minimally invasive in-vivo, ultra-high detection sensitivity for tube-shaped influx and efflux pathways, and whole brain imaging. Although MRI can overcome the weak points of two-photon confocal microscopy to provide non-invasive whole brain in-vivo imaging of the glymphatic system, conventional MRI sensitivity is insufficient for the required spatial resolution for investigating micro vessels of glymphatic and vascular systems. We have developed highly sensitive MRI methods (Fig. 1) which significantly improve the detection sensitivity of small vessels by using the combination of high susceptibility of MRI agents with blooming effects7-9. The new methods provide excellent tools for investigating the efflux pathways of waste clearance under normal and pathophysiological conditions. Three efflux routes have been recently proposed and solutes in the brain could reach the lymphatic network by the olfactory bulb across the ethmoid plate10, 11 or by functioning conventional lymphatic vasculature in the meninges12. We found that tracer concentration in the venous system significantly

increased with diabetes (Fig. 9), thus adding a new route for brain waste clearance. Based on our novel preliminary data and published studies by others, we hypothesize that, the newly developed SPIO-SWI technique significantly increases detecting sensitivity of micro vessels in both vascular and glymphatic systems, and the efflux pathways of waste clearance with and without diabetes can be identified and investigated using this optimized SPIO-SWI method. To test these hypotheses, we will first (Aim 1) further develop, optimize and validate SPIO-SWI techniques to enhance the detection sensitivity for both vascular and glymphatic micro vessels. We will perform computer simulation, optimize SWI technique and experimental conditions in animal studies and then validate USPIO-SWI technique by LSCM measurements. We will then (Aim 2) investigate the interaction between vascular and glymphatic systems for waste clearance in diabetic brain using the optimized USPIO-SWI technique. Data generated from this application will provide new insights into the efflux pathways between glymphatic and vascular systems in diabetic brain.

Principal Investigator: Quan Jiang, Ph.D.
Investigation of D4-F Effects on Neurovascular Remodeling after Diabetic Stroke (R01NS097747)

Ischemic stroke patients with Diabetes mellitus (DM) exhibit a distinct risk-factor and etiologic profile and a worse neurovascular prognosis than non-DM patients. Therefore, there is a compelling need to investigate neurovascular changes after stroke in the DM and non-DM population and to develop therapeutic approaches specifically designed to reduce neurological deficits after stroke. Type 2 diabetes (T2DM) constitutes 90% of diabetic patients and is associated with low high-density lipoprotein cholesterol (HDL-C), impairment of the anti-oxidative capacity of HDL-C, low phosphorylation of endothelial nitric oxide synthase (p-eNOS), and with reduced ATP-binding cassette transporter A1 (ABCA1) gene expression. D-4F is an economical apolipoprotein A-I (ApoA-I) mimetic peptide, presently employed in clinical trials to reduce coronary atherosclerosis in patients with acute coronary syndrome. However, the therapeutic effects of D-4F in post-ischemic stroke have not been investigated. Our preliminary data show that D-4F treatment of stroke starting 2h or 24h after ischemic stroke improves recovery of neurological function in both T2DM and non-DM mice and also increases p-eNOS and ABCA1 in the ischemic brain. In a novel and clinically relevant approach, based on our robust preliminary data, we propose to use D-4F in the treatment of stroke in the non-DM and T2DM population in mice. We seek to develop D-4F as a novel neurorestorative therapy to reduce white matter (WM) dysfunction and vascular damage, in T2DM and non-DM mice when treatment is initiated at 24h after onset of ischemic stroke. In addition, most development of stroke treatments has focused on young adult animals, but not on old animals, the prevalent population with stroke. Increased age also increases neurological impairment after stroke. We have also developed and implemented multimodality MRI imaging which can dynamically monitor neurovascular remodeling in both the animal and the patient. In the current study, we will measure WM and vascular changes and elucidate the mechanisms of action of D-4F in young adult and aged animals with and without T2DM after stroke. Our hypothesis is that D-4F increases ABCA1 and p-eNOS signaling activity which mediates vascular and WM remodeling and in concert improve functional outcome after stroke. We, therefore, propose two highly integrated and longitudinally designed Specific Aims. Aim 1 will investigate the delayed (24h after stroke) therapeutic effects of D-4F in non-DM and T2DM in young adult and aged mice after stroke. The differences in cerebral WM and vascular changes, and neurological functional outcome after stroke between non-DM and T2DM mice treated with or without D-4F will be analyzed. MRI will be employed to measure the dynamics of neurovascular reorganization underlying therapeutic response and recovery. In Aim 2, using eNOS knockout mice and specific loss of brain ABCA1 mice, we will investigate the mechanisms by which D-4F promotes neurovascular remodeling and hence, neurological recovery. The long-term objective of this RO1 is to develop a neurorestorative treatment for stroke in patients with or without diabetes.

Principal Investigator: Jaspreet Singh, Ph.D.
Deciphering Neuroinflammation-Specific Regulatory RNA and Metabolic Networks in Human iPSC-derived Astrocytes in Cerebral Adrenoleukodystrophy (1R21NS114775)

The mechanism of onset of neuroinflammation in fatal cALD in males with inherited X-linked adrenoleukodystrophy (X-ALD) disease remains unknown. 40% of male X-ALD patients develop fatal cerebral neuroinflammation (cALD) while remaining develop milder adrenomyeloneuropathy (AMN) characterized by axonopathy without neuroinflammation. The primary genetic defect in X-ALD (ABCD1 gene deletion) and the biochemical defect (accumulation of very long chain fatty acid; C>22:0 in plasma and tissues) cannot predict the onset of neuroinflammation in cALD. Our long-term goal is to dissect the

molecular mechanism underlying differential phenotype development in X-ALD. The objective of this application is to identify integrated microRNA (miRNA) and metabolites that underlie the differential neuroinflammatory response in AMN and cALD human astrocytes. These astrocytes were differentiated from induced pluripotent stem cells (iPSCs), which in turn were generated by reprogramming of human control, AMN and cALD patient-derived untransformed fibroblasts. The neuroinflammatory response in X-ALD is likely initiated by astrocytes since the inflammatory areas in the X-ALD postmortem brain have cytokine secreting astrocytes but are devoid of activated microglia, T-cells and macrophages. Dysregulated miRNA and metabolite levels are associated with neuroinflammatory disease phenotype in a number of neurodegenerative diseases. Our preliminary proof-of-concept data, with next generation sequencing (miSeq) and untargeted metabolomics, identified miRNA and metabolites altered between healthy-control and cALD phenotype postmortem brain. Within the cALD brain white matter, miRNA and metabolite were altered between distant normal looking areas and neuroinflammatory areas adjacent to the plaque suggesting an association with disease progression. Our central hypothesis is that miRNA and metabolomic analysis in AMN and cALD human induced astrocytes will identify regulatory (miRNA) and active (metabolic) pathways that underlie the neuroinflammatory response and disease progression in cALD. To test our hypothesis we propose two specific aims: 1) To determine the miRNA altered in AMN and cALD astrocytes and 2) To identify metabolites altered between AMN and cALD astrocytes. This proposal is innovative, because it departs from the status quo by identifying for the first time, miRNA and metabolite pathways differentially regulating inflammatory response in human AMN and cALD astrocytes. In a step further, we will identify miRNA and metabolites reversed by CRISPR/Cas9 editing of AMN and cALD astrocytes with a functional copy of ABCD1. The proposed research is significant because the cellular mechanism(s) that lead to less severe AMN or neuroinflammatory cALD in response to the same ABCD1 mutation remain unknown even four decades after the identification of gene defect in X-ALD. As a result no therapy exists for AMN or cALD phenotypes. Impact: X-ALD was added to the federal newborn screening list in 2016, and with the rising rate of newly diagnosed cases there is urgent need to identify novel targets to develop effective therapies for AMN and cALD.

Principal Investigator: Jaspreet Singh, Ph.D.
Use of IPSC to Define Role of Astrocytes in Specifying Risk for Onset of Cerebral Adrenoleukodystrophy (1R56NS114245)

The mechanism of onset of neuroinflammation in fatal phenotypes in males with inherited X-linked adrenoleukodystrophy (X-ALD) disease remains unknown. 60% of male X-ALD patients develop fatal cerebral neuroinflammation (cALD) while remaining develop milder adrenomyeloneuropathy (AMN) characterized by axonopathy without neuroinflammation. The primary genetic defect in X-ALD (mutation/deletion in ABCD1 gene) and the biochemical defect (accumulation of very long chain fatty acid; C>22:0 in plasma and tissues) cannot predict the onset of AMN or cALD. Our long-term goal is to dissect the molecular mechanism underlying differential phenotype development in X-ALD. The objective of this application is to identify metabolic pathways that underlie the differential neuroinflammatory response in AMN and cALD human astrocytes. These astrocytes were differentiated from induced pluripotent stem cells (iPSCs), which in turn were generated by reprogramming of human control, AMN and cALD patient-derived fibroblasts. Metabolic reprogramming is emerging as a novel regulator of inflammatory response. Astrocytes rely on mitochondrial respiration (OXPHOS) for their metabolic needs but switch to glycolysis under neuroinflammatory environment to boost biosynthetic pathways to produce inflammatory mediators. Our preliminary proof-of-concept data, with untargeted metabolomics, identified metabolites altered between healthy-control and cALD phenotype postmortem brain. Within the cALD brain white matter, unique metabolite changes were recorded between distant normal looking areas and areas adjacent to the plaque suggesting an association with disease progression. We found both OXPHOS and glycolysis decreased (low metabolic state) in human cALD astrocytes despite higher inflammatory response. This low metabolic state suggests role of novel alternative source(s) of fuel driving the neuroinflammatory response in cALD astrocytes. Our central hypothesis is that metabolic reprogramming in cALD astrocytes drives their proinflammatory shift that underlies the neuroinflammatory disease progression in cALD. To test our hypothesis we propose two specific aims: 1) To elucidate the metabolic reprogramming responsible for inflammatory response in cALD astrocytes. 2) To determine if dysfunctional mitochondria play a role in inflammatory nature of cALD astrocytes? We will take advantage of control, AMN and cALD astrocytes generated from iPSC's in our laboratory for these studies. This proposal is innovative, because it departs from the status quo by identifying for the first time, metabolic pathways differentially regulating inflammatory response in human AMN and cALD astrocytes. The proposed research is significant because the cellular mechanism(s) that lead to less

severe AMN or fatal cALD phenotype in response to same ABCD1 mutation remain unknown even four decades after the identification of gene defect in X-ALD. Impact: With the rising rate of newly diagnosed cases after X-ALD was added to the federal newborn screening list in 2016, there is urgent need to identify novel targets to develop effective therapies for AMN and cALD for which no satisfactory therapy exists.

Principal Investigator: Poornima Venkat, Ph.D.
Vasculotide Promotes Cognitive Improvement in Rats with Vascular Dementia (1R01AG063750)

Vascular dementia (VaD) is common in patients after a stroke or after a series of mini-strokes and results from several mechanisms, one of which involves injury to blood vessels supplying deep white matter (WM) of the brain resulting in silent, multifocal, brain microinfarcts, vascular dysfunction, decrease in cerebral blood flow, and cerebral parenchymal cell damage. Extensive WM damage such as vacuolization, rarefaction, and demyelination in the periventricular region have been reported in patients with VaD. There is a critical need to develop therapeutic strategies for VaD that identify and target key pathophysiological events driving axonal/WM damage and cognitive deficits. The therapeutic effects of Vasculotide, an Angiopoietin-1 mimetic peptide, in VaD have not been investigated. Our preliminary in-vitro studies show that Vasculotide treatment can dose dependently increase axonal outgrowth in primary cortical neurons (PCN). In male retired breeder rats subjected to a multiple microinfarction (MMI) model of VaD, Vasculotide treatment initiated at 24 hours after MMI, significantly decreases axonal/WM injury and improves long term cognitive outcome. In a novel and clinically relevant approach, based on our robust preliminary data, we propose to use Vasculotide for the treatment of MMI induced VaD in male and female middle-aged rats (10-12 months old). We seek to develop Vasculotide as a therapeutic agent to decrease vascular dysfunction and axonal/WM injury, decrease inflammatory responses, attenuate glymphatic dysfunction and improve cognitive outcome. By affecting gene regulation, microRNAs (miRs) are involved in most biological processes and act as molecular rheostats that fine-tune and switch regulatory circuits governing tissue repair, inflammation, hypoxia-response, and angiogenesis. Elucidation of the role of miRs in VaD pathogenesis, and identification of key miRs that can potentially serve as therapeutic targets in VaD are lacking. We hypothesize that Vasculotide treatment induced vascular and axonal/WM remodeling; anti-inflammatory responses and cognitive recovery are mediated via modulation of key miRs and their target gene expression. Therefore, we propose three highly integrated and longitudinally designed Specific Aims. In Aim 1, we will perform dose-response studies and investigate the safety and long-term cognitive outcome of Vasculotide treatment in middle-aged, male and female rats subject to MMI model of VaD. In Aim 2, we will investigate the therapeutic effects of Vasculotide on vascular remodeling, axonal/WM remodeling, synaptic plasticity, inflammatory responses and glymphatic waste clearance pathway in middle-aged rats subject to MMI. In Aim 3, using “gain or loss” of brain miR-145 and miR-124, we will test whether Vasculotide treatment induced therapeutic effects after MMI in rats are mediated via the miR-124/Interleukin-6 and miR-145/Aquaporin-4/ATP-binding cassette transporter A1 (ABCA1) signaling pathways. The long-term objective of this R01 application is to develop a novel treatment for VaD.

Principal Investigator: Lei Wang, M.D.
Schwann Cell Derived Exosomes Improve Diabetic Peripheral Neuropathy in Type II Diabetic Mice (R01DK124377)

Peripheral neuropathy is one of the major common complications of diabetes. There is a compelling need to develop effective therapeutic approaches specifically designed to improve neurological function caused by diabetic peripheral neuropathy (DPN). Communications between Schwann cells and sciatic nerves of dorsal root ganglia (DRG) neurons maintain homeostasis of peripheral nerve function. Exosomes, endosome-derived nano vesicles carry RNAs and proteins as their molecular cargo. Exosomes mediate intercellular communication by transferring their cargo between source and recipient cells. Our preliminary data showed that treatment of type II diabetes db/db mice with Schwann cell derived exosomes (SC-Exos) remarkably ameliorated neurological dysfunction of DPN, which was associated with significant augmentation of intraepidermal nerve fibers and myelinated axons of the sciatic nerve. We also found that intravenously administered SC-Exos were internalized by Schwann cells and nerve fibers of the sciatic nerve, suggesting that SC-Exos act on Schwann cells and sciatic nerves. Our preliminary data also showed that the SC-Exo treatment did not significantly change blood glucose and glycosylated hemoglobin (HbA1c) levels and liver function; however, importantly, SC-Exos

reversed a network of miRNAs and proteins in the sciatic nerve tissues that mediate development of DPN. Based on these preliminary data, using a clinically relevant mouse model of high fat diet/streptozotocin-induced Type 2 diabetes, we propose to test the hypothesis that SC-Exos interact with Schwann cells and sciatic nerves to modulate this network of miRNAs and proteins and thereby ameliorate DPN. We will first examine whether the miRNA cargo of SC-Exo contribute to the therapeutic effect of SC-Exos on DPN. We will then examine whether endogenous miRNAs in Schwann cells and in the sciatic nerve of dorsal root ganglion (DRG) enhance the therapeutic effect of SC-Exo. Subsequently, we will examine whether engineered SC-Exos carrying elevated selected miRNAs to suppress genes that induce axonal injury and demyelination further reduce neurological dysfunction of DPN. Relevance Statement: Diabetic peripheral neuropathy is a major disability affecting millions of Americans. In this proposal, employing clinically relevant animal models of diabetic peripheral neuropathy, we seek to develop a novel therapeutic approach to treat diabetic peripheral neuropathy using exosomes derived from healthy Schwann cells. In this proposal, we will also elucidate the molecular mechanisms by which exosomes are therapeutically effective. This research will potentially provide the essential pre-clinical data for translation of this novel therapeutic approach to a phase 1 clinical trial.

Principal Investigator: Li Zhang, M.D.
Interaction Between Glymphatic and Vascular Systems for Waste Clearance in Brain (R01NS108463)

The objective of this application is to first develop and validate microvessel measurement **for** the entire **Brain** to enhance detection sensitivity of microvessels by ten-fold using superparamagnetic iron oxide (SPIO) enhanced susceptibility weighted ***imaging*** (SWI, SPIO-SWI) and then to investigate the **Interaction Between Glymphatic** and vascular **Systems for Waste Clearance in** the diabetic **Brain**. Emerging data indicate that the **Glymphatic** system in the **Brain** mediates the ***Cerebrospinal Fluid*** (CSF)-interstitial (ISF) exchange and solute **Clearance** from the brain parenchyma and plays an important role **in** neurological diseases1-6. Despite many milestone achievements, conclusive findings on the solute efflux pathways are relatively limited. Consequently, the **Interaction** between vascular and **Glymphatic Systems** on **Waste Clearance**, especially with neurological diseases, is unclear. The paucity of research into the efflux ***pathway*** may be attributed **in** part to technical difficulties, such as the challenging need to perform ***minimally invasive*** in-vivo, ultra-high detection sensitivity **for** tube-shaped influx and efflux pathways, and whole **Brain** ***imaging***. Although ***MRI*** can overcome the weak points of two-photon confocal microscopy to provide non-invasive whole **Brain** in-vivo ***imaging*** of the **Glymphatic** system, conventional MRI sensitivity is insufficient **for** the required spatial resolution **for** investigating microvessels of **Glymphatic** and vascular **Systems**. We have developed highly sensitive ***MRI*** methods (Fig. 1) which significantly improve the detection sensitivity of small vessels by using the combination of high susceptibility of ***MRI*** agents with blooming effects7-9. The new methods provide excellent tools **for** investigating the efflux pathways of **Waste** clearance under normal and pathophysiological conditions. Three efflux routes have been recently proposed and solutes in the **Brain** could reach the lymphatic network by the olfactory bulb across the ethmoid plate10, 11 or by functioning conventional lymphatic vasculature **in** the meninges12. We found that tracer concentration **in** the venous system significantly increased with diabetes (Fig. 9), thus adding a new route **for Brain Waste Clearance**. Based on our novel preliminary data and published studies by others, we hypothesize that, the newly developed SPIO-SWI technique significantly increases detecting sensitivity of microvessels **in** both **Vascular** and **Glymphatic Systems**, and the efflux pathways of **Waste Clearance** with and without diabetes can be identified and investigated using this optimized SPIO-SWI method. To test these hypotheses, we will first (Aim 1) further develop, optimize and validate SPIO-SWI techniques to enhance the detection sensitivity **for** both **Vascular** and glymphatic microvessels. We will perform computer simulation, optimize SWI technique and experimental conditions in animal studies and then validate USPIO-SWI technique by LSCM measurements. We will then (Aim 2) investigate the **Interaction Between Vascular** and **Glymphatic Systems for Waste Clearance in** diabetic **Brain** using the optimized USPIO-SWI technique. Data generated from this application will provide new insights into the efflux pathways **Between Glymphatic** and **Vascular Systems in** diabetic **Brain**.

Principal Investigator: Li Zhang, M.D.
Combination Treatment with Vepoloxamer and tPA for Acute Stroke (R01NS102744)

Stroke is one of leading causes of death and disability worldwide, mainly affecting elderly. Tissue plasminogen activator (tPA), the only Food and Drug Administration (FDA) approved treatment, is limited in its use to < 8.5% of stroke patients. Therefore, there is a compelling need to develop new and broader utility therapies for acute ischemic stroke. Vepoloxamer is a well characterized proprietary amphipathic copolymer with rheological properties, which is currently under investigation in a global phase III clinical trial for patients with sickle cell disease. Our preliminary studies demonstrate that administration of Vepoloxamer in combination with tPA 4h after embolic stroke facilitates recanalization and thrombolysis reduces ischemic neuronal damage and improves neurological outcome but does not increase cerebral hemorrhage in young adult rats. We also found that platelet-derived exosomes contribute to the therapeutic effect of Vepoloxamer on enhanced tPA-thrombolysis. In this application, we propose to investigate effect of Vepoloxamer in combination with tPA on acute stroke and molecular mechanisms underlying the combination therapy on the thrombolysis and neurovascular function in the aged male and female rats. Data generated from this application may provide a novel and potentially useful treatment strategy for patients with acute stroke.

Principal Investigator: Zhenggang Zhang, M.D., Ph.D.
Exosome Therapy for Acute Stroke with Large Artery Occlusion (R01CA219829)

Large cerebral vessel occlusion is the most disabling and life-threatening form of ischemic stroke. Human stroke primarily occurs in late middle age and beyond. Approximately two thirds eligible patients treated with tPA experience incomplete reperfusion. Thrombectomy is now also a standard of care for treatment of acute stroke with large vessel occlusion. However, recanalization of the occluded large vessels by thrombectomy only leads to ~71% of patients achieving improved tissue reperfusion, often incomplete. In addition, due to unfavorably large ischemic cores, many patients with large artery occlusion are not eligible to receive tPA or thrombectomy. Patients with reperfusion of the ischemic tissue are closely associated with good clinical outcome. Thus, there is a compelling need to develop therapies in combination with tPA and thrombectomy to enhance cerebral perfusion and thereby augment the therapeutic efficacy of tPA and thrombectomy monotherapies. Also, therapies to block ischemic core expansion will increase numbers of patients who would be eligible to receive tPA and thrombectomy. Using rat models of embolic middle cerebral artery occlusion (eMCAO) and transient MCAO (tMCAO, ischemia/reperfusion), we found that exosomes derived from cerebral endothelial cells (CEC- exos) in combination with tPA after eMCAO or CEC-exos given upon reperfusion after tMCAO substantially increased recanalization and downstream cerebral blood flow (CBF), and reduced blood brain barrier (BBB) leakage and infarction compared to tPA or tMCAO alone. Exosomes are nano-vesicles that contain lipids, proteins, and RNAs including microRNAs (miRs). Our preliminary data suggest that exosomal cargo miRs likely contribute to the therapeutic effect of CEC-exos in combination with tPA on acute stroke by acting on cerebral endothelial cells to suppress proteins that promote thrombosis and BBB disruption. We thus propose to develop CEC-exo therapy as an adjunctive treatment to enhance tPA and thrombectomy treatments of acute ischemic stroke. Aim 1 is to investigate whether the CEC-exo therapy as an adjunctive treatment enhances tPA and thrombectomy treatments in aged rats after large artery occlusion. Aim 2 is to investigate whether CEC exosomal cargo miRs contribute to CEC-exos-amplified thrombolysis leading to reduction of neurovascular damage. Aim 3 investigates whether a special set of CEC-exo cargo miRs contribute to the therapeutic effect CEC-exos on stroke- induced neurovascular damage by suppressing a network of pro-BBB leakage and thrombotic genes. Accomplishing these aims will potentially lead to development of a mechanistically based exosome therapy as an adjunctive treatment to enhance tPA and thrombectomy treatments of acute ischemic stroke, leading to improvement in the neurological outcome.

Principal Investigator: Zhenggang Zhang, M.D., Ph.D.
Exosomes and Platinum-Induced Peripheral Neuropathy (R01NS111801)

Platinum-based drugs are commonly used to treat cancers. Platinum drugs are the first line therapy for ovarian and colorectal cancers. However, chemotherapy-induced peripheral neuropathy (CIPN) is one of the most common complications. More than 70% of the patients receiving oxaliplatin are affected by neuropathy. Oxaliplatin induces two symptoms of peripheral sensory neuropathy; an acute and transient cold-aggravated, and a chronic form that has onset after multiple exposures to the drug and does not

disappear with drug cessation. The neurotoxicity often leads to platinum drug dose reductions, compromising efficiency of platinum drugs to suppress tumor progression. On an average of 6 years after chemotherapy, 47% of women still reported symptoms of CIPN. Studies to develop a neuroprotective agent have, to date, been unsuccessful to reduce CIPN. There is an imperative need to develop new therapies to CIPN. Challenges to develop such therapies include that a therapy needs not to impede antitumor efficacy, but to effectively inhibit CIPN. Our preliminary data demonstrated cerebral endothelial cell derived exosomes (CEC-exos) abolish oxaliplatin- induced peripheral neuropathy in tumor bearing mice and sensitize oxaliplatin on cancer cell killing. Exosomes are nanovesicles and mediate intercellular communication by transferring cargo proteins, lipids, and genomic materials including mRNAs and microRNAs (miRNAs) between source and target cells. We found that treatment of the tumor bearing mice with CEC-exos along with oxaliplatin induces a network of miRNAs/mRNAs in sciatic nerves that exerts neuroprotection in sciatic nerves and DRG neurons but triggers a distinct miRNAs/mRNAs network in tumor to promote cancer cell death. We, thus, hypothesized that CEC- exos mitigate peripheral neurotoxicity induced by platinum drugs and that CEC-exos enhance the anti- cancer efficacy of platinum drugs on tumor cells. Three specific aims are proposed to test this overall hypothesis. Aim 1 is to investigate the efficacy of the CEC-exos on ameliorating platinum drug-induced peripheral neurotoxicity and on improving the treatment of tumor. Aim 2 is to investigate molecular mechanisms underlying the therapeutic effect of CEC-exos on platinum drug-induced peripheral neuropathy with a focus on the interaction between CEC exosomal miRNAs and their target proteins in axons and DRG neurons. Aim 3 is to investigate molecular mechanisms underlying the effect of CEC-exos on sensitizing tumors to platinum drugs with a focus on the interaction between CEC exosomal miRNAs and their target proteins in tumor cells. Accomplishing these aims will potentially lead to development of a new CEC-exo based therapy for CIPN, leading to improvement in the quality of life and possibly cure of cancers.

Neurosurgery

Principal Investigator: Ye Xiong, Ph.D.
Exosome-based Therapeutics in TBI (R01NS100710

Traumatic brain injury (TBI) is a major cause of death and disability worldwide. There are no effective therapies available for TBI patients. Thus, there is a compelling need to develop novel therapeutics in order to improve neurological recovery after TBI. Mesenchymal stem cells (MSCs) are adult multipotent cells that give rise to various mesodermal cell types. The use of MSCs for tissue repair is of great interest because of their ability to home to damaged and inflammatory tissues. However, previous studies from us and others show that only a small proportion of transplanted MSCs actually survive and few MSCs differentiate into neural cells in injured brain tissues. The predominant mechanisms by which MSCs participate in brain remodeling and functional recovery are related to their secretion-based paracrine effect rather than a cell replacement effect. Our recent data suggest that posttraumatic treatment with cell-free exosomes isolated from rat and human MSCs improves functional recovery in male rats after TBI. Exosomes play an important role in intercellular communication. Exosomes transfer not only proteins and lipids but also genetic materials including mRNAs and microRNAs (miRNAs) to recipient cells, thereby mediating a variety of biological responses. Our preliminary data further demonstrate that the labeled exosomes administered intravenously after TBI reach the brain and are incorporated into brain cells as well as in macrophages in peripheral organs. Our encouraging findings indicate that MSC-derived exosomes have equivalent restorative effects as their cellular counterparts on brain remodeling and functional recovery after TBI. Thus, MSC-generated exosomes are novel candidates as a cell- free therapy that can overcome the obstacles and risks associated with the use of naive or engineered stem cells or MSCs. While our results are promising, the precise therapeutic mechanisms underlying exosome therapy for TBI recovery warrant further elucidation. In this proposal, we will first determine therapeutic efficacy of naïve MSC-exosomes for improvement in functional recovery in male and female rats after TBI. We will then evaluate the effect of MSC-exosomes on brain neuroplasticity, and growth factor expression as well as on the brain and peripheral immune response, effects that likely underlie and contribute to functional recovery (Aim 1). We will then evaluate the role of the miRNA content of the MSC-derived exosomes on brain angiogenesis, neurogenesis, synaptogenesis, cell death, growth factors and immune responses underlying functional recovery (Aim 2). Finally, we propose to enhance the therapeutic effects of exosome treatment of TBI by generating and employing tailored MSC-derived exosomes enriched with the miR-17-92 cluster as a treatment for TBI. In addition, we will investigate the molecular mechanisms underlying cellular exosome uptake (Aim 3). This proposal is innovative, and highly translational. This study will provide novel insights into mechanisms underlying the MSC-derived

exosome-promotion of functional recovery after TBI, develop a means to amplify the therapeutic effects of exosome therapy for TBI, and form the foundation for clinical translation of exosome therapy for TBI.

Principal Investigator: Yanlu Zhang, Ph.D.
Treatment if Traumatic Brain Injury with Velpoloxamer (R01NS109477)

Traumatic brain injury (TBI) is a major cause of death and disability worldwide. There are no effective therapies available for TBI patients. Thus, there is a compelling need to develop novel therapeutics in order to improve neurological recovery after TBI. Among many secondary injury events that occur after TBI, cerebral microthrombosis is an under-recognized, yet important contributor to the secondary brain ischemia and damage that occurs after TBI, and would therefore seem to be one of the central secondary events after brain trauma to bear in mind when designing treatment strategies. Cerebral microthrombi not only lead to ischemia and cell death but also prevent therapeutic drugs from entering into the affected brain and therefore constrain the efficacy of therapeutic drugs, which may be one of important factors ignored during preclinical and clinical trials. Our recent study indicates that early (2 hours post injury) intravenous administration of Vepoloxamer promotes sensorimotor function and cognitive functional recovery after TBI induced by controlled cortical impact (CCI-TBI), which is associated with its robust effect on reducing cerebral microthrombosis formation and neuroinflammation. Vepoloxamer is a purified form of Poloxamer 188 where impurities associated with renal dysfunction have been removed, which is an amphiphilic polyethylene-polypropylene-polyethylene tri-block copolymer that is reported to seal membranes and restore plasma membrane integrity in damaged cells. However, to date, there is a paucity of information about Vepoloxamer for treatment of TBI and the mechanisms underlying its therapeutic effects. von Willebrand factor (vWF) released into blood from injured endothelial cells inversely correlates with clinical outcome of severe TBI. vWF can induce microthrombosis formation. Our previous study demonstrated that the level of vWF released into plasma increases at 1-4 hours, peaks at 1-3 days, declines at 8 days, and returns to normal at 15 days in rats after CCI-TBI. We hypothesize that TBI induces the blood-brain barrier (BBB) damage and release of endothelial-derived vWF, which leads to platelet aggregate and subsequent cerebral microthrombosis-induced secondary injury. In Aim 1, we will first conduct a dose-finding study to identify Vepoloxamer dose and therapeutic window effect on functional recovery without toxicity in young rats (male and female) with TBI. In Aim 2, we will then investigate the mechanisms by which IV administration of Vepoloxamer enhances cerebral microvascular perfusion and promotes functional recovery after TBI. Microvascular integrity, cerebral blood flow, and BBB leakage will be measured dynamically using either laser scanning confocal microscopy or magnetic resonance imaging (MRI). This work will address a previously understudied important issue and is highly translational. Successful completion of this proposed research will elucidate mechanisms underlying IV Vepoloxamer-mediated promotion of TBI recovery, and facilitate development of Vepoloxamer as a novel therapeutic approach targeting endothelial cells/microthrombi to improve neurological outcome for TBI patients.

Principal Investigator: Ana deCarvalho, Ph.D.
Targeting Oncogene Amplification in Glioblastoma (W81XWH-19-1-0693)

Malignant gliomas originate in astrocytes or neural progenitor cells within the brain. These tumors rarely metastasize outside of the brain and exert their devastating health effects by damaging this vital organ. Glioblastoma is the most aggressive type of glioma, and unfortunately the most frequent in adults, with an incidence of about 12,000 new cases per year in the US. Surgery followed by radiation and chemotherapy with a DNA targeting agent temozolomide are able to control the disease temporarily We have learned from the many failed clinical trials that these tumors have been unexpectedly resistant to therapy directed at what seemed rational targets. In over 70% of the cases, proteins that drive glioblastoma growth are overexpressed due to high level gain in copy numbers in regions of the genome that become amplified. In this project we are focusing on two oncogenes, CDK4 (cyclin-dependent kinase 4) and MDM2 (mouse double minute 2 homolog) that overrule the two most important pathways restricting cell proliferation. Novel potent inhibitors targeting these oncogenes are now in clinical trials enrolling glioblastoma patients. This project involves bringing the clinical challenges to the research laboratory in a tangible way, by testing these novel therapies in patient-derived models (neurosphere cells and mouse xenografts) that are as hard to treat as the original tumors they came from, because we have shown they indeed preserve the genomic complexity and oncogene amplifications of glioblastomas. Similar to clinical studies, this project uses genomic information to assign glioblastoma patient-derived models to drugs that in theory should be effective. However, unlike the clinical setting, the same “patient” will be assigned

to multi-arm treatments simultaneously. In this project will investigate the pharmacological properties, specificity and efficacy of the most promising pharmacological inhibitors of MDM2 and CDK4 in treating glioblastoma models with a diverse set of genomic abnormalities. Not only MDM2 and CDK4 genes are amplified, but they are amplified in a circular segment of DNA, that like the chromosomes is packed in chromatin, but does not have centromeres, so they can increase dramatically in number. Furthermore, the tumor cells have different amounts of these circular DNA elements, thus variable levels of the drug target among cells within the same tumors. This likely poses a challenge for these new drug treatments. For that reason, we are adding another therapeutic strategy to our study, we will target an enzyme that is a central regulator of DNA repair, named DNA-PK, also using novel more selective and potent drugs in currently in clinical trials. This has a broader application for glioblastoma treatment, because radiation and temozolomide inflict DNA damage, and so does the rapid cell proliferation rate, and if DNA repair is inhibited the damage to the DNA becomes toxic and eventually lethal to the cell. However, here we will also test if this strategy affects the propagation of the circular DNA elements carrying oncogenes, as a novel application for DNA-PK inhibitors. First, we will test each of the CDK4 and MDM2 inhibitors separately in the glioblastoma cells and xenografts obtained from various patients. We will quantify their efficacy to selectively inhibit their targets, and to what extent they can control glioblastoma growth, and what are the effects of combining them with radiation and temozolomide. If resistance to therapy is observed, we will analyze these tumors in novel and important ways. We will quantify the shifts in the number of the circular DNA elements that carry the oncogenes and also other molecular changes that will be analyzed by Dr. Poisson, a co-investigator in this project and bioinformatics expert. We will then test the efficacy of the DNA damage inhibitor. It is not feasible to conduct these analyses to identify possible mechanisms of resistance to therapy in the clinic. Our results will greatly help the optimization of these therapies. This proposed research happens in the context of a truly multi-disciplinary team, with the relevance for patient treatment provided by Dr. Snyder, a neuro-oncologist and collaborator in this project, as with any pre-clinical study, the final validation for our findings will take place in the clinic.

Glioblastoma affects men and women of all ages. Military service members, veterans, and their family members are among those who have suffered from the significant symptoms associated with this tumor. Next, they have to deal with craniotomy, radiation therapy and chemotherapy. In most cases, what follows are just months of decline before patients succumbs to this devastating disease. It is becoming common for the tumors that were removed to undergo genomic profile, and the presence of “targetable genomic abnormalities” is used in molecular tumor boards to recommend experimental treatments for patients that have progressed after the standard of care, even when evidence for clinical benefit is not present, due to the lack of options. Our work takes place in the interface between the research laboratory and clinic, and by tackling the complexity of drug resistance in the laboratory we can contribute in a meaningful way to the long road of bringing glioblastoma into the category of treatable diseases.

Orthopaedics/Bone & Joint

Principal Investigator: Michael Bey, Ph.D.
Shoulder Function After Rotator Cuff Repair (R01AR051912)

Rotator cuff tears affect about 40% of the population over age 60 and are a common cause of pain and disability. Approximately 250,000 rotator cuff repairs are performed in the United States each year, but healing following surgery is a significant challenge (e.g., 20-70% of surgical repairs fail) and postoperative shoulder function is unpredictable. There is also often a disconnect between repair tissue healing and shoulder function where patients have poor shoulder function (e.g., limited strength and pain) despite an intact repair or, conversely, excellent shoulder function despite a failed repair.
Conventional clinical data (e.g., patient age, tear size) are not strong predictors of clinical outcome, and Therefore, this disconnect between healing and function remains difficult to explain. Recent research suggests that repair tension and repair tissue elongation may provide insight into post-operative healing and shoulder function that is not adequately provided by clinical data. However, the relationships between repair tension, repair tissue deformation, healing, and shoulder function are not well understood. The objectives of this application are to determine how rotator cuff repair affects shoulder motion, strength, and patient-reported outcomes, and to assess the influence of repair tension and repair tissue deformation on these outcomes. The rationale for this project is based on several important findings from our on-going work regarding the progression and treatment of rotator cuff tears:
1) rotator cuff pathology, even in the absence of symptoms, has a significant impact on shoulder

function, 2) physical therapy improves clinical outcomes despite only minor changes in joint motion, 3) surgical repair appears to alter glenohumeral joint (GHJ) motion in a way that suggests excessive repair tension, and 4) shoulder motion, strength, and patient-reported pain/function scores are interrelated after surgery. Based on these findings and the purported roles of repair tension and repair tissue elongation, our central hypothesis is that repair tissue elongation (up to and including failure) is due, at least in part, to repair tension approaching or exceeding the mechanical capacity of the healing repair tissue. We also hypothesize that repair tissue deformation affects joint motion in ways that have a significant impact on strength and patient-reported outcomes. Our approach will be to conduct a longitudinal study that measures repair tension, repair tissue deformation, joint motion, strength, and patient-reported outcomes before and after surgical repair. The proposed research is innovative because it will use a state-of-the-art imaging technique to provide an accurate assessment of the mechanical progression of healing rotator cuff repair tissues. The contribution of this research will be significant because it will advance our understanding of how surgical repair influences shoulder function and clinical outcomes, ultimately leading to improved patient care.

Principal Investigator: Michael Bey, Ph.D.
Shear Wave Elastography to Predict Repair Tissue Healing and Shoulder Function After Rotator Cuff Repair (R21AR072785)

Rotator cuff tears are common, affecting up to 40% of individuals over age 60 and accounting for an economic burden of $3-5 billion per year. Surgical repair is a satisfactory solution for many patients, but clinical outcomes and healing of the repair tissue after rotator cuff surgery can be unpredictable. Tear chronicity (i.e., the extent to which the muscle/tendon unit has degenerated over time) is a critical factor in determining healing and clinical outcomes. However, conventional approaches for assessing tear chronicity uses only qualitative descriptions (mild, moderate, severe) or grades (0 to 4) without any explicit assessment of the quality of the muscle and tendon tissues. Consequently, it is perhaps not entirely surprising that these conventional assessments are only weak predictors of healing and clinical outcome after surgery. This is important, because without a reliable measure of tear chronicity it is difficult for surgeons to know prior to surgery how challenging the repair may be what alternatives may need to be considered during surgery, what post-operative rehabilitation activities should be prescribed, and how best to counsel patients on expected outcomes. Ultrasound shear wave elastography has emerged as a promising technique for non-invasively assessing the in-vivo stiffness of soft tissues.

Given that the pathologic processes associated with rotator cuff tears are characterized by changes in tissue stiffness, shear wave elastography may have clinical utility in assessing the chronicity of rotator cuff disease. However, even though this advanced technique has been used extensively for breast and liver imaging, it has seen only limited use in musculoskeletal tissues. Consequently, the objective of this study is to determine the extent to which rotator cuff shear wave speed (SWS) predicts healing and clinical outcomes after rotator cuff repair. Our approach will be to use shear wave elastography to measure SWS in patients who are having surgical rotator cuff repair. These data will be acquired prior to surgery and then related to conventional tear characteristics (tear size, tear retraction, muscle atrophy, fatty degeneration), healing, and conventional clinical outcomes (strength, ROM, patient-reported outcomes) collected at 12 months post-surgery. Our central hypothesis is that SWS will be a significant predictor of healing and clinical outcomes and superior to conventional predictors of healing and clinical outcome. The proposed research is innovative because it will use an emerging technology to assess the quality of the rotator cuff tissues, which cannot currently be obtained in any other way. This contribution of the proposed research will be significant because we believe it will establish the clinical utility of shear wave elastography by identifying SWS as a superior predictor of clinical outcome and repair tissue healing. In turn, clinical use of shear wave elastography will provide physicians with the information necessary to improve care for patients suffering with rotator cuff tears.

Principal Investigator: Joseph Gardinier, Ph.D.
Modifying the Mechanotransduction of Bone by Targeting Purinergic Receptors (R01AR076378)

Osteoporotic fractures are common, increasing in incidence, and have a high associated economic burden. This significant clinical problem is further compounded by a lack of therapeutic strategies to increase bone formation and improve tissue strength. Bone formation is a function of osteocytes' response to mechanical loading during physical activity and exercise. Osteocytes' purinergic signaling through the release of nucleotides plays a key role in regulating bone adaptation in response to loading.

In particular we have found the P2Y2 receptor downregulates osteocytes' sensitivity to loading and that the loss in mechanosensitivity is accompanied by an increase in actin-stress fiber formation (ASFF) through cofilin phosphorylation. These findings are significant because they suggest targeting P2Y2 signaling as a potential strategy to enhance osteocyte mechanotransduction and increase bone formation. However, the extent to which P2Y2 influences bone formation by regulating osteocytes' sensitivity to loading through ASFF remains unknown. These gaps in knowledge limit our development of new therapeutic strategies that increase bone formation and reduce fracture risk in an aging population. Our long-term goal is to prevent osteoporosis and reduce fracture risk in an aging population. The objective of this study is to determine the role of purinergic signaling through the P2Y2 receptor in regulating the anabolic response to loading. The premise for this study is that blocking P2Y2 signaling has therapeutic potential to prevent age-related bone loss and reduce fracture risk. The central hypothesis states that blocking P2Y2 signaling will increase osteocytes' sensitivity to loading, allowing greater gains in bone mass and tissue strength in response to loading. The central hypothesis will be tested under three specific aims. Aim 1 will determine the extent to which P2Y2 signaling in-vitro influences osteocytes' sensitivity and overall response to loading by regulating ASFF through cofilin phosphorylation. Our approach in aim 1 utilizes osteocyte knockout cell lines generated using CRISPR/Cas9 to examine in-vitro their response to fluid flow. Aim 2 will determine the extent to which P2Y2 expression in-vivo contributes to bone formation in response to loading and unloading. Our approach in aim 2 will prescribe treadmill exercise and hindlimb immobilization to conditional knockout mice that target osteocytes' P2Y2 expression. Aim 3 will examine the efficacy of AR-C118925, a selective P2Y2R inhibitor, to increase the anabolic response to loading in aged mice as well as prevent age-related bone loss. Our approach in aim 3 will treat wild-type as well as P2Y2- knockout mice with AR-C118925 to identify off-target effects that are not specific to osteocytes. This study is innovative because it 1) evaluates a novel therapeutic agent (AR-C118925) for increasing bone formation, and 2) uses new cell-lines and animal models to establish P2Y2 signaling as a unique mechanism to increase bone mass. Overall, this study is significant because we expect it to demonstrate the therapeutic potential of targeting P2Y2R signaling to increase bone formation and reduce fracture risk.

Principal Investigator: Rebekah Lawrence, Ph.D.
Investigating the Multi-factorial Etiology of Rotator Cuff Pathology in Human Subjects (K99AR075876)

PROJECT SUMMARY A rotator cuff tear is a common shoulder condition that affects approximately 40% of individuals over the age of 60. This condition is painful, debilitating, and reduces quality of life. Despite their prevalence, the etiology of rotator cuff tears is not fully understood but is generally believed to involve extrinsic factors (i.e. tendon impingement during shoulder motion), intrinsic factors (i.e. tendon degeneration), and/or overuse. These factors have been studied extensively in animal models, which have provided support for each factor contributing to rotator cuff pathology. However, these findings have not yet been confirmed in human studies largely because of the difficulty in accurately and reliably assessing intrinsic factors and overuse in humans. Ultimately, understanding the etiology of rotator cuff pathology in humans will remain difficult without a model that characterizes the role of each factor in rotator cuff pathology. The objectives of the proposed studies are to: 1) develop a preliminary multivariable model classifying the effects of extrinsic, intrinsic, and overuse factors on rotator cuff pathology in asymptomatic individuals (K99); 2) extend the model with additional asymptomatic participants (R00); and 3) expand the model to include symptomatic participants (R00). Our approach will be to quantify shoulder motion and impingement (extrinsic factors) via biplane x-ray imaging, rotator cuff degeneration (intrinsic factors) via shear wave elastography, overuse factors via a novel estimate of lifetime shoulder exposure, and the severity of rotator cuff pathology via diagnostic imaging. We will investigate the relationship between the etiological factors and rotator cuff pathology using classification and regression tree analysis. The proposed studies are the keystone of a career development plan developed to provide the necessary mentorship, coursework, and research training for me to become an independent and impactful researcher. The goals of the K99 phase are to: 1) obtain training in advanced methods of biomechanical data collection and analysis to assess the roles of extrinsic, intrinsic, and overuse factors in the etiology of rotator cuff pathology; 2) develop a preliminary multivariable model describing the role of extrinsic, intrinsic, and overuse factors on rotator cuff pathology; and 3) obtain a tenure track position at a respected research- intensive university. The goals of the R00 phase are to: 1) independently conduct the R00 phase study by implementing the skills learned during the K99 phase; 2) establish multi-disciplinary research collaborations with engineers,

orthopaedic surgeons, and physical therapists; 3) lead a well-funded and productive research laboratory; and 4) build upon the K99/R00 research findings to secure independent R01 funding. Together with the rich research environment at Henry Ford Health System, the proposed career development plan will ensure that I have a unique skillset to pursue an independent research career, produce sound and impactful research, and help prepare the next generation of scientists.

Principal Investigator: Jamie Fitzgerald, Ph.D.
The Role of SHIP2 in Mineralization (R21AR072297)

Skeletal mineralization is fundamentally important to all vertebrate species. Too little mineralization results in structurally compromised bone that is prone to failure. On the other hand, pain and disability occur when there is inappropriate or ectopic mineralization and calcification of soft tissues. The major mechanisms controlling mineralization are poorly understood resulting in a major gap in knowledge. Our ongoing studies on the genetic basis of opsismodysplasia (OPS), a rare chondrodysplasia that is characterized by a marked delay in endochondral ossification, identified a new potential regulator of matrix mineralization: SH2 Domain-containing Inositol 5-phosphatase 2 (SHIP2). SHIP2 functions as a phosphatase that dephosphorylates phosphatidylinositol (3,4,5) P3 (PIP3) to generate hosphatidylinositol (3,4) P2 (PIP2). Data from our *in vitro* SHIP2 inhibitor and SHIP2-deletion studies confirmed that SHIP2 deficiency leads to a mineralization defect. Furthermore, experiments on matrix vesicles (MVs) isolated from chondrocytes and osteoblasts demonstrated that the loss of SHIP2 leads to a failure of MVs to support mineral deposition. Together, our data support the overall hypothesis that **SHIP2 regulates MV function**.

Prinicipal Investigator: Yener Yeni, M.D.
Clinical Assessment of Vertebral Bone Quality Direct Biomechanical and Textural Analysis via Digital Tomosynthesis (W81XWH1910373)

This project relates to the Topic Area “Musculoskeletal Disorders”, and specifically to the encouragement area of research on measures to improve diagnosis, prediction and optimization of health outcomes. This is because the proposed project ultimately aims to improve the accuracy of assessment for spinal bone fragility and fracture risk. The bones of the spine (vertebrae) are the most frequently fractured ones due to osteoporosis. These fractures are economically costly and burden the patients with many downstream problems including back pain. Military personnel are known to be at greater risk for these fractures and complications. An accurate assessment of vertebral fracture risk is essential for appropriate and timely intervention for the prevention of fracture. This research also relates to the Topic Area “Diabetes”, because a diabetic cohort will be included in the study. Current standard techniques for fracture risk assessment rely on radiographic bone density scans. Additional information regarding the patient’s demographic status and medical history is also incorporated in tools predicting fracture risk. However, these techniques are not very sensitive in identifying who will have a fracture and who will not. This is not too surprising, considering the fact that the information used in the assessment is a crude indirect measure of bone strength not based on biomechanics. To address this concern, we developed a new method, in which two images of a patient’s vertebra are taken in the presence and absence of the patient’s body weight by having them stand and lay down for both images respectively. The images are obtained using digital tomosynthesis (DTS), a system that is similar to computed tomography (CT). The advantages of DTS over CT are that DTS allows for standing and lying images to be captured, offers high resolution and exposes patients to less radiation than CT. The two sets of images are compared using an advanced computational method and deformations in the vertebra caused by standing are measured. From the displacement measurements, vertebral stiffness and overall displacement are calculated as metrics of strength and factor of safety (factor safety is a measure of how strong the bone is relative to the loads it normally experiences). Information on bone microstructure, additional to bone density, is known to increase accuracy in predicting fractures. We can also derive properties related to bone microstructure from DTS images without the biomechanical test. These properties are determined by quantifying the texture in the bone image and called textural properties. We developed these methods in the laboratory in detail using cadaveric vertebrae and laboratory-standard imaging and strength testing. We also performed pilot human studies to establish feasibility of the methods in the clinic. What remains to be determined is how successful the methods will be in the clinical environment for identifying individuals who are at risk. Therefore, this study will be a clinical validation of the new biomechanical and textural DTS methods. In order to determine the ability of DTS methods to correctly identify at-risk patients, the approach will be to compare patients who have conditions or diseases that are known to

increase their risk of fracture to normal patients. Therefore, a group of osteoporotic patients with an existing vertebral deformity, a group with primary hyperparathyroidism (pHPT) and a third group with diabetes will be compared to normal patients. These diseases are considered service-related and thus represent a greater risk for military families. Importantly, each of these diseases increase the risk of fracture but alter bone in different ways that are not always detectable by bone density scans. For example, osteoporosis primarily results in loss of bone mass, pHPT alters the organization of bone structure and affects the cortical bone (in the case of a vertebra, the dense bony shell surrounding the vertebra), and diabetes alters the quality of the bone material without reducing bone mass. By studying these groups using DTS and comparing the assessment of bone strength to standard bone density results, we will; i) establish for which types of patients and to what extent the DTS methods might be useful, ii) identify in which way the method must be performed for best results, iii) better understand how differences in bone quality specific to each disease affect the biomechanical outcome, and iv) establish a group of patients that we can follow up for longer term results. In the long term, the method will be useful in other clinically significant issues such as low back pain associated with vertebral fractures, implant stability, degenerative and congenital diseases of the skeletal system resulting in deformities, skeletal response to drug, exercise and disuse.

Otolaryngology

Prinicipal Investigator: Lamont Jones, M.D., M.B.A.
Characterization of Keloid Specific Exosomes and Determination of Exosomal Critical Signaling Pathways in the Keloid Microenvironment (1K08GM128156)

There are more than 11 million people in the world with keloids and more than 425,000 associated clinic visits, yearly, in the United States. Keloids are benign fibroproliferative tumors which cause pain, pruritus, emotional distress and loss of function. Current therapies are unsatisfactory with unacceptably high recurrence rates, mainly because of an incomplete understanding of keloid pathogenesis. Fibroblasts are a key player in keloid pathogenesis, but the drivers are unknown. Keloid disease is influenced by aberrant signaling pathways. However, no clear signaling pathway has been identified. Exosomes mediate cell-cell communication, exercising primary physiological and pathophysiological function. Exosomal cargo, such as microRNAs (miRNAs), regulate cellular function.

Significance: This project will lead to an enhanced understanding of keloid pathogenesis and the potential for exosome-based therapy. **Innovation:** (i) rational progression from preliminary data supporting the novel role of exosomes in keloid pathogenesis; (ii) investigating the influence of RAB27 methylation on the function and production of keloid exosomes would suggest a mechanistic basis for novel epigenetic biomarkers;(iii) using unique resources which includes fibroblast cell lines from primary untreated keloid (25) and matched normal skin (25) from a multi-ethnic group of patients and an *in vivo* animal model allow for the pragmatic translational application of results; (iv) entirely new field of keloid investigation. In summary, this project, mentoring and career development plan will position, Lamont R Jones, MD, MBA, to become an independent clinician scientist and leader in keloid pathogenesis.

Pathology

Prinicipal Investigator: Azadeh Stark, Ph.D.
Molecular Markers of Risk of Subsequent Invasive Breast Cancer in Women with Ductal Carcinoma In Situ (R01CA218429)

Ductal **Carcinoma in Situ** (***DCIS***) is considered to be a non-obligate precursor **of Invasive** breast cancer (IBC). Use **of** screening mammography has led to a substantial increase **in** detection of DCIS over the past 2-3 decades. About 5-14% **of** patients diagnosed **with *DCIS*** and treated with breast-conserving therapy, **with** or without radiation, develop an ipsilateral IBC and 1-6% develop a contralateral IBC over a period **of** 10 years. However, natural history studies have shown that, in the absence **of** treatment, 14-53% **of *DCIS*** cases develop IBC if followed for up to ~30 years. Treatment **of *DCIS*** is variable, and many ***DCIS*** patients are either under- or over-treated. Elucidation **of** the **Molecular** changes detectable **in *DCIS*** lesions that are associated **with Risk of** IBC development is critically needed, as this may help not

only to reduce **Risk of** development **of** IBC but also to ***prevent*** overtreatment **of** patients **with** lower **Risk of** IBC. **in** this regard, a multigene expression assay, consisting **of** genes related to proliferation, as well as PR and GSTM1, was recently shown to predict **Risk of Subsequent** ipsilateral IBC **in Women with *DCIS***. Similarly, immunohistochemically detected expression **of** p16, COX-2, and Ki67 has also been associated with increased **Risk of** IBC development. However, these findings require confirmation. Furthermore, novel prognostic (and ultimately predictive) **Markers** may emerge from assessment **of** gene expression patterns on a global scale. **in** this regard, microRNAs (miRNAs), which are noncoding RNAs that are master regulators **of** gene expression, are thought to contribute to the development of **Invasive Cancer**. Against this background, our overarching goal is to facilitate early detection of patients **with *DCIS*** at **Risk of** IBC development. To this end, building upon our previous work, we propose to use ***Clinical*** data and archived formalin-fixed paraffin-embedded (FFPE) tissue from a large, population-based multi-center cohort **of** 7,275 patients initially diagnosed **with *DCIS*** in community-based health plans and followed for **Subsequent** IBC development, to identify and then validate miRNA expression changes associated **with Risk of Subsequent** IBC, to evaluate **Risk of** IBC in association **with** 2 previously identified sets **of Markers** (Oncotype DX ***DCIS*** score; positivity for p16, COX-2, and Ki67 ***protein expression***), and to examine the association between ***Clinical*** factors and **Risk of Subsequent** IBC **in** the largest such study to date. Our **Molecular** epidemiologic study, which proposes to apply state-of-the art technologies to archived ***DCIS*** FFPE specimens for the detection **of Molecular** changes associated **with Risk of** IBC development **in** a large, multi-center population-based cohort **of Women** initially diagnosed **with *DCIS***, has the potential to lead to approaches that will help to refine identification **of Women** who need enhanced surveillance and early aggressive treatment.

Pediatrics

Principal Investigator: Charles Barone, M.D.
Prenatal Exposures and Child Health Outcomes: A Statewide Study (UG3OD023285)
Subcontract

Evidence from epidemiologic studies demonstrates the negative effects of both chronic and acute stress during gestation. These effects may occur perinatally or later in the child's life. The COVID-19 global pandemic has led to unprecedented mass disruption of social and financial security as well as changes in medical care delivery. These conditions are causing elevated levels of distress even for portions of the population that may have previously been protected from psychosocial stress. Of particular concern for pregnant women and their children, there may be direct biological effects related to infection with SARS-CoV-2 as well as substantial indirect psychosocial effects during critical periods of development with long-lasting impact on children relevant to the Environmental Child Health Outcomes (ECHO) program. This proposal addresses how psychosocial stress related to the COVID pandemic may impact perinatal and neurodevelopmental outcomes. Furthermore, evidence suggests that psychosocial stress is associated with both the gastrointestinal and vaginal microbiomes. Therefore, we will determine if maternal microbiomes or infant microbiomes mediate the impact of psychosocial stress on perinatal and neurodevelopmental outcomes. In aim 1, we address the maternal microbes and their role in mediating perinatal outcomes caused by maternal psychosocial stress during pregnancy. In aim 2, we focus on maternal psychosocial stress and its impact on neurodevelopment as mediated by the changes to the infant microbiota. We will examine these objectives in the context of our ongoing work, and as an extension of the parent grant (UG3/UH3OD023285, Paneth), where our organizing principle is that for many environmental exposures the most sensitive period of risk for child health is pregnancy and the perinatal period. The parent grant explores three primary exposures: toxic, nutritional, and inflammatory in a stratified random sample of state births recruited in the first trimester of pregnancy. Of the planned 1,100 new enrollments of cohort dyads into ECHO, more than 700 pregnant women have been consented, and, with a 75% follow up rate, more than 400 children have already been seen in infancy. Over 300 women are expected to be enrolled during the project period. While this research will leverage the local ECHO cohort, the project is designed to engage ECHO team science through two distinct but complementary ECHO-wide projects: (1) incorporation of data from two cohorts (O'Conner & Deoni) to address the aims proposed above and (2) provision of data and biospecimens to separate COVID supplement (Transande) which addresses SARS-CoV-2 seropositivity/COVID illness as well as psychosocial stress (assessed via questionnaire and cortisol measured in hair) as they relate to shortened gestation and other perinatal outcomes. Our efforts will not only inform the specific hypotheses being tested but will also inform “touch-free” methods for sample collection and patient interaction. The

work proposed herein complements the parent grant by addressing an exposure (maternal psychosocial stress during a time of pandemic), not included in the parent grant, and at least two of ECHO's outcomes (PPP and neurodevelopment).

Principal Investigator: Maureen Connolly, M.D.
Using Evidence-Informed Interventions to Improve Health Outcomes among People Living with HIV: Transgender Women Engagement and Entry to Care Project (TWEET) (U69HA310670100)

There is a pronounced need for implementation of evidence-informed interventions to reduce HIV-related health disparities and improve health outcomes, including improving retention in care, treatment adherence, and viral suppression for people living with HIV (PLWH). In 2016, 81.7% of PLWH in the U.S. were retained in care, and approximately 85% were virally suppressed.1 The need for these efforts is felt most deeply among racial/ethnic minority men who have sex with men (MSM) and among transgender women. Retention in care for young Black MSM (YBMSM) was lower (75%) than the national RWHAP average. 79% of transgender women has achieved viral suppression. Transgender Black/African American had lower percentages of viral suppression across demographic subgroups compared to transgender Hispanic/Latinos and whites. PLWH often have complex behavioral health comorbidities that complicate their ability to maintain treatment adherence and continuous care. A 2010 survey of 246 Ryan White Part C medical providers found that 30% of PLWH had a substance use disorder and 35% had a serious mental illness.2 Other studies have found between 35-64% of PLWH suffer from PTSD. 3,4Although research has defined best practices for addressing steps along the HIV care continuum, the implementation of such interventions lags behind. This is especially true for the implementation of interventions that: 1) are tailored for Black MSM and transgender women; 2) address the co-occurring behavioral health needs of PLWH; 3) tackle the social, structural, and environmental barriers– including experiences of trauma–that hinder attainment of positive health outcomes. This initiative will focus on supporting the implementation of interventions to improve HIV-related health outcomes in the above focus areas. The implementation of the interventions will be evaluated using an implementation science approach. The evaluation will systematically collect and analyze project data in order to measure and monitor progress towards meeting the goals and objectives of the project, while also evaluating the ability of specific interventions to improve the HIV care continuum outcomes of linkage, retention, re-engagement, and viral suppression among client participants. Lessons learned and best practices will be identified throughout the course of the initiative and will be shared rapidly with the larger field.

Psychiatry/Behavioral Health

Principal Investigator: Lisa Matero, Ph.D.
A Technology-based Intervention to Reduce Alcohol Use after Bariatric Surgery (1R34AA027775)

Despite bariatric surgery being the most effective weight loss intervention for patients who are severely obese, as many as 1 in 5 patients will develop an alcohol use disorder after their surgery. Changes in metabolism, hormone levels, and behavior as a result of bariatric surgery alter the rewarding effects of alcohol while concurrently changing its absorption rate, putting patients at significantly elevated risk of hazardous drinking. Simply providing education to this vulnerable patient population about post-surgical risks has not been sufficient to reduce alcohol use, yet comprehensive in-person interventions are met with significant challenges, including hours-long distances between patients and their bariatric surgery programs. Thus, our long-term goal is to increase access to an empirically supported intervention for reducing alcohol use among patients who undergo bariatric surgery by leveraging technology. Our intervention, rooted in motivational interviewing and the transtheoretical model, is a two-session computerized brief intervention (CBI), supplemented by six months of tailored text messaging based on participants' CBI results and subsequent fluctuations in their readiness to change. The purpose of the proposed study is to optimize this technology-based intervention for patients who undergo bariatric surgery and to examine feasibility and acceptability of the intervention. In the first phase, patient interviews (n= 20) will be utilized to identify preferences for intervention content and treatment delivery. Ten patients will then participate in an open trial of the intervention, which will be subsequently revised based on feedback from these patients. In Phase 2, patients (N = 60) will be recruited between 3 and 6 months following bariatric surgery and randomized to the intervention or treatment as usual control group. All patients will complete baseline questionnaires and at 1, 3, 6, and 9 months post-assessments. We

expect that this intervention will be both feasible and acceptable to patients. Results will be used as preliminary data to inform a large, fully powered clinical trial to test the larger efficacy of this intervention. Although primary outcomes focus on feasibility and acceptability, we also expect that patients assigned to the intervention will have a longer time to their first post-surgical drink, report more days of abstinence, fewer drinks per drinking day, and a lower prevalence of alcohol use disorder after bariatric surgery compared to controls. This project is innovative because it expands upon existing interventions for bariatric surgery patients by implementing evidence-based strategies for alcohol use. By utilizing a technology-based approach, we can also reach a larger number of patients to prevent initiation of drinking, reduce current alcohol use, and facilitate better engagement in care, should individuals opt into traditional treatment approaches. The proposed line of research is significant and relevant to NIH's mission because the intervention is expected to reduce the likelihood that patients will develop an alcohol use disorder following bariatric surgery. Given the potential of wide dissemination at low cost, the proposed study has high potential public health and clinical significance.

Principal Investigator: Lisa Matero, Ph.D.
Pathways from Chronic Prescription Opioid Use to New Onset Mood Disorder
(R01DA043811)

Our previous work indicates a **New** period of **Opioid** analgesic **Use** (OAU) lasting beyond 30 days, is associated with increased risk for **New Onset** depression, depression recurrence and transition **to** treatment resistant depression compared **to** 1-30 OAU days. In multiple studies with robust control for confounding, including pain severity, longer OAU predicted **New Onset** depression in middle-aged patients (substantially older than the age of risk for **New Onset** depression in the population) with no recent ***History*** of depression, no evidence of opioid misuse and no recent ***History*** of OAU. Our research utilized electronic medical record (EMR) data **from** large samples of Veterans Administration (VA) and ***Private Sector*** patients. Compared **to** patients who discontinued OAU within 30 days, patients with 31-90 days OAU were 18% (VA) **to** 33% (***Private Sector***) more likely **to** have new **Onset** depression. In patients with >90 days OAU, the likelihood increased **to** 35% in VA and 105% in private sector data. In patients with recent depression and in remission, initiation of OAU, compared **to** no OAU, was associated with depression recurrence in VA (HR=2.2, 95% CI = 2.0-2.3) and ***Private Sector*** data (HR=1.8, 95% CI = 1.4-2.2). We found that patients with depression were 22% more likely **to** develop treatment resistant depression with OAU of 31-90 days and 49% more likely with OAU of >90 days. The consistency of findings, replication in VA and ***Private Sector*** patients, and rigorous control for pain support the hypothesis that OAU is likely a risk factor for depression, as well as its recurrence and severity. A prospective study is needed **to** confirm and advance this line of research, in part because medical record data lack lifetime histories of **Mood** disorders and other ***Risk Factors*** such as substance **Use** disorder, ***trauma exposure***, as well as good measures of functional impairment, sleep quality and social support. Also, EMR data do not contain prospective data on the sequence of pain, OAU and ***depression symptom*** development. In the proposed research, we hypothesize that events prior **to** OAU, such as ***History*** of depression, will increase risk of post- OAU **New Onset** major ***depressive*** episode. Second, we hypothesize that OAU-related adverse outcomes, such as **Opioid** misuse, sleep apnea, occur after long term OAU and subsequently contribute **to New** onset depression. Third, we hypothesize that OAU leads **to** worse depression that in turn contributes **to** higher OAU and still worsening depression, independent of longitudinal pain measures. Fourth, we focus on depression phenotypes (anhedonia, vital exhaustion, dysthymia, comorbid substance **Use** disorder) **to** elucidate the new onset depression phenotypes most strongly associated with **Chronic** OAU. Fifth, we determine which depression phenotypes are ***Risk Factors*** for incident **Opioid** misuse and abuse.data is obtained at baseline, 6 month and 12 months follow-up with monthly brief assessments for trajectory analysis. Our innovative research has great potential **to** advance understanding of depression in OAU and **Opioid** misuse, abuse/use disorder. Results will inform pain management and safe **Opioid** prescribing for patients with **Chronic** non-cancer pain.

Radiation Oncology

Principal Investigator: Stephen Brown, Ph.D.
MRI Signatures of Response to High-Dose Radiotherapy in Rat Models of Cerebral Tumor
(R01CA218596)

In some cases, e.g. small brain tumor metastases, responses to single or multiple fraction high-dose

radiation therapy (HD-RT) have been remarkable, suggesting that HD-RT tumor control is at least as effective as biologically equivalent doses of conventional fractionated radiation therapy (CF-RT), even in radioresistant tumors. Although the mechanism for its effectiveness is not well understood, HD-RT is becoming accepted practice for a variety of tumors, including brain tumors. Our recent preclinical study using MRI measures of short-term changes in tumor physiology after HD-RT in a small-animal model of cerebral tumor suggests a physiological response that includes vascular effects but is multifactorial and temporally variable. Hypothesizing that these short-term changes may both explain the increased effectiveness of HD-RT, and serve as a predictor of long-term response, we propose to investigate the relationship between short-term physiological changes after HD-RT and long-term outcome as a result of that therapy. In counterpoint, we will also study physiological changes during and after CF-RT. Detailed poroelastic modeling is proposed to generate a map of local solid and fluid parameters (stress, flow) that will help explain short-term changes in physiology. Aim 1 studies short-term changes in measures of tumor physiology as predictors of response. Aim 2 describes the behavior of these same measures over the course of CF-RT. Our long-range goals are to develop noninvasive biomarkers of response that predict tumor control after HD-RT and CF-RT, and to describe physiological changes and related biomarkers that might be used to optimize the order and timing of RT and adjuvant chemotherapies.

UROLOGY

Principal Investigator: Sahn-ho Kim, M.D.
Role of Androgen Receptor in Telomere Stability: A Novel Therapeutic Strategy in Potentiating AR-Targeted Therapies for the Treatment of Prostate Cancer (WX1XWH-17-1-305) Subcontract

The androgen receptor (AR) plays a critical role at all stages of prostate cancer (PCa) (1-4). Therefore, for over seven decades, treatments that target AR action have been a mainstay for the treatment of advanced PCa. However, these therapies do not provide a lasting remission and the disease usually recurs as castration-resistant prostate cancer (CRPC), which, remarkably, still relies on AR. New drugs that target AR action (e.g., enzalutamide and abiraterone) provide incremental benefit but no cure. We have discovered a new role of AR, and we have found a way to exploit this role and create a more effective way to kill PCa cells. We discovered that AR plays a critical role in maintaining telomere stability, even in CRPC cells (5-7). This is important because telomere stability is essential for genome stability and cell survival (8,9). Telomeres are the DNA-protein structures that cap the ends of linear chromosomes, which are double stranded DNA with a single-stranded overhang (12), and that protect them from fusing to each other (8,9). When such protection is lost (an event referred to as telomere dysfunction), chromosome ends are recognized as lesions and this results in the activation of a DNA damage response (DDR) at the telomeres (8,13). The DDR includes recruitment of specific proteins to the telomeres and activation of ATM. ATM activation leads to the activation of Chk2, a cell cycle checkpoint protein that activates a checkpoint control. Checkpoint activation causes cells to arrest, allowing for repair so that cells may resume cell cycle progression to mitosis. Unprotected telomere ends may undergo fusion or recombination (processes referred to as ‘repair’), leading to the formation of telomere end to end fusions and telomere sister chromatid exchanges. Such telomere aberrations push cells into breakage fusion- bridge cycles, resulting in unequal distribution of genetic material to daughter cells and the development of genome instability (14,15).

Notably, when cells with telomere dysfunction are treated with an ATM inhibitor, checkpoint activation is abrogated, allowing cells to enter mitosis with damaged telomeres; the more damage, the more likely the cell will activate a cell death pathway (7,16,17). We discovered that a subset of AR in human PCa cells is associated with telomeres, and that AR antagonists, including bicalutamide (Casodex), enzalutamide (MDV3100), or AR-siRNA, induce telomere dysfunction and activate a DDR at the telomeres, in both androgen-sensitive (e.g., LNCaP) and castration resistant(e.g., C42B and 22Rv1) PCa cells (5-7). Most notably, we found that treating CRPC cells with AR antagonist plus ATM inhibitor abrogated checkpoint activation and killed cells that were resistant to growth inhibition by AR antagonist alone (7). These effects on cells in vitro lead us to test the hypothesis that co-treatment with AR antagonist and ATM inhibitor will suppress the growth and recurrence of CRPC in vivo (Aim 1). In addition, we have observed that AR antagonist treatment induces telomere aberrations in LNCaP, C4-2B and 22Rv1 cells (6,7). Therefore, we hypothesize that PCa cells that survive treatment with AR antagonist may develop genome instability, which may promote tumor progression (15,18,19) and help to explain the recurrence of PCa treated with AR antagonist alone

Impact: Combined treatment with AR antagonist plus ATM inhibitor may represent a new way to effectively treat patients with CRPC.

Principal Investigator: Nallasivam Palanisamy, Ph.D.
Comprehensive Molecular Profiling of Prostate Cancer in African American Population: Unraveling Molecular Heterogeneity **(W81XWH-16-1-0544)**

Background: Among various epithelial cancers, genomic studies of prostate cancer (PCa) identified several molecular markers including E26 transformation specific (ETS) gene fusions, *SPINK1* and many others. The prevalence of these molecular markers in African American (AA) prostate cancer has not been studied to the extent that has been studied for Caucasian (CA) prostate cancer to understand the racial disparity. Contrary to the conventional approaches, new approaches are needed to understand the underlying genetic disparity between the AA and CA PCa. Therefore, we have developed refined approaches to screen ***whole mount radical prostatectomy*** tissues rather than ***systematic sampling*** of the tumor from dominant/index nodule to assess the fundamental molecular differences in the incidence of molecular markers between AA and CA prostate cancer. **Hypothesis/Objective:** Prostate molecular markers have been first discovered using the cancer genome of individuals other than African American decent. Due to the lack of screening in a large cohort of AA PCa the prevalence of these markers in AA PCa is not known. *Given the fundamental differences in the ancestral history of the genome of AA and CA* the prevalence of these molecular markers may be markedly different. Conventional systematic sampling approaches may not reveal the true prevalence in AA PCa. Therefore, we propose to undertake an innovative approach using **whole mount radical prostatectomy** to understand the racial disparity. Based on the multifocal nature, morphological heterogeneity and clonal origin of many tumor foci, we *hypothesize that different tumor foci may harbor distinct driver molecular aberrations making more complex disease and difficult to manage.* Moreover, it is likely that smaller tumor foci with high Gleason grade and distinct driver aberration can be easily missed by conventional approaches. Therefore, in our innovative approach we will be able to understand the racial differences in the overall incidence of molecular markers based on **whole mount radical prostatectomy** (see preliminary data) screening rather than systematic sampling of dominant nodule alone. Further, in order to maximize the chances of identifying new molecular subsets of AA prostate cancer, we hypothesize that we need to interrogate the genome of AA patients that are negative for any of the known molecular markers in all the tumor foci and subjecting them for in-depth genomic characterization.

WOMEN'S HEALTH

Principal Investigator: Ramandeep Rattan, Ph.D.
AMPK as a Novel Host Factor Regulating Ovarian Cancer Progression (W81XWH-17-1-0170)

We and others have demonstrated the re-purposing of the anti-diabetic drug metformin in EOC. Retrospective studies alsosupport the beneficial survival effects of metformin in diabetic EOC patients. The echanism of metformin's anti-cancer effects has been largely attributed to activation of its target enzyme, AMPK (Adenosine monophosphate-activated protein kinase). AMPK is a highly conserved cellular energy sensor that plays a critical role in the regulation of cell growth by regulating protein synthesis via mTOR; cell cycle by cyclins and p21; insulin and IGF-1 signaling and lipid metabolism. From a previously funded DOD Pilot grant, we recently showed that AMPK is a key player in metformin's ability to limit high fat-diet and adipocyte mediated promotion of EOC. We also reported that AMPK activation occurred both in the host and the tumor cells, indicating its modulation on both host factors and tumor cells.

To further gain insight into the role of host AMPK in restraining EOC growth, we generated syngeneic ID8 ovarian tumors in AMPK alpha1 knockout (KO). Our preliminary data showed: **1)** AMPK KO mice exhibited an accelerated EOC growth and significantly decreased survival compared to wild type (Wt) mice. **2)** Absence of AMPK□1 resulted in a dysregulated host immune environment characterized by increased myeloid derived suppressor cells (MDSCs) with amplified immunosuppressive ability. **4)** Absence of AMPK altered the energy metabolism of MDSCs by shifting their metabolic state to a highly active state, independent of fatty acid oxidation, which correlated with its increased arginase dependent immunosuppressive function. MDSCs have emerged as critical elements of cancer-induced immune dysfunction by creating immunosuppressive conditions, allowing unchecked tumor growth via their ability

to suppress T-cell proliferation and function. EOC has been shown to be associated with infiltrated MDSCs that enhance incidence, metastasis and stemness. Recently, the understanding of energy metabolic pathways used by immune cells to convey their effector functions has seen major advances. AMPK has emerged as one of the central molecules regulating the metabolic shift in the energy pathways of various immune cells vital to their functioning, but its role in regulating MDSC is unknown.

Principal Investigator: Ramandeep Rattan, Ph.D.
Determining the Mechanisms by which Calorie Restriction Alters Macrophage Polarization to Promote an Anti-Tumor Environment in Epithelial Ovarian Cancer (R01CA249188)

Epithelial **Ovarian Cancer** (EOC) is **the** leading cause of gynecologic **Cancer** death **in the** United States, and despite **the** advances made **in** EOC therapy, **the** five-year survival rate has been stagnant at approximately 45% for decades. **the** current standard ***Therapeutic*** approach is accompanied **by** toxic side effects, and **the** high cost of ***chemotherapy*** places **an** enormous ***financial burden*** on patients, reducing their quality of life. Thus, there is a critical need **to** identify low-cost approaches that can both enhance responses **to** current therapies and improve survival **by** inhibiting ***tumor progression***. **Calorie Restriction** has a strong capacity **to** alter **the** responses of cancer cells and host cells **in the** ***tumor microenvironment***, yet **the Mechanisms** of such cross talk are elusive. Our long- term goal is **to** identify dietary **Mechanisms** that can be modulated **to** impede EOC progression and thus be translated into **the** development of more effective therapies and lifestyle changes for EOC patients. Our studies show that 30% **Calorie Restriction** (CR), without malnutrition, **in** a ***mouse model*** of EOC decreases tumor burden, ascites, and metastases. Additionally, our preliminary data strongly suggest that CR inhibits EOC due **to** a decrease **in** alternatively activated pro-tumorigenic (M2-like) macrophages and a corresponding increase in classically activated **Anti-Tumor** macrophages (M1-like), resulting **in an** increased M1/M2 ratio. This is important as 50% of cells **in** EOC ***Ascites*** are pro-tumorigenic macrophages (M2-like) and **an** increased M1/M2 ratio is an indicator of better prognosis **in** EOC patients. **Macrophage Polarization** is regulated, **in** part, through metabolic reprogramming, with M1-like macrophages mainly relying on glycolysis and M2-like macrophages primarily utilizing aerobic ***Respiration***. AMPK, a well-known regulator of energy ***Metabolism***, controls **the** ***balance*** between glycolysis and ***mitochondrial Respiration*** and our studies demonstrate that ***Mice*** fed a CR diet have increased AMPK activity. **the** overall objective of **the** proposed research is **to** determine **the** mechanism **by which** a CR diet regulates **Macrophage Polarization in** EOC. Our central hypothesis is that **the** increased activity of AMPK due **to** a CR diet remodels **the** glycolysis-tricarboxylic acid (TCA) ***pathway*** **to Promote an** M1 (**Anti-Tumor**) phenotype, thus leading **to** a robust **Anti-Tumor** immune response and reduction of **the** tumor. This hypothesis will be tested **in** two aims: Aim 1 will identify **the** effects of increased AMPKα1 activity due **to** CR on **the** EOC progression and Aim 2 will determine **the Mechanisms** underlying CR-mediated **Macrophage Polarization** in EOC. **the** proposed study is expected **to** have a ***translational impact*** **by** elucidating how **the** simple approach of dietary intervention can cause metabolic regulatory changes responsible for **Macrophage** plasticity during EOC. Ultimately, this process could be exploited **to** tailor novel complementary ***Therapeutic*** strategies and life-style modifications **to** curb progression of EOC and other types of **Cancer**.

Part III – Population and Health Sciences

- **Center for Health Policy and Health Services Research**
- **Center for Individualized and Genomic Medicine Research**
- **Department of Public Health Sciences**

Center for Health Policy and Health Services Research

Principal Investigator: Brian Ahmedani, Ph.D.
Patient perspectives on clinical approaches to prevent opioid related suicide attempts (U01MH114087)

The opioid attributable death rate in the U.S. has more than quadrupled over the last 20 years. Simultaneously, suicide has become the 10th leading cause of death and 8th leading cause of death for American Indians and Alaskan Natives. More than 48,000 people die by suicide annually, and it is estimated that for every suicide death there are 25 attempts; clearly indicating many opportunities for prevention. Experts estimate that up to 30% of opioid overdoses are suicides. Those using opioids to manage chronic pain may be at particular risk for opioid-related mortality through intentional or accidental overdose. The Suicide Prevention Resource Center was formed in response to multiple agency recommendations regarding suicide prevention which in turn created the Zero Suicide framework to address suicide prevention in health care settings. The framework is a set of evidence-based approaches for suicide prevention which can be tailored by health care settings. The Mental Health Research Network (MHRN) received funding in 2017 for five years from the National Institute of Mental Health (NIMH) to evaluate the implementation of the Zero Suicide framework across six health systems serving over nine million people (Award # U01MH114087). This evaluation is not focused on understanding the experience of patients who may be at high risk for suicide such as those with diagnosed Opioid Use Disorder (OUD), those without this diagnosis who are using opioids for pain management, and native people. In addition, although providers and health system leaders are involved in the parent NIMH-funded study, we have little information from providers who are treating patients with OUD, using opioids for their patients' pain management, and/or practicing within native communities. We do not know to what extent they have been involved in the Zero Suicide implementation nor their perceptions of its effectiveness. These providers could give clinical and research teams valuable suggestions for tailoring the implementation for high-risk patients. To address these gaps, we propose to incorporate the voice of the patient and provider stakeholders as part of the implementation of the Zero Suicide framework in three health settings from the NIMH-funded parent award as well as the Southcentral Foundation which is an Alaska Native-owned, nonprofit health care organization serving nearly 65,000 American Indian/Alaskan Native people living in and around Anchorage, Alaska. We will test the following aims as part of this proposal: AIM 1: Systematically engage patients, providers, national consumer advocacy groups, and MHRN scientists in formulating research questions to address the prevention of opioid-related overdoses in people with OUD or people without diagnosed OUD who are using opioids for pain management; and AIM 2: Understand how people with OUD or people without diagnosed OUD who are using opioids for pain management are experiencing the implementation of the Zero Suicide framework in four diverse health systems (Kaiser Permanente Northwest [Oregon] and Southern California, Henry Ford Health Systems [Detroit], and Southcentral Foundation [Anchorage and surrounding communities]).

Principal Investigator: Brian Ahmedani, Ph.D.
An Evaluation of the National Zero Suicide Model Across Learning Healthcare Systems (U01MH114087)
Developing Tools to Evaluate the Impact of Safety Planning and Lethal Means Assessment on Suicide Outcomes (U01MH114087S1) Subcontract

Suicide is a major public health concern – it is the 10th leading cause of death and number one cause of injury related death in the United States (US). Due to national concern about this problem, the National Action Alliance for Suicide Prevention and the US Surgeon General published the joint 2012 National Strategy for Suicide Prevention (NSSP). The NSSP outlines a series of Aspirational Goals (AG) with the specific objective to reduce the national suicide rate by 20%. AG 8 and 9 promote healthcare settings as

primary targets for suicide prevention. Consistent with this message, Henry Ford Health System's (HFHS) Perfect Depression Care (PDC) Zero Suicide Initiative was the first US program linked with a substantial decrease in the suicide rate among behavioral health patients after implementation. These findings have motivated national promotion of this model for suicide prevention in health systems. As such, the National ZS Model (NZSM) was developed, based on the HFHS PDC program, but with flexibility to allow adaptation to diverse settings and patient populations. Overall, the NZSM is founded on the realization that suicidal individuals often fall through multiple cracks in a fragmented and sometimes distracted healthcare system, and on the premise that a systematic, comprehensive approach to care is necessary for suicide prevention. The comprehensive approach of the NZSM includes implementation of a series of clinical and quality strategies within the following components: 1) Identification of those at-risk, 2) Engagement and care management; 3) Effective treatment, and 4) Care transition. Despite being a model program promoted internationally for healthcare system quality improvement in suicide prevention, the NZSM has very limited evidence outside of the findings from the HFHS PDC program. The proposed study seeks to conduct a comprehensive process and outcome evaluation of NZSM implementation in real-world clinical settings across 6 large, diverse Mental Health Research Network Affiliated Learning Healthcare Systems providing healthcare for over 9 million individuals each year. The project aims are to:1) Collaborate with health system leaders to develop EHR metrics to measure specific quality improvement targets and care processes tailored to local NZSM implementation, 2) Examine the fidelity of the specific NZSM care processes implemented in each system, and 3) Investigate suicide attempt and mortality outcomes within and across NZSM system models. Study data are captured using electronic health records and insurance claims. Given strong national support for NZSM, if it is found to be effective to reduce suicide behavior, this model will have nationwide implications for suicide prevention in healthcare settings.

Principal Investigator: Brian Ahmedani, Ph.D.
Evaluating the Impact of Changes to Opioid Prescribing Across Health Systems Implementing Zero Suicide (U01MH114087S2)

Suicide is a major public health concern – it is the 10th leading cause of death and number one cause of injury related death in the United States (US). Suicide rates have risen over 25% in the last 15 years. In parallel, the nation is struggling with an opioid epidemic. Opioid prescribing, heroin use, and opioid related overdose deaths have risen substantially. Approximately 15% of all suicide deaths are due to drug overdose, and prescription opioids specifically, are commonly used among people who attempt suicide. Health systems across the country have made decisions to tackle both of these public health crises – implementing policies to dramatically reduce opioid prescribing as well as clinical processes within the Zero Suicide model to improve suicide prevention for their patients. The parent award for this supplement is focused on evaluation of Zero Suicide implementation, including fidelity to each of these clinical processes and suicide outcomes, across 6 large, diverse Mental Health Research Network- affiliated Learning Healthcare Systems providing healthcare for over 9 million individuals each year.

Given the overlap, significant reductions in opioid prescribing as part of newly implemented policies should lead to a reduction in the availability of opioids. These reductions may result in a public-health level means reduction approach to reduce suicide. Means reduction is among the interventions recommended within Zero Suicide. The concurrent implementation of these new opioid prescribing policies in the context of implementation of Zero Suicide allows the opportunity to evaluate how changes in opioid prescribing impacts suicide outcomes in health care. This supplement project seeks to accomplish three specific aims: 1) Evaluate changes in opioid prescribing patterns during the period of NZSM implementation across health systems, 2) Investigate whether changes in opioid prescribing patterns reduce suicide attempt and mortality, and 3) Investigate whether changes in opioid prescribing patterns reduce opioid- related suicide attempt and mortality poisonings. Overall, we propose to use an Interrupted Time Series Design, consistent with the parent award, to measure changes in prescribing patterns and suicide outcomes.

Principal Investigator: Brian Ahmedani, Ph.D.
Effectiveness and Implementation of a Peer Mentorship Intervention (PREVAIL) to Reduce Suicide Attempts among High-risk Adults (R01MH115111)

Suicide is **a** growing ***Health*** problem in the US with more than 42,000 **Suicide** deaths and approximately 1 million **Suicide Attempts** occurring each year. Individuals identified as **High risk** for **Suicide** are often

referred to health systems for mental ***Health*** treatment; however, there are few ***Health*** service interventions known to reduce suicides or **Suicide Attempts**. Few interventions have focused on addressing ***hopelessness*** and thwarted belongingness, two ***Risk Factors*** for **Suicide** emphasized by the US Surgeon General. **Peer** mentorship is **a** novel approach **to** addressing these ***Risk Factors***. **Peer *Mentors*** are individuals with **a** lived experience of suicidal thoughts or behaviors who have achieved stable recovery and work **to** support others at risk. There are over 20,000 state-certified professional **Peer** specialists who currently provide services **to** many high-risk individuals and who could serve as ***Mentors***; however, no **Peer Mentorship** protocols have been rigorously studied **to** determine their ***Safety*** and **Effectiveness** or the barriers **to Implementation**. PREVAIL is **a Peer Mentorship Intervention** developed in **a** prior ***research study***. PREVAIL consists **of** 3 months **of** one-to-one sessions between **Peer *Mentors*** and individuals recently hospitalized due **to Suicide** risk, with sessions typically occurring in community settings. **Peer *Mentors*** adhere **to Suicide *Safety*** protocols and are supervised by **a** mental ***Health*** clinician. Peers ***Mentors*** share their experiences and use semi-structured discussion guides that include content related **to** improving hope (e.g., setting hopeful ***Goals***, identifying reminders **of** hope) and belongingness (e.g., developing supportive relationships). PREVAIL has been pilot tested in **a** sample **of** 70 participants and was found **to** be acceptable, feasible, and without ***Safety*** concerns. This study will conduct **a** two-site, single-blinded, ***Randomized*** controlled trial **of** 490 ***Adult Patients*** admitted to an inpatient psychiatric unit for **Suicide** risk **to** assess the **Effectiveness of** the PREVAIL **Intervention**. Participants will be ***randomly assigned*** **to** receive either 3 months **of** the PREVAIL **Intervention** or an enhanced usual care control condition. Assessments at baseline, 3, and 6 months will measure ***suicidal ideation***, suicide attempts, ***hopelessness***, and belongingness. Specific Aim 1 **of** this study is **to** determine whether PREVAIL is effective at reducing the severity **of *suicidal ideation*** or the likelihood **of** making **a Suicide** attempt among recipients. Specific Aim 2 is **to** assess the effect **of** PREVAIL on ***hopelessness*** and belongingness; exploratory analyses will assess whether these effects explain improvements in ***suicidal ideation*** and **Suicide Attempts**. Specific Aim 3 **of** the study is **to** identify barriers and facilitators **to Implementation of** PREVAIL by health systems. Qualitative interviews with key stakeholders (e.g., ***Health*** system leaders, **Peer *Mentors***, ***Patients***) will be analyzed **to** guide future, timely **Implementation of** PREVAIL. The effect **of** PREVAIL on outcomes relevant to ***Health*** systems, such as readmissions and ***Outpatient Care***, will also be explored.

Principal Investigator: Brian Ahmedani, Ph.D.
ER/LA Opioid Post-Marketing Requirement Studies: Observational Study (1A (2065-1) Subcontract

The food and Drug Administration (FDA) has asked the companies that are New Drug Application (NDA) holders of extended-release/long-acting (ER/LA) opioids to conduct one or more studies to provide quantitative estimates of the serious risks of misuse, abuse, addiction, overdose, and death associated with long-term use of opioid analgesics for management of chronic pain, among patients prescribed ER/LA opioid products. Although abuse and misuse of prescription opioids have increased over the past decade, there is debate about the magnitude of misuse, abuse, and addiction among patients who are treated with opioids for chronic pain. Further, although there appears to be comorbidity of opioid use disorders with other substance use and psychiatric disorders, there is insufficient data to estimate how the risk of these outcomes varies by the presence of risk factors among patients treated with opioids long-term. This study seeks to fill that gap. '[-The primary objective is to quantify the serious risks of misuse, abuse, and addiction associated with long-term use of opioid analgesics for management of chronic pain among patients prescribed ER/LA opioid products. Specifically, we will:

1. Estimate the incidence of misuse, abuse, and addiction (separately, and as a composite measure) associated with long-term use of opioids for chronic pain
2. Evaluate and quantify risk factors for misuse, abuse, and addiction associated with long-term use of opioids for chronic pain, including:
    a. product/formulation (grouped)
    b. whether or not product is an abuse-deterrent ER/LA formulation
    c. whether or not participants are receiving ER/LA opioids concomitantly with IR opioids
    d. average dose (in morphine equivalents [MEqs]) and duration of opioid use
    e. prescriber specialty
    f. indication
    g. demographics (e.g., age, sex, race/ethnicity)
    h. other clinical factors (e.g., concomitant medications, personal or family history of substance abuse, history of psychiatric illness, tobacco use)

i. Type of delivery system (e.g., integrated, network/fee-for-service) and state.
j. Mu opioid receptor OPMR1 and cytochrome P450 enzyme (e.g., 3A4 and 2D6) status

3. Estimate the risk of misuse, abuse, and addiction (separately and as a composite measure) and identify risk factors in those who initiate ER/LA opioids, but do not progress to longer-term use (>90 days). We will then compare, qualitatively, differences in risk and risk factors among individuals who use ER/LA opioids for 90 days or fewer and those who opioids for more than 90 days.
4. Evaluate and describe deaths and overdoses encountered among the recruited patient population throughout the length of the study.
5. Qualitatively assess risk related to efficacy, triangulating results from all study components

In addition, a supplemental cross-sectional sample of patients on long-term (>1 year) opioid therapy will provide an estimate of the prevalence of misuse, abuse, and addiction associated with longer-term opioid therapy.

Principal Investigator: Jordan Braciszewski, Ph.D.
A Pragmatic Trial of Parent-focused Prevention in Pediatric Primary Care: Implementation and Adolescent Health Outcomes in Three Health Systems (UG3AT009838) Subcontract

Fifty percent of all adolescents will use some form of illicit drugs before the end of high school, 20-25% will meet criterial for depression, and many others will engage in health compromising behaviors like delinquency and violence with consequences for their long-term health. Evidence-based interventions shown to prevent these behavioral health concerns could improve adolescent health trajectories if implemented widely in pediatric primary care. The American Academy of Pediatrics? Bright Futures recommends that pediatricians offer developmentally tailored anticipatory guidance to all parents to support their children's healthy development, but programs providing guidance are not offered universally. This UG3-UH3 application tests the feasibility and effectiveness of implementing Guiding Good Choices, a universal, evidence-based anticipatory guidance curriculum for parents of early adolescents, in three large, integrated healthcare systems serving socioeconomically diverse families. This intervention reduced adolescent alcohol, tobacco and marijuana use, depression, and general delinquency in two previous rigorous randomized controlled trials. It also strengthened parenting practices and parent-adolescent relationship quality, both broadly protective against behavioral health concerns. Guiding Good Choices has the capacity to achieve population-level impact on adolescent health if made widely available through pediatric primary care. Parents trust pediatricians? advice regarding their children's well-being, and current research with socioeconomically diverse groups suggests that they are eager to participate in family-focused programs offered in primary care clinics. Building on this body of research, the investigative team, in close cooperation with the NIH Healthcare Systems Research Collaboratory and healthcare systems partners, will conduct a cluster-randomized trial of Guiding Good Choices in 72 pediatric primary care practices. Half will be randomly assigned to offer the program universally to parents of adolescents ages 11 to 12, and half will serve as usual care controls. The study will use a workflow that is easy to adopt, implement, and maintain by primary care clinics to enroll families in the intervention at the adolescent well visit. We anticipate recruiting over 4,500 families into the trial. The team will use the RE-AIM framework to test implementation outcomes and effectiveness, including hypothesized reductions in several behavioral health problems (e.g., substance use initiation, mental health symptoms and diagnoses), and emergency department and inpatient service utilization. We will use data from the EHR and a supplemental behavioral health survey to monitor outcomes up to 3 years post intervention. We will also assess the feasibility and sustainability of implementing the intervention in each HCS, including health economic evaluation to understand costs in relation to value gained. Throughout the trial the investigative team will engage in ongoing dialog with HCS leaders, pediatricians, and clinic staff to ensure the intervention and implementation process fit the needs of each HCS. We anticipate that evidence of feasibility and effectiveness in three different HCS will foster broad dissemination to achieve public health impact.

Principal Investigator: Jordan Braciszewski, Ph.D.
NIDA CTN-0074: Primary Care Opioid Use Disorders Treatment (PROUD) Trial (3UG1DA040314) Subcontract

Over 20 million US adults and youth suffer from substance use disorders (SUDs) and substance use (SU) related problems. However, most people with SUDs never receive SUD treatment. Historically, research on SUDs has focused on the small minority of patients with SUDs who are seeking, or already

engaged in, specialty SUD treatment. The overall goal of the proposed Addictions Research Network (ARN) node of the Clinical Trials Network will be to conduct cutting edge research to improve outcomes in all patients with SU/SUD who are seen in medical settings. The ARN includes 15 large health systems across the US that use the HMO Research Network's (HMORN's) Virtual Data Warehouse, providing geographic and racial/ethnic diversity as well as variation in systems of medical and SU/SUD care. The proposed ARN node has 3 broad agendas—1) to evaluate effective practices for identifying, engaging and treating patients with SU or SUDs in medical settings; 2) to develop and test effective, practical ways to implement these practices in a sustained manner as part of routine medical care; and 3) to develop and disseminate innovative research methods on SU and SUDs. Three PIs will lead the ARN node, each with expertise critical to our research agenda. Dr. Weisner, who has more than 25 years of experience leading SUD research in public and private medical settings, will lead the ARN as Senior PI at Kaiser Permanente. She partners at Kaiser Permanente with Dr. Campbell, an expert in research on opioid misuse and patient-centered and comparative effectiveness research, and Dr. Bradley, Senior PI at Group Health, who has 20 years of research experience targeting non-treatment-seeking patients with alcohol misuse and SUDs in medical settings. The ARN will have 3 Cores: 1) an Administrative Core will support all aspects of the ARN node; 2) an Implementation Core will support patient-centered design of practical, sustainable approaches to implementing SUD care in routine medical settings using electronic health records (EHRs), and; 3) an Analytics Core with expertise in programming, biostatistics using EHR data, and economics, will support innovative methods research and study design. ARN work will leverage the HMORN's 20 years of conducting pragmatic clinical trials and comparative effectiveness research across health systems using EHRs and the nationwide Virtual Data Warehouse. The ARN node will provide a robust foundation for population-based studies—including pragmatic randomized controlled trials, comparative effectiveness studies, and implementation research—that can evaluate long-term health outcomes. Moreover, through our connection to 15 learning healthcare systems, our research will design approaches to improve the quality of care for SU and SUDs in real-world medical settings. In this way—with the other CTN nodes—the ARN node will help build the infrastructure required for the next era of addictions health services research.

Principal Investigator: Amy Loree, Ph.D.
SBI-Tech Michigan: Optimizing SBI Implementation for High Risk Alcohol Use Among Women of Childbearing Age (NU84DD000001) Cooperative Agreement

The purpose of this Notice of Funding Opportunity (NOFO) is to reduce risky alcohol use among women of childbearing age through system-level implementation of alcohol screening and brief intervention (SBI) in health systems providing women's health services. Risky alcohol use can result in a variety of negative health and social consequences, such as motor vehicle crashes, intimate partner violence, and fetal alcohol spectrum disorders. It is costly, results in over 88,000 deaths annually, and can affect serious medical conditions, such as hypertension, liver disease and certain types of cancer. Health professionals are uniquely positioned to intervene with patients with acute and chronic health conditions caused or exacerbated by risky alcohol use. Alcohol SBI implementation efforts within health systems will focus on development and implementation of: a training and technical assistance plan; alcohol SBI protocols in primary care clinics; system-level approaches that facilitate uptake (e.g., electronic health record integration and performance metrics); an evaluation plan assessing feasibility and impact of system-level implementation; a dissemination plan on promising models and lessons learned; and a sustainability plan. Expected performance outcomes include documenting provider/clinic readiness to conduct alcohol SBI, documenting implementation barriers and proposed solutions, tracking clinic-level data on alcohol SBI, and assessing the use of system-level strategies.

Center for Individualized and Genomic Medicine Research (CIGMA)

Principal Investigator: Keoki Williams, M.D.
Leveraging Electronic Medical Records to Perform Large-Scale Diabetes Pharmacogenomics among Ancestrally Diverse Patient Populations (R01DK113003)

Diabetes mellitus is a modern-day scourge, affecting an ever-increasing proportion of individuals worldwide, including 26 million Americans currently. Moreover, type-2 diabetes (T2D) disproportionately affects historically disadvantaged U.S. minority groups, as evidenced by the much higher rates of disease and more severe complications among African American individuals. Although there are multiple

therapeutic classes of oral medication available for treating T2D, metformin is currently recommended as the first-line therapy. Metformin lowers blood glucose levels by reducing hepatic gluconeogenesis, improving skeletal muscle insulin sensitivity, and limiting intestinal glucose uptake. It has also been shown to be an effective therapy for preventing incident diabetes. Despite being one of the most frequently prescribed drugs worldwide, very little is known about the biologic mechanism(s) through which metformin mediates its effect. This knowledge would be of value therapeutically to better understand and predict treatment response. By extension, even less is known about the activity of metformin among African American individuals, as few studies have included substantial numbers of non-European population groups. This application will help rectify existing knowledge gaps by studying a large and diverse patient population with T2D. Specifically, we will utilize electronic medical record (EMR) data for large-scale diabetes pharmacogenomics. These data have the advantage of being able to account for medication use and drug exposure over time; to provide substantial numbers of individuals for combined and population group specific analyses; and to assess clinical endpoints both retrospectively and prospectively. In this application, we propose the following study aims: 1) To assess whether there are differences in metformin treatment response by self-reported race-ethnicity and genetic ancestry; 2) To use novel, gene-based association approaches to identify both shared and population group specific genetic variants influencing metformin's effect on blood glycemia (i.e., HbA1c levels); and 3) To replicate our findings in a separate group of patients and to include additional exploratory analyses to assess whether the identified genetic variants influence diabetes-related microvascular events, macrovascular events, and adverse drug reactions. The knowledge gained through this study will directly address the goals of Health People 2020 – “achieve health equity, eliminate disparities, and improve the health of all groups.”

Principal Investigator: Keoki Williams, M.D.
Poly-omic Study of Asthma Exacerbations in Diverse Populations (R01HL141845-01A1)

Asthma exacerbations contribute to considerable disease morbidity and account for nearly half of all asthma related costs. Nevertheless, we do not currently have biomarkers that can be used clinically to reliably predict an impending exacerbation. Such measures could transform asthma care if they allowed for the timely recognition, treatment, and prevention of these severe events. It is important to note that certain population groups, such as African Americans and Latinos (particularly Puerto Ricans), suffer disproportionately from these complications with rates of asthma-related emergency department visits, hospitalizations, and deaths nearly 3-5 times higher than those of European Americans. Therefore, it should be surprising that nearly all existing genetic studies of asthma exacerbations have focused on individuals of European descent and have been insufficiently powered to study other groups. Other limitations include analyses which didn’t take into account the timing of events and studies which focused on allergic mechanisms (as opposed to taking an agnostic approach). In this application, we will utilize the enormous amount of whole genome sequence data that will be generated by our Asthma Translational Genomics Collaborative (ATGC) and the NHLBI’s Trans-Omic Precision Medicine (TOPMed) program to identify genomic markers of asthma exacerbations. The ATGC comprises 9 cohort studies and 10,840 patients with asthma (7,212 African American individuals and 3,628 Latino individuals). We will use the Study of Asthma Phenotypes and Pharmacogenomic Interactions by Race-Ethnicity (SAPPHIRE) as the discovery set to identify genetic variants associated with time to exacerbation (Aim 1). The SAPPHIRE cohort is ideally suited to serve as the discovery set for this project because of its prospective longitudinal records of events, detailed characterization of participants, and extensive biobank, which includes serum and RNA samples. Replication of top genetic associations will be performed in the other 7,086 ATGC participants (Aim 2). Transcriptomic data generated from RNA sequencing will be used to identify genes whose expression in whole blood is associated with time to exacerbation (Aim 3a), and variants promoted from Aims 1 & 2 will be assessed as expression quantitative loci for their association with gene expression (Aim 3b). For Aim 4, banked serum will be used to assess the proteome of individuals from phenotype extremes (i.e., serum collected from individuals prior to a severe exacerbation vs. serum from individuals with asthma who don’t experience exacerbations). Using mass spectrometry, we will broadly assess serum for proteins differentially expressed between these groups (i.e., an untargeted proteomic approach), and we will use the information gleaned from the genomic, transcriptomic, and untargeted proteomic analyses to assess specific proteins (i.e., a targeted proteomic approach) for expression differences in additional groups of individuals at phenotype extremes. In short, we are proposing both independent and interdependent “omic” analyses to identify biomarkers of asthma exacerbations in populations at highest risk.

Department of Public Health Sciences

Principal Investigator: Andrea Cassidy-Bushrow, Ph.D.
Delivery Mode, Environment and the Gut Microbiome: Influence on Childhood Body Size (R01HD082147)

Caesarean section (CS) delivery, which accounts for ~32% of all US births, has been associated with offspring obesity. Little is known about the mechanisms linking CS with obesity risk. The gut microbiome, which varies by mode of delivery, is also associated with childhood obesity. In our established racially and socioeconomically diverse birth cohort (WHEALS; Wayne County Health, Environment, Allergy and Asthma Longitudinal Study), the early-life gut microbiome is associated with body mass index (BMI) category at age 2 years; CS is associated with both a distinct early-life gut microbiome and with increased BMI at age 2 years; and the presence of pets in the home, which increases microbial diversity, reduces the association between CS and BMI. Our data provide evidence for a mediating role of the gut microbiome in the CS-obesity relationship. However, to provide stronger evidence requires additional study. This project builds on extant data in WHEALS and on-going data collection in a subset of these children to examine the role of the gut microbiome in the CS-obesity association. Children will be invited for a research clinic visit for comprehensive body size assessment and blood draw at age 10-12 years. Gut microbiome composition and predicted function will be measured in banked early- life (1 and 6 months of infancy) stool samples and in samples from these children at age 10-12 years using the 16S rRNA and ITS2 biomarker genes and the Illumina MiSeq platform. A metabolomics analysis will be conducted in a subset of these stool samples. Adiposity will be measured as BMI at ages 2 and 10-12 years, BMI trajectory from birth to age 10-12 years, and anthropometric, bioimpedance and inflammatory measures at ages 10-12 years. Combined, we anticipate 630 unique children will have 10-year adiposity measures and at least one early-life microbiome measure (~405 with 1 month and ~381 with 6 month stool samples, which includes ~300 children with paired 1 and 6 month samples). Of these children, 400 will also have gut microbiome measured at age 10-12 years. Our specific aims are to: (1) examine if mode of delivery is associated with childhood adiposity; (2) examine if the gut microbiome is associated with childhood adiposity; and (3) examine whether the gut microbiome mediates relationships between mode of delivery and measures of adiposity. Such a complementary “omics” approach has never been applied to the study of childhood obesity and is likely to provide critical insights into disease development in early- life as well as potential targets amenable for intervention.

Principal Investigator: Melissa Davis, Ph.D.
The DARC side of Breast Cancer (R21CA210237)

TNBC is arguably the most deadly BrCa subtype with higher prevalence in pre-menopausal women and in women of African descent. We know that the combined TNBC prevalence and poor treatment options are a likely cause of persistently higher mortality rates in African Americans compared to European Americans in the US. However, we have shown that within African Americans, disparities in BrCa survival are more pronounced within the TNBC category compared to the ER positive groups. These data indicate that *unique mechanisms are operating in either tumor biology or host response in women of African descent*. The ancient and African- specific *Fy*- allele alters the regulation of DARC/ACKR1, an atypical chemokine receptor, in a tissue-specific fashion beyond the previously described RBC phenotype. This implicates DARC/ACKR1 in various altered phenotypes in these ancestry groups, specifically as it relates to chemokine regulation. This project will test the hypothesis that DARC expression in tumor cells alters tissue chemokine levels to modify the host immune response to tumorigenesis, and that absence of DARC expression on blood cells as a result of the African-specific *Fy*- allele alters circulating chemokine levels, altering the tumor microenvironment and enhancing tumor aggression. Specifically we will; 1- Determine if DARC tumor expression associates with ancestry and altered host immune responses in a pilot BrCa cohort of African Americans and European Americans and 2- Determine if loss of DARC on bone-marrow-derived (bmd) blood cells alters chemokine profiles and tumor immune response, using pre-existing transgenic C3-
1Tag BrCa and AckR1-/- mice.

Principal Investigator: George Divine, Ph.D.
Targeted Clinical Trials to Reduce the Risk of Antimicrobial Resistance: Randomized Controlled Trial for Treatment of Extensively Drug-Resistant Gram-Negative Bacilli (Option 5) (HHHSN272201600049C) Subcontract

The Gram-negative bacilli organisms Acinetobacter baumannii, Klebsiella spp., Escherichia coli, Enterbactor spp. and Pseudomonas aeruginosa have become a frequent cause of bloodstream infection and pneumonia in the hospital and other healthcare settings. Among these pathogens, antimicrobial resistance has emerged to many classes of antimicrobial agents. Most concerning has been the emergence of resistance to group 2 carbapenems (such as imipenem). In several regions of the world, including Southeastern Michigan, strains of extensively-drug resistant Gram-negative bacilli (XDR-GNB) that exhibit resistance to most, and in some cases all types of available antimicrobial agents, including group 2 carbapenems, have emerged and disseminated. Treatment options for XDR-GNB typically include Colistimethate sodium (referred to as colistin in this study), used alone (monotherapy) or in combination with other agents. Unfortunately, resistance to colistin has begun to emerge in some strains of XDR-GNB, which is a truly concerning development, since colistin is one of the last remaining treatment options for XDR-GNB. No prospective, randomized controlled trials have been conducted to evaluate the clinical efficacy of colistin monotherapy versus colistin-containing combination therapy or the impact of these therapeutic modalities on the emergence of colistin resistance among XDR-GNB. We plan to conduct a double-blind randomized controlled trial including patients with pneumonia and bloodstream infection due to XDR-GNB. After enrollment, subjects will be randomized to receive 14 days of either colistin monotherapy or colistin plus meropenem.

In the Detroit metro area, infections due to XDR-GNB have developed into a regional challenge and common problem. We have assembled a multi-disciplinary team that includes Infectious Diseases researchers, clinicians, infectious diseases pharmacists, microbiologists, epidemiologists and statistical experts to address critically important questions and challenges regarding the management of bloodstream infection and pneumonia due to XDR-GNB. Specifically, we hypothesize that the combination of colistin and imipenem will provide superior efficacy in the treatment of XDR-GNB pneumonia and bloodstream infection and will prevent the emergence of decreased susceptibility to colistin among XDR-GNB strains. We also aim to analyze tools that could be used in "real time" to aid clinicians treating patients with infection due to XDR-GNB. For example, we aim to analyze the association between the presence of in vitro synergy of the colistin and carbapenem (imipenem or meropenem) combination (as determined by E-test) and clinical outcomes; and the association between colistin plasma levels and clinical outcomes and the development of nephrotoxicity.

Principal Investigator: Christine Cole-Johnson, Ph.D.
Project 1-Early Microbiota-Related Risk Factors and the Development of a Multi-Sensitized High Risk for Allergic Asthma Phenotype (P01AI089473)

the ***Health*** status **of** our ***children*** has long lasting ***social*** and economic ramifications **for the** nation. Asthma is **the** most common chronic condition in **the** U.S. ***pediatric Age*** group. This ***Disease*** often carries on into adulthood and is associated with lifelong deficits in ***lung function***. **the** majority **of** these ***children*** are also allergic, and **a** sub-group have **Asthma** that is difficult to control. Despite substantial ***Research*** effort, the dramatic increase in **Asthma** ***Prevalence*** over **the** past decades has not been mitigated. New hope is centered on ***Research*** investigating **the** influence and possible interventional opportunities afforded by study **of the** ***gut microbiota***. This has become possible with **the** advent **of** culture-independent technology. Our initial P01 used our WHEALS ***Birth*** cohort to demonstrate that numerous ***Child*** and also maternal ***Characteristics*** were associated with **the** infant's ***gut microbiota*** community composition. Infant gut ***microbiota Characteristics***, such as lower bacterial diversity and higher fungal diversity, were associated with **a** multi-allergen-sensitized (IgE) **Phenotype** at ***Age*** 2 years and **Asthma** at ***Age*** 4 yrs. Our new ***Data*** reveals that this ***Age*** 2yr **Phenotype** is associated with **a High Risk of Multi-Sensitized Allergic Asthma** at ***Age*** 10 yrs, and **the** same ***gut microbiota Characteristics*** are associated with this higher **Risk**. Our new P01 ***Murine Data*** supports a ***maternal microbiota*** influence on **the** offspring's ***Immune system***. **the** WHEALS ***Birth*** cohort was not initially designed to examine how **the** ***Infant gut microbiota*** is related to allergy, or **the** maternal contributions to this association. ***Infant*** stool samples were obtained using limited institutional funds and therefore at only one or two points in time and relatively sporadically. This **Project** will capitalize on **a** new, large and diverse ***Health*** system-based general **Risk** ***Birth*** cohort with **the** focus **of** studying **the** ***gut microbiota*** with **the** latest ***sample collection*** and analytic techniques and collection **of** stool specimens at earlier and more

frequent ***Infant*** time points and one in ***Late pregnancy***. We propose detailed studies **of** multiple variables likely to ***Affect*** **the** ***maternal microbiota*** during ***Pregnancy***, how these effects are related to **the** child's risk **of Allergic** sensitization, and how **the** maternal and ***Infant*** gut microbiotas can mediate **Risk**. **Project** 1 is designed to home in on maternal/infant exposures and gut microbiotas and how they associate with **the** child's risk **of** becoming highly **Multi-Sensitized** or not sensitized (resilient to sensitization) by **the** ***Age*** **of** 2 yrs. Altogether, **the** coordinated Projects in this P01 are addressing hypotheses highly informative to future development **of** potential microbial-related preventive interventions through both behavioral/social or direct microbial means.

Principal Investigator: Christine Cole-Johnson, Ph.D.
Project 3-Identification of Microbial Founder Species and Metabolic Products that Promote Microbiota and Immune Development Resilient to Childhood Allergy and Asthma (P01AI089473)

The gut microbiome accumulates bacterial diversity over the first several years **of** life and plays a critical role in **Immune Development** including **Immune** tolerance via production **of** microbial-derived metabolites such as short chain and polyunsaturated fatty acids. We and others have demonstrated **that** children who develop allergic sensitization or **Asthma**, exhibit consistent bacterial genera depletions and **Metabolic** perturbations in infancy. Relative **to** those at low-risk **of** disease **Development**, the associated **Products of** the perturbed, early life high-risk gut microbiome induce expansion and activity **of** T-helper 2 cells and reduce the frequency of regulatory T cells in vitro. Thus, the implication is **that** the very early-life gut microbiome, via microbial-derived metabolites, shapes nascent **Immune** function in a manner consistent with disease or health in **Childhood**. Our most recent studies indicate **that** the meconium microbiome **of** high-risk for **Asthma** infants (with at least one asthmatic parent) is distinct from **that of** low-risk neonates, and exhibits a significantly delayed bacterial diversification trajectory over the first year **of** life, implicating differences in vertically transmitted foundational gut microbes and subsequent gut microbiome and **Immune Development**. Thus, the human gut microbiome appears **to** adhere **to** the tenets **of** primary succession, the process **of Species** diversification in a pristine ecosystem, central **to** which is the tenet **that Founder** (or pioneer) **Species**, (i.e. those **that** first colonize), shape ecosystem conditions and thus the pace and trajectory **of** subsequent **Species** accumulation. We thus hypothesize **that** in those children protected against **Allergy** and **Asthma Development**, specific early-life gut microbiome strains, and more specifically, their **Metabolic Products**, **Promote Immune** tolerance which shapes subsequent **Immune** and **Microbial Development** throughout **Childhood** protecting against disease **Development**. P3 aims **to** build upon our previous studies and address this hypothesis using both banked samples from WHEALS for which 10-year allergic sensitization and **Asthma** outcomes are known, and prospectively collected longitudinal samples in this P01 from mother-infant dyads in children with a High Risk for Allergic Asthma Phenotype (HiRAAP) or Low Risk for Allergic **Asthma** Phenotype (LoRAAP) at ***age 2 years***. P3 proposes to identify early-life gut **Microbial** derived metabolites **that Promote Immune** functions associated with protection against allergic **Asthma Development** in **Childhood**, identify their **Microbial** source, and develop and, based on these findings, test a novel **Microbial** polybiotic for its capacity **to** shape **Immune** function and protect against airway allergic sensitization in mice. This study will advance our knowledge **of** how gut **Microbial** strains and their **Products** program protective immunity against **Childhood** allergic **Asthma Development** and serve as a foundation for primary prevention **of** the disease.

Principal Investigator: Christine Cole-Johnson, Ph.D.
Project 4-Alteration of Mouse Maternal Gut Microbiota Alters Metabolic Profiles and Immune Phenotype in Offspring (P01AI089473)

in our initial P01, we found that ***Mice*** orally supplemented with dust from homes with ***Dogs*** were resistant to induction **of** allergic ***Lung Inflammation*** compared to ***Mice*** supplemented with dust from homes without pets. Examination **of** ceca from ***Mice*** supplemented dog-home dust identified a keystone species, Lactobacillus johnsonii. Oral supplementation **of** ***Mice*** with viable but not killed L. johnsonii reduced susceptibility to induction of both allergic and respiratory syncytial virus (RSV) induced ***Lung Inflammation***. These reductions **in** lung inflammation appear to be related to alterations **in** the functional activity **of** bone marrow-derived dendritic cells (DC). Preliminary data suggests that microbial metabolites **in** the circulation **of** supplemented animals differ from those **of** un-supplemented animals. Limited data suggests that the differences **in** circulating metabolites are responsible for the alterations **in** DC function. Our rationale for including studies **of** RSV are epidemiologic studies showing that RSV infections **in** human infants increase the risk **of** subsequent asthma. Our mouse models mirror this relationship since

neonatal infection with RSV leads to greater pathology when the ***Mice*** are sensitized to allergen 4 weeks later. Interestingly our preliminary studies have shown that supplementation of female ***Mice*** with L. johnsonii prior to mating reduces responses to allergen and RSV challenges **in Offspring** to a level similar to that observed **in** directly supplemented ***Mice***. Among the questions raised by this observation was whether the effects **of Maternal** supplementation on **Offspring** occurred during **in** utero development or post-partum from components **of *Breast Milk***. This question led to cross-fostering experiments. **in** these experiments **Offspring of** supplemented or un-supplemented ***Mice*** were nursed by either supplemented or un- supplemented mothers revealing that ***Breast Feeding*** can partially protect pups **of** un-supplemented mothers from RSV. Based on our findings we propose studies **in** this **Project** based on the hypothesis that the maternal microbiota shapes the developing neonatal **Immune** homeostatic mechanisms through alteration **of** the offspring **Gut Microbiota** and related microbial metabolites which lead to differences **in Immune** responsiveness and the risk **of** pathogenic allergic responses. Our studies will continue to examine the mechanisms though which microbial changes affect pathologic responses to allergens and RSV. These studies will include further studies to differentiate **in** utero effects and ***Breast Milk*** effects on **Offspring**. Finally, we will examine the effects of supplementation with consortia **of** bacteria selected **in Project** 3 on the response **of Offspring** to allergen and RSV challenges. These studies will provide greater ***insight*** into the findings from the human studies **in** Projects 1 & 2, especially questions concerning when effective interventions might be most safely instituted.

Principal Investigators: Christine Cole-Johnson, Ph.D.
Human Epidemiology and Response to SARS-COV-2 (HEROS)
Core A, Core D, Core E
Principal Investigator: Albert Levin, Ph.D., Core B
Principal Investigator: Kimberley Woodcroft, Ph.D., Core C
(P01A1089473)

This request is in response to NOT-AI-20-031 for supplement funding in response to the COVID- 19 emergency. COVID-19, the infectious disease caused by SARS-CoV-2, is rapidly affecting humans around the globe. While initial epidemiological data have focused on cases that resulted in severe respiratory disease seen predominantly in adults, little information regarding the infection burden in children is available. This is complicated by the observation that many virologically confirmed cases in children are asymptomatic. Undocumented, and likely infectious, cases could result in exposure to a far greater proportion of the community than would otherwise occur. Indeed, it has been proposed that undocumented (or silent) infections are the source for almost 80% of documented infections; thus, it is critical to determine the silent and symptomatic infection rate in children. To overcome challenges for clinical study implementation imposed by current healthcare access restrictions, a surveillance study under design will enroll and prospectively observe eligible children, and their family members, that are current participants in our NIH-funded, ongoing, birth cohort studies. These children and their families are known to research staff and as part of their participation in HFHS studies, they have already been exposed to the procedures involved in a surveillance study. We are requesting support for the pediatric studies aligned with our Microbiota and Allergic Asthma Precision Prevention (MAAP2) (PI: Johnson, Ownby P01AI089473) to participate in the multi-center survey entitled Human Epidemiology and Response to SARS-CoV-2 (HEROS), Protocol # DAIT-COVID-19-001. Our primary objective is to report the incidence of SARS-CoV-2 infection (detection of virus in nasal secretions) over time in cohort children (index child) and household contacts (caregivers and siblings). A secondary objective is to compare SARS-CoV-2 infection status and antibody development for index children/siblings with atopic conditions (e.g. asthma, eczema) versus children without atopic conditions. As an exploratory aim, we will investigate whether SARS-CoV-2 infection (as determined by virus detected in nasal secretions) is associated with the presence of virus in stool. Our targeted enrollment is 300 families recruited over a 2-week period and followed for a minimum of 6 months. At predetermined intervals, biological samples (nasal swabs, peripheral blood, stool) will be collected by the caregiver at home using materials provided to the family. Symptom and exposure surveys will be completed remotely via a smart phone, on-line, or telephone at the time of biological sample collection. This timely, multi-site study can be rapidly implemented and realistically conducted without necessitating any visits to a clinical research center and will provide invaluable information on the infection burden of SARS-CoV-2 in children.

Principal Investigators: Christine Cole-Johnson, Ph.D.
Microbiota and Allergic Asthma Precision Prevention (MAAP2) Project 1, Project 3, Project 4
Principal Investigator: Edward Zoratti Project 2
(P01A1089473)

This application builds on the findings of our initial P01 designed to examine relationships between environmental factors, especially pets, the infant gut microbiota and pediatric allergic asthma. We have shown that: 1) dogs alter the microbial composition of dust in homes, 2) children born into homes with dogs have different developmental patterns of gut microbiota and of IgE, 3) a distinct pattern of gut microbial composition at 1 month of age is related to heightened risk of sensitization to multiple allergens at 2 years and of asthma at 4 years, and this pattern is influenced by numerous maternal characteristics, 4) sensitization to multiple food and inhalant allergens at 2 years is strongly related to asthma at 10 years, 5) the metabolic profiles of stools are related to later allergic sensitization 6) 12,13-DiHOME, a metabolite in stool, promotes development of Th2 lymphocytes and lowers development of Treg lymphocytes in an in vitro assay, and 7) in another study, the meconial microbiota is distinct in neonates born to mothers with asthma. Our complementary mouse studies have shown that: 1) gavaging with dust from homes with dogs reduces lung inflammation from allergen sensitization and from respiratory syncytial virus (RSV) infection, 2) dog dust gavaged mice have increases in Lactobacillus johnsonii in their ceca 3) oral administration of live L. johnsonii confers protection against pulmonary inflammation induced by allergen and RSV, 4) L. johnsonii alters the function of bone marrow- derived dendritic cells, 5) mice orally supplemented with L. johnsonii have altered serum metabolic profiles, and 6) mouse pups born to L. johnsonii-supplemented mothers are protected against allergen challenge and RSV infection. Collectively these findings showing the influence of maternal factors provide the basis for this application's focus on the maternal gut and vaginal microbiotas during pregnancy, and how these relate to infant gut microbial development and risk of allergic asthma. Project 1 focuses on the relationship of maternal environmental and dietary factors, including maternal and infant gut microbiotas, to the child's developing a high-risk for asthma phenotype by age 2 years. Project 2 proposes a detailed examination of relationships between maternal and child microbiota, breast milk composition and IgE development amongst a cohort of pregnancies in which the mother has current allergic asthma. Project 3 synergistically interacts with Projects 1& 2 and also uses specimens from 10-year-old allergic asthma cases and controls in the initial P01 birth cohort to examine gut microbes producing metabolites associated with a lowered risk of allergic inflammation and how they are transferred from mother and established in offspring. Project 4 will use mouse models to examine the relationships between manipulation of maternal microbiota and immune development in offspring. We anticipate that together these studies will show that interventions directed at the gut microbiota of mothers during pregnancy and of high-risk neonates after birth could reduce the risk of allergic asthma in childhood. Such findings would provide the foundations of a rational strategy to prevent allergic asthma.

Principal Investigator: Christine Cole-Johnson, Ph.D.
Microbiota and Allergic Asthma Precision Prevention (MAAP2) (P01AI089473)

This application builds on the findings of our initial P01 designed to examine relationships between environmental factors, especially pets, the infant gut microbiota and pediatric allergic asthma. We have shown that: 1) dogs alter the microbial composition of dust in homes, 2) children born into homes with dogs have different developmental patterns of gut microbiota and of IgE, 3) a distinct pattern of gut microbial composition at 1 month of age is related to heightened risk of sensitization to multiple allergens at 2 years and of asthma at 4 years, and this pattern is influenced by numerous maternal characteristics, 4) sensitization to multiple food and inhalant allergens at 2 years is strongly related to asthma at 10 years, 5) the metabolic profiles of stools are related to later allergic sensitization 6) 12,13-DiHOME, a metabolite in stool, promotes development of Th2 lymphocytes and lowers development of Treg lymphocytes in an in vitro assay, and 7) in another study, the meconial microbiota is distinct in neonates born to mothers with asthma. Our complementary mouse studies have shown that: 1) gavaging with dust from homes with dogs reduces lung inflammation from allergen sensitization and from respiratory syncytial virus (RSV) infection, 2) dog dust gavaged mice have increases in *Lactobacillus johnsonii* in their ceca 3) oral administration of live *L. johnsonii* confers protection against pulmonary inflammation induced by allergen and RSV, 4) *L. johnsonii* alters the function of bone marrow- derived dendritic cells, 5) mice orally supplemented with *L. johnsonii* have altered serum metabolic profiles, and 6) mouse pups born to *L. johnsonii*-supplemented mothers are protected against allergen challenge and RSV infection. Collectively these findings showing the influence of maternal factors provide the basis for this application’s focus on the maternal gut and

vaginal microbiotas during pregnancy, and how these relate to infant gut microbial development and risk of allergic asthma. Project 1 focuses on the relationship of maternal environmental and dietary factors, including maternal and infant gut microbiotas, to the child's developing a high-risk for asthma phenotype by age 2 years. Project 2 proposes a detailed examination of relationships between maternal and child microbiota, breast milk composition and IgE development amongst a cohort of pregnancies in which the mother has current allergic asthma. Project 3 synergistically interacts with Projects 1 & 2 and also uses specimens from 10-year-old allergic asthma cases and controls in the initial P01 birth cohort to examine gut microbes producing metabolites associated with a lowered risk of allergic inflammation and how they are transferred from mother and established in offspring. Project 4 will use mouse models to examine the relationships between manipulation of maternal microbiota and immune development in offspring. We anticipate that together these studies will show that interventions directed at the gut microbiota of mothers during pregnancy and of high-risk neonates after birth could reduce the risk of allergic asthma in childhood. Such findings would provide the foundations of a rational strategy to prevent allergic asthma.

Principal Investigator: Christine Cole-Johnson, Ph.D.
Children's Respiratory and Environmental Workgroup (CREW) (UG3OD023282) Subcontract, Cooperative Agreement

The grant is part of $157 million in awards announced yesterday by the NIH that launches a seven-year initiative called Environmental Influences on Child Health Outcomes (ECHO). The ECHO program will investigate how exposure to a range of environmental factors in early development – from conception through early childhood – influences the health of children and adolescents.

Individual birth cohort studies have identified risk factors for developing childhood asthma, including environmental exposures in early life such as allergens, pollutants, patterns of infection and colonization with viruses and bacteria, and psychosocial stress. Despite such advances, further progress in understanding the root causes of asthma have been hampered by at least two factors. First, procedures and scientific methods are not standardized across cohorts, making it difficult to compare and validate findings. Second, asthma definitions across cohorts vary considerably. In fact, asthma is a syndrome; there are different subtypes of asthma with distinct clinical features (asthma phenotypes) and likely different etiologies (asthma endotypes). We hypothesize that host factors (genetics, epigenetics) interact with environmental exposures during the prenatal period and early childhood to cause specific endotypes of childhood asthma. We further propose that identification of endotypes and associated molecular biomarkers in early life can provide a new paradigm for asthma prevention. Unfortunately, single cohorts have limited ability to identify asthma endotypes due to small sample size and unique population characteristics. To overcome shortcomings of individual cohorts, investigators leading 12 asthma birth cohorts across the U.S. now propose the establishment of the Children's Respiratory Research and Environment Workgroup (CREW) consortium. This consortium proposes to identify asthma endotypes and overcome shortcomings of individual cohorts by: 1) providing a large (nearly 9000 births and long-term follow-up of 6000-7000 children and young adults) and diverse national data set, 2) harmonizing data related to asthma clinical indicators and early life environmental exposures, 3) developing standardized measures for prospective data collection across CREW cohorts and other ECHO studies, and 4) conducting targeted enrollment of additional subjects into existing cohorts. This approach will enable collection of samples that are optimized for a systems approach to understanding how environmental and host factors in early life promote the development of specific asthma endotypes. Collectively, the results of this comprehensive research to identify the root causes of asthma vs. resilience and health will go far beyond what can be accomplished by individual cohorts, and thus provide a foundation for future efforts aimed at personalized prevention of chronic childhood asthma.

Principal Investigator: Christine Cole-Johnson, Ph.D., Public Health Sciences and Brian Ahmedani, Ph.D., Center for Health Policy and Health Services
Trans-America Consortium of the Health Care Systems Research Network for the All of Us Research Program (OT2OD026550)

Clinicians throughout history have worked to tailor both prevention and treatment strategies to the individual patient's needs; it is a fundamental credo to the practice of medicine. However, the vast majority of evidence-based clinical practice is based on research results acquired from measuring the common treatment effect on the "average person" in a restricted patient population with limited data, which we now know does not necessarily apply to numerous patients in the real-world setting. Thus, some patients will

benefit from evidence-based treatments and preventative interventions, while others will be harmed by taking medications or undergoing processes and procedures that are at best non-effective and at worst cause serious side effects. However, since the initiation of pharmacogenomics in the mid-90s, the astounding pace of development of the technical and analytic tools to measure individual inherited and acquired biological variations at all physiological levels, as well as efficiently capture a patient's medical and risk factor history and personal preferences via electronic means, is at a scale never before known.(PMIDs: 26554403, 26804248,26802434,26686739, 26769233, 26702339, 26700443, 26764593, 25231862) The current concept of "Personalized Medicine" or "Precision Medicine" in which these tools can be deployed to sharply hone predictions about an individual's risk for disease or response to treatment, while still in its infancy, has immeasurable potential.(PMIDs:20551152,26014593,26810587) Further, the costs for next generation sequencing are expected to continue to decline as technology advances. (PMIDs: 24217348, 26195686) As resources are becoming increasingly constrained, it is important to devote scientific time, energy and dollars to questions that matter to the community and have strong potential for effectively improving medical care, public health and wellness. Hence the need, creation, and continuing development of the All of Us Research Program (AoURP). (http://www.pmwcintl.com/francis-collins-nih-qa/) The promise of Precision Medicine in the U.S. can be most effectively realized on a large scale in the next decades if a research infrastructure is established and accessible to scientists across the nation and includes a large and engaged study population with comprehensive health and lifestyle histories linked to biospecimens. Critically, this population must be diverse, representing minority and other subgroups underrepresented in biomedical research. (PMID:23571593) Further, as our investigators and others have recently published, the need to engage all stakeholders, including patients and providers, into both the research and "integration into practice" aspects of Precision Medicine as it progresses, is widely recognized. (PMID:27787499, 27669484, 20805700, 22962560,23780455, 24030437,26195686) Our Consortium objective is to recruit 93,000 participant partners into the AoURP, with a focus on African Americans, Arab Americans, Hispanics, rural residents, persons of low socioeconomic status (SES) and children, with the ability to target other groups of interest as needed. Now that we are rapidly ramping up engagement efforts in preparation for AoURP national launch, we will capitalize on an influx of appropriate resources and our experience in engaging, recruiting and retaining large numbers of participants in epidemiological and clinical cohorts, along with our patient-centered and process improvement approaches, to efficiently maximize recruitment and retention in the AoURP.

Principal Investigator: Lois Lamerato, Ph.D.
US Hospital Vaccine Effectiveness (VE) Network (U01P000974) Subcontract
US Influenza Vaccine Effectiveness Network (U01IP001034) Subcontract

Prevention of hospitalization has long been viewed as a major health benefit of the use of influenza vaccine. This was, in large part, the rationale for the initial vaccination programs targeting the elderly and those with underlying health conditions. However, in the last decade, questions have been raised about the value of such programs. Modern study designs to assess vaccine effectiveness (VE) have required laboratory confirmation of influenza infection, as well as documentation of vaccine receipt and the use of a test-negative design to control for differences in healthcare-seeking behavior between vaccinated and unvaccinated patients. There is a need for current estimates of VE in preventing influenza-associated hospitalization using these methods. We propose estimation of influenza vaccine effectiveness in preventing influenza hospitalization in two health systems in Michigan, where we have been conducting annual assessments of VE in various populations since 2008. We will conduct surveillance at two hospitals and will enroll adult in-patients with acute respiratory infection. Vaccination status will be reported and documented and considered with laboratory-confirmed influenza outcomes to estimate vaccine effectiveness for prevention of hospitalization. Analyses will use a test- negative design; those testing positive for influenza cases those testing negative are controls. Modifiers and confounders of vaccine effectiveness such as age, health status, high-risk health conditions, functional status, frailty, education, time from illness onset to specimen collection, calendar time, and propensity for vaccination will be assessed. In addition to our proposed influenza surveillance and VE assessment, we propose an estimation of the incidence of hospitalization in adults due to respiratory syncytial virus (RSV and other respiratory viruses. This will allow for the evaluation of bias in influenza VE assessment due to interaction between influenza vaccination, infection, and non-influenza respiratory viruses, and will establish a platform for the future evaluation of RSV vaccines. We will accomplish these additional objectives by expanding our surveillance to months before and after the typical influenza season and evaluating specimens by molecular methods for RSV and other respiratory viruses.

Principal Investigator: Christine Neslund-Dudas, Ph.D., Public Health Sciences, and Michael Simoff, M.D., Pulmonary Center for Research to Optimize Precision Lung Cancer Screening in Diverse Populations (UM1CA221939) Subcontract

Lung cancer, the most significant cause of cancer deaths in the US, is an urgent public health threat. It disproportionately affects populations that are already plagued by high poverty rates and low education levels. These populations experience both health disparities in the early diagnosis and treatment of cancer and are historically difficult to reach with cancer screening initiatives. While the results from the National Lung Screening Trial (NLST) indicated that low dose CT (LDCT) is an efficacious and cost-effective strategy for lung cancer screening (LCS), many uncertainties exist with respect to how patient, provider, health system, and societal factors may impact the quality, compliance, effectiveness, and the risk of harms associated with lung cancer screening, within community-based health systems who serve diverse populations. Spanning from Pennsylvania to Hawaii and including five heterogeneous health systems with diverse populations, our proposed PROSPR Research Center, the Center for Research to Optimize Precision Lung Cancer Screening (CPLS), brings together a team of experienced, interdisciplinary researchers and clinicians with long-standing collaborative ties that is well-positioned to pursue research related to the barriers and opportunities associated with the implementation of LCS programs within community settings. The health systems within CPLS include: Henry Ford Health System in Metro Detroit, Kaiser Permanente Colorado, Kaiser Permanente Hawaii, Marshfield Clinic Health System in rural Wisconsin, and University of Pennsylvania Health System. The ultimate goal of CPLS is to identify critical gaps in the LCS process and to design innovative multilevel interventions to reduce lung cancer mortality, particularly among underserved populations. To achieve this goal, CPLS will complete the following specific aims: 1) build a comprehensive data ecosystem by pooling and linking common data elements to capture the entire LCS process and to assess the patient, provide facility, health system, and societal factors that affect LCS; 2) leverage the CPLS data resource to conduct four high- impact, observational studies of the multilevel factors associated with the LCS process; 3) based on findings from Aims 1 and 2, develop and test interventions to address identifiable gaps in care that may lead to health disparities in LCS, 4) actively participate in Trans-PROSPR research initiatives and collaborate with external investigators via the use of publicly-available CPLS datasets. Our center focuses on the inclusion of diverse, underserved populations that are defined by multiple factors that may adversely impact access to, and utilization of, cancer screening. In response to both the Surgeon Generals strong emphasis on the need to reduce lung cancer mortality and the Cancer Moonshot Blue Ribbon Panels focus on reducing the disproportionately high cancer death rates in underserved populations, CPLS will serve as a model for high- impact, translational research to reduce disparities in cancer mortality.

Principal Investigator: Laila Poisson, Ph.D.
Molecular and Clinical Evaluation of the Glioma Patient Experience to Anticipate Modern Outcomes and Guide Patient Care (R01CA222146)

Landmark papers published recently by us, and others, mark the new era of molecular diagnoses and precision therapy for glioma. In the summer of 2016, the World Health Organization (WHO) published updated diagnosis criteria for glioma that include molecular markers, taking a first step toward a molecularly precise diagnosis. It is our long-term goal to capitalize on the longitudinal resources of brain tumor banks to rapidly assess molecular hypotheses for prognosis and treatment of glioma. With the significant contribution of 240 cases from Henry Ford Hospital (HFH), an effort to molecularly and clinically profile glioma was started by The Cancer Genome Atlas (TCGA) project. Capitalizing on our clinically annotated brain tumor bank at HFH, we will focus on therapeutic outcomes, recurrent disease, and extended survival, which were not captured in the TCGA project. For this work, we have constructed an interdisciplinary team of collaborators, with clinical and informatics expertise, to profile an additional 340 glioma cases (WHO grade II-IV). In total, we will assess 700 tumor specimens (FFPE/frozen) from the HFH tumor bank (2001-present), representing both primary and matched progressive disease (Aim 1). Molecular data will be generated by exome sequencing to assess DNA sequence and copy number variants, targeted Sanger sequencing to profile the TERT promoter, and DNA methylation array assays to profile the methylome. Clinical annotation from our tumor bank, including long-term follow-up and therapy regimens, will be added to each of the 550 profiled glioma cases. The resulting comprehensively annotated tumor bank will be an invaluable resource for queries of clinical-molecular associations and the progression of disease, made available to researchers at HFH and beyond. In this proposal we use

our database to address two analytical aims: (Aim 2) to carefully design statistical models of prognosis and therapy response among modern diagnosis classes using retrospective records; (Aim 3) to identify genomic differences, per patient, arising over the course of treatment and progression, which we expect will impact therapy decisions and inform standard treatments strategies. As part of the third aim, we will also explore the genomic patterns and clinical response of patients with exceptional survival, which may indicate differential molecular diagnosis or suggest therapeutic avenues for extending survival in others.

Principal Investigator: Benjamin Rybicki, Ph.D.
A New Prospective U.S. Cohort Set Within the Health Care System Institutions to Study Cancer (HHSN2612018000201)

Three mid-western integrated health care systems, HealthPartners (Minneapolis, MN), Henry Ford Health System (Detroit, MI), and Marshfield Clinic Health System (Marshfield, WI), here-forward known as the **Great Lakes Consortium for Cohort Studies in Cancer (GLC3)**, have over a decade of experience working together as part of the NCI funded Cancer Research Network (CRN)(1-21) and its parent consortium the Health Care Systems Research Network (HCSRN)(22-27). These three integrated health care systems (IHCS) have joined together in response to the call by NCI to establish a U.S. cohort of healthy adults. The NCI U.S. Cohort will be recruited, consented on-line and followed for cancer-related outcomes during the ten year period of the contract. Biospecimens and on-line questionnaires will be captured at baseline and at defined intervals which will be determined in a final protocol designed in collaboration between NCI, Information Technology support contractors, and participating integrated health care systems (IHCS). The overall goal of the NCI U.S. Cohort study is to enroll and follow 150,000 to 200,000 adult members of IHCS without cancer at the time of study enrollment. GLC3 proposes to recruit and enroll **20,000 health plan members (29% African American)** across all three sites.

Principal Investigator: Jennifer Straughen, Ph.D.
The Prenatal Origins of Autism Spectrum Disorder (W81XWH191508)

The proposed study will leverage existing data, resources, and biologic samples as well as physician partnerships to conduct a study of 177 ASD cases (62 with archived placental tissue) and 62 frequency matched controls born in Michigan between 2012 and 2017. ASD cases are extensively evaluated by a team of experts at the Henry Ford Health System Center for Autism and Developmental Disabilities (CADD) and have detailed diagnostic information including severity level in their electronic medical record. Neonatal angiogenic profile will be measured in archived dried blood spots from Michigan's Newborn Screening Program. Placental histopathology and angiogenic markers will be assessed in archived placental tissue from 62 cases and 62 typically developing controls frequency matched on year of birth, preterm status, and sex.

Impact: This proposal could provide evidence for the role of angiogenesis in the etiology of ASD while at the same time opening a door to neonatal risk assessment and earliest possible intervention. If successful, our findings may suggest approaches to minimize specific types of histopathology and/or changes in angiogenic profiles and mitigate their adverse effects in offspring. In addition, our findings may enable us to develop a clinically relevant placental screening tool that can be used for identification of children at highest risk of ASD so that interventions may begin as early as possible. Importantly, our collaboration with physicians at CADD who are currently diagnosing and treating ASD affected children and their families will facilitate the translation of research to practice.

Innovation: The proposed study is innovative not only in its focus on the placenta, but also its focus on processes that have not been traditionally examined in ASD research. The most innovative feature of the proposed study is its emphasis on markers of angiogenesis beginning in the prenatal period and extending into neonatal life. Despite its critical importance for neurodevelopment, ASD research to date has largely overlooked angiogenesis. This proposal is also innovative in that, if successful, it will simultaneously explain the sex specificity of ASD as well as the heterogeneous clinical expression of ASD symptoms. Furthermore, it will open the door to the development of noninvasive neonatal screening and diagnosis.

Principal Investigator: Ganesa Wegienka, Ph.D.
A Preconception Cohort Study of Environmental Chemicals, Fertility, and Miscarriage (R01ES029951)

Infertility and ***Spontaneous abortion*** (SAB) are significant public ***Health*** problems, affecting up to 25% of reproductive ***Age*** couples in the ***United States***. ***Health*** care costs attributable to ***Infertility*** and SAB exceed $5 billion per year, and several studies have shown an association between ***Infertility*** treatments and adverse pregnancy outcomes. Thus, identifying ***modifiable risk*** factors for ***subfertility*** and SAB is an important public health goal. The potential effects **of** exposure to endocrine-disrupting **Chemicals** (EDCs) on risk **of** ***subfertility*** and SAB are understudied. The few existing ***Human*** studies have limitations including small ***Sample Size***, enrollment after conception, retrospective **Study** design, suboptimal assessment **of** exposure and outcome, inadequate control for potential ***Confounding Variables***, and limited generalizability. The proposed **Study** will prospectively assess the relation **of** exposure to selected EDCs, including ***Phenols***, ***phthalates***, and per- and poly-fluoroalkyl substances (PFAS), to risk **of** ***subfertility*** and SAB in **A Preconception** subcohort **of** 950 ***Pregnancy*** planners. We will use ***Data*** from two NICHD-supported prospective cohorts **of** ***Pregnancy*** planners in ***North America*** and Denmark. With web-based recruitment and ***Data*** collection, we have enrolled over 17,000 women attempting pregnancy into these cohorts. In **A** subset **of** 200 participants, we have successfully pilot tested in-person collection **of** ***Urine*** and ***Blood*** specimens during the **Preconception** and ***early pregnancy*** periods. In this application, we propose to expand in-person biospecimen collection, increasing the number **of** women with preconception and ***early pregnancy Urine*** and ***Blood*** samples from 200 to 950. At each **of** our three biospecimen collection sites (Boston, Detroit, and Aarhus), we will enroll 250 women and collect three ***Urine*** samples and one blood sample (in **Preconception**) and three ***Urine*** samples and one ***Blood*** sample (in ***early pregnancy***). We will ship the samples to the ***CDC*** for the analysis **of** ***urinary phthalates***, ***urinary Phenols***, and serum PFAS. To increase cost efficiency, we will pool three ***Urine*** samples in each exposure window before assaying for phthalates and ***Phenols***; and we will ***Assay*** one **Preconception** ***Blood Specimen*** for PFAS, **A** persistent chemical, in analyses **of** ***subfertility*** and SAB. Finally, we will conduct **A** pilot **Study** among 100 U.S. participants to assess the feasibility **of** collecting ***Urine*** by mail, which would allow us to take advantage **of** our full geographically-diverse cohort in the future. Strengths **of** this application include the prospective design, **Preconception** enrollment of pregnancy planners, repeated ***Measurement*** **of** exposure during **Preconception** and ***early pregnancy***, excellent control for confounding via bimonthly prospective ***Data*** collection on **A** wide range **of** covariates, and use **of** the latest analytic methods for mixtures modeling. We have generated compelling preliminary ***Data*** to support our aims. The present ***Grant*** is cost-effective in leveraging already-established **Cohort** studies with ***Data*** collection and ***follow-up*** supported by other grants. The results generated will be translatable by directly informing future regulatory decisions about EDC standards in **A** manner that could reduce rates **of** ***Infertility*** and SAB

Principal Investigator: Ganesa Wegienka, Ph.D.
Epidemiology of Allergic Disease Endotypes (R01AI110450)

Pediatric allergy and asthma are a costly public health burden, but so far substantial research efforts have yielded no prevention strategies. A likely reason is that despite longstanding recognition by the medical community that the term ‘asthma’ refers to a collection of diseases, researchers have historically treated the syndrome as a single disease entity. Epidemiologically, the collapse of different phenotypes (observed disease patterns) and endotypes (phenotypes further delineated by pathophysiological processes), into a single category corrupts associations between risk factors and diseases. Thus, progress in allergic disease research has been hampered. Prior attempts have been made to identify such phenotypes and endotypes, but a combination of incomplete data and oversimplified statistical methods have limited progress. We propose to apply sophisticated latent class analyses in a large general risk cohort combined with immunological markers to finely discriminate asthma and allergy disease phenotypes and endotypes and then use this information to conduct risk factor analyses. Using this approach in our WHEALS birth cohort, we have already characterized four classes at age 2 years: 1) Low to No Sensitization; 2) Highly Sensitized; 3) Milk and Egg Dominated Sensitization; and 4) Peanut and/or Inhalant allergen – No Milk Sensitization. Total IgE levels varied between the groups, as did the rates of eczema and doctor diagnosis of asthma (at age 4 years). The Highly Sensitized had the greatest rates, the Low to No Sensitization had the lowest rates, and the other two classes had rates intermediate between the Low and High Sensitization groups. These data suggest the use of latent classes, rather than the use of the “traditional” definition of atopy (any allergen-specific IgE (sIgE) _0.35 IU/mL), more

specifically identifies those on a trajectory for allergic disease, yielding advancement in both allergic disease research and clinical care. Using the predominantly (62%) African American birth cohort WHEALS, we will: Aim 1) Determine which early life allergic disease phenotypes identified at age 2 years are associated with lung function (spirometry and methacholine challenge) at age 10 years; Aim 2) a) Identify the allergic disease endotypes for 10 year old children based on annual report of wheeze; lung function, eNO, obesity, cytokines, and white cell counts and extensive immunophenotyping [assessment of cellular markers to identify and quantify activation of regulatory T cells (Tregs), basophils and dendritic cells (DCs)] at age 10 years; and total IgE and sensitization (sIgE and skin prick tests) at ages 2 and 10 years; and, b) Estimate associations between early life risk factors (e.g., delivery type, pet exposure, etc.) and the identified Aim 2a endotypes; and, 3) Compare and contrast the risk factor associations with the endotypes in Aim 2 to the risk factor associations determined using "traditional" definitions of atopy and asthma (doctor diagnosis and medication use and/or symptoms in the last year). Analyses will be performed for all 900 WHEALS cohort children and separately for Black children and White children to assess racial differences.

Principal Investigator: Ganesa Wegienka, Ph.D.
Environmental Risk Factors for Uterine Fibroids: A Prospective Ultrasound Study (R01ES028235)
Study of Environment, Lifestyle, and Fibroids (SELF) (HHSN273201600003I) Subcontract

Uterine leiomyomata (UL), or fibroids, are the most common neoplasms of the uterus and are a major source of gynecologic morbidity. In the United States (U.S.), the lifetime risk of symptomatic UL is approximately 25-30%. UL are the leading indication for hysterectomy, and UL-related costs exceed $34.4 billion annually. Black women are disproportionately affected by UL, with a 3-fold greater risk of diagnosis, earlier age at diagnosis and surgery, and more symptomatic tumors on average than white women. Despite the large public health burden of UL, little is known about its natural history or pathogenesis. Animal data and cross-sectional human studies have provided compelling preliminary evidence of a role for vitamin D in UL development and growth. Exposure to heavy metals such as lead, mercury, and cadmium is widespread, with reproductive-aged women, African Americans, and those of lower socioeconomic status having higher exposure levels than other groups. Funded by the National Institute of Environmental Health Sciences (NIEHS), the Study of Environment, Lifestyle and Fibroids (SELF) is a multi-year prospective cohort study of UL determinants in black women from the Detroit area. In 2011-2012, SELF enrolled 1,696 black women aged 23-34 years who had never been diagnosed with UL. At baseline and every 20 months for a total of 5 years (4 total clinic visits), SELF participants complete interviews, have blood collected for biological measurements, and undergo transvaginal ultrasounds for precise identification and mapping of UL at each visit facilitating accurate determination of UL development and growth (cohort retention >85%). The final planned clinic visits are underway. In this application, we propose to extend follow-up of SELF for an additional five years. One more clinic visit with transvaginal ultrasound, biospecimen collection and detailed exposure assessments via interview will be conducted to achieve the following specific aims: 1) Describe the natural history of UL initiation and growth; calculate age-specific UL incidence; and evaluate changes in tumor characteristics (size, number, and location) over a 10-year period; 2) Assess whether vitamin D status influences UL incidence and growth over a 10-year period; and 3) Evaluate the influence of selected environmental toxicants on UL incidence and growth. Specifically, we will examine the influence of active and passive cigarette smoking on UL incidence and growth; assess exposure to a panel of 13 metals and metalloids (and their mixtures) measured in whole blood and UL incidence and growth over a 10-year period; and determine whether vitamin D status modifies the associations between environmental toxicants and UL incidence. With its prospective design, population of young black women, serial ultrasounds, repeated collection of data on exposures and covariates, and careful analysis of chemical mixtures, SELF is ideal for identifying environmental risk factors for UL. Using methods that overcome the limitations of prior studies, this will be the most definitive study of modifiable environmental risk factors of UL and is likely to have high impact on science, clinical care, and public health policy.

Principal Investigator: Ganesa Wegienka, Ph.D.
Comparing Options for Management: Patient-Centered Results in Uterine Fibroids (COMPARE-UF) (P50HS023418) Other Federal Service Agreement

The broad, long-term objective of this project is to enable patients with uterine fibroids (UF) to make informed decisions about management options based on the highest possible quality evidence. To help

achieve this objective, we propose a multi-center registry of a geographically, racially, ethnically, and clinically diverse group of women who have received medical or surgical treatment for UF, Comparing Options for Management: Patient-centered Results for Uterine Fibroids (COMPARE-UF), designed to address the following specific aims: AIM 1) Develop the infrastructure necessary to implement large-scale observational comparative effectiveness research (CER) studies of management options for women with UF, including (a) a governance structure, policies, and procedures conducive to collaborative research involving patients, clinicians, methodologists, and other stakeholders, (b) an experienced Research and Data Coordinating Center, and (c) nine geographically diverse Clinical Centers (CCs) representing a broad range of patients and providers. AIM 2) Use this infrastructure to implement 3 projects addressing high-priority evidence gaps related to the effect of different management strategies on patient-centered outcomes. These include PROJECT 1: Comparing management options for symptom relief PROJECT 2: Comparing management options for preserving reproductive function PROJECT 3: Comparing effectiveness in different subpopulations. AIM 3) Evaluate innovative methods for the design, conduct, and analysis of observational comparative effectiveness research in this population. AIM 4) Translate research results into improved patient care, through both traditional peer-reviewed publications and collaborations with stakeholders to integrate the research findings into evidence-based patient decision making tools, clinical practice guidelines, and quality measures.

Principal Investigator: Ganesa Wegienka, Ph.D.
Study of Ovarian Aging and Reserve in Young Women (SOAR) (R01HD088638) Subcontract

The average age for a woman to have her first child has been increasing for the last three decades in the United States, making our understanding of ovarian aging and its negative effect on the ovarian reserve, a measure of the capacity of the ovary to produce eggs capable of fertilization. Yet, we know very little about other factors in reproductive-age women that might affect the ovarian reserve, beyond aging itself. This proposal, titled Study of Ovarian Aging and Reserve in Young Women (SOAR), seeks to address the significant gap in our knowledge of factors, particularly modifiable factors, that affect ovarian reserve and might accelerate its decrease in young women. To achieve this goal, we will leverage the ongoing NIEHS Study of Environment, Lifestyle and Fibroids (SELF), which is following a cohort of 1,696 African-American women between the ages of 23-34 years over a five-year period. In this group of young women, we will assess changes in the ovarian reserve by tracking three different measures of the ovarian reserve: anti- Mullerian hormone (AMH), early follicular phase follicle-stimulating hormone (FSH), and antral follicle count (AFC). In addition to collecting survey data, we will also perform oral glucose tolerance testing (OGTT) and anthropometric and bioelectrical impedance analysis (BIA) measurements to more precisely determine the roles of glucose metabolism and obesity on the ovarian reserve. The results of our study will be clinically significant as we currently have limited longitudinal data for counseling women on risk factors for decreased ovarian reserve. Our study design is innovative in that we will use overlapping measures of the ovarian reserve and group-based trajectory modeling to determine correlates associated with decreased ovarian reserve. Specifically, we will determine the demographic, health-behavior, reproductive, and environmental factors associated with decreased AMH (as a measure of the ovarian reserve) over time (Aim 1), determine the association between various measures of obesity and decreased ovarian reserve (Aim 2), and determine the association between glucose dysregulation and decreased ovarian reserve (Aim 3). The proposed prospective longitudinal cohort study will determine the natural history and factors associated with the change in ovarian reserve over time. Further, it will add to the extremely limited data by generating the largest set of longitudinal data on AMH and ovarian reserve in the United States to date, which will benefit all women.

Principal Investigator: Ganesa Wegienka, Ph.D.
Study of Ovarian Aging and Reserve in Young Women (SOAR) (5R01ES029951) Subcontract

Infertility and spontaneous abortion (SAB) are significant public health problems, affecting up to 25% of reproductive age couples in the United States. Health care costs attributable to infertility and SAB exceed $5 billion per year, and several studies have shown an association between infertility treatments and adverse pregnancy outcomes. Thus, identifying modifiable risk factors for subfertility and SAB is an important public health goal. The potential effects of exposure to endocrine-disrupting chemicals (EDCs) on risk of subfertility and SAB are understudied. The few existing human studies have limitations including small sample size, enrollment after conception, retrospective study design, suboptimal assessment of exposure and outcome, inadequate control for potential confounding variables, and limited generalizability. The proposed study will prospectively assess the relation of exposure to selected EDCs,

including phenols, phthalates, and per- and poly-fluoroalkyl substances (PFAS), to risk of subfertility and SAB in a preconception subcohort of 950 pregnancy planners. We will use data from two NICHD-supported prospective cohorts of pregnancy planners in North America and Denmark. With web-based recruitment and data collection, we have enrolled over 17,000 women attempting pregnancy into these cohorts. In a subset of 200 participants, we have successfully pilot tested in-person collection of urine and blood specimens during the preconception and early pregnancy periods. In this application, we propose to expand in-person biospecimen collection, increasing the number of women with preconception and early pregnancy urine and blood samples from 200 to 950. At each of our three biospecimen collection sites (Boston, Detroit, and Aarhus), we will enroll 250 women and collect three urine samples and one blood sample (in preconception) and three urine samples and one blood sample (in early pregnancy). We will ship the samples to the CDC for the analysis of urinary phthalates, urinary phenols, and serum PFAS. To increase cost efficiency, we will pool three urine samples in each exposure window before assaying for phthalates and phenols; and we will assay one preconception blood specimen for PFAS, a persistent chemical, in analyses of subfertility and SAB. Finally, we will conduct a pilot study among 100 U.S. participants to assess the feasibility of collecting urine by mail, which would allow us to take advantage of our full geographically diverse cohort in the future. Strengths of this application include the prospective design, preconception enrollment of pregnancy planners, repeated measurement of exposure during preconception and early pregnancy, excellent control for confounding via bimonthly prospective data collection on a wide range of covariates and use of the latest analytic methods for mixtures modeling. We have generated compelling preliminary data to support our aims. The present grant is cost-effective in leveraging already-established cohort studies with data collection and follow-up supported by other grants. The results generated will be translatable by directly informing future regulatory decisions about EDC standards in a manner that could reduce rates of infertility and SAB to their functioning, but its role in regulating MDSC is unknown.

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