2019 RESEARCH ANNUAL REPORT

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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.

Cardiology/Cardiovascular Research

Principal Investigator: Steven Keteyian, Ph.D.
The Improving ATTENDance to Cardiac Rehabilitation (iATTEND) Trial (R61HL143099)

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 andits

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.

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 US, 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: 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.

Infectious Disease

Principal Investigator: Marcus Zervos, M.D.
Improving antimicrobial use at hospital discharge through a collaborative pharmacist-led transition-of-care intervention (75D30118C02928) Other Federal Contract

Traditional antimicrobial stewardship interventions in the hospital utilize core strategies such as formulary preauthorization and audit and feedback, while decisions about the choice and duration of discharge antibiotics may be limited to more passive interventions of guidelines and education. Pharmacists practicing in TOC focus specifically on optimizing rnedication management and patient education at discharge to home, long-term care facilities, or other settings. Using the existing infrastructure of the TOC model to extend the scope of ASP interventions, we can address this gap. The goal of this project will be to implement and evaluate a multi-disciplinary ASP bundle for patients discharged from general medical and/or surgical units on antibiotics. At the time of collaborative discharge rounding, the TOC team will identify patients on, or transitioning to, oral antimicrobials to prepare for hospital discharge and applicable follow-up. Optimal antimicrobial selection, dose, duration, and dispensing location will be determined, and the pharmacist will enter the prescription in the discharge queue, which the provider will send for the patient at the time of discharge. This early entry of discharge orders into the queue also provides opportunity to facilitate medication access for those with financial barriers or insurance plans that do not align with medications. The primary outcome will be appropriate duration of therapy prescribed at hospital discharge.
Aim l: Implement a multi-disciplinary antibiotic discharge assessment for adults with respiratory, urinary, intra-abdominal, and skin infections
Aim 2: Evaluate the impact on appropriate durations of therapy, infection-related readmissions, and subsequent antibiotic-associated harms
Aim 3: Describe burden of illness and opportunities for improvement with discharge antibiotic prescribing patient access, resistance, and antibiotic-related adverse effects
The long-term objective of this work is to improve patient outcomes such as clinical resolution and reduced development of resistan[pathogens adverse drug events (ADE). Inappropriate antibiotic prescribing contributes [.0 medica[ion-rclated adverse events, hospital readmissions, and thc development oc drug-resistant pathogens. Novel strategies involving all healthcare providers are sorely needed to address these concerns.

Pulmonary

Principal Investigator: Bruno DiGiovine, 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.

Principal Investigator: Jennifer Swiderek, M.D.
Testing a Nurse-led Algorithmic Approach to Terminal Ventilator Withdrawal (R01NR015768)
Subcontract

Terminal ventilator withdrawal is a process that entails the cessation of mechanical ventilatory support with patients who are unable to sustain spontaneous breathing and is commonly performed in the ICU. Ventilator withdrawal is undertaken to allow a natural death. Opioids and/or benzodiazepines are administered before, during, and after as an integral component of the ventilator withdrawal process to prevent or relieve respiratory distress, but there are few guidelines to determine how much to administer or when. Insufficient opioid and/or benzodiazepine administration places the patient at risk for unrelieved respiratory distress and preventable suffering. Conversely, excessive medication administration may hasten death, an unintended consequence, and one that concerns clinicians. The effective doses of medications given during ventilator withdrawal are unknown. We hypothesize that an algorithmic approach to ventilator withdrawal, relying on a biobehavioral instrument to measure and trend distress, will ensure patient comfort, and guide effective opioid and/or benzodiazepine administration. We plan to use a stepped wedge cluster randomized controlled trial with all clusters providing unstructured usual care until each cluster is randomized to implement the algorithmic approach (intervention). The proposed study is innovative because there is no standardized, evidence-based approach guided by an objective measure of respiratory distress to this common ICU procedure. The study has broad clinical significance to provide knowledge that can potentially reduce patient suffering.

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 in insomnia 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: Michelle Schreiber, M.D.
eASSIST A Post-Visit Patient Portal Tool to Promote Colorectal Cancer Screening (R01CA197205) Subcontract

Colorectal rectal cancer (CRC) is the third most common cancer in the US with over 50,000 individuals dying annually from the disease. Despite multiple effective screening tests, CRC screening remains underutilized relative to other cancer screening. A driving factor behind this underutilization among insured populations is the gap that exists between a physician recommendation for care and the patient's receipt of screening. How best to support patients in CRC screening once they have a physician recommendation for care remains unknown. The proposed project will test the effectiveness and impact of a post-visit, patient portal tool, e-Assist, for engaging and supporting primary care patients in their decision making regarding, and ultimately in their obtaining, CRC screening. The tool purposely leverages the cue to action provided by a physician recommendation for care as well as the secure patient portal platform now commonly found within primary care practices. It seamlessly combines important patient-physician decision making content with assistance in removing personal and structural barriers to screening. Our research will answer four overarching questions: (1) Can a post-visit, patient portal tool, e-Assist, increase adherence to physician-recommended CRC screening? (2) How does e-Assist engage primary care patients in the CRC screening decision making process? (3) Are there subgroups of the primary care population for whom e-Assist is more engaging and effective? and (4) What adaptations are needed to e-Assist to improve its reach, and ensure its adoption, implementation, and ultimately its impact on evidence-based CRC screening use among diverse primary care patients and clinics? These questions will be addressed using a two-arm, practical randomized trial supplemented with findings from focus groups and in-depth interviews with patients, clinicians and other clinic staff to ensure a comprehensive understanding of not only program effectiveness and implementation, but the factors driving overall program impact. Results will illustrate how e-tools can be used following an office visit to support both patient decision making, and the dissemination and implementation of evidence-based cancer screening services in primary care.

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 end-points 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."

Part II – All Other Clinical Departments

- **Dermatology**
- **Neurology**
- **Neurosurgery**
- **Orthopaedics/Bone & Joint**
- **Otolaryngology**
- **Pathology**
- **Pediatrics**
- **Radiation Oncology**

Dermatology

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 DPT-1 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-17-92 cluster, 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.

Neurology

Principal Investigator: Jieli Chen, Ph.D.
Neurorestorative Therapy of Stroke with HUCBC in Type Two Diabetic Mice (R01NS083078)

Diabetes mellitus (DM) leads to a 3-4 fold higher risk of experiencing ischemic stroke. Hyperglycemia and diabetes instigate a cascade of events leading to vascular endothelial cell dysfunction, increased vascular permeability and poor recovery after ischemic stroke. Diabetic animals exhibit more severely injured white matter (WM) than non-DM animals after stroke. There is lso a differential response to treatment of stroke between DM and non- DM subjects. Effective therapy of stroke in the non-DM population may not necessarily transfer to the DM population, prompting the need to develop therapeutic approaches specifically designed to reduce neurological deficits after stroke in the DM population. Our preliminary data show that T2DM significantly decreases microRNA- 126 (miR-126) and Angiopoietin-1 (Ang1) expression in the circulation and in the ischemic brain of mice.Human umbilical cord blood cell (HUCBC) treatment of stroke in T2DM mice starting at 3 days after stroke significantly improves recovery of neurological function as well as increases miR-126 and Ang1 expression in the ischemic brain. Therefore, based on our robust preliminary data, we propose to use HUCBCs for the treatment of stroke in the T2DM mice and to investigate the role of intercellular communication via miR-126 encapsulated within Exosomes/Microvesicles (EMVs) in mediating the therapeutic benefit on HUCBCs for ischemic stroke. This application includes three Aims. Aim 1 will test if miR-126 mediates HUCBC treatment induced neurorestorative effects after stroke in T2DM mice. We hypothesize that miR-126 mediates HUCBC treatment–induced vascular integrity, axonal outgrowth, and WM remodeling, and improves functional outcome after stroke in T2DM mice. Aim 2 will test whether miR-126 generated by HUCBCs is transferred to brain endothelial cells (BECs) and parenchymal cells via EMVs. We hypothesize that HUCBCs secrete EMVs containing miR-126 which are taken up by BECs and parenchymal cells. Aim 3 will investigate whether miR-126 regulation of Ang1 promotes the HUCBC-induced neurorestorative effects after stroke in T2DM mice. We hypothesize that: 1) HUCBC treatment of stroke in T2DM mice increases Ang-1 signaling activity in the ischemic brain; 2) miR-126 regulates Ang1 expression and thereby regulates vascular remodeling, axonal outgrowth and oligodendrocyte survival and differentiation; 3) Restoration of Ang1 with an Ang1 mimetic peptide will rescue the neurorestorative effects of knockdown of miR-126 in HUCBC after stroke in T2DM mice. In this application, we are the first to propose that, generation of miR-126 encapsulated in EMVs by HUCBCs contributes to its robust therapeutic restorative effects and that miR-126 and its regulation of Ang-1 mediate HUCBC- induced neurovascular and WM remodeling, and thereby improve stroke functional recovery in T2DM mice. This proposal is highly clinically relevant and if successful, will significantly impact the treatment of diabetic and possibly all stroke patients.

Principal Investigator: Jieli Chen, Ph.D.
MiR-126/ABCA1 Mediates Exosome Induced Neurorestorative Effects after Stroke in T2DM Mice (1R01NS099030)

Diabetes mellitus (DM) leads to a 3-4 fold higher risk of experiencing ischemic stroke. Stroke in type two DM (**T2DM**) patients and in animal models increases vascular and white matter (**WM**) damage in the ischemic brain, and stroke in T2DM patients has a distinct clinical pattern and a poor prognosis compared to non-DM stroke. Exosomes (Exo), are active nano-size biological lipid containers, which transport regulatory genes and proteins between cells and form a major biological communication conduit, facilitating a plethora of biological responses. The regulatory molecules contained in the exosome include microRNAs (miRs), which regulate gene translation and play primary roles in mediating a vast range of

biological functions. MicroRNA-126 (**miR-126**) is an angiogenic microRNA and primarily expressed in endothelial cells (**EC**). Specific conditional knockout of EC miR-126 (**miR-126EC-/-)** mice have significantly worse functional outcome after stroke as well as decreased brain miR-126 and ATP-binding cassette transporter A1 (**ABCA1**) expression. Exosomes derived from EC (**EC-Exo**) have a high level of miR-126. Based on our robust preliminary data, in this pioneering study, we propose that treatment of stroke with EC-Exo will enhance neurorestorative effects after stroke in T2DM mice, possibly, via the miR-126/ABCA1 signaling pathway. This application includes three Aims. **Aim-1:** To test the therapeutic effects of EC-Exo on cerebral ischemic stroke in adult male and female T2DM mice. **Aim-2:** To evaluate whether miR-126 mediates EC-Exo treatment induced neurorestorative effects, we will evaluate the therapeutic effects of treatment of stroke in specific conditional knockout of EC miR-126 (**MiR-126EC-/-)** and in non-miR-126 knockout control (**miR-126fl/fl**) T2DM mice with EC-Exo derived from miR-126EC-/- brain ECs (**miR-126EC-/- EC-Exo)** or EC-Exo derived from wild type miR-126fl/fl brain ECs **(miR-126fl/fl-EC-Exo)** on vascular and axonal/WM remodeling and neurological and cognitive functional outcome. **Aim-3:** To test whether ABCA1, an indirect target of miR-126, contributes to EC-Exo treatment induced neurorestorative effects after stroke in adult male T2DM mice, mice with specific knockout of brain ABCA1 (***ABCA1*-B/-B**) and WT ABCA1 knockout control (**ABCA1fl/fl**) mice will be employed. In this application, we are the first to propose that, generation of miR-126 encapsulated in EC-Exo contributes to its robust therapeutic restorative effects and that miR- 126/ABCA1 pathway mediates EC-Exo-induced neurovascular and WM remodeling, and thereby improves stroke neurological and cognitive functional recovery in T2DM mice. This proposal is highly clinically relevant and if successful, will significantly impact the treatment of diabetic stroke, and possibly all stroke patients.

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 Investigator: Michael Chopp, Ph.D.
miR-17-92 Exosome Treatment of Stroke (R01NS088656)

Exosomes, small lipid microvesicles (30-150 nm), are active biological containers, which transport regulatory genes and proteins between cells and form a major biological communication conduit, facilitating a plethora of biological responses. The regulatory molecules contained in the exosomes include microRNAs (miRNAs), short (22-25 nt) non-coding RNAs which regulate gene translation and play primary roles in mediating a vast range of biological functions. In this proposal, based on strong preliminary data, we propose to manufacture a distinct exosome population which contains increased levels of the miR- 17-92 cluster as a proof-of-principle and a mechanistic demonstration of a new method of treating stroke and possibly other neurological diseases and injury. We test the premise, that by modulating their miRNA content, exosomes can be designed to enhance plasticity of axons and thereby further promote neurological recovery post stroke. Success of this novel approach may lead to a new designer-based paradigm for the treatment of stroke and neurological disease. The following Specific Aims and associated Hypotheses are proposed: Specific Aim 1: To employ exosomes derived from multipotent mesenchymal stromal cells (MSCs) to treat stroke in order to enhance neurovascular remodeling and thereby, functional recovery post stroke. Hypothesis: Exosomes, derived from MSCs when administered to rats after stroke promote neurovascular remodeling which improves functional outcome. Specific Aim 2: To alter specific miRNAs contained within exosomes generated by MSCs as a means to enhance axonal plasticity and neurological recovery post stroke. Hypothesis: Administration of exosomes withincreased miR-17-92 cluster to rats post stroke promotes axonal remodeling and enhances functional outcome. There are multiple layers of innovation in our application: we generate biological exosome carriers tailored for specific miRNAs; we use these exosomes to treat stroke, without the administration of exogenous cells; we employ electrophysiological methods, lasercapture, fiber track tracing, a battery of neurological tests, and an array of novel approaches, e.g. microfluidic chambers, and ex vivo slice cultures, to mechanistically determine the molecular pathways of the target exosomes which mediate axonal outgrowth. Development of this designer exosome-based therapy, also serves as a prototype for capitalizing on the characteristics of exosomes to transport specific miRNAs and for the manufacture of designer exosomes. Developing a therapy for stroke that is exosome-based opens up a wide variety of means to deliver targeted regulatory genes to enhance multifaceted aspects of central nervous system (CNS) plasticity and to amplify neurological recovery for neural injury and neurodegenerative diseases.

Principal Investigator: Xu Cui, Ph.D.
ABCA1 Regulates White Matter Remodeling and Oligodendrogenesis after Stroke (R01NS092917)

Stroke is a major cause of white matter (WM) damage which induces long-term disability. There is limited WM remodeling in the adult brain. Many neuroprotective treatments of stroke have failed in clinical trials because they cannot protect WM. Therefore, there is a compelling need to investigate the mechanism underlying WM remodeling and oligodendrogenesis of the adult brain and to develop effective long-term stroke therapy. Cellular cholesterol modulates axonal and dendritic outgrowth and is required for myelination. The level of HDL-cholesterol is related to the progression and recovery of stroke patients. ATP-binding cassette transporter A 1 (ABCA1) is a major cholesterol transporter and plays critical roles in regulation of HDL- cholesterol and ApoE synthesis and metabolism in the central nervous system. Brain specific- ABCA1 deficient (*ABCA1-B/-*mice have very low brain HDL-cholesterol/ApoE level, and exhibit neuronal ultrastructure changes and functional deficits. Both HDL-cholesterol and ApoE increase neurite outgrowth in culture conditions. Our preliminary study shows that *ABCA1-B/-B* mice exhibited increased WM damage and reduced oligodendrogenesis and exacerbated neurological functional deficits after stroke. Primary cultured neurons derived from *ABCA1- B/-B* mice show decreased neurite outgrowth, which can be attenuated by HDL treatment. *ABCA1-B/-B* astrocyte-conditioned media also decreased wild type neurite outgrowth after hypoxic ischemia. Therefore, we propose the following three specific aims: **Aim1** To investigate whether brain-deficient in ABCA1 exhibits decreases in WM-

remodeling and axonal growth after stroke. *ABCA1-B/-B* and floxed-control mice will be subjected to stroke, WM-changes and oligodendrogenesis will be measured. **Aim2** To investigate molecular mechanism underlying ABCA1 in regulation of WM-remodeling and oligodendrogenesis after stroke, we will examine whether ABCA1 regulates brain HDL and ApoE level, and whether brain HDL and ApoE levels mediate ABCA1-induced WM-remodeling and oligodendrogenesis after stroke. **Aim3** To investigate cellular mechanisms of ABCA1 in regulation of WM- remodeling and oligodendrogenesis, we will examine neurons and oligodendrocytes and the cross talk of astrocytes with neurons and oligodendrocytes on ABCA1-induced WMremodeling and oligodendrogenesis in vitro and in vivo. We expect that ABCA1 deficient brain will exhibit significant decreases in HDL and ApoE level,and decreases WM-remodeling and oligodendrogenesis as well as reduced functional outcome after stroke. The level of HDL/ApoE in brain or cerebrospinal fluid will, at least partially, mediate ABCA1-induced WM- remodeling and oligodendrogenesis in the ischemic brain after stroke. To our knowledge, no one has investigated the functional effect of ABCA1 on oligodendrogenesis and WMremodeling post- stroke recovery, especially by using *ABCA1- B/-B* mice. The new insights gleaned from this study will contribute to our understanding of the beneficial role of ABCA1/HDL-C/ApoE in brain plasticity which will impact development of rational restorative approaches to improve neurological outcome for stroke patients.

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.
Role of AMP-Activated Protein Kinase in Bacterial Endophthalmitis (R01EY026964) Subcontract

The role of myeloid cells such as neutrophils in providing host defense to microbial infections is well-established; however, the contribution of monocytes/macrophages (M¿) to the pathophysiology of bacterial endophthalmitis is less clear. Our preliminary studies revealed that M¿ depletion results in increased inflammatory mediators at the resolution phase, suggesting their involvement in the resolution of endophthalmitis. The M¿ perform multiple tasks, including sensing pathogens, tissue repair, and, in response to host-derived mediators, they differentiate into distinct functional phenotypes; a feature termed "plasticity". The "classically activated" M¿ (M1) produce inflammatory cytokines and nitric oxide, contributing to host tissue damage. Conversely, the "alternatively activated" M¿ (M2) mediate tissue repair through the elimination of damaged cells/tissue and the production of anti-inflammatory molecules to resolve inflammation. Therefore, understanding the mechanisms governing the phenotypic switch of M¿

can be utilized to develop novel therapeutic strategies. Our transcriptome and metabolomics analyses of the bacteria-infected retina directed us to the identification of adenosine monophosphate-activated protein kinase (AMPK), a metabolic gene, which modulates the infiltrating myeloid cell phenotype in endophthalmitis. We discovered that mice with global deletion (knockout) of AMPKa1 (KO) developed severe endophthalmitis and pathology compared to wild type (WT) mice. M¿ lacking AMPKa1 maintained a low metabolic state, even in the hyper-inflammatory state. To precisely examine the role of AMPK in myeloid cells, we induced endophthalmitis in myeloid cell specific KO of AMPKa1 (LysM-KO) and observed that LysM-KO displayed exacerbated inflammation and reduced retinal function compared to WT mice, suggesting an essential role of AMPK in myeloid cells in the pathogenesis of bacterial endophthalmitis. Building on these findings, we propose to test our central hypothesis that AMPK exerts protective effects in bacterial endophthalmitis by modulating the polarization of infiltrating monocytes/M¿ to promote inflammation resolution and that metabolic reprograming is an underlying mechanism of the monocytes/M¿ phenotype switch. To test our hypothesis, in Aim 1, we will investigate the mechanisms underlying reduced AMPK activity in bacterial endophthalmitis by examining the modification of LKB1 via nitrosylation or chemical adduct formation. Aim 2 tests the hypothesis that AMPKa1 ablation enhances the activation state of myeloid cells and maintains their proinflammatory (M1) state during the resolution phase of the disease. In Aim 3, we will decipher the bioenergetic events, regulated by AMPK in M¿, that polarize and maintain their pro-inflammatory nature. The anticipated results of this study will demonstrate that defective AMPK activity in myeloid cells, mainly in monocytes/M¿¿ impacts the resolution of endophthalmitis via regulation of cellular metabolism. Also, it may provide novel therapeutic targets for the development of anti-inflammatory therapies for endophthalmitis and other microbial infections.

Principal Investigator: Quan Jiang, Ph.D., and Jieli Chen, 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 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: Xianshuang Liu, M.D.
Translational Study of miR-146a Gene Therapy for Diabetic Peripheral Neuropathy (R01DK102861)

Peripheral neuropathy is the major complications of diabetes. There is a compelling need to develop effective therapeutic approaches specifically designed to improve neurological function in the damaged peripheral nervous system after diabetes. MicroRNA-146a (miR- 146a) has been implicated in the regulation of multiple immune diseases. However, the role of miR-146a in diabetic peripheral neuropathy (DPN) has not been investigated. In a novel set of experiments, our preliminary data show that intravenous administration of miR-146a remarkably improved sciatic nerve vascular function, axonal myelination and peripheral nerve function in diabetic mice, indicating that miR-146a may have a beneficial effect on the clinical treatment of DPN. In this application, we therefore seek to investigate the mechanisms underlying the therapeutic effects of miR-146a on DPN. We propose that miR- 146a by improving vascular function and suppressing pro-inflammation factors ameliorates DPN. The associated hypotheses are: 1. Treatment with chemically engineered miR-146a improves neurological outcomes in DPN in dose and therapeutic window dependent manners. 2. Elevation of miR-146a levels suppresses its target genes, IRAK1/TRAF6 and their down-stream pro-inflammatory factors in vascular endothelial cells and monocytes of type II diabetic mice, thereby, leading to the improvement of neurovascular function and consequently ameliorating peripheral neuropathy. To investigate the effect of miR-146a on neurological outcomes, type II diabetic mice which develop severe peripheral neuropathy will be treated with miR-146a at various time points and doses after onset of DPN. To investigate the underlying molecular mechanisms, the effects of miR-146a overexpression and knockdown on target genes and inflammatory genes that mediate miR- 146a-enhanced neurovascular function will be determined. These studies are innovative and will provide novel insights into mechanisms underlying the neurological dysfunction of DPN and likely lead to the development of a new miRNA-based genetherapy.

Principal Investigator: Jaspreet Singh, Ph.D.
Plasma Biomarkers of Cerebral Disease in X-Linked Adrenoleukodystrophy (R21NS104560)

There are no biomarkers to predict the onset of fatal demyelinating phenotypes in males with inherited X-linked adrenoleukodystrophy (X-ALD) disease. 60% of male X-ALD patients develop demyelination in childhood (5-12 years; ALD) or in adulthood (25-35 years; cerebral adrenomyeloneuropathy, cAMN). On average, cAMN and ALD are fatal in 2 to 5 years of onset. 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 ALD or cAMN. Our long-term goal is to dissect microRNA (miRNA) and metabolic pathways underlying neurodegeneration in X-ALD. The objective of this application is to identify plasma miRNA and metabolite biomarkers predictive of progression to fatal cAMN and ALD in X-ALD males. Plasma exosome miRNAs and circulating metabolites have been used as diagnostic and prognostic biomarkers for neurodegenerative diseases.

No plasma (or other biofluid) miRNA and metabolite biomarkers have been explored for the fatal X-ALD phenotypes. Our preliminary proof-of-concept data, with next generation sequencing and untargeted metabolomics, identified differential miRNA and metabolites between healthy-control and ALD-phenotype postmortem brain. Within the ALD brain white matter, unique miRNA and metabolite changes were recorded between distant normal looking areas and areas adjacent to the plaque suggesting an association with disease progression. Our central hypothesis is that metabolomic and miRNA analysis in retrospective plasma samples, collected both before and at the time of detection of demyelination in the patients, will provide biomarker(s) predictive of fatal cAMN and ALD progression in X-ALD males. To test our hypothesis we propose two specific aims: 1) Identify a plasma metabolome and miRNA signature for the cAMN phenotype. 2) Define a plasma metabolome and miRNA signature for the ALD phenotype. We will take advantage of a large cohort of control, non-converting AMN, cAMN (pre and post) and ALD (pre and post) plasma samples already available in the biorepository of the Moser Center for Leukodystrophies, Kennedy Krieger Institute, Baltimore, for discovery and validation. This proposal is innovative, because it departs from the status quo by identifying novel plasma regulatory (miRNA) and active (metabolite) biomarkers predictive of cAMN and ALD. The proposed research is significant because our pre-post design will identify plasma biomarkers able to predict disease course before the onset of fatal cAMN or ALD. X-ALD was added to the Recommended Uniform Screening Panel in February 2016, a federal list of all genetic diseases recommended for state newborn screening programs. This study will nominate and validate plasma biomarkers that have the potential to provide an effective monitoring tool for identified X-ALD infants. This study will lay the foundation for our future, large-scale, prospective clinical trial studies using novel plasma miRNA and metabolite biomarkers to predict fatal cAMN and ALD phenotypes. In collaboration with Kennedy Krieger Institute we will apply for funding for these future clinical trials from NINDS.

Principal Investigator: Lei Wang, M.D.
Schwann Cell Derived Exosomes Improve Diabetic Peripheral Neuropathy in Type II Diabetic Mice (R56DK115601)

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. Exosomes, endosome-derived nano vesicles carry proteins and RNAs as their molecular cargo. Exosomes mediate intercellular communication by transferring their cargo between source and target cells. Exosomes are presently in clinical trials for treatment of type 1 diabetes, cutaneous wound healing and several cancers. However, the effects of Schwann cell-derived exosomes as a treatment of diabetic peripheral neuropathy have not been investigated. In a novel set of experiments, our preliminary data show that intravenous administration of Schwann cell-exosomes remarkably improves sciatic nerve neurovascular function and peripheral nerve function in diabetic mice, indicating that exosomes have a beneficial effect on the treatment of diabetic peripheral neuropathy. In this application, we therefore propose to investigate the mechanisms underlying the therapeutic effects of Schwann cell-exosomes on diabetic peripheral neuropathy, with a focus on neurovascular remodeling. Our hypotheses are: 1) Treatment of diabetic peripheral neuropathy with Schwann cell-exosomes improves neurological outcome in mice with diabetic peripheral neuropathy by transferring exosomal microRNA (miR)-9, -21, and -27a into the sciatic nerves of dorsal root ganglion (DRG) neurons, Schwann cells, and endothelial cells, leading to suppression of their target proteins, SEMA6A, RhoA, PTEN and pNF-?B. And 2) Treatment of diabetic peripheral neuropathy with engineered exosomes carrying elevated miR-9, -21, and -27a enhances neurovascular function and improves neurological outcome compared to nave-Schwann cell-exosome treatment. The effect of the exosomes on expression of miR-9, -21, and -27a and their target proteins SEMA6A, RhoA, PTEN and pNF-?B in the sciatic nerve of the DRG neurons, Schwann cells, and endothelial cells will be examined. The neurovascular function and neurological outcomes will be measured. These studies are innovative and will provide new insight into mechanisms underlying the neurological dysfunction of diabetic peripheral neuropathy, and may lead to the development of a new treatment using Schwanncell-exosomes. Relevance Statement: Diabetic peripheral neuropathy is a major disability affecting millions of Americans. In this proposal, employing preclinical studies in the diabetic animal, I seek to develop a novel treatment for diabetic peripheral neuropathy using exosomes derived from Schwann cells. In this

proposal, I will also elucidate the molecular mechanism by which exosomes are therapeutically effective. This research will provide the essential pre-clinical data for translation to a phase 1 clinical trial.

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.
Exosomes and Platinum-Induced Peripheral Neuropathy (R01CA219829)

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.

Principal Investigator: Zhenggang Zhang, M.D., Ph.D.
Novel Highly Regenerative and Scalable Progenitor Cell Exosomes for Treating Stroke (R41NS105263) Subcontract

Stroke is a leading cause of disability worldwide. Globally, there are 15 million stroke survivors each year who have significant neurological deficits including sensory and motor disability, leading to excessive socioeconomic burden. Cell therapy using primary MSCs has been actively explored by us and others as a therapeutic solution to this unmet medical need. However, since the therapeutic efficacy of MSCs has been shown to be dependent on intercellular communication between administered cells and brain parenchyma, we reasoned that the exosomes (nanoscale extracellular vesicles) that cells secrete to transmit this information could be used instead of and perhaps more efficiently than the cells themselves. Indeed, we were the first to report that systemic delivery of exosomes released by mesenchymal stromal cells (MSCs) to rats subjected to stroke or traumatic brain injury (TBI) substantially improves recovery of neurological function. A key rationale for using exosomes is that they obviate many of the risks associated with cell therapy because their use concentrates the active component of stem cells in a simpler compact non-replicative form. Importantly, they can cross the BBB allowing systemic delivery for treating the injured brain. Moreover, exosomes have low risk of immune rejection and are likely to be more cost-effective to produce and convenient to use and store and thus have potential for an ?off- the-shelf? treatment. However, there are significant roadblocks to translation of our preclinical results using MSC exosomes because of inherent limitations of MSCs for industrial scale production. MSCs are a poorly defined heterogeneous cell population with low proliferative capacity which can hinder batch consistency and limit scale- up for exosome production. Thus to overcome these roadblocks, we propose here to use exosomes derived from BioTime?s clonally pure PureStem progenitor cell lines, which have a high degree of homogeneity and are highly proliferative and regenerative because of their early embryonic origin. The goal of this phase I proposal is to conduct a feasibility study that would demonstrate neurological recovery in our rat stroke model using PureStem exosomes. We propose to identify 2 PureStem derived candidate exosomes based on angiogenic activity and micro RNA and protein content because increasing angiogenesis in the ischemic brain facilitates improvement of neurological function after stroke. The 2 lead candidates will be tested for efficacy measured by neurological function in our rat middle cerebral artery occlusion (MCAO) model. Evidence of feasibility using our well-established model to mimic human stroke and using exosomes from a scalable PureStem cell line will pave the way for further preclinical development and IND enabling studies in phase II.

Neurosurgery

Principal Investigator: Meser Ali, Ph.D.
Treatment of Glioma with Nanocombretastatin with MRI Monitoring (R01CA206190)

Glioblastoma (GBM) is a highly aggressive hypervascularized brain tumor characterized by high recurrence rates and poor prognosis despite advanced treatment. The vasculature of GBM is fundamentally different from that of normal vasculature and offers a unique target for anti-cancer therapy. Therefore, direct targeting of tumor vasculature with vascular disrupting agents (VDAs) is distinctly different from anti-angiogenic strategies, and offers a complementary approach to standard therapies. Combretastatin A4 (CA4) is a potent vascular disrupting drug. CA4 induces rapid shutdown of tumor blood supply, typically promoting a necrosis at the core of the tumor, but leaves a rim of viable tumor cells at the periphery which can then rapidly re-grow. However, CA4 is not effective in inducing necrosis at the core of GBM tumor. The ineffectiveness of small molecule chemotherapy drugs in treating malignant brain tumors has been attributed to the blood-brain barrier (BBB) being a significant impediment to the transvascular extravasation of drug fraction across the barrier into the extravascular compartment of tumor tissue and the high tumor interstitial fluid pressure also presents an additional delivery barrier. Nanotechnology is already benefiting to deliver drugs across the BBB and into brain tumors. We have engineered a nano-sized polymeric CA4 conjugate which demonstrates high water solubility. Preliminary intravenous (i.v.) delivery of G3-CA4 in an orthotopic glioma model demonstrated necrosis at the core of the tumor leaving a rim of viable tissue. By applying the designed nano-prodrug

strategy and tumor-specific prodrug activation mechanism, we observed the true success of inducing necrosis at the core of the tumor in an orthotopic U-251 glioma animal model first time. Tumor-VDAs have significant potential when combined with cytotoxic chemotherapy and radiotherapy in treating other tumor models. Combined treatment with radiation is attractive, as radiation therapy (RT) represents a standard of care and RT should effectively kill the well-oxygenated cancer cells in the well-perfused tumor rim. We have shown that GBM cancer stem cells are sensitive to radiation exposure in culture and a single dose of 50Gy irradiation yielded necrosis in primary GBM rat model. Therefore, this study is extended to include SRS and standard cytotoxic temozolomide (TMZ) therapies with G3-CA4. We hypothesize that the combination of G3-CA4 with SRS and TMZ will show synergistic cytotoxic effect in clinical relevant primary GBM model. Our objectives of the proposed research are A) To incorporate CA4 molecules with dendrimer-based nanoparticles (G3-CA4) that demonstrates full solubility in aqueous media, B) To determine the efficacy and safety of small molecule CA4, CA4-P and G3-CA4 nano-prodrug in U251 glioma tumor model, C) To determine the efficacy and safety of G3-CA4 alone or in combination with SRS in primary GBM, D) To determine the efficacy and safety of a combined G3-CA4 and standard TMZ therapy in primary GBM model. The overall therapeutic effect from G3-CA4 alone or in combination with SRS/TMZ will be evaluated by image-guided MRI monitoring of long-term survival rats.

Principal Investigator: Meser Ali, Ph.D.
Extracellular pH Mapping as Therapeutic Readout of Nanoparticle-based Drug Delivery in Glioblastoma (R01EB023366) Subcontract

Extracellular acidosis (i.e., low pHe) is a tumor microenvironment hallmark, caused by atypical metabolism and perfusion. Acidic pHe enhances cancer growth, proliferation, and builds therapy resistance. The prognosis remains dismal for most brain tumor patients. Malignant gliomas, including glioblastoma multiforme (GBM), fail treatments because gliomas invade outside tumor boundaries conventionally demarked by MRI contrast and the blood-brain barrier (BBB) blocks most drugs. Furthermore conventional MRI methods are insensitive to physicochemical parameters like pHe and mainly track intratumoral volume. Among the primary MRI methods are paramagnetic agents for longitudinal (T1) contrast, where assessment of treatment response involves 2D or 3D measurement with Gd3+ enhanced MRI contrast. However, such methods are not reliable in distinguishing pseudoprogression and pseudoresponse from actual changes in tumor status. Thus there is an urgent need for alternative MR techniques sensitive to metabolic changes, which can aid in effective monitoring of therapeutic response in addition to measuring the tumor size. Because acidic pHe milieu is conducive to tumor growth and builds resistance to therapies, simultaneous mapping of pHe inside and outside the tumor (i.e., intratumoral-peritumoral pHe gradient) is an important cancer imaging need. A novel way to map intratumoral-peritumoral pHe gradient is using lanthanide III (Ln3+) agents with BIRDS methods, where physicochemical factors like pHe contribute to shifts of non-exchangeable protons. To meet the need for MR readouts of the tumor physicochemical state, we developed BIRDS to map the intratumoral-peritumoral pHe gradient, and found that it is a sensitive readout of cancer growth and treatment. Based on preliminary data obtained from GBM models (e.g., U251), including patient-derived xenograft (PDX) models, we will validate high-resolution pHe mapping with BIRDS as therapeutic readout of chemotherapy drugs delivered into human GBM models. Although we detected 1-2 mm diameter tumors with BIRDS using non-methylated agents, higher resolution mapping of intratumoral-peritumoral pHe gradients will be reached with a novel methylated multiplexed agent in Aim 1.
In **Aim 2** we will validate intratumoral-peritumoral pHe gradient mapping by BIRDS with fluorescent pHe probes. We will use BIRDS to examine how intratumoral-peritumoral pHe gradients change with tumor aggression (Aim 3). We will also test compatibility of pHe mapping with BIRDS for tracking response to chemotherapy drugs (e.g., Temozolomide and Sorafenib) being used to treat GBMs (Aim 4). Both of these drugs are known to cross the BBB and are used in GMB therapy. Temozolomide activates apoptosis by alkylating DNA to stall cell replication and Sorafenib is a multiple kinase inhibitor targeting several oncogenic pathways and enhances glycolysis. If successful, pHe mapping by BIRDS will enable monitoring of therapeutic response of various chemotherapy drugs for preclincal PDX models to potentially be translated clinically.

Principal Investigator: Houtan Noushmehr, Ph.D.
Epigenomic Master Regulators that Define IDH1/2 Mutant Glioma Tumor Progression (W81XWH1810540) Department of Defense

Diffuse gliomas are a heterogeneous group of primary brain tumors that develop from glial cells, such as glioblastoma, astrocytoma, and oligodendroglioma. Although environmental risk factors for glioma and glioblastoma remain poorly defined, with the exception of exposure to ionizing radiation, evidence has shown that traumatic brain injury may predispose service members to gliomagenesis via inflammation and stem cell transformation. Despite advances in surgical techniques and clinical regimens, treatment of gliomas remains challenging and the tumors usually progress or recur. Therefore, understanding the mechanisms involved in glioma progression and recurrence is essential and will have clinical implications. The hypothesis tested here is based on our recent reports that defined and characterized glioma subgroups as related to specific genetic alterations associated with *IDH1/2* (*IDH*) mutations and chromosome 1p and 19q deletions (codel) and distinct epigenetic alterations. Within the *IDH* -mutation subgroup, two novel subtypes were defined by intact codel status and DNA methylation levels, namely Glioma-CpG Island Methylator Phenotype (G-CIMP)-low and -high. G-CIMP-low patients were younger at diagnosis (<39 yo) and exhibited a shorter overall survival compared to G-CIMP-high. Longitudinal analyses of matched glioma samples revealed that 12% of G-CIMP-high cases progressed to G-CIMP-low and the molecular changes occur at candidate non-coding functional elements, suggesting the existence of a potential master regulator affecting brain tumor progression in young adults.

Objective/Hypothesis: The hypothesis that G-CIMP-high tumors may relapse as G-CIMP-low gliomas with no NA methylation and other epigenomic events could be a key determinant of the mechanisms that drive glioma progression. Using advances in next generation sequencing and insights of the relationship between transcription factor (TF), histone modifications and DNA methylation, the goal of this project is to investigate the functional genomic elements (e.g. enhancers or silencers) that define brain cancer progression between G-CIMP-high and G-CIMP-low.

Specific Aims: In aim 1, we will screen our nationally recognized brain tumor bank for G-CIMP-high to G-CIMP-low transformation using our published signatures. We will obtain matched recurrent pairs, isolate the molecular DNA/RNA and chromatin and generate high-throughput next generation sequencing of distinct epigenomic profiles such as whole-genome bisulfite sequencing, chromatin immunoprecipitation of H3K27ac, CTCF followed by sequencing and RNA-sequencing. In aim 2, we will define candidate master regulators in non-coding functional genomic elements by integrative bioinformatics approach and integrate the most significant findings with publicly available data from ENCODE/Roadmap to define candidate functional genomic elements that will discriminate subgroups during tumor progression. In aim 3, we will validate the identified genomic functional elements in appropriate *IDH* -mutant cell culture using targeted CRISPR/Cas-9 to delete the genomic elements, and shRNA to do s of the targeted alterations on gene expression will be assessed by RNA-seq.

Principal Investigator: Ye Xiong, M.D., 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.

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 use 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: 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**.

SPECIFIC AIM 1: To investigate the regulation of cell surface phosphoinositides (PIs) by SHIP2 in mineralizing cells. Hypothesis 1 states that SHIP2 controls MV formation by regulating PI cell surface levels. We have generated new tools to address this hypothesis including ATDC5 chondrocyte and SaOs-2 osteoblast cell lines engineered using CRISPR to eliminate SHIP2 protein, and several highly specific, fluorescently-labeled PI-binding proteins. These reagents will be used to track the sub-cellular and cell surface PIP3, and metabolites of PIP3 in the presence or absence of SHIP2.
SPECIFIC AIM 2: Investigate the control of MV composition by SHIP2. Hypothesis 2 states that SHIP2 regulates mineralization by controlling MV composition. This hypothesis will be addressed in two Sub-Aims:

Sub-Aim 2.1: Define the proteins recruited to the cell surface by PIs: PI pull-down experiments will be conducted separately on cell lysate, MV, and membrane preparations using reagents against biosensor proteins. We will focus on PIP3 initially and then other PIPs that have disrupted distribution in the absence of SHIP2. Immunoprecipitated proteins will be identified by mass spectroscopy and confirmed by immunoblotting.

Sub-Aim 2.2: Define how SHIP2 controls MV composition: A comprehensive proteomic analyses will be conducted on MV fractions from both skeletal cell types. Mass spectroscopic findings will be confirmed in immunohistochemical and immunoblot experiments.

The proposed studies have the potential for new insight into the mechanisms of the fundamental, but poorly understood, process of matrix mineralization. Significantly, these studies may identify new, rational targets for the clinical treatment of bone mineralization defects and other mineralization-associated disorders.

Principal Investigator: Yener Yeni, Ph.D.
A Clinically Viable Noninvasive Method for Direct Measurement of Mechanical Strains in Vertebral Bone (R21AR070363)

Mechanical strains experienced at the tissue level are intimately related to the mechanical integrity of whole bones and their response to environmental and interventional stimulus. Techniques for measurement of trabecular strains in an entire cancellous bone volume have been developed for laboratory studies of excised bones that can be performed using high resolution imaging systems such as microcomputed tomography (μCT). These methods involve comparing high-resolution images of bones taken under loaded and no-load conditions and, through advanced mathematical computations, calculation of tissue strains (digital volume correlation, DVC). Thus far, it has not been possible to apply DVC methodologies to human spines in vivo, due to issues with resolution, radiation exposure and the need for a safe, yet effective mechanical loading protocol within the clinical imaging modality. Modern digital tomosynthesis (DTS) systems have the characteristics needed to be able to perform strain mapping in vivo but this has never been tried. With the overall hypothesis that DTS-based strain mapping is feasible and informative, the following aims are proposed to rigorously optimize, validate and demonstrate utility in human spine through a set of in vitro and in vivo experiments: **Aim 1:** Identify the strain levels that can be measured with DTS-DVC under physiologically relevant load magnitudes by using human cadaveric vertebral bodies and comparison to μCT based DVC μCT will serve as the gold standard for optimizing values of DVC analysis parameters for a DTS application, determining thresholds for admissible strain values to maximize measurement accuracy and precision and identifying strain components to be further validated in the later stages of the research. **Aim 2:** Determine the extent to which DTS-DVC performed under a clinically applicable loading protocol predict vertebral strength and energy to failure independent from bone density. Using in vitro destructive mechanical test results as gold standard outcome, this aim will determine the relative efficacy of strain components for improving prediction of mechanical failure in cadaveric vertebrae and define margins of error for these predictions. **Aim 3:** Determine the range of in vivo vertebral strains measured with DTS-DVC in a sample of human subjects with a vertebral deformity and those without. Testing the ability of DTS-DVC to discriminate between cases with a predictable strain outcome from those that are normal will provide in vivo proof of concept. Through comparison of in vivo and in vitro strains, it will be possible to estimate the error in predictive models to be tested in future clinical studies. Development of the DTS-DVC methodology through the proposed aims is expected to substantially improve understanding of etiologies of age- and disease-related bone and joint degeneration,

assessment of fracture risk and assessment of efficacy of therapeutic and surgical interventions aiming to restore bone function.

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.
Our group identified RAB27, important for exosome secretion, as being differentially hypomethylated in keloid compared to normal skin. Our group has isolated keloid-specific exosomes. To date, there are no published studies on keloid exosomes or the contribution of RAB27 methylation on exosome function. We propose to test the **central hypothesis** that exosomes communicate critical signaling events in the keloid microenvironment mediated by RAB27 gene methylation. **Aim 1: To determine the effect of RAB27 methylation on keloid exosome production and miRNA cargo profiles.** Hypotheses: (1) Keloid exosomal production correlates with RAB27 gene methylation. (2) Keloid exosomal cargo miRNA expression profiles correlates with RAB27 gene methylation. (3) Keloid exosome miRNA's putative target genes lie within pathways essential for wound healing and/or fibrosis. **Aim 2: To determine the effects of keloid exosomes on the keloid microenvironment.** Hypothesis: Keloid fibroblast exosomes compared to normal fibroblast exosomes will cause pro-fibrotic phenotype changes in normal fibroblasts. **Aim 3: To determine the effect of keloid exosomes on scar formation *in vivo***. Hypothesis: Exosomes generated in aim 1 and tested in aim 2 will increase scar formation in a rabbit ear scar model.
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

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

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: 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.

Radiation Oncology

Principal Investigator: Carri Glide-Hurst, Ph.D.
Development of Anatomical Patient Models to Facilitate MR-only Treatment Planning (1R01CA204189) Subcontract

Accurate delineation of targets and organs at risk for radiation therapy planning (RTP) remains a challenge due to the lack of soft tissue contrast in computed tomography (CT), the standard of care imaging for RTP. Radiation Oncology has addressed this limitation by registering magnetic resonance images (MRI) to CT datasets to take advantage of the superior soft tissue contrast afforded by MRI. MRI brings considerable value to RTP by improving delineation accuracy which, in turn, has enabled dose escalation to improve local control while maintaining or reducing normal tissue toxicities. However, the current integration of MRI as an adjunct to CT has significant drawbacks as it requires image registration and contour transfer between datasets. This process introduces systematic geometric uncertainties that persist throughout treatment and may compromise tumor control. Thus, we propose to translate MR-only RTP into clinical use, with the ultimate goal of improving patient outcomes accomplished via improved treatment plan design. MR-only RTP will eliminate redundant CT scans (reducing dose, patient time, and costs), streamline clinical efficiency, entirely circumvent registration uncertainties, and fully exploit the benefits of MRI for high-precision RTP. Yet, MRI is not routinely used alone for RTP, largely due to its known spatial distortions, lack of electron density, and inability to segment the bone needed for online image guidance and electron density mapping for dose calculation. The **central hypothesis** is that the innovative technologies that our multi- disciplinary academic/industrial (Henry Ford Health System/Philips Healthcare) collaboration develop will yield geometrically accurate patient models built from MRI data across several platforms/field strengths with CT-equivalent densities that can be used in confidence throughout the entire RTP workflow. In Aim 1, we will perform geometric distortion corrections, determine distortion variability with changing anatomy, benchmark the results in a novel modular phantom, and develop an image processing toolkit. In Aim 2, we will fully automate MR image segmentation in the brain and male/female pelvis to yield accurate synthetic CT patient models derived from novel MRI sequences, including provisions for metal implants, and benchmark the results in phantom. In Aim 3, we will conduct end-to-end testing to characterize the uncertainties in the MR-only RTP workflow. We will perform a virtual clinical trial of MR-only RTP for brain and male/female pelvis and compare to the standard of care. Final translation will include developing physician-physicist practice guidelines, end-user validation of all translational steps, and dissemination of image processing tools into the Radiation Oncology community. This research will systematically address the major challenges limiting MR-only RTP and lay the groundwork for multi-institutional clinical trials across MRI platforms. It will support future work related to MR-guided RT, functional MRI for biologically adaptive RT, and focal RT to areas of high tumor burden.

Principal Investigator: Brent Griffith, M.D.
A Pragmatic Trial of Lumbar Image Reporting with Epidemiology (LIRE) (UH3AR066795) Subcontract

Low back pain, an Institute of Medicine priority condition for comparative effectiveness research, is of major public health importance. It is one of the most common reasons for physician visits and an important cause of functional limitation and disability. Imaging is frequently performed as part of the diagnostic evaluation and is an important contributor to the cost of back pain care, which totaled more than $86 billion in 2005. It is well known that, even without back pain, magnetic resonance (MR) imaging of the lumbar spine frequently reveals findings such as disc desiccation or bulging. Patients and their providers may attribute greater importance to these findings, which are often age-related, than they should, because they do not have an appropriate frame of reference in which to interpret the findings. These incidental findings may initiate a cascade of events leading possibly even to surgery, without improving patient outcomes. We propose a pragmatic, randomized controlled trial (RCT) to determine the effectiveness of inserting epidemiological benchmarks into imaging reports at reducing subsequent tests and treatments. Our rationale is that providing a context for both physicians and patients to better interpret imaging findings may reduce concern about incidental findings and reduce unnecessary further diagnostic tests and treatments. Our intervention is simple, inexpensive and easy to deploy. We propose

an efficient, novel, cluster randomized design referred to as a stepped wedgedesign, permitting longitudinal comparisons while controlling for temporal trends. As called for in the Request for Applications, we plan to passively collect primary outcome measures of healthcare utilization both pre- and post-intervention, using robust electronic medical records at our participating sites. We hypothesize that for patients of primary care providers, inserting epidemiological benchmarks in lumbar spine imaging reports will reduce subsequent diagnostic and therapeutic interventions, including MR and CT, opioid prescriptions, spinal injections and surgery. The rationale is that the epidemiologic data may provide a context for both physicians and patients to better interpret imaging findings. The long-term public health significance is high. Not only may this simple, inexpensive intervention substantially reduce unnecessary and expensive care for back pain; thee intervention can easily be generalized to all diagnostic tests, and could become the dominant paradigm for communicating diagnostic information.

Principal Investigator: Jae Ho Kim, M.D.
Improving the Radiation Therapeutic Ratio by Inhibiting Proinflammatory Cytokines (R21CA205660)

The goal of the proposal is to determine the potential of a new class of compounds, selective inhibitors of dysregulated proinflammatory cytokines/chemokines, to increase the therapeutic gain for cancer patients receiving radiotherapy for tumors that are known to be radiation resistant (e.g. malignant brain tumors, pancreatic cancer, and lung cancer - stage III/IV). The exploratory proposal builds on our previous discoveries that the lead cytokine/chemokine inhibitor mitigates radiation injury in multiple tissues and the same compound confers an enhancement in radiation tumor growth delay. Two specific aims are planned to confirm the studies in orthotopic tumor models and to elucidate the mechanism of action. In Aim 1, we test the hypothesis that the inhibitor when administered to rats with brain tumors enhances the radiotherapy efficacy and reduces the radiation injury to normal brain. We aim to determine if the therapeutic ratio of a cytokine inhibitor combined with radiation is superior to radiation alone. In Aim 2, we test the hypothesis that the inhibitor suppresses acute proinflammatory cytokines including those produced by activated macrophage in the tumor tissue and reduces neuroinflammation caused by activated microglia in the normal brain following radiation. We aim to elucidate the mechanism of action of a cytokine inhibitor's effects on the radiation response of tumor and normal brain following single and fractionated radiation. At the completion of these studies we expect that a new paradigm for improving radiation therapy will have been initiated.

Part III – Population and Health Sciences

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

Center for Health Policy and Health Services Research

Principal Investigator: Brian Ahmedani, Ph.D.
Treatment Utilization Before Suicide (TUBS) (R01MH103539)

Adult suicide rates in the United States rose by almost 30 percent between 1999 and 2010. These rates have not markedly improved in decades. To date, previous suicide attempts and psychiatric diagnoses are largely the only known clinical risk factors for suicide death. Recent research shows that most individuals who die by suicide make a health care visit in the weeks and months prior to their death. Most of these visits occur in primary care or outpatient medical specialty settings. However, over half of these visits do not include a psychiatric diagnosis.

Thus, there is limited evidence available from health care users in the US general population to inform targeted suicide screening and risk identification efforts in general medical settings. New research is needed to investigate the general medical clinical factors associated with increased suicide risk among individuals without a known risk factor. This research project uses data on more than 4000 individuals who died by suicide and made health care visits to one of eight health care systems across the United States in the year prior to their death. These health systems are members of the Mental Health Research Network and have affiliated health plans. They are able to capture nearly all health care for their members via the Virtual Data Warehouse (VDW). The VDW consists of electronic medical record and insurance claims data organized using standardized data structures and definitions across sites. These data are matched with official regional mortality data. This project includes the following specific aims:

1) identify clinical factors from general medical visits prior to suicide across sites, 2) compare clinical factors to a matched sample of health care users across sites, and 3) investigate indications of psychiatric and other concerns in general medical chart notes prior to suicide. This is the first study with a large enough sample in the US general population to be able to study general medical treatment utilization prior to suicide death. This project will allow the identification of previously unknown factors that increase risk of suicide death, including general medical diagnoses, medications, health care procedures, and types of visits. These results will inform decisions about how to focus suicide prevention efforts in general medical settings.

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.
Mental Health Research Network II (U19MH092201) Subcontract

This application requests five years of funding to sustain and expand the Mental Health Research Network (MHRN), a consortium of 13 research centers affiliated with large integrated health systems. Two new members will join the network, increasing the diversity of health system organization and adding a larger rural population. Established data and informatics infrastructure will be maintained (with reduced levels of funding). New infrastructure development will aim to address the opportunities and challenges described above and to address specific goals described in RFA MH-14-110: * Dissemination of MHRN

tools and resources to the broader mental health research community * Facilitating new research collaborations with investigators outside of MHRN member institutions * Improving capacity for ongoing surveillance of mental health treatment patterns and outcomes MHRN will be governed by a steering committee of participating investigators and representatives from NIMH. An Administrative Core will house specific infrastructure activities, including: The Informatics Unit will maintain and expand data infrastructure to support multi-site research. The Organizational Unit will continue work to streamline administrative and regulatory processes. The Outreach and External Collaboration Unit will continue engagement with health system partners and external stakeholders while expanding outreach to external investigators. Four specific research projects are proposed: Reducing cardiovascular risk in adults with SMI using EMR-based clinical decision support - This cluster randomized trial will test an informatics-based intervention to reduce risk factors for cardiovascular disease among people living with severe mental illness. Maximizing biospecimen collection from children with mental health conditions - This pilot study will evaluate strategies for collecting biospecimens from families affected by early-onset mental disorders. Next-generation assessment using mobile devices - This pilot study will evaluate the feasibility, acceptability, and potential utility of direct assessment of behavior and neuropsychological performance (including NIMH RDoC constructs) for prediction or early detection of depression treatment response. Automated outreach for depression treatment dropout - This pilot study will evaluate the feasibility and acceptability of population-based outreach to address early dropout from depression treatment.

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.

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: 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: 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: 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 ar 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, provider,

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 General?s strong emphasis on the need to reduce lung cancer mortality and the Cancer Moonshot Blue Ribbon Panel?s 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: 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 of 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.

PUBLICATIONS

Note: Publications in Red denote Epubs ahead of print

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