Abstract — Administrative Core

The goal of the Administrative Core is to establish tightly interacting Projects that interface with Technology, Modeling, and Data Management and Informatics Cores to achieve the scientific and administrative goals of the Omics for TB (OTB) consortium. The Core will organize and manage projects, their integration and progress, as well as communication among the groups. The Core is also responsible for implementation of a Training and Outreach Program, Systems Biology of Pathogens and Their Hosts Short-Course, to promote the use of the systems biology approach in the study of infectious disease. The Administrative Core will: • Continuously monitor the scientific progress of each component of the program. • Facilitate communication across OTB program, other U54 Centers, and within NIH. • Coordinate meeting scheduling and travel with the PIs and the NIH (Annual Programmatic Meeting). • Provide fiscal oversight to ensure that all financial resources are used appropriately. • Coordinate production of annual progress reports and updates to the Resource Sharing Plan.

Abstract — Project 1

Project 1: Human Mtb infection results in a large variety of clinical outcomes, ranging from bacterial eradication, to controlled latent infection, to progression and active disease with a range of clinical phenotypes. We recently discovered a blood transcriptional signature that predicts TB risk in Mtb-exposed individuals up to 18 months before they exhibit clinical symptoms, a landmark contribution to the field. Still, the mechanisms that underlie TB disease progression remain poorly understood, in large part because the key immune responses within the human lung cannot be readily monitored. Furthermore, TB is a highly heterogeneous disease in which individuals progress to active disease due to a variety of mechanisms. In this project, we will conduct comprehensive, multi-scale integration of transcriptomic, cytokine, chemokine and eicosanoid profiles from lung and blood during Mtb infection in order to identify and model molecular mechanisms and pathways that determine the outcome of infection. First, we will use multiple experimental strategies to recapitulate the heterogeneity of human Mtb infection in the mouse. These include a novel “ultra low dose” (ULD) infection model that we have pioneered in which mice are infected with 1–3 bacteria and subsequently exhibit a broad range of outcomes, ranging from immune control to progression. We will also employ mice from the Collaborative Cross project that have demonstrated extreme TB phenotypes and Mtb strains that span a range of pathogenicity. Second, we will interrogate and model the host-Mtb interaction in these mouse models using a variety of systems biology approaches in order to uncover the molecular regulators, pathways, and networks pulmonary innate and adaptive immune cells. We will test the predicted role of critical regulatory molecules by genetically perturbing them in vivo and examining the impact on control of Mtb infection. We will also apply machine-learning approaches to define multi-omic blood-based signatures in mice that predict TB progression. In our preliminary work, we have defined an early blood-based signature that predicts the late-time bacterial burdens in ULD-infected mice. We will correlate this signature with systems-level measurements of immune function in the lung to uncover mechanisms of Mtb control. Third, we will translate these findings to humanized mouse models. Through the Africa Health Research Institute, we will leverage a large-scale program that will obtain genomic sequence data as well as associated epidemiological and clinical metadata on 50,000 individuals living in a TB-endemic region. We will conduct a candidate gene genetic association analysis to validate regulatory molecules identified in mice to determine whether mutations in human orthologs are associated with altered risk of TB. In addition, we will use several existing non-human primate and human datasets to refine the blood based multi-omic progression signatures defined in mice and test their ability to predict TB progression in humans.

Abstract — Omics for TB: Response to Infection and Treatment

Omics for TB: Response to Infection and Treatment

Abstract — Technology Core

Technology Core

Abstract — Overview

With about 10 million new cases of active disease and 1.8 million deaths annually, TB is a global health emergency. A distinguishing feature of TB disease is its biological heterogeneity, which manifests at the cellular level chiefly in 2 forms: disease progression and treatment response. The premise of this Program is that the heterogeneous outcomes of TB infection and treatment are determined by the interplay of competing regulatory networks between the pathogen and the host. Our primary goal is to apply systems biology approaches to elucidate the biological control underlying the variability of disease outcome and response to treatment. Our first specific aim is to define novel host regulators of TB disease progression in vivo, and their innate and adaptive networks they control. We will also seek to define novel Mtb regulators of TB treatment response, and the Mtb regulatory networks that they control. This work will allow us to produce and validate host and Mtb models of TB disease progression and treatment response. Altogether, this program addresses key unanswered questions that stymie efforts to combat the TB pandemic. Our team has perfected the required platforms and scientific approaches to execute this ambitious research plan in a timely and cost-effective manner. All the participating investigators have strong records of interacting productively, and of disseminating their data and reagents to the scientific community.

Abstract — Technology Core

This U19 proposes a novel and fully integrated approach to understanding disease progression and treatment response in tuberculosis (TB). We will combine traditional approaches to understanding the pathophysiology of TB, bacterial genetics and immunology, with systems biology approaches including genomics, transcriptomics, metabolomics, lipidomics, proteomics, and computational modeling. We will apply HTP “Omics” technologies and systems analysis to murine and mycobacterial studies and to human field studies in tuberculosis. Data generated by HTP and targeted state-of-the-art approaches will be integrated through bioinformatic and modeling approaches, which, combined with domain expertise, will lead to a deep understanding of the molecular networks that underlie the progression of TB infection and response to treatment, and predict complex biological behaviors that lead to either containment or active disease; treatment cure or relapse. Multiple innovative HTP technologies will be leveraged and enhanced through this program. These technologies have been established in multiple experimental systems and will be customized and further developed for their application to this program and to clinical samples as appropriate. These developments will ensure data quality.
and maximize the efficiency of data generation. The approaches chosen are innovative and state-of-the-art and will maximize our ability to complement and extend the existing comprehensive work and provide spatial perspective for models generated as part of the original OTB program. We will disseminate modifications and improvements to extant technologies and detailed protocols broadly to enhance adoption and further development in the community.

**BEIER, DAVID R.**
Center for Developmental Biology and Regenerative Medicine (CDBRM)

**Screening for modifiers of PKD severity using ENU Mutagenesis**

**PROJECT SUMMARY/ABSTRACT**

There is abundant evidence from the analysis of human populations and mouse models that the severity of Polycystic Kidney Disease (PKD) can be modified by interacting genetic loci. The identification of these loci could provide insight into our understanding of the basic pathobiology of cystogenesis and disease progression. Importantly, they can potentially reveal novel pathways of therapeutic intervention. We have extensive experience in the characterization of a mouse model of cystic kidney disease, and specifically the investigation of strain-specific modifiers of its severity. However, the yield of proven causal genes in mouse studies of this type has been low. In contrast, we have shown that this modifier approach for novel disease discovery, namely mutants generated with the chemical ethyl-nitrosourea (ENU). We have recently modified this method so that we can do our screen entirely on an inbred background, using WholeGenome Sequencing methodology for positional cloning. The recent characterization of the PKD1/RC mutant mouse as having slowly progressive PKD, which is sensitive to strain-specific modifiers, compels our proposal that we use ENU mutagenesis for the generation and discovery of modifiers of PKD1/RC-induced cystic kidney disease. To complement this phenotype-driven approach, we will also pursue an analysis of candidate loci that modify PKD severity. We have data to suggest that Sonic Hedgehog (SHH) signaling plays a role in cystogenesis, and we will test whether the deletion of genes in this pathway affects disease severity in the PKD1/RC mouse model.

**BJORNSON, KRISTIE F.**
Center for Child Health, Behavior and Development (CHBD)

**Short-Burst Interval Treadmill Training to Improve Community Walking Activity and Mobility in Cerebral Palsy**

**Project Summary/Abstract:**

Ambulatory children with cerebral palsy (CP) walk predominately in low intensity stride rates with limited variability, thus limiting their walking activity and participation in daily life. In contrast, typically developing (TD) children engage in short bursts of intense walking activity interspersed with varying intervals of low intensity walking within daily life. In order to optimize motor learning, active participation, task-specific training and multiple repetitions or massed practice is required to learn new motor skills. Short bursts of vigorous intensity locomotor treadmill training (SLBTT) alternating with low/moderate intensity was specifically designed to mimic activity patterns of TD children in a massed practice format. Pilot data suggests that SLBTT is feasible and enhances walking capacity and performance in daily life for children with CP. The objective of this application is to examine the effect of SLBTT versus an equivalent dosage of traditional locomotor treadmill training (TLTT) on the primary outcomes of walking capacity and performance in children with CP. This protocol will also examine whether the effects of SLBTT on walking capacity and performance are mediated by improvements in muscle power generation. The scientific premise is that SLBTT, that approximates the walking intensity patterns of typically developing (TD) children through a clinically feasible and detailed massed practice protocol, will be more effective than TLTT in improving walking capacity and performance. We hypothesize that SLBTT strategies for children with CP modeled on activity patterns in TD children will be positively mediated by muscle power generation and subsequently improve walking capacity and community walking performance and mobility. We will test the following specific aims. Aim #1. Determine the immediate and retention effects of short-burst interval TLTT (SLBTT) on walking capacity in ambulatory children with CP. Walking capacity will be measured by self-selected gait speed and the one minute walk test. Aim #2. Examine the effects of treatment on community-based walking activity and mobility. Walking activity performance will be captured by accelerometer. Community mobility will be measured by home versus community locations, which will be measured with a novel combination of global positioning system and accelerometer. Aim #3. Explore whether the effects of SLBTT on walking capacity and performance are mediated by musclepower generation. This project is innovative because it focuses novel task-specific approach for addressing walking limitations in children with CP and implements an individualized community locations outcome. The proposed research is significant because it will be the first step in a continuum of research that is expected to directly influence training protocols and rehab strategies across pediatric disabilities and positively affecting the community walking performance and mobility for children with CP. Increased understanding of the muscular mechanisms by which children with CP respond to SLBTT are expected. Such knowledge has relevance to the health and functional benefits of enhanced mobility and physical activity across the lifetime span.

**CHERRY, TIMOTHY JOEL**
Center for Developmental Biology and Regenerative Medicine (CDBRM)

**Non-Coding Genetic Vulnerabilities in Human Photoreceptor Function and Disease**

**PROJECT SUMMARY/ABSTRACT**

The cis-regulatory elements (CREs) are critical sites of transcription factor (TF) binding to the genome that orchestrate the expression of genes necessary for normal cellular function. Mutations within CREs can disrupt TF binding and cause inherited human diseases including disorders of vision. The genomic location and function of CREs that are necessary for human vision is largely unknown. This gap in knowledge is asignificant obstacle toward understanding the genetic regulation of normal human vision and to identifying disease-causing mutations with CREs. The long-term goal for our research is to understand how genetivariation within CREs shapes the structure and function of the retina and contributes to human vision. The focused objective of this proposal is to determine the mechanisms by which CREs regulate essential gene expression in photoreceptor cells and to determine how genetic mutations within CREs lead to retinal disease. The central hypothesis driving this work is that discrete DNA sequences within CREs are required to regulate essential photoreceptor gene expression and that CRE mutations that disrupt evolutionarily conserved TF binding sites contribute to inherited visual disorders. To test this hypothesis we are pursuing the following specific aims: 1) Determine the activity of human photoreceptor CRPs in human retinal organoids using ATAC-Seq, CHIP-Seq and RNA-Seq to compare them to CREs we have previously identified from adult and developing human retinas. This will demonstrate the utility of organoids for studying photoreceptor CREs and their native cellular-epigenetic context. 2) Test the function of patient-derived variants in human photoreceptor CREs. Using high-throughput AAV-based reporter assays we will determine which CREs are essential to the expression of retinal genes in mouse retina and human retinal organoids and determine the consequence of sequence variants on CRE activity. 3) Test the mechanisms by which multiple CREs regulate the expression of a critical photoreceptor transcription factor, NRL. CRISPR/Cas9-based approaches will target specific CREs at the NRL locus to reveal the contribution of each CRE to the expression of the essential gene and to serve as a case study for the regulation of other essential genes. The contribution of this research will be to elucidate the mechanisms by which CREs regulate genes that are necessary for human photoreceptor function and survival. This work will enable the systematic identification and interpretation of genetic variants within CREs and therefore improve genetic diagnostics for unexplained retinal disease. By opening up the
non-coding genome to functional analyses it will be possible for the first time to determine the mechanisms by which individual CREs regulate specific genes that are critical for photoreceptor cell function in a high-throughput and comprehensive manner. This will enable discovery of genetic contributions to human vision and inherited visual diseases that have thus far been inaccessible.

**CHRISTAKIS, DIMITRI A**

Center for Child Health, Behavior and Development (CHBD)

**Attentional attributes of early child media usage**

PROJECT SUMMARY Increasingly, early childhood includes electronic media. It is not just the age at which children begin to view regularly that is concerning anymore; mobile and interactive media platforms (e.g., tablets) have changed the way media is consumed. Current viewing metrics suggest that tablet media is beginning to replace traditional TV viewing, but that passive content (“watching video”) remains the primary component of early media use. Tablets allow several important differences in use. First, apps for tablets allow both passive viewing as well as interactive gameplay, providing a more diverse set of content choices. Second, the personalized use of these smaller devices allows the content to be delivered directly to the child, and adults are less likely to control or supervise tablet use. On-demand access also allows programs to be controlled, changing patterns of consumption and use and has extended out of the home into more environments. Observational studies of TV use have linked excessive early media use with attention problems, language delay, and cognitive delays. It is unclear if the interactive nature of tables and apps might approximate more traditional exchanges or even promote interactions. Given the changing media landscape, a better understanding of how tablet-based media influences early joint learning is important for supporting better child media use with early risk. To address these issues, we will investigate: (1) the immediate impact of different tablet media content on infant joint attention behaviors, engagement, and cardiophysiological responses related to regulation and attention. As well, we will explore parents' ability to predict their child's difficulty disengaging from touchscreen technologies.

**COKER, TUMAINI RUCKER**

Center for Child Health, Behavior and Development (CHBD)

**Well-Child Care Clinical Practice Redesign: A Parent Coach-Led Model of Care for Young Children**

Project Summary Well-Child Care (WCC) visits for child preventive health care during the first three years of life are critical because they may be the only opportunity before a child reaches preschool to identify and address important social, developmental, behavioral, and health issues that could have significant impact and long-lasting effect on children's lives as adults. Unfortunately, this opportunity is often missed for children in low-income communities. The structure of WCC in the U.S. cannot support the vast array of WCC needs of these vulnerable children and their families. Key structural problems include (a) reliance on physicians for basic, routine preventive care services, (b) limitation to a 15-minute face-to-face clinician-directed well-visit for the wide array of education and guidance services needed, and (c) lack of a systematic, patient-driven method for visit customization to meet families' needs. These structural problems contribute to the wide variations in processes of care and preventive care outcomes, resulting in poorer quality of WCC and perhaps worse health outcomes. We previously used a rigorous, structured community-based participatory approach guided by key WCC stakeholders and expert panel methods to develop and test a new, innovative model of WCC delivery to meet the needs of children in low-income communities: ParentCoach2 (PARENT). PARENT is a team-based approach to care using a health educator (“Parent Coach”) to provide the bulk of WCC services, address specific needs faced by families in low-income communities, and decrease reliance on the clinician as the primary provider of WCC services. In an initial pilot randomized controlled trial of PARENT among 251 low-income families in two urban area pediatric practices, we found strong and consistent intervention effects on the quality of preventive care provided to families, and reducing emergency department (ED) utilization. A larger trial of PARENT with multiple clinics is needed to position PARENT as an evidence-based, financially sustainable model for WCC delivery that can be implemented by practices and clinics nationwide. In a clinic randomized controlled trial of PARENT, we will examine parent-reported quality of care and healthcare utilization (e.g., ED utilization), conduct a cost analysis, and use direct observations to assess changes in physician time allocation with Parent Coach-led well-visits. The study will be conducted in partnership with 12 clinics and their health plan payers, and address the following Specific Aims: Aim #1: Measure the effect of PARENT on receipt of nationally-recommended WCC services and parent experiences of care. Aim #2: Determine the effect of PARENT on WCC, urgent care, and ED utilization, and on net costs. Aim #3: Examine the effect of PARENT on physician time allocation for WCC and urgent care visits. Aim #4: Assess the effect of PARENT on parent-focused outcomes in an exploratory analysis.

**FRENKEL, LISA M**

Center for Global Infectious Disease Research (CGIDR)

**Defining HIV reservoirs that rebound following suspension of ART**

ABSTRACT In the twenty years since effective HIV treatments became available, the lifespan of HIV-infected adults in high-resource settings has increased to within a decade of uninfected individuals. Nevertheless, antiretroviral treatments (ART) fall short in restoring health, and if therapy is discontinued virus usually rebounds atopretreatment levels due to the persistence and reactivation of proviruses. Curative therapies are being sought, including therapeutic vaccines, chemotherapies paired with stem-cell transplant, chimeric antigen receptor T-cells, neutralizing and immune modulating antibodies, gene therapies, cytokines and initiation of ART during acute infection. While some of these approaches have reduced the "reservoirs" of infectious viruses and in one case may have cured HIV infection, a better understanding of the mechanisms underlying HIV persistence is needed to develop an effective, safe and economical cure. HIV reservoirs are primarily established early in infection, and while they decay and change in composition during ART, the mechanisms that sustain reservoirs are only partially known. We hypothesize that HIV reservoirs are maintained by: (1) Integrated proviruses that modulate gene expression to promote survival of these cells, allowing infected cells to persist by proliferatorinor latency; (2) HIV-specific immune responses become exhausted due to dysregulation of T-regulatory (T-reg) cell function; and (3) Epigenetic marks repress expression of proviral DNA, allowing infected cells to persist due to “deep” latency of proviruses. We propose studies to explore the role of these mechanisms in sustaining HIV reservoirs using specimens collected prospectively from a unique Belgian cohort of chronically infected individuals sampled during ART-suppression as well as during and after ananalysed treatment interruption (ATT). Samples for this study include blood, cerebral spinal fluid, bone marrow, bronchoalveolar lavage fluid, lymph node, duodenum, ileum, and colon. The knowledge gained from the proposed studies should point to interventional strategies that could be tested and potentially contribute to the goal of developing an intervention to cure HIV infection.

**FRENKEL, LISA M**

Center for Global Infectious Disease Research (CGIDR)

**Drug Resistance Genotypic and Phenotypic Correlates of**

Abstract Antiretroviral therapy (ART) is critical to improving the health of people living with HIV (PLHIV), to reducing HIV transmission and to maintaining the effectiveness of the current ART programs in resource-limited settings (RLS). However, HIV antiretroviral drug resistance (HIVDR) can hamper global efforts to control the AIDS epidemic and achieve the UNAIDS 90-90-90 targets. Pretreatment drug resistance (PDR) and on treatment-acquired drug resistance (ADR) are associated with virologic failure (VF) and increased morbidity and mortality. The correlates of PDR and ADR are not fully understood, and the level and breadth of HIVDR mutations (DRM) circulating in
Efavirenz and Dolutegravir based Treatment Outcomes across Non-B HIV-1 subtypes

individuals and populations, especially in children, have been derived from developed countries where the predominant circulating HIV strain is HIV-1 Group M subtype B. However, in communities with the highest prevalence of HIV-1 infection across the world, PLHIV are infected by non-B subtypes, such as subtypes A, C, D and circulating recombinant forms CRF01_AE and CRF02_AG. HIVDR and the correlate of viral suppression and outcome for these HIV-1 strains has been poorly studied. The success in regimens in RLS such as TDF-3TC-Efavirenz (TLE) and the planned roll-out of dolutegravir (DTG) based regimens (TLD) could be impeded by emerging evidence of widespread HIVDR. This proposal seeks to expand our understanding of HIVDR and its correlates and consequences in low-and-middle income countries where HIV-1 non-B subtypes circulate. To accomplish these goals, we propose the following three Specific Aims.1. Determine the number and breadth of PDR mutations that correlate with virologic failure to TLE or TLD in adults and children infected with HIV-1 subtype A, C, D, CRF01_AE or CRF02_AG.2. Determine DRM at the time of VF in adults and children, including DRM frequencies across cohorts by regimen (TLE and TLD) and by HIV-1 subtype 3. Determine the correlations between in vitro phenotypic drug resistance testing against genotypic DRMs across HIV-1 non-B subtypes (A, C, D, CRF01_AE and CRF02_AG). To address these aims we will use state-of-the-art and innovative assays to quantify the frequency of DRM in individuals’ pre-ART quasispecies and use phenotypic assays to better understand DRM interactions. Our novel studies will determine (1) the risks of specific PDR DRM and minority variants across HIV-1 non-B subtypes for VF; (2) interactions between DRM that determine phenotypic resistance associated with VF; and (3) mutations selected by TLE and TLD regimens at VF. The long-term goal of this proposal is to provide data to enable the best practices for HIV care across a range of resource-limited settings.

FRENKEL, LISA M Center for Global Infectious Disease Research (CGIDR)

Mechanisms controlling the persistence of infectious HIV reservoirs in children

Project Summary/Abstract Twenty years ago effective treatments for HIV became available, and the lifespan of HIV-infected adults in high-resource settings has increased dramatically. However, if treatment is interrupted, regain of virus occurs in the blood to pretreatment levels, due to viruses that persist and reactivate from the “HIV Reservoir”. Curative therapies suitable for the millions of infected individuals have yet been sought, including strategies using therapeutic vaccines, chemotherapies paired with stem-cell transplant, chimeranticigen receptor cells, gene therapies, cytokines and antiretroviral therapy during acute infection. While many of these have reduced the HIV reservoir and in one case may have cured HIV infection, a better understanding of the mechanisms that allow persistence of the reservoir are needed to develop an effective, safe antenatal cure. The HIV reservoir of perinatally infected children is primarily established early in infection when their immune system is tolerogenic to foster a healthy gestation, postnatal colonization with commensal bacteria and tolerance of foods. We propose to examine four mechanisms that could contribute to sustaining the HIV reservoirs and compare the contribution of each in children versus adults. We hypothesize that (1) mechanisms of self-tolerance (1) immune tolerance of HIV due to “perinatal tolerance” in the early weeks of life when immune tolerance to non-self antigens including non-inherited maternal antigens (NIMA) and oral tolerance to foods are established; and (2) “cross immune tolerance” to HIV generated by increased levels of maternal microchimerism (MMCs), as observed with allografts.15 In both adults and children, we hypothesize that the HIV reservoir is maintained by (3) modulation of gene expression by HIV integration (in utero or postnatal), via PI3K/AKT pathway, which inhibits the Hippo pathway and stimulates YAP signaling pathway to mediate cell growth. We will investigate the mechanisms underling the effects of cadherin homophilic adhesion and signaling at different levels of analysis, from basic biochemical/biophysical/structural mechanisms, through cell biological process controlling adhesion (especially the role of p120-catenin), to evaluating the roles of adhesion regulation and cadherin signaling in tissue development and physiology. Classical cadherins are cell-cell adhesion proteins that regulate tissue morphogenesis and cell junctions during physiologic processes. They are highly regulated at the cell surface, controlling dynamic interactions between cells. Although much is known about the basic functions of cadherin-mediated adhesion, an understanding of the mechanisms underlying dynamic cell surface regulation, has not yet been achieved, nor is it well understood how such regulatory mechanisms control physiological processes in vivo. Cadherins also transduce signals into the cell to convey information about the state of the tissue. One way they do this is by stimulation of the Hippo-YAP signaling pathway to mediate contact inhibition of growth. This process is antagonized by growthfactor signaling, via the PI3-kinase (PI3K) signaling pathway, which inhibits the Hippo pathway and stimulates YAP. We will investigate the mechanisms underlining the effects of cadherin homophilic adhesive binding at different levels of analysis, from basic biochemical/biophysical/structural mechanisms, through cell biological process controlling adhesion (especially the role of p120-catenin), to evaluating the roles of adhesion regulation and cadherin signaling in tissue development and physiology. We've found that cancer- and cleft lip-associated mutations in E-cadherin specifically interfere with the regulation of adhesion at the cell surface, and these will provide valuable tools for these studies. In vivo studies of cadherin regulation will focus on their roles in physiological control of barrier function in both epithelia and endothelia, especially during inflammatory processes where control of these function are especially important. Studies on endothelial junctional regulation will require us to develop tools for studying VE-cadherin regulation, including activating antibodies, and models for endothelial barrierfunction; these will be compared to our studies of E-cadherin in epithelia. We’ll also investigate the mechanisms by which cadherins transduce various signals into the cell. A major focus will be on the regulation of the Hippo-YAP pathway and associated TEAD transcription factors by cadherin-mediated contact and bygrowth factors and PI3K signaling. The goals are both to understand how they function and to enable us to develop genetic approaches to selectively perturb these interactions in vivo to evaluate their importance. Hippo signaling by formation of cadherin contacts will be compared to signaling by tight junctions as well as signals produced by mechanical tension at the cadherins. The Hippo-YAP pathway may be an important new branch of the PI3K signaling pathway that regulates tissue growth in addition to the well-known Akt-TOR pathways. This hypothesis will be tested in vivo both by studies on tissue overgrowth diseases caused by somatic mosaic constitutively active PI3K mutations and by studies of mouse models of mammary tumorigenesis. This project should reveal how
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<th>Name</th>
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<td>HORSLEN, SIMON</td>
<td>Center for Clinical and Translational Research (CCTR)</td>
<td>Continuation of the Childhood Liver Disease Research Network Seattle Clinical Center</td>
<td>Project Summary/Abstract Biliary atresia (BA) and the other childhood cholestatic liver diseases are significant causes of chronic liver disease in children, and the leading causes for liver transplantation in pediatrics. The initial funding period leading to the current Childhood Liver Disease Research Network (ChiLDReN) has resulted in unprecedented collections of well phenotyped subjects and banked data and biological specimens. Although ongoing recruitment of subjects with these rare conditions is needed to allow full attainment of many of the individual study aims, the collection of subjects, data, and biospecimens is now sufficient to support meaningful investigation into the pathogenesis of these diseases and allow thorough genomic screens to elucidate etiologies and modifiers of disease phenotypes. This next funding period will allow completion of ongoing studies and add study of primary sarcoïd cholangitis. Furthermore, in conjunction with, identification of predictors of liver disease development will be possible before the phase III trial of non-invasive markers of disease (led by Dr. Murray). The Seattle Clinical Center (CC) has the experience, expertise, and proven track record to continue participation in ChiLDReN, and has the experienced patients over time to support the ongoing and all studies. Dr. Murray and her CC team have additionally proposed a Pilot and Feasibility Trial to study the impact of parenteral nutrition, with standard intralipids versus Omegaven, on marmoset children with end-stage liver disease due to BA. This study has the potential to Indian the standard of care for these vulnerable patients, improve their outcomes, and enhance our understanding of the pathogenesis of this obliterative cholangiopathy. Furthermore, to better understand the etiology of BA and potentially identify unique underlying candidate genes, Dr. Murray also proposes to perform whole genome sequencing and rare-variant analysis on a subset of the ChiLDReN BA trios to identify variants of both large and small effects in families with BA and a platelet configuration.</td>
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<td>JACKSON, SHAUN WILLIAM</td>
<td>Center for Immunity and Immunotherapies (CIIT)</td>
<td>B cell costimulatory signals in the pathogenesis of SLE</td>
<td>Project abstract Despite modern immunosuppressive therapies, patients with systemic lupus erythematosus (SLE) remain at high risk for progressive organ damage, emphasizing the need for better, targeted treatments for this disease. In addition to the production of pathogenic autoantibodies, recent studies have demonstrated that B cells can promote lupus pathogenesis by initiating immune tolerance breaks and facilitating the generation of spontaneous germinal centers (GC). In this context, distinct costimulatory receptor families have been linked with the pathogenesis of autoimmunity. However, despite compelling preclinical data in SLE and clinical benefit of other autoimmune diseases, costimulatory blockade with CTLA4-Ig (Abatacept) failed to control disease in lupus clinical trials. These data emphasize our understanding of the cell-intrinsic mechanisms whereby B:CD28 costimulatory signals impact autoreactive B cell activation in lupus is incomplete. In this project, we will use well-characterized murine lupus models and the novel application of chimeric antigen receptor (CAR) T cell technology to dissect the immune mechanisms underlying the initiation, propagation, and cellular output of GC-like follicular (EF) vs. GC B cell activation pathways in SLE. In Aim 1, we will study whether pathogenic autoantibodies can be generated via an EF B cell activation pathway in a T cell-dependent, but CD28-independent, manner. In Aim 2, we will test whether B cell costimulatory signals promote the initiation or maintenance of autoreactive GC responses. Finally, in Aim 3, we will test whether another costimulatory receptor pair, ICOS/ICOS ligand, compensates for loss of CD28 signals during lupus pathogenesis. Together, these studies promise to advance our understanding of lupus pathogenesis and may inform the design of future human clinical trials of costimulatory blockade in SLE and other humoral autoimmune diseases.</td>
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<td>JAMES, RICHARD</td>
<td>Center for Developmental Biology and Regenerative Medicine (CDBRM)</td>
<td>Role of Dock8 in Mucosal Immunity</td>
<td>PROJECT SUMMARY/DOCK8 deficiency in humans leads to severe immunodeficiency. The clinical manifestations of Dock8 immunodeficiency include recurrent infections, allergies, and malignancies. Dock8-/- deficient patients suffer from recurrent bacterial infections of the mouth or skin, C. rodentium infection, which is suggestive of TH17 cell dysfunction. Although it has been suggested that Dock8 might coordinate cytokine/costimulatory signals, cellular activation, and regulation and track Dock8 deficiency in humans. Dock8 is essential for the protective immunity against C. rodentium. Dock8 deficient mice succumb rapidly to C. rodentium infection. Dock8 deficient mice have very low numbers of IL-22-producing RORγt+ ILCs in WT mice. Dock8 deficient RORγt+ ILCs are important for Th17 cell development and the generation of TH17 cells in C. rodentium infection is selectively impaired, whereas the generation of TH1 cells is dramatically increased in Dock8-deficient mice in comparison to WT mice. Dock8 is a very large protein that has been shown to function as a guanine nucleotide exchange factors (GEFs) that binds and activates small GTPases of the Rho/Rac/Cdc42 family. In order to determine whether Dock8 function in the generation of TH17 cells independent on its GEF activity for CDC42, or its interaction with WASp, a protein that plays an important role in the organization and function of the actin cytoskeleton, we infected mice in which CDC42 was wasspecifically eliminated in T cells. Dock8 deficient mice were unable to mount a robust TH17 cell response upon infection, CDC42 T cell-deficient mice developed a TH17 cell responses robust as WT mice. From this study, we concluded that at least for the development of TH17 cells, Dock8 is likely acting as a scaffolding protein rather than a GEF for CDC42, or via its interaction with WASp. Thus, it is possible that Dock8 might act as a scaffolding protein that is important for the activation of unknown factors necessary for the differentiation of TH17 cells. Here we hypothesize that Dock8 regulates the function of TH17 cells by interacting with a specific set of proteins selectively expressed in TH17 cells and not in TH1 cells. In order to identify proteins that interact with Dock8 in vivo, we have generated a novel knockin mouse in which endogenous Dock8 was fused to Avi tag. Flag and GFP reporter. The Avi tag technology will allow us to perform proteomics and Mass spectrometry analysis in a relatively small number of primary T cells, whereas the GFP will allow us to perform confocal microscopy and track Dock8 localization in both ILCs and TH17 cells. Overall, our proposed studies will help us understand why Dock8 deficiency has a profound effect on the immune system.</td>
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<td>JOHNSON, SIMON C</td>
<td>Center for Developmental Biology and Regenerative Medicine (CDBRM)</td>
<td>The role of mTOR in mitochondrial encephalopathy</td>
<td>Project Summary/Abstract Our overarching goal is to define the molecular mechanisms underpinning the pathogenesis of mitochondrial disease. Our overall objective in the studies proposed here, which represent the next step in pursuing this goal, is to characterize the pathogenesis of subacute necrotizing encephalopathy and define thereof of mTOR in this disease using the Ndufs4 KO model Genetic mitochondrial diseases include an array of symptoms, may affect one organ or present as a multisystem disorder, and are remarkably heterogeneous in severity. There are few models for these diseases and no effective treatment options for mitochondrial disease of postnatal life.</td>
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any etiology. A clear understanding of the pathogenesis of individual mitochondrial diseases is severely needed; the molecular mechanisms underlying their multiple distinct clinical manifestations are currently unknown. Subacute necrotizing encephalomyelopathy, or Leigh syndrome (LS), is a fatal pediatric mitochondrial disease. Characteristic features of LS include specific necrotizing lesions of the brain. Though these lesions are the major defining feature of LS, virtually nothing is known of initiating events, what underlies the spatial and temporal specificity of the disease, or why some regions of the CNS are inexplicably spared. Recent work has shown that inhibition of the nutrient sensing signaling complex mTOR attenuates LS in a mouse model, but the mechanisms underlying the benefit are unknown. The goal of this proposal is to define the pathogenesis of LS and the role of mTOR in this disease. We hypothesize that the neurological lesions characteristic of LS result from region and cell-type specific effects of mitochondrial dysfunction, and that mTOR inhibition acts through a discrete downstream neurotoxic pathway. Our experiments will take advantage of the Ndufs4 KO (KO) mouse model of LS, a paradigm model which closely resembles human LS. Using this model, we will use characterize the cellular and molecular pathogenesis of neurological lesions in LS by i) identifying the earliest type of cell death and ii) the CNS cell types first lost in lesion formation, iii) defining the region, and cell compartments specificity of phospho-proteome changes during CNS lesion formation, and iv) testing the role of key mTOR regulated pathways in LS using pharmacological approaches. Ultimately, this work will expose basic molecular features of LS and mitochondrial disease in general. In addition, the career development and components of this proposal will provide key elements for my successful transition to an independent career.

KALUME, FRANCK K  
Center for Integrative Brain Research (CIBR)  
Mechanisms of epilepsy-related death in Leigh syndrome  
Loss-of-function mutations in NDUFS4, the gene that encodes a subunit of the protein complex I in the mitochondrial electron transport chain, are strongly associated with Leigh Syndrome (LS). LS, or subacute necrotizing encephalopathy, is a debilitating progressive neurodegenerative disorder. It typically presents with multi-systemic clinical symptoms which result in disability and ultimately death by 3 years of age. Mouse models of LS, generated with specific Knock Out (KO) of Ndufs4, exhibit several key clinical features of human LS, including failure to thrive, growth retardation, ataxia, hypotonia, visual problems, breathing irregularities, and spontaneous seizures and deaths. Our preliminary studies of conditional Ndufs4 KO mice reveal that the epilepsy phenotype can be dissociated from most of the other features of LS, using genetic approaches. Selective KO of Ndufs4 in GABAergic (not glutamatergic) neurons causes spontaneous seizures and leads to sudden death in mice. Therefore, in this proposal we will explore the intriguing possibility that Ndufs4 is a SUDEP gene and GABAergic neuron dysfunction, caused by its KO, is the principal cause of epilepsy and SUDEP in LS mice. Indeed, these mice present a unique opportunity for functional studies of SUDEP risks associated with LS-causing Ndufs4 mutation in particular and interneuron dysfunction in general. Interestingly, current gene discovery studies have postulated that a SUDEP gene might be best identified as amutation or pathogenic variant that causes epilepsy and increases SUDEP risk via central or peripheral nervous system or end-organ effects on respiratory, cardiac, or other autonomic functions. The forebrain includes regions known to be involved in seizure generation and the brainstem encompasses control centers for autonomic functions, commonly affected in SUDEP. In this project, we will examine the role of interneurondysfunction in mechanisms SUDEP in Leigh syndrome epilepsy using mouse models. We will: (1) Compare the contributions of Ndufs4 KO in excitable (by Vglu2zcre) and inhibitory (by Gad2zcre) neurons to SUDEP susceptibility in LS mice; (2) compare the contributions of Ndufs4 KO in forebrain (by Dicer1s2) and brainstem (by viral cre) GABAergic neurons to SUDEP susceptibility in LS mice; and (3) determine changes in intrinsic synaptic functions of forebrain interneurons that contribute to epilepsy and SUDEP pathophysiology in Lsmice. Findings from these studies will provide insights into the mechanisms of sudden death in Leigh syndrome, the most common form of mitochondrial disorder in children.

KAPPE, STEFAN HI  
Center for Global Infectious Disease Research (CGIDR)  
Molecular Determinants of Sporozoite / Host Cell Interactions  
PROJECT SUMMARY/ABSTRACT Malaria, caused by Plasmodium parasites, continues to be a major global health problem, with more than 200 million new infections and nearly 500 thousand deaths annually. Infection initiates when sporozoites stages are inoculated into the skin by the mosquito vector. Sporozoites then move in the skin tissue, enter the bloodstream and reach the liver. Sporozoites traverse cells before eventually infecting a hepatocyte within an aperiphasic parasite phagosome (PV). Enshrouded in the PV membrane (PVM), a single sporozoite will transmigratory into a liver stage that replicates and then forms thousands of merozoites. These are released from the liver and infect and replicate in blood cells, which causes all clinical symptoms of malaria and enables further parasite transmission. Mice specific Knock Outs (KO) of Ndufs4, the gene that encodes a subunit of the protein complex I in the mitochondrial electron transport chain, are strongly associated with Leigh Syndrome (LS). LS, or subacute necrotizing encephalopathy, is a debilitating progressive neurodegenerative disorder. It typically presents with multi-systemic clinical symptoms which result in disability and ultimately death by 3 years of age. Mouse models of LS, generated with specific Knock Out (KO) of Ndufs4, exhibit several key clinical features of human LS, including failure to thrive, growth retardation, ataxia, hypotonia, visual problems, breathing irregularities, and spontaneous seizures and deaths. Our preliminary studies of conditional Ndufs4 KO mice reveal that the epilepsy phenotype can be dissociated from most of the other features of LS, using genetic approaches. Selective KO of Ndufs4 in GABAergic (not glutamatergic) neurons causes spontaneous seizures and leads to sudden death in mice. Therefore, in this proposal we will explore the intriguing possibility that Ndufs4 is a SUDEP gene and GABAergic neuron dysfunction, caused by its KO, is the principal cause of epilepsy and SUDEP in LS mice. Indeed, these mice present a unique opportunity for functional studies of SUDEP risks associated with LS-causing Ndufs4 mutation in particular and interneuron dysfunction in general. Interestingly, current gene discovery studies have postulated that a SUDEP gene might be best identified as an amutation or pathogenic variant that causes epilepsy and increases SUDEP risk via central or peripheral nervous system or end-organ effects on respiratory, cardiac, or other autonomic functions. The forebrain includes regions known to be involved in seizure generation and the brainstem encompasses control centers for autonomic functions, commonly affected in SUDEP. In this project, we will examine the role of interneurondysfunction in mechanisms SUDEP in Leigh syndrome epilepsy using mouse models. We will: (1) Compare the contributions of Ndufs4 KO in excitable (by Vglu2zcre) and inhibitory (by Gad2zcre) neurons to SUDEP susceptibility in LS mice; (2) compare the contributions of Ndufs4 KO in forebrain (by Dicer1s2) and brainstem (by viral cre) GABAergic neurons to SUDEP susceptibility in LS mice; and (3) determine changes in intrinsic synaptic functions of forebrain interneurons that contribute to epilepsy and SUDEP pathophysiology in Lsmice. Findings from these studies will provide insights into the mechanisms of sudden death in Leigh syndrome, the most common form of mitochondrial disorder in children.

KAPPE, STEFAN HI  
Center for Global Infectious Disease Research (CGIDR)  
Inducing durable, protective  
Project Summary The goal of generating a licensed vaccine that can provide long-lived immunity against infection with Plasmodium falciparum, the protozoan parasite that causes the most lethal form of malaria, is yet unrealized. Currently, the malaria vaccine candidate that has undergone the most extensive clinical testing is RTS,S, a subunit vaccine based on the circumsporozoite protein (CSP), expressed on the surface of the infectious sporozoite stage of the parasite. Yet, as seen with many other vaccine strategies, protection induced...
| **KAUSHANSKY, ALEXIS**  | **Center for Global Infectious Disease Research (CGIDR)** | **Pathogens must successfully navigate the complex interaction networks of their hosts to survive.** During malaria parasite liver stage infection, parasites use several hepatocytes to protect their host cells from immune system identification for growth and development. The host hepatocyte molecular signaling landscape that facilitates successful liver stage replication has not been elucidated, yet it is highly medically relevant. During the first year of this grant, we have made significant strides towards elucidating this complex hepatocyte signaling pathway upon which the malaria parasite relies and also identified the critical host receptor which parasites engage. In 2021, we will focus on developing and testing a decision support tool for clinicians to facilitate within-sport decision making about sport participation post-concussion. Furthermore, we will test the hypothesis that the tumor suppressor P53 is the regulator of ferroptosis in infected cells and how increasing P53 levels can eliminate liver-stage parasites. Finally, we will conduct a randomized trial in healthy volunteers to examine the impact of perturbation on the hepatocyte signaling landscape that regulates the success or failure of the infected hepatocyte. Accomplishing our aims opens the possibility of altering key host factors with small-molecules that could prevent a wild-type parasite from progressing to symptomatic erythrocyte infection. Such a host-based approach for prophylaxis is novel and will circumvent the massive problem of continuous development and resistance to anti-malarial drugs. This approach is further fostered by the fact that many hepatocyte proteins are already targets of known therapeutic inhibitors. A more detailed understanding of the complex perturbations elicited by this important intracellular pathogen might also reveal new aspects of how does previous malaria infection alter the generation of sporozoite vaccine immunity, as well as murine malaria models, which will be studied using a process of user-centered design. | 5R01GM101183-09 |

| **KROSHUS, EMILY GRACE**  | **Center for Child Health, Behavior and Development (CHBD)** | **PROJECT SUMMARY**Every year, more than one million U.S. youth sport participants ages 6 to 18 are diagnosed with a concussion. After acute post-concussive symptoms have resolved, clinicians often struggle with how to discuss returning to sport with families. The decision to cease sport is very individualized and cannot be reduced to a single numeric cut-off. Influential factors in this decision include clinical variability (e.g., injury severity, recovery trajectory, type of functional impairment post-injury), the interval between prior injuries, the age at which injuries occurred, and premorbid health conditions). Different family tolerance for the uncertain risk of harm associates with sustaining an additional concussion relative to what they see as the benefits of returning to sport, the risks and benefits of substitute activities, and psychosocial readiness for sport retirement. Further complicating this conversation is the lack of definitive evidence about how does previous malaria infection alter the generation of sporozoite vaccine immunity, as well as murine malaria models, which will be studied using a process of user-centered design. To address these challenges, we seek to develop and evaluate a decision aid that helps adolescents and their parents/guardians with their clinician’s informed and value-driven decision about sport participation post-concussion. We will focus on helping shared decision making in two situations: (1) the clinician believes there is equipoise in the decision about whether to return to or cease participation in contact/collision sport, and (2) the clinician believes contact/collision sport cessation would be medically beneficial but sport disqualification is not mandatory based on current consensus guidelines. This will be accomplished with a (1) a web-based module to be developed separately by parents and adolescents pre-visit, sharing risk information and supporting values clarification related to sport participation, and (2) implementation support for clinicians to facilitate within-visit discussion, prioritization, and decision-making. To develop an optimally useful decision aid, we will engage a diverse group of families and clinicians in the development process and will focus on meeting the needs of families with low health literacy. The following aims will be addressed: (1) Develop a decision aid to support shared decision-making about sport participation post-concussion using a process of user-engaged content specification and design; (2) Conduct usability testing of the decision aid in a clinical setting; (3) Conduct a pilot test of the efficacy of this decision aid in a diverse sample of families presenting to Seattle Children’s Hospital for post-concussion care. The primary outcome of the pilot clinical trial will be decision regret. Secondly, the impact of the tool on sport participation, physical activity and psychosocial outcomes three months post-decision will be explored. Achieving the proposed aims will result in a decision aid that is acceptable to the target populations. | 1R21HD098355-01A1 |
and that has preliminary evidence of its efficacy facilitating higher quality decision making post-concussion. This will provide the foundation for a subsequent R01 application to evaluate the effectiveness of the tool in a multicenter cluster randomized controlled trial.

LAW, EMILY F
Center for Child Health, Behavior and Development (CHBD)

**Enhancing Efficacy of Migraine Self-Management in Children with Comorbid Insomnia**

**PROJECT SUMMARY**

Migraine is a major pediatric health problem impacting 10-12% of youth. Poor sleep is a common comorbidity, particularly insomnia symptoms, which are reported by 65-71% of adolescents with migraine. Insomnia contributes to greater headache-related disability, higher pain intensity, greater anxiety, and depression, poorer quality of life, and increased healthcare use. History of childhood insomnia places youth at risk for a lifelong pattern of migraine and disability and high health care costs in adulthood. Thus, finding effective methods that support youth in the self-management of migraine is a priority. Cognitive-behavioral therapy (CBT) for pain is an established treatment approach for youth with migraine; however, improvements in sleep are inconsistent. In fact, our preliminary data suggest that poor baseline sleep is a risk factor for youth to achieve less improvement in pain outcomes with CBT for migraine. Sleep and migraines share a cyclical relationship, and data indicate that insomnia symptoms increase migraine severity in adults and children. CBT for insomnia has demonstrated efficacy for improving insomnia symptoms in adults with migraine and other pain conditions, however, effects on pain have been inconsistent. Post-hoc analyses suggest that changes in pain may occur only after there are sustained improvements in sleep, but this has never been empirically tested. In the proposed study, we will address these gaps in knowledge by using an innovative 2-Phase trial design to: 1) test efficacy of CBT insomnia intervention for youth with migraine and comorbid insomnia, and 2) investigate how changes in sleep may modify response to CBT pain intervention. We will study a cohort of 180 youth, ages 11-17 years, with migraine (with or without aura, chronic migraine) and comorbid insomnia. In Phase 1, youth will be randomly assigned to receive internet-delivered CBTinsomnia intervention or internet sleep education control over 4-weeks. In Phase 2, all youth will receive internet-delivered CBT pain intervention over 8-weeks. Assessments will occur at baseline, immediately after Phase 1 intervention, and repeated 6 months post-intervention. The primary outcome for Phase 1 is insomnia symptoms. The primary outcome for Phase 2 is headache-related disability. Secondary outcomes are sleep quality and sleep patterns, headache frequency and pain intensity, anxiety and depressive symptoms, and quality of life. Sleep hygiene and pre-sleep arousal will be assessed as potential mediators. We will use a comprehensive multidimensional assessment of sleep and headache including self-report questionnaires, ambulatory actigraphy monitoring, and 14-day daily diaries. Given the high prevalence of insomnia in adolescents with migraine, extension of CBT insomnia intervention to this population will address an important gap in clinical practice and in conceptual understanding of the relationship between sleep and migraine. By testing a separate CBT insomnia intervention, we will be able to apply this treatment to a wide range of other pediatric populations (e.g., cancer, arthritis) who commonly experience comorbid insomnia.

MCELRAH, MARGARET JULIANA
Center for Global Infectious Disease Research (CGIDR)

**Immune Responses to Malaria and HIV Infection and Immunization - Clinical Core**

The Clinical Core, based within the Vaccine and Infectious Disease Division (VIDD) at Fred Hutchinson Cancer Research Center (FHCRC), will provide relevant expertise in clinical medicine, human subjects research, vaccine trials, and human immunology in support of the scientific aims of the Collaboration focused on the prevention and control of malaria and HIV. In order to most efficiently support the diverse aims of Collaboration projects, the CC will utilize a variety of resources including: 1) archived samples (e.g., cryopreserved PBMC, serum, plasma) from study participants in relevant completed or ongoing clinical trials, which comprise the primary source of clinical samples for Collaboration projects; 2) newly obtained samples from existing clinical cohorts and from specifically designed sub-studies to enable more comprehensive analysis within ongoing or planned vaccine or treatment trials; and 3) samples acquired through the clinical expertise of collaborating clinicians accomplished in the safe sampling of specialized immunological reservoirs (e.g., bone marrow; lymph nodes; mucosa) using minimally invasive procedures in human volunteers.

MCELRAH, MARGARET JULIANA
Center for Global Infectious Disease Research (CGIDR)

**Immune Responses to HIV virus immunization - Project 2**

Pre-exposure prophylaxis and HIV infection can lower HIV infection rates but nearly 70 million new infections still occur worldwide each year. A vaccine that can elicit long-lived protective immunity against HIV infection offers the best prospect to end the AIDS epidemic. While no licensed HIV vaccine is available, the modest efficacy observed in the RV144 Thai trial raises hope that a preventive vaccine is possible. To improve on this efficacy, a deeper understanding of the underlying immune mechanism of vaccine protection is crucial. Our proposed studies aim at generating critical insights on how to improve anti-HIV T cell function, induce enhanced immunopotentcy and durability, and develop pathways to elicit broad neutralizing antibodies. We are in a unique position to address these topics with access to an exceptional set of samples from several HIV vaccine trials and well-characterized HIV infection cohorts. Our proposed studies include the assessment of the kinetics of the vaccine-induced immune response in relevant anatomic compartments (lymph nodes, bone marrow and gut) and access to cutting-edge analytical methods to generate linked datasets that are ideally suited for our proposed, comprehensive systems biology approach. Our project team is uniquely suited to conduct these studies, and of well-established collaborators focused on HIV vaccine research, translational immunology, systems approaches to understand immunological memory, and immune correlates analyzes. We expect that our work will reveal testable hypotheses on the underlying mechanistic interplay between key components of innate and adaptive immune response that are responsible for protection against HIV by vaccination.

MENDOZA, JASON A
Center for Child Health, Behavior and Development (CHBD)

**Fit 5 Kids Screen Time Reduction Curriculum for Latino Preschoolers: A RCT**

**ABSTRACT**

Screen time is a major risk factor for childhood obesity and inadequate physical activity, both of which are determinants of type 2 diabetes (T2D), cardiovascular disease, and mortality. Latinos are the largest and fastest growing minority in the US. Because US Latino children have more screen time and higher rates of obesity than their non-Latino White peers, interventions to reduce screen time adapted for Latino preschoolers are necessary to reduce health inequities related to obesity and T2D in the US. However, asymptomatic review reported no successful screen time reduction interventions among Latino preschoolers. Our team’s pilot study tested the culturally adapted Fit 5 Kids screen time reduction curriculum among Latino preschoolers in Head Start. This short term cluster randomized controlled trial (RCT) is the only successful screen time reduction program for Latino preschoolers, having significantly reduced screen time by over 25 minutes/day. Our culturally adapted, multi-level intervention consists of lessons taught by study staff directed to preschoolers, a weekly parent newsletter, child-tailored goal setting with parents, a lending library (books, games, arts/crafts, etc.) and parenting tips via text messages several times/week. We will use a social ecological model and consider multiple levels of influences for analyses: (1) individual-level influences, e.g., acculturation and social cognitive theory, (2) families, e.g., screen time parenting practices, (3) schools, and (4) macro-environmental influences, e.g., neighborhood disorder. Building on this pilot work, we propose a long term, efficacy, cluster RCT of the culturally adapted Fit 5 Kids among Latino preschoolers in Head Start from three US setting: Seattle, Seattle, Seattle.
MIAO, CAROL H  
Center for Immunity and Infectious disease (CIIT)  

Ultrasound-mediated gene delivery to achieve therapeutic correction of hemophilia A  

Project Summary  
The goal of this proposal is to achieve long-term therapeutic correction of hemophilia A (HemA) via a noninvasive ultrasound-mediated gene delivery (UMGD) of factor VIII (FVIII) plasmids in the dog model. HemA is a genetic disorder characterized by a deficiency of the blood clotting FVIII. Patients are treated acutely prophylactically by protein replacement therapy, which is very costly and inconvenient for treating HemA patients by delivering FVIII transgene into targeted cells to persistently produce therapeutically relevant levels of FVIII protein. Recent clinical trials for HemA gene therapy using recombinant adenovirus-mediated therapy (rAAV) vectors have shown nominal yet promising results. However, significant obstacles remain to prevent treatment to a significant portion of patients especially patients who have high-titer anti-AAV antibodies. Repeated treatment is also prohibited. UMGD has emerged as an effective gene transfer approach with great clinical relevancy and translational potential. In comparison to viral gene transfer, UMGD transfers plasmid vectors that are easier to prepare and more cost-effective; it also elicits less immune response and toxicity due to specific tissue targeting, prevents random integration, and allows for repeated delivery of the vectors. Other nonviral gene delivery method such as DNA-polyplex nanoparticles encounters the challenge of crossing the nuclear envelope for DNA transcription. We have established a minimally invasive, transhepatic venous approach to efficiently deliver plasmid DNA (pDNA)/microbubble (MB) mixture into the target liver lobe combined with transcutaneous US applications in large animal models. We showed that high levels of Luciferase reporter gene expression was achieved in swine and therapeutic levels of FVIII expression was detected in canine using the clinically feasible protocol. Only transient tissue damages were observed and repaired quickly and returned to normal within short time. However, in order to translate this novel technology to clinics, we recognize that several major problems need to be solved. (i) higher FVIII expression levels are needed to achieve a long-term therapeutic effect, (ii) persistence of therapeutic FVIII expression needs to be evaluated and maintained, (iii) consistently high efficiency of US treatment on targeted liver tissue is needed to achieve reproducible and effective transfection. (iv) Better functional FVIII expression and reduced liver damage are desired. This may be achieved by targeting FVIII transfection in liver sinusoidal endothelial cells (LSECs), the native site of FVIII synthesis or by using newly synthesized nanobubbles (NBs). Thus, we propose to continue improving the transcutaneous UMGD instrument, transducers, US protocols, FVIII plasmid constructs, and MBs/NBs in mice and swine. Furthermore, we will deliver FVIII gene using the best transcutaneous UMGD protocol combined with optimal plasmid constructs and MBs/NBs to achieve persistent and therapeutic levels of FVIII expression in normal and HemA dogs. These progress will promote the eventual translation of this novel technology into human application, bringing significant benefit for treating HemA patients, and potentially other genetic diseases.

PARK, JULIE R  
Center for Clinical and Translational Research (CCTR)  

Accelerate cellular immunotherapy development for treatment of life-threatening childhood disorders  

Project Summary & Abstract  
Dysregulated or dysfunctional immunity is well documented in human disease states ranging from autoimmunity to infection and cancer. A deeper understanding of the role of the immune system in human disease brings with it the real potential of immune-directed cellular therapies. However, the complexity and expenses associated with the generation of cell therapies that are both patient- and disease-specific prohibit broad application at the current time. Progress in the setting of rare pediatric conditions is further hampered by the fact that financial returns on investment are in many cases not considered favorable for industry-sponsored research and development. This U01 Innovative Award application is designed to accelerate the translation of cellular immunotherapies to treat disorders that affect children and adolescents through the establishment of the Consortium for Pediatric Cellular Immunotherapy comprised of quaternary care pediatric hospitals affiliated with their Clinical and Translational Science Award (CTSA) programs. We aim to accelerate the implementation of engineered cellular therapeutic products for cancer (including chimeric antigen receptor-T cell therapy and NK cell therapy) or selected immune cellular therapies for treatment of lymphoproliferative disorders, and viral diseases (viral-specific T cell therapy). In addition, we will also accelerate the novel implementation of engineered regulatory T-cells to invoke immune tolerance as a therapeutic modality for a wide range of disorders that include graft vs. host disease following allogeneic hematopoietic cell transplantation, rejection after solid organ transplantation and pediatric auto-immune diseases. We propose an multi-pronged approach to spearhead the development of cellular immunotherapy clinical trials in pediatric medicine. We aim to expand cGMP manufacturing programs with the capacity to supply products through multi-center clinical trials, to establish a centralized clinical trials/regulatory affairs coordinating office to facilitate clinical research for rare pediatric diseases, to increase efficiency/can reliability of analytic assays to monitor safety and clinical efficacy of cellular immunotherapy trials and to develop collaborations necessary to sustain this infrastructure beyond the life span of the U01 grant mechanism. There will be a directed focus on training the translational workforce at each participating CTSA Hub and establishing standard processes and procedures that can be easily disseminated to other hubs in the future. Moreover, within we will establish key collaborations between academia and pharma to ensure long-term sustainability and to broaden our advantages towards applicable adult disease states. While our initial work will develop a limited consortium of CTSA sites, our long-term goal is to expand these processes for enabling the development of cellular immunotherapy trials to all CTSA sites and ultimately beyond.

PILIPONSKY, ADRIAN M.  
Center for Global Infectious  

Critical Role of Basophils in the Enhancement of...  

PROJECT SUMMARY  
There are approximately 850,000 new cases of sepsis each year with mortality rates ranging from 240,000–375,000. An impaired innate immune response can aggravate the septic condition by compromising the patient’s ability to combat an infection. However, the cells and mediators that enhance the innate immune response in sepsis are still unknown. Basophils account for less than 1% of peripheral blood leukocytes, which makes them the rarest known granulocytes. Basophils are evolutionarily conserved in many...
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<th>Disease Research (CGIDR)</th>
<th>The Innate Immune Response during Sepsis</th>
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<td>animal species. suggesting a beneficial rather than deleterious role of basophils. Nevertheless, it is unknown whether basophils play any role in the host’s defense against bacteria that can potentially prevent sepsis development. Oursuprimary studies support such a role by showing that basophils are one of the very first cells to accumulate at the infection site at early stages of infection, and can improve survival and bacteria clearance in the polymicrobial model of sepsis induced by cecal ligation and puncture (CLP). We think that our findings in the murine system may be translatable to humans because we observed that trauma patients show increased numbers of basophils in circulation when a nosocomial infection was circumscribed to local tissues (early stages of infection) while basophil numbers decreased or remain unchanged when a patient developed systemic infection (bacteremia) and was therefore at high risk of developing sepsis. Based on these studies, we hypothesize that basophils play a protective role in sepsis by enhancing the innate immune responses against infection. Accordingly, we propose a research plan aimed at investigating the contribution of basophil to the innate immune response against bacteria. In Aim 1, we will identify mechanisms involved in basophiliactivation during an infection. We will use a genetic approach to investigate whether basophil stimulationthrough the TLR and MyD88 pathways is required to induce basophil activation and to confer protection during infection; and we will examine whether the epithelial cell-derived cytokine, thymic stromal lymphopoietin (TSLP), can enhance the ability of basophils to respond to an infection. In Aim 2, we will define the mechanisms by which basophils confer protection against bacterial infections. Specifically, we will investigate interactions between basophils, the endothelium, and circulating leukocytes in a microvascular system and will use mice with basophil-specific TNF deficiency to study these interactions during CLP. In Aim 3, we will establish the relevance of basophils in human infections and sepsis. Specifically, we will use mass cytometry (CyTOF) to assess basophil immune functions in samples collected from patients that develop nosocomial infections, mainly pneumonia, and we will establish whether these immune functions associate with clinical outcomes. We think that the studies proposed will expand our knowledge of basophils and their role in host defense against bacterial infections. Specifically, our studies will provide, for the first time, evidence for a critical role for basophils in theheemnecrosis of the innate immune response against bacteria, an unexpected role for this rare cell population.</td>
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<th>PORTMAN, MICHAEL A</th>
<th>Center for Integrative Brain Research (CIBR)</th>
<th>Genetic Prediction for Treatment Resistance in Kawasaki Disease</th>
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<td>PROJECT SUMMARY/ABSTRACT Kawasaki Disease (KD) is a major contributor to cardiovascular morbidity in children. Poor response to IVIGremains one of the critical determinants of coronary artery risk in KD. The inability to predict this response and the potential for developing persistent coronary artery aneurysms serves as a major impediment topopgresst and development of intensified therapy. Currently available data indicate that KD susceptibility and treatment response, as well as the propensity for coronary artery disease, depend on an individual patient’s genetic background. Studies directed at identifying appropriate genetic biomarkers have been impaired by: 1) phenotyping lacking rigor, 2) use of genome wide association studies often employing chips or arrays for detection of common variants rather than low frequency or rare variants, 3) lack of clarity for the mechanisms of IVIG anti-inflammation in KD (necessary for guiding most pharmacogenomics studies) 4) focus on gene candidates, which are impractical for clinical testing, and 5) vague racial assignment methodologyconfounding pharmacogenomics. Furthermore, exome sequencing and analyses likely would miss potential important variants as IVIG anti-inflammatory mechanism includes transcriptional regulation at intergenic regions. We hypothesize that, by using improved and rigorous phenotyping techniques in combination with whole genome sequencing (WGS) and analyses, we will be able to identify select biomarkers for accurate prediction of KD treatment response and development of coronary aneurysms. The Pacific Northwest Kawasaki Disease Data Bank, established mainly through funding via PI Portman, R21HL090558, Thrasher Research Foundation; and PI, Shrestha, Southeastern AHA has accumulated DNA and clinical data from over 800 KD patients, eligible for pharmacogenomics analyses. We will leverage this wealth of data and clinical data along with recently updated AHAclinical KD criteria in order to identify rare and common variants, which determine IVIG treatment response. WGS will also allow a) identification of individual private SNPs (rare variants), b) identification of population-specific private SNPs, c) building a complete picture of genetic variations including structural variants (CNVs and insertions/deletions), d) gene-based analysis of both common and rare variants, and e) identification of actual functional SNPs as opposed to common or SNPs in linkage disequilibrium (LD). Additionally, we will account for race, an important variant in KD, by rigorous racial assignment using ancestry information markers and principalcomponent analyses. We will use rigorous methodology to achieve the following specific aims 1) Perform whole genome sequencing to identify genetic variations, which could serve as clinical biomarkers for IVIG resistance in KD patients. 2) Determine novel genomic variants associated with giant coronary artery aneurysms (GCA) among children with KD. 3) Prepare to assess if IVIG resistance is greater among African Americans and if this response depends on racial based differences in the frequency of genetic variations.</td>
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<th>RABBITTS, JENNIFER</th>
<th>Center for Clinical and Translational Research (CCTR)</th>
<th>Mechanisms of transition from acute to chronic pain in youth undergoing musculoskeletal surgery</th>
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| Chronic postsurgical pain (CPSP) has been recognized as a major health concern across the lifespan. Adolescents undergoing invasive surgical procedures are particularly at risk for CPSP, which occurs in about 20% of youth after surgery. CPSP is associated with significant functional, psychosocial, and psychosocial health-related quality of life. Chronic pain in adolescence also places individuals at risk for chronic pain in adulthood, as well as risk for substance use disorder, generating potential lifelong consequences on functioning, productivity, and quality of life. In our own preliminary studies in a small sample of adolescents having major surgery, we demonstrated that 1) youth who develop CPSP may follow distinct recovery patterns that emerge within the first 2 weeks following surgery, and 2) baseline psychosocial risk factors predict development of CPSP. However, further studies are needed to understand recovery during the first 30 days following surgery when acute postsurgical pain begins to transition to CPSP. Although there is indication from prior studies that psychosocial factors may play a role in CPSP, there has been limited data on further biopsychosocial mechanisms that influence the transition from acute to chronic pain in adolescents. These gaps in knowledge have limited the development and implementation of perioperative interventions targeted at the mechanisms of the transition from acute to CPSP. In this project, we aim to 1) develop valid and reliable outcome indices using short-term trajectories of pain, sleep quality, mood, and physical function over the first 30 days following spinal fusion surgery, and 2) determine the psychosocial and psychophysic mechanisms contributing to the transition from acute to chronic postsurgical pain. To address these aims, we propose a 2-site prospective longitudinal cohort study in 160 youth aged 10 to 18 years undergoing spinal fusion surgery, and their parents. Prospective assessments of pain, health, and functional outcomes will be conducted before surgery and at three time points during the 6 months after surgery. Immediately after surgery, adolescents will complete daily monitoring of pain, sleep quality, mood, and physical function in the first 30 days following hospital discharge using ecological momentary assessment tools.
assessment. These data will be used to develop valid and reliable acute recovery indices that predict CPSP at 3 and 6 months post-surgery. We will measure two potential sets of mechanisms underlying the transition from acute to CPSP, psychosocial variables and labatory-based psychophysical pain responses, before surgery and at 8-weeks post-surgery in order to determine the temporal influence on subsequent development of CPSP. This study will increase understanding of the transition from acute to chronic postsurgical pain and the causal mechanisms program is to develop effective periphero-peripheric interventions to reduce exposure to opioids and decrease incidence of CPSP in adolescents undergoing musculoskeletal surgeries.

RAJAGOPAL, LAKSHMI Center for Global Infectious Disease Research (CGIDR) Role of the hyaluronidase in GBS Virulence PROJECT SUMMARYMorbidity and mortality of preterm and newborn infants remain significant public health concerns. Streptococcus agalactiae or Group B Streptococcus (GBS) are a leading cause of bacterial infection-associated preterm births, stillbirths and early onset sepsis. We recently showed that increased expression of the hyaluronidase can be associated with GBS invasive disease and induces fetal demise in pregnancy. The goal of this proposal is to understand how the GBS hyaluronidase subverts the function of triple host innate immune cells to induce fetal injury and systemic infection. Aim 1 will define mechanisms by which the GBS hyaluronidase subverts immune cell function and immune cell recruitment. Aim 2 will establish how expression of the GBS hyaluronidase promotes microbial invasion of the amniotic cavity and fetal injury in the pregnant nonhuman primate model that closely emulates human pregnancy. These studies will provide novel insight into mechanisms of immune evasion during GBS infections. These results are essential and invaluable for development of novel therapeutic approaches to reduce the risk of GBS infection associated fetal injury, stillbirth, preterm births and neonatal infections.

RAJAGOPAL, LAKSHMI Center for Global Infectious Disease Research (CGIDR) Immune Control of Group B Streptococcal Placental PROJECT SUMMARYABSTRACTIntra-amniotic infection and inflammation remain a significant cause of preterm birth, stillbirth and neonatal morbidity and mortality. The objective of this proposal is to define early maternal and placental immuneresponses that are critical for resolution of bacterial infections at the maternal-fetal interface. Elucidation of immune events occurring at the maternal-fetal interface in human pregnancy is complicated by the inability of maternal and fetal compartments, which also imposes limitations on our understanding of the nature of the invading organism and cells that direct breathing control. We have overcome these challenges by using a unique chronically catheterized pregnant nonhuman primate (NHP) model that closely emulates human pregnancy. In this proposal, we will elucidate early immune mechanisms that result in bacterial clearance and define the key immune-cell types and host defense networks that protect the fetus from invasive bacterial infections. We will use the established NHP model of GBS infection to study bacterial clearance at the maternal-fetal interface using innovative methods including: 1) multidimensional flowcytometry to quantitate immune cell populations at the maternal-fetal interface, 2) single cell RNA-Seq to generate a transcriptional map of cell types and regulatory gene networks, 3) reverse phase protein array to analyze signaling cascades and host translational networks and 4) sophisticated computational modeling to link clinical metadata (e.g. bacterial burden, peak uterine activity) with single cell RNA-Seq and protein array data. The proposed aims will thus establish the temporal and spatial nature of immune responses and hosttranscriptional and translational networks essential for bacterial clearance at the maternal-fetal interface during pregnancy.

RAMIREZ, JAN M. Center for Integrative Brain Research (CIBR) Unraveling respiratory rhythm generation in the medullary network PROJECT SUMMARYBreathing is vital for survival, and failure to breathe is fatal. This has become tragically evident in the current opioid crisis. Breathing disturbances are also the cause of sleep apnea, which is another health issue of epidemic proportions. At the core of all these disturbances located within the brainstem. Two of these networks, the preBötzinger complex (preBötC) and the parafacial respiratory group (pFRG) are thought to give rise to inspiration and active expiration, respectively. During the initial funding period of this grant, we identified a third excitatory microcircuit, the postinspiratory complex (PiCo), which gives rise to a third breathing phase: postinspiration – the expiratory phase that follows inspiration. Based on our discovery, we proposed the triple oscillator hypothesis: i.e. three excitatory microcircuits (preBötC, pFRG, PiCo) give rise to the three phases of breathing. However, the discovery of PiCo raised an important unresolved issue: what is the role of the so-called Bötzinger complex (BoTC), a fourth region that contains respiratory neurons, and that is located rostral of the preBötC? Here we test the overarching hypothesis that the preBötC is not a small microcircuit, as previously thought, but that this network forms a dynamically regulated column contiguous with the BoTC. The extent of this column is dynamically regulated by synaptic inhibition, chemosensory inputs, and mechanosensory afferents. The project tests this hypothesis in three specific aims: Aim 1 maps the extent of respiratory activity along the medullary column. We will use electrophysiological, calcium imaging and optogenetic approaches to characterize the neuronal discharge patterns within this column. Aim 2 investigates the cellular determinants that control the extent of this column using intracellular and optogenetic recordings. We specifically test the hypothesis that a balance between synaptic inhibition, and excitation regulates the regularity, frequency and spatial extent of the column. To conduct aims 1 and 2, we will employ horizontal brainstem slices that isolate the entire ventral medulla and that are amenable to a rigorous cellular and network analysis. Aim 3 explores the dynamic regulation of the column in alert and anesthetized in vivo animals. We test the hypothesis that vagal and chemosensoryafferents play a critical role in regulating the spatial extent of this column by activating inhibitory neurons that are capable of shrinking and extending the inspiratory rhythmogenic network. The proposed research may lead to a better understanding of the fundamental question: how the brain generates rhythmic motor activity and how it integrates sensory information. Insights gained will also have important implications for understanding the cellular and systems level mechanisms underlying the mortality and morbidity associated with breathing disorders.

RAMIREZ, JAN M. Center for Integrative Brain Research (CIBR) Unraveling the dynamic mechanisms underlying opioid respiratory depression PROJECT SUMMARYThe opioid epidemic claims more than 50,000 lives every year and contributes to a significant drop in overall life expectancy in the USA. The primary cause of death associated with opioid-based analgesics and drugs of abuse is Opioid-mediated Respiratory Suppression (ORS). Although, the mortality risk increases in a dose-dependent manner, opioid use is particularly dangerous because it is unpredictable. Many conditions can lead to opioid use disorders, which is very common among opioid users. Opioids cause respiratory depression and terminal apnea by inhibiting rhythmic networks within the ventrolateral medulla. This project has 4 aims to explore the medullary mechanisms underlying ORS. Aim 1 employs a variety of electrophysiological, pharmacological, and optogenetic approaches in vitro and in vivo to explore how opioids inhibit the inspiratory rhythmogenic network. This opioid-sensitive network forms a column that dynamically extends beyond the well-known preBötzinger complex, a microcircuit that is essential for breathing. Aim 2 will obtain horizontal slices from this rhythmogenic column to dissect the pre- and postsynaptic mechanisms that are responsible for the cessation of inspiratory activity. Aim 3 will investigate how opioids inhibit
ROSENBERG, ABBY R  Center for Clinical and Translational Research (CCTR)  The Promoting Resilience in Stress Management (PRISM) Intervention: a multi-site randomized controlled trial for Adolescents and Young Adults with advanced cancer  PROJECT SUMMARY Cancer among Adolescents and Young Adults (AYAs) is particularly difficult because age-related developmental challenges of identity, relationships, and vocation may add to the burden of cancer. Compared to other age groups, AYAs have poorer psychosocial outcomes. Among AYAs with advanced cancer, too many miss opportunities to express their hopes, worries and end-of-life preferences, translating to both patient and parent distress. A potential barrier to improving these experiences may be that AYAs have few opportunities to develop the personal resources needed to handle adversity and articulate their needs. We have previously described the "Promoting Resilience in Stress Management" (PRISM) intervention for AYAs with cancer. This manualized, brief intervention is delivered in 4, 30-60 minute, one-on-one sessions, followed by a family meeting. It targets skills in stress-management and mindfulness, goal-setting, positive reframing, and meaning-making. All of these skills are associated with improved patient well-being in other populations, and findings from a recent pilot randomized controlled trial among AYAs with newly diagnosed cancer suggest PRISM is associated with improved perceptions of resilience, lower psychological distress, and higher health-related quality of life (HRQOL). This application proposes to build on our prior experience and add three critical knowledge gaps: (1) PRISM’s impact among AYAs with advanced cancer (as opposed to early stage cancer); (2) Associations between AYA-PRISM participation and parent outcomes; and, (3) Associations between PRISM skills and patient engagement in clinical decision-making. This funding opportunity seeks to test AYA-specific palliative care interventions designed to positively randomize controlled trial among N=144 AYAs (n=72 PRISM, n=72 Usual Care; ages 12-21) with the primary trial outcome of patient-reported HRQOL 3 months following enrollment. Secondary outcomes will include patient- and parent-reported anxiety and depression, and family “palliative care activation” (advocacy on behalf of AYA’s hopes and worries, engagement in goals-of-care conversations, and utilization of formal palliative care and psychosocial services). We hypothesize that AYAs who receive PRISM and their parents will report higher HRQOL, lower anxiety/depression, and higher activation when compared to those who receive usual care. This application offers an opportunity to expand the body of knowledge regarding methodologically rigorous evidence-based palliative care interventions and standards of care for AYAs with advanced cancer and their families. Ultimately, this research has the potential to reduce the burden of cancer in these vulnerable populations.

ROTH, CHRISTIAN LUDWIG  Center for Integrative Brain Research (CIBR)  Brain systems and behaviors underlying response to obesity  PROJECT SUMMARY Given the high prevalence of childhood obesity in the U.S. and the lack of durable weight loss without existing obesity interventions, new options that improve pediatric weight management are needed. Intensive-family-based behavioral treatment (FBT) is the gold-standard intervention for children with obesity and is focused on changing food environments and parenting around children’s eating. The proposed research is arenewal of the Brain Activation and Safety in Children (BASIC) study which used functional Magnetic Resonance Imaging (fMRI) to better understand if neurobiological factors impact success in FBT. In this study, 55% of children with obesity treated with FBT showed clinically significant reductions in BMI z-score, and evenafter successful treatment, over two-thirds of children increased their BMI z-score 6–12 months after ending FBT. At baseline pre-FBT, children with obesity, compared to children of healthy weight, exhibited an attenuated central response to a satiating meal in which they did not reduce activation by high-calorie meals.
food cues across a set of a priori appetite-regulating brain regions. This pattern also was associated with worse FBT outcomes among obese children undergoing FBT, specifically, less reduction in BMI z-score during treatment. Further, greater BMI z-score reduction during FBT was associated with a decreased neural satiety response after treatment. These findings implicate neurobiological factors as a negative input onto children’s ability to achieve and maintain clinically significant improvement in weight status via FBT. The proposed follow-up project builds upon these findings and investigates the hypothesis that adding a glucagon-like peptide-1 receptor agonist (GLP-1RA) once weekly drug intervention to FBT will augment BMI z-score reduction, even among children who seem initially resistant to FBT, by promoting greater reductions in neural activation in response to a meal. In a double-blinded randomized placebo-controlled clinical trial among 64 children aged 10-12 years old, Specific Aim 1 will test the effect of adding GLP-1RA to FBT on change in BMI z-score over atotol GLP-1RA treatment duration of 24 weeks and a subsequent 1-year observational follow-up period aftertreatment cessation. To provide mechanistic insight, Specific Aim 2 will test whether adding GLP-1RA to FBT impacts neural activation by food cues. Finally, the proposed research will investigate the role of a cellular inflammatory process in the mediodisal hypothalamus—so-called glia—which might contribute to impaired hypothalamic function, attenuated satiety responsiveness, and potentially to worse weight-management outcomes. Specific Aim 3 will test if hypothalamic glia is modified by FBT and/or GLP-1RA in children and in reduction of glia’s associated with better long-term outcomes. This research builds upon the team’s prior findings to test a pharmacologic intervention with potential to modify neurobiological barriers to treatment success. The long-term objective is to translate these findings to improve obesity interventions and sustain better long-term results.

SARKAR, SUROJIT
Ben Towne Center for Childhood Cancer Research (BTCCC)

Mechanisms of T Cell Memory Quiescence

ABSTRACT Cytotoxic T lymphocytes (CTLs) are a key element of adaptive immunity against intracellular infections and cancers. They are potent antigen-directed killer cells that specifically eliminate infected or diseased target cells. Following pathogen control, a subset of effector CTLs gradually shutdown functions, while retaining heightened responsiveness to future encounters with the same pathogen. Long-term persistence of quiescent CTL memory sentences is deemed a highly desirable outcome for vaccines and modern adoptive cell immunotherapies, to maintain their protective integrity. The goal of this proposal is to gain mechanistic insight into how functionally active effector CTLs convert into quiescent memory. The classic paradigm is that effector-to-memory conversion is the default differentiation pathway for memory precursor effector cells (MPECs) after antigen clearance. This is believed to occur simply because effusion of T cell receptor (TCR) stimulation. However, recent studies from our group and others that Treg exerts a critical role in the process of effector-to-memory transition following antigen clearance. Tregs promote the development of quiescent, yet functionally poised memory cells by suppressing effector and proliferative programs in memory-fated CTLs. In the absence of Tregs during effector-to-memory transition, critical metabolic and transcriptional remodeling does not occur, thus leading to memory failure. Based on tight expression levels of CTLA-4 on Tregs (amongst all immune cells), and recent human studies noting dysregulation of Tregs and hyperactivation of T and B cells in CTLA-4 haplosufficient individuals, we hypothesized that CTLA-4 is a critical mediator of Treg-dependent reprogramming of MPECs from effector-to-memory state. We further hypothesize that CTLA-4 mediates its effects through direct as well indirect effects on D8 T cells. Our preliminary studies showing that exogenous administration of CTLA-4 reduces homeostasis and fully reverses the defects in D8 T cell memory associated with the notion that CTLA-4 is the primary initiating signal for Treg-mediated memory D8 T cell quiescence. Using unique mouse models, and high throughput cellular and biochemical readouts, the goal of this proposal is to rigorously investigate the interplay of cellular networks, and transcriptional and metabolic programs in orchestrating Treg-mediated help during memory D8 T cell development. Over the last decade, there has been significant progress in understanding when and how memory fate commitment occurs. However, less is known about how quiescent memory cells are formed from effector CTLs once a filter is cleared, and how quiescent memory cells are maintained for life. Successful completion of proposed studies will uncover novel strategies for augmenting D8 T cell memory during immunization, and will also afford new immunotherapeutic approaches against cancers and for maintaining homeostasis in autoimmune disorders.

SATHER, D. NOAH
Center for Global Infectious Disease Research (CGIDR)

Development of a pre-erythrocytic P. vivax vaccine to prevent clinical relapse

ABSTRACT More than 3 billion people are at risk for contracting malaria caused by Plasmodium vivax (Pv). Pv infection differs from other Plasmodium species in that it develops dormant liver stage forms called hypnozoites. Hypnozoites can reactivate and cause blood stage malaria months to years after primary infection. As such, the dormant form is a major driver of P. transmission and accounts for nearly the entire clinical disease burden. Therefore, a vaccine that reduces or eliminates the formation of hypnozoites, and thus reduces relapse infection, would have a significant impact on both disease burden and transmission rates. Importantly, models suggest that this can be accomplished even in the absence of feteralizing immunity, because hypnozoites only form in a fraction of the infected hepatocytes. Current vaccines to prevent P. infection or relapse. Development efforts have been hampered by the inherent difficulty of working with P. in the lab, the lack of non-CSP antigens, and the lack of a biologically relevant system that mimics P. infection. Our long term goal is the development of a pre-erythrocytic vaccine against P. by creating novel PvCSP vaccines and P. falciparum genetically attenuated parasite (GAP) vaccines that can reduce or prevent relapse infection. To this end, we put together a research program that addresses all the major roadblocks of Pv vaccine development. We propose to evaluate the efficacy of PvCSP vaccines against relapse, and will study novel, non-CSP vaccine candidates that were recently identified to be part of the surface proteome. We will identify those that augment anti-PvCSP-mediated immunity and reduce or block the formation of hypnozoites. Finally, we will engineer P. (and potentially novel antigens) into the existing PfGAP platform that is currently under clinical evaluation. Importantly, we have partnered with Mahidol University in Thailand to enable us to work with wildtype P. sporozoites. Additionally, we propose to conduct our vaccine development in a completely humanized model system. We will evaluate our vaccines in humanized immunoglobulin mice and test efficacy in humanized liver mouse models of relapse infection, allowing for a more reliable translation of results in this study toward the eventual deployment of the vaccine into the clinic. Our ultimate goal is to develop a next generation relapse vaccine that is effective in reducing or eliminating relapse infection.

SATHER, D. NOAH
Center for Global Infectious Kinetics, evolution, and effector function

ABSTRACT A safe, effective vaccine remains the best hope for eradicating HIV-1, which now infects 37 million people worldwide and results in more than 1.2 million deaths and 1.8 million new infections each year. The only HIV-1 vaccine clinical trial to show vaccine efficacy was RV144, which achieved 31% protection from infection. Follow-on analyses identified immunological correlates of reduced risk of infection, which might contribute to improved hypothalamic function, attenuated satiety responsiveness, and potentially to worse weight-management outcomes. Specific Aim 3 will test if hypothalamic glia is modified by FBT and/or GLP-1RA in children and if reduction of glia’s is associated with better long-term outcomes. This research builds upon the team’s prior findings to test a pharmacologic intervention with potential to modify neurobiological barriers to treatment success. The long-term objective is to translate these findings to improve obesity interventions and sustain better long-term results.
| **Disease Research (CGIDR)** | **Project 2: Response to Treatment** | **Abstract** — Project 2The responses of Mtb to individual drugs and regimens and their relationship to treatment outcome remainstable and poorly understood. Our premise is that knowledge of bacterial networks and their regulatory controls constitute a powerful but underexplored window to novel targets and treatment strategies. The major goal of this project is to identify strain-independent and strain-specific bacterial networks associated with varying drug responses in Mtb, and their regulation. In Aim 1 this project will utilize carefully selected clinical drug-sensitive Mtb isolates exhibiting varying drug responses to characterize the genetic, transcriptional, and metabolic differences revealed by exposure to important anti-TB drugs, and then to map those changes to condition-specific drug tolerance phenotypes. We will subject each strain to detailed analyses including transcriptomics, metabolomics, and regulator-based genetic screens in response to frontline antibiotics and in conditions that promote tolerance. In Aim 2 we will employ the data from Aim 1 to build and refine regulatory network models that elucidate both common and strain-specific Mtb strategies to subvert drug action. These models will be refined by testing model-driven predictions through an iterative series of multi-omic analyses and perturbations including more focused experiments such as targeted protein interaction studies, bacterial cell sorting and solid-phase time-lapse microscopy. Ultimately, in Aim 3 we will test the extent to which the drug-response network models generated in Aims 1 and 2 predict clinical treatment outcomes, and identify potential strategies to interfere with adaptive drug-response network states to improve the efficacy of chemotherapy. We will further test the relevance of identified drug response networks in targeted studies of Mtb isolates from treatment failures in humans. The outcome of this project will be the identification and validation of the specific cellular networks associated with varying drug responses in Mtb and their regulation. | **SHERMAN, DAVID R**<br>Center for Global Infectious Disease Research (CGIDR) | **SU19AI135976-04** |
| **SHIC, FREDERICK**<br>Center for Child Health, Behavior and Development (CHBD) | **Complex versus Essential Autism: A Developmental Study of Risk** | **PROJECT SUMMARY/ABSTRACT** In recognition of the developmental heterogeneity of ASD, Miles and colleagues divided ASD into two groups: 'complex autism' and 'essential autism'. The label 'complex autism' grouped together children with ASD who had overt evidence of abnormalities of early morphogenesis, e.g. as signaled by the presence of multiple dysmorphic features and/or microcephaly and associated with lower IQ, motor delay, and lower brain volumes. In contrast, children with 'essential autism', by comparison, had fewer dysmorphofeature scores, had higher male to female ratios, and showed greater heritability of autism features within families. An implication of this work was that in complex autism, autism was expected to arise as the result of broad developmental insult that also impacted social function, whereas essential autism was viewed as the result of specific neural social systems dysfunction. In this study, we use this conceptualization of complex versus essential autism to longitudinally track from 6 to 36 months of age two groups of infants with distinct etiologies but common elevation of autism symptoms: very low birthweight (VLBW, n=100) infants, who, like children with complex autism, are expected to evidence a broad range of delays in multiple domains, and high-risk infants or siblings of children with ASD (HR-Sibs, n=100), who, as in essential autism, show heightened heritability of ASD symptoms and greater risk for social and communicative challenges. These groups are compared against control group of low-risk typically developing children (LR, n=100). We take promising eye tracking (ET) and EEG paradigms that have been associated with the emergence of ASD in HR-Sibs in the first year after lifeafter birth, and which were primarily developed to capture social dimensions of function, and extend them inorder to investigate analogous nonsocial information processing. We hypothesize that VLBW infantsevidencing ASD symptoms will show decreased performance in both social and nonsocial tasks, highlighting generalized difficulty with information processing consistent with broader developmental risk, whereas we hypothesize that difficulties in HR-Sibs with similar ASD symptoms will show more specific social (c.f. nonsocial) atypicalities. By adapting and extending paradigms which have shown strong or unique signal for later ASD in HR-Sibs, we will further our understanding of mechanisms underlying ASD risk and inform potential biomarker discovery; by pairing this with different etiologic risk groups, we will elucidate multilevel vulnerabilities that can shape developmental trajectories and the emergence of the disorder. In summary, this work will advance our understanding of developmental trajectories of risk associated with ASD, elucidate mechanisms underlying later emergence of core autism features, and help to test and refine the sensitivity and specificity of putative early neurobehavioral and neurocognitive biomarkers for ASD. | **SR01MH115913-03** |
| **SHIH, ANDY Y**<br>Center for Developmental Biology and Regenerative Medicine (CDBRM) | **Deciphering the Cerebral Microinfarct and Its Role in Vascular Cognitive Impairment** | **Project Summary** Numerous clinical studies have shown that cerebral microinfarcts are likely contributors to vascular cognitive impairment and dementia (VCID). However, the mechanism by which these small, but prevalent lesions lead to brain-wide neural dysfunction remains unknown. Our central hypothesis is that microinfarct injury leads to neural impairments that extend well beyond the restricted lesion cores seen during histological and radiological examination. These remote effects, when accumulated, are a mechanism by which microinfarcts cause large-scale disruption of brain function and cognitive decline. The rationale of the proposed research is to use mouse models where the timing and location of microinfarcts can be controlled in order to better understand how they cause brain dysfunction. We plan to examine: i) the spatial extent and chronicity of functional impairments induced by individual microinfarcts, ii) the cumulative effects of multiple microinfarcts, and iii) the cellular/ molecular changes that underlie their remote effects. Our model uses state-of-the-art methods for controlled optical occlusion of targeted cortical penetrating arterioles, individually and in multiples, to precisely and non-invasively form small regions of ischemic injury that mimic human microinfarcts. The associated injury processes can lead to tissue remodeling and the development of white matter abnormalities, as well as remote brain dysfunction. In Aim 1 we will use our model to test whether the systemic administration of antiplatelet drugs will interfere with the development of white matter abnormalities and remote brain dysfunction associated with cerebral microinfarcts. In Aim 2, we will establish a mouse model of microinfarcts that develop at multiple sites in the brain in a controlled manner, allowing us to examine how the location and distribution of microinfarcts in the brain influence the extent and chronicity of functional impairment. In Aim 3, we will establish a mouse model of microinfarcts that develop at multiple sites in the brain in a controlled manner, allowing us to examine how the location and distribution of microinfarcts in the brain influence the extent and chronicity of functional impairment. We will use this model to test whether the systemic administration of antiplatelet drugs will interfere with the development of white matter abnormalities and remote brain dysfunction associated with cerebral microinfarcts. In Aim 4, we will establish a mouse model of microinfarcts that develop at multiple sites in the brain in a controlled manner, allowing us to examine how the location and distribution of microinfarcts in the brain influence the extent and chronicity of functional impairment. We will use this model to test whether the systemic administration of antiplatelet drugs will interfere with the development of white matter abnormalities and remote brain dysfunction associated with cerebral microinfarcts. | **5R01NS097775-04** |
then be studied in vivo over time using parallel high-resolution two-photon fluorescence calcium imaging and 7T MRI to reveal detailed aspects of brain pathophysiology that are potentially invisible to MRI or histopathology. We further use behavioral paradigms that are sensitive to microinfarcts to uncover their effects on sensory perception and cognitive function. Aim 1 of the project tests the hypothesis that cortical microinfarcts induce sustained neuronal deficits beyond their lesion core following their strategic induction within the mouse vibrissa sensory system. It further examines whether aberrant changes to these deficits. Aim 2 of the project tests the hypothesis that the accumulation of multiple microinfarcts, spatially distributed throughout the cortices of both cerebral hemispheres, is sufficient to cause subcortical white matter degeneration (assessed invivo with diffusion MRI tractography and ex vivo with histology) and impairment in cognitive tasks. This work will complement clinical research on VCD in several ways. First, it will provide detailed mechanistic information on how, and to what extent, microinfarcts impair remote brain tissues. Second, it will clarify what aspects of microinfarct injury are viewed or invisible to MRI during life. This will provide unique in vivo MRI ex vivo histopathology comparisons to reveal the underlying biological processes that cause MRI signal change during gray and white matter injury. Fourth, it will establish first-of-its-kind in vivo experimental platform to study mechanisms of microinfarct-induced pathology and to gauge the utility of new therapeutic agents.

**SHNOHAVORIAN, MARGARETT**

Center for Clinical and Translational Research (CCTR)

**Testicular effects of modern chemotherapy regimens in osteosarcoma survivors**

**DESCRIPTION (provided by applicant):** Among the most important challenges faced by male childhood and adolescent and young adult (AYA) cancer survivors is the reproductive toxicity of cancer chemotherapy. Cisplatin and ifosfamide form the backbone of chemotherapy for some of the most common childhood and young adult cancers, but there is a gap in knowledge regarding the effects of cisplatin and the effects of ifosfamide without cyclophosphamide, on spermatogenesis and steroidogenesis in male AYA survivors of childhood non-germ cell cancer populations. DNA methylation changes are a possible mechanism of action of these drugs on testicular function.

A better understanding of these high-risk patients and better prevention strategies for testicular toxicity is expected to be developed for pediatric and AYA cancer treatment protocols. This study will comprehensively evaluate the effects of cisplatin with or without ifosfamide on spermatogenesis and steroidogenesis among childhood and AYA survivors treated with modern chemotherapies for osteosarcoma. Specifically, our aims are: 1) Determine whether infertility and/or biomarkers of spermatogenesis and steroidogenesis differ in male osteosarcoma survivors treated with cisplatin with or without ifosfamide compared to male controls without a history of cancer; 2) Evaluate whether cisplatin with or without ifosfamide for the treatment of osteosarcoma is associated with sperm DNA methylation patterns. Osteosarcoma survivors will be recruited from two COG therapeutic trials [COG AOST0331 and INT0133 (CGG9721 and PG9351)], and controls identified and recruited through address-based sampling. Subjects will complete questionnaires and provide blood, saliva, and semen samples through a mail protocol. Blood samples will be analyzed for testosterone, FSH, LH, inhibin B, androgen, and estrogen. Genomic DNA extracted from saliva and stored for future studies of host genetic variation in metabolism of chemotherapeutic drugs storage; and sperm DNA will be assayed using genome-wide methylated DNA immunoprecipitation followed by next generation sequencing. The assembled team and consortium brings together multi-disciplinary expertise in urology, andrology, oncology, endocrinology, biostatistics, and epigenomics. Completion of the proposed research will be a major step towards informing male childhood and AYA cancer patients receiving similar regimens about the adverse effects of their treatment and towards identifying high-risk groups that would benefit from targeted strategies for fertility preservation.

**SMITH, JOSEPH DOUGLAS**

Center for Immunity and Immunotherapies (CIIT)

**Molecular Mechanisms in Pediatric Cerebral Malaria Pathogenesis and Immunity**

**PROJECT ABSTRACT:** The human malaria parasite Plasmodium falciparum remains one of the most important causes of child mortality in the world. Cerebral malaria, the most severe complication of P. falciparum infection, is caused by by sequestration of infected red blood cells in cerebral microvasculature. The var gene or P. falciparum erythrocyte membrane protein 1 (PfEMP1) is the major cytoadhesion ligand for the parasite. While progress has been made in understanding the structure and function of PfEMP1 proteins, the key parasite ligand-receptor interactions involved in cerebral binding remain unestablished. Our recent studies have shown that specific parasite adhesion types are increased in the blood of cerebral malaria patients, and that parasite adhesion to endothelial protein C receptor (EPCR) may impair a key anticoagulant and injury protective pathway. Moreover, we have shown that hyperlactemia increases fatality risk in pediatric cerebral malaria. However, large knowledge gaps remain in parasite sequestration in brain, in large part due to its inaccessible and the lack of appropriate in vitro models. We have recently developed an innovative technology using 10 human brain microvessels that recapitulates physiological flow characteristics in health and disease. We are able to fabricate 3D microvessels with different geometries and lumen dimensions, which allow us to study parasite adhesion across a range of flow velocities in a single device, as well as to investigate factors that contribute to microvascular obstruction in malaria. In this project, we will use 3D human brain microvessels in combination with parasites isolated from pediatric cerebral malaria cases to investigate parasite tropism for brain, to identify the precise steps by which parasites adhere to brain endothelial cells, and to characterize potential interactions between lactemia and parasite adhesiveness, and to investigate antibody protective mechanisms in cerebral malaria. The proposed studies will advance our understanding of the molecular mechanisms of P. falciparum binding in cerebral malaria and immune mechanisms in anti-disease immunity.

**SMITH, STEPHEN**

Center for Integrative Brain Research (CIBR)

**Quantitative protein network profiling to improve CAR design and efficacy**

**PROJECT SUMMARY:** This grant is in response to PAR-18-206, Bioengineering Research Grants (BRG). Our goal is to adapt acute edge proteomic network analysis platform, Quantitative Multiplex co-Immunoprecipitation or QMI, to chimeric antigen receptor (CAR) T cell signaling. We will then use CAR-QMI to characterize signal transduction network activation downstream of the CAR, to both understand how the CAR instructs a T cell to attack and destroy cancerous targets, and to make batch-specific predictions about efficacy and side-effect profiles of CAR T cell products. CAR T cells are a breakthrough anti-cancer therapy that recently won FDA approval for relapsed B cell lymphomas. A true “personalized medicine”, CAR T cells are manufactured for each patient’s own T cells by transducing T cells collected by leukopheresis with a viral vector encoding a CAR. However, since each batch is unique, some batches perform better than others in terms of producing remissions and/or deleterious and sometimes fatal side effects including cytokine storms and neurotoxicity. The goal of this project is to develop a “personalized signal transduction network analysis platform” that can screen each batch of CAR T cells and predict the efficacy and side-effect potential of that specific batch. Because signal transduction networks integrate information from multiple input sources—forexample costimulatory and immunosuppressive cell surface receptors, patient genetic background, and T-cell specific history of activation— we hypothesize that this readout will be a powerful predictor of function.
Our preliminary data show that small changes in CAR design parameters such as scFV binding domain affinity produce measurable changes in signal transduction network state that correlate with functional variables such as target killing ability and cytokine release. Further, we show that there exists considerable individual-to-individual variation in batches of CAR T cells produced from different donors. Therefore, the two prerequisites for an individualized predictive assay are present—variation in our measurement across the population, and the functional relevance of our measurement to outcome parameters. Our interdisciplinary team consists of experts in CAR development, signal transduction, proteomics, and bioinformatics. Our ambitious but achievable goals are to expand the QMI panel to include CAR-specific components; to understand how CAR design parameters influence both signal transduction network states and functional performance measures; and to develop a predictive machine learning algorithm that translates QMI-derived signal transduction network states into a functional biomarker of in vivo clinical efficacy. Successful completion of these aims will (1) identify specific proteins or protein interactions that determine clinically relevant outcomes such as target killing ability, allowing CAR designers to rationally modify the design of CARs to target specific signaling outcomes; (2) provide clinicians with a test to predict the clinical performance of CAR T cells on a batch-to-batch basis; and (3) provide the community with a novel analytical platform to measure CAR activity.

SMITH, STEPHEN
Center for Integrative Brain Research (CIBR)
Investigating the synaptic pathology of Autism

PROJECT SUMMARY: Genetic mutations that confer autism risk often occur in genes that are expressed at the glutamate synapse. The protein products of these genes form a highly interconnected protein interaction network (PIN), and are present at diverse therapeutic targets since they are expressed throughout the lifespan and can be acutely targeted with small molecule drugs. However, the dynamic, network-scale behavior of this PIN in normal or diseased states is poorly understood. Here, we apply a novel PIN-mapping technology, quantitative multiplexed immuno-precipitation, to explore the input-output relationships of an autism-linked PIN at the glutamatergic synapse as it responds to physiological inputs. Our target system is a 20-member PIN, consisting of glutamate receptors, scaffolds, and signal transduction molecules; mutations in the genes encoding all target proteins have been genetically linked to autism. We first show that, in wild-type animals, our target PIN changes its pattern of co-associations in a stereotyped manner in response to acute stimulation with KCl or glutamate, using cultured neurons or acute slices. We then model the input-output relationships of the PIN system, and demonstrate that the PIN produces specific, recognizable signatures in response to stimulation through the GluR or NMDA receptors. In the context of physiological glutamate stimulation, the PIN integrates the two inputs to produce a coordinated cellular response—potentiation or depression. Based on these and other preliminary observations, we propose that mutations that contribute to autism risk disrupt information flow through this PIN, such that the balance between LTP-like potentiation and LTD-like depression is altered, ultimately leading to an organism-level imbalance between excitation and inhibition. We will test this hypothesis by modeling the PIN response to mGluR or NMDA stimulation in three distinct well-characterized animal models of autism: the Fragile X knockout, Shank3 knockout, and Ube3aoverexpressing models. We will characterize the input-output relationships for mGluR or NMDA stimulation and statistically model their integration using a vector transformation model in principal component space. We will define specific mechanisms by which autism-linked mutations disrupt either input-output relationships, or disrupt signal integration in the context of physiological stimulation. In addition, we will treat two of our animal models (Shank3 and Fragile X) with drugs that have been previously demonstrated to rescue autism-like behaviors. We will model the response of the PIN to the drug with or without concurrent stimulation to define a PIN signature associated with behavioral rescue. In summary, we propose to (1) define normal information flow through a PIN consisting of the protein products of autism-linked genes; (2) define how information flow is disrupted in mouse models of autism, with the goal of understanding the system sufficiently to design targeted drug treatments and (3) define how the PIN responds to drugs that correct behavior, which could serve as a template for the design of PIN-modifying treatments to restore normal synaptic function.

SODORA, DONALD L
Center for Global Infectious Disease Research (CGIDR)
Mediators of fatty liver disease during HIV/SIV and cART treatment

Liver disease is currently the most common cause of non-AIDS morbidity and mortality in developed countries amongst HIV-infected people. Indeed, non-alcoholic fatty liver disease (NAFLD) is more prevalent during HIV infection compared to the uninfected population occurring in 30-40% of HIV-infected individuals. Critically, fatty liver disease is becoming an increasingly recognized precursor to non-alcoholic steatohepatitis (NASH), which can further develop into cirrhosis and liver failure. Progression toward NASH and steatohepatitis is multifactorial, and includes metabolic changes, cytokine release associated with TLR stimulation and oxidative stress. With regards to HIV infection, the precise drivers and mechanisms of liver disease are not well defined. This proposal will utilize the pathogenic SIV infection of rhesus macaques and in vitro human cell cultures to delineate the early mediators that drive liver disease during SIV/HIV infection. Our previous study assessing livers from SIV-infected and SIV-infected-cART-treated macaques (assessed at necropsy) identified increased levels of bacterial 16s DNA in the livers of both groups. Importantly, an unexpected finding from this study was the enrichment of Mycobacterial 16s DNA in the liver of infected macaques, which we have subsequently identified as Mycobacteria smegmatis, a commensal or potentially opportunistic pathogen. These data, as well as previously published findings, have led to the hypothesis that translocation of bacteria and bacterial products to the liver (including Mycobacteria-associated dysbiosis) are key mediators of liver inflammation during cART-treated HIV/SIV infection and can initiate the early events that trigger fatty liver disease. This hypothesis will be tested through three specific aims: (1) identify the first two Aims to assess immune and microbiome changes within the liver, lymph node and blood in SIV-infected-cART-treated macaques. Aim 3 will evaluate the mechanisms underlying changes observed in human macrophages or hepatocytes utilizing in vitro experiments following exposure to HIV, cART and bacteria/PAMPs. Our goal is to delineate the role of bacterial translocation and microbiome dysbiosis in HIV/SIV-associated liver inflammation, with particular focus on mycobacteria. To undertake these aims, this study will be led by Dr. Sodora, who has 12 years of experience evaluating immune inflammation during HIV/SIV disease including previous studies assessing liver inflammation during SIV infection and cART-treatment. In addition, the team consists of Drs. Burwitz, Sacha and Smedly at the Oregon National Primate Research Center who have the necessary expertise to successfully undertake the outlined experiments. Collectively, these approaches will allow us to undertake a mechanistic assessment of the precise contributions of HIV/SIV virus, cART drugs and gut-derived microbes in liver inflammation as well as identify potential synergistic effects of these mediators when combined in a macaque or in vitro. Our long-term goal is to identify an immune therapeutic strategy to reduce incidence and/or severity of liver disease in HIV-infected and cART-treated individuals.
STUART, KENNETH D
Center for Global Infectious Disease Research (CGIDR)

Abstract: This project will determine how three closely related editosomes, precisely edit mRNAs and do so differentially between life cycle stages in Trypanosoma brucei. We hypothesize that insertion and deletion editosome compositional and structural differences enable differential binding and catalysis of specific gRNA/mRNA substrates during editing and the differential editing between developmental stages. We will: 1. Determine the high resolution structures of insertion and deletion editosomes, subcomplexes thereof, and RNA association by cryoEM. Samples for cryoEM will be purified from cells with one type of functional or catalytically arrested editosome. This will determine detailed editosome architecture, protein stoichiometry, RNA location and differences between these editosomes. 2. Determine the roles non-catalytic editosome proteins/domains. We will determine if endonuclease partner proteins function as heterodimers and if non-catalytic proteins function insubstrate RNA binding and positioning. The catalytic function of recombinant heterodimers will be assayed by crosslinking, mutagenesis and sequencing and functional RNA-protein interactions will be identified in vivo. 3. Determine how editosomes progress from one editing site (ES) to the next and test whether editing is either processive or progresses non-sequentially 3’ to 5’ and if endonuclease subcomplexes exchange between editosomes or not as they encounter different ESs. Cognate gRNA/mRNA pairs engaged in editing in cells with single or multiple types of functional editosomes will be identified and sequenced to resolve whether editing is processive or not. Proximal RNA editing signals that contain all three, combinations of two, one, or no functional editosomes that have specific tags and assayed to determine if editosome components exchange or not during editing. These results along RNAseq analysis of their edited RNAs will elucidate how the three different editosomes collaborate to edit multiple ESs specified by a single gRNA, including gRNAs that specify insertion and deletion. 4. Identify key aspects of developmental regulation of RNA editing. We will determine the order during development, if these are accompanied by differences in the abundances and editosome associations, of specific 3’ initiating gRNAs and cognate gRNA/mRNA pairs, and if these are impacted by mutations that differentially affect editing. Selected structures of BF and PF editosomes will be compared by CXMS, SILAC and cryoEM based on Aim 1. These studies will determine whether specific cognate gRNA/mRNA pairs are differentially bound and utilized in stage specific editosome differences. The project will provide key insights into how three poly-protein complexes function in an integrated fashion to precisely edit mt mRNAs and differentially regulate this process between life cycle stages which adapts energy metabolism to the different environments in the host cell the vector.

STUART, KENNETH D
Center for Global Infectious Disease Research (CGIDR)

Immune Responses to Malaria and HIV Infection and Immunization - Administrative Core

Within the context of this large multi-project and multi-center proposal it is imperative to establish a centralized administrative system necessary for tracking progress, documentation, communications and reporting and importantly for enhancing synergies among the components of the project and with external partners. The Administrative Core (1) will allow for centralization of all the practical matters to be handled by an experienced Project Manager in support of the scientific goals of Projects 1 and 2 and the Clinical (2), Data Management (3), and System Biology (4) Cores. The Administrative Core will facilitate exchange of information, tools and analytical approaches between the projects and the cores. It will serve as the main hub within the structure of the overall project for all non-scientific and non-analytical aspects of this research consortium, as well as the scientific components that emerge from individual research projects. The Administrative Core Project Manager will work under the direct supervision of the program PI as guided by the Scientific Leadership Committee and act as a liaison for individual project managers or P.I.s of Projects 1 and 2 and cores 2-4 and will ensure smooth operations and overall increased efficiency. This will entail assistance in managing budgets, organizing planning and discussions meetings, arranging necessary travel for project PIs, and relocating communications to/from the Steering Committee, SLC, ESAC, HIPC and NIH officials.

STUART, KENNETH D
Center for Global Infectious Disease Research (CGIDR)

Immune Responses Associated with Malaria Infection and Immune Protection - Project 1

Scientific Project 1: Immune Responses to Preerythrocytic Malaria

This project will comprehensively define human immune responses during the pre-erythrocytic stage of Plasmodium falciparum infection and vaccination with attenuated sporozoites which prevents infection and thus target this stage. It will identify immune signatures during the pre-erythrocytic, immunization and postvaccination periods in complementary vaccine trials that assess protection by controlled human malaria infection (CHMI). The primary endpoint is a correlation of vaccine-induced protection from infection, secondary endpoints are the effects of prior malaria exposure and differences in immunization protocols, vaccines and CHMI methods. The exploratory endpoints are the identification of signals that impact immune process that contribute to protection. The approach combines formal statistical methods, well-defined endpoints and system biology analyses. It systematically integrates high-dimensional with diverse immunological analyses to generate an expansive immunological view to identify and assess signals that are associated with protection. It aims to: 1. Identify immune responses during the pre-erythrocytic stages of infection. Virtually nothing is known about this stage of the infection in the liver during which there is extensive parasite replication and synthesis of new antigens. We will identify signals of infection and of immunization with attenuated SPZ during this stage and compare variable results among trials. 2. Determine immune response kinetics over the immunization period. We will identify the
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<th>Name</th>
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<td>STUART, KENNETH D</td>
<td>Center for Global Infectious Disease Research (CGIDIR)</td>
<td>Immune Responses to Malaria and HIV Infection and Immunization Malaria and HIV/AIDS are two of the most devastating infectious diseases, impacting millions of people worldwide. Effective vaccines against the pathogens that cause these diseases (HIV and Plasmodium falciparum) have proven elusive and traditional vaccine approaches are unlikely to succeed in eradicating either disease. In addition to our limited understanding of the desired immune responses to confer protection against the pathogens and our limited ability to elicit such responses, vaccine efficacy is also confounded by the diversity of pathogens, human populations, environmental exposures, and health status. The projects described hereinafter designed to support the identification of immune profiles that correlate with vaccine efficacy and are of potential relevance to protection against HIV-1 and P. falciparum infection. Beyond the importance of combating these diseases, the strategies for profiling immunity in response to infection and vaccination hold promise for garnering fundamental insights into the complexity of the immune system as a whole. Such insights will have potential for impacting strategies for vaccine development and for treating immune-related diseases more broadly.</td>
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<td>URDAHL, KEVIN B</td>
<td>Center for Global Infectious Disease Research (CGIDIR)</td>
<td>Eliciting lung-localized CD4 T cell responses against Mycobacterium tuberculosis in preventive and post-exposure settings SUMMARY An effective vaccination strategy is urgently needed to combat the global scourge of tuberculosis (TB). Due to the known importance of CD4 T cells and IFNγ in immunity against TB, current vaccine efforts are focused on boosting bulk IFNγ-producing CD4 T cell numbers (i.e., Th1 cells). These efforts, however, have been only moderately successful in conferring protection against TB in animal models, and a recent human efficacy trial of a novel candidate vaccine failed to confer measurable protection despite significantly boosting a Th1 response. Thus, there is growing concern that TB vaccines that target Th1 cells may not be adequately effective. We, and others, have recently discovered that adoptive transfer of fully differentiated Th1 cells, expressing high levels of the Th1-promoting transcription factor T-bet, provide little or no protection against murine TB, at least in part because they localize poorly to the lung parenchyma, the primary site of infection. In contrast, less differentiated CD4 T cells, expressing intermediate levels of T-bet and sharing properties with Th1 and central memory T cells, readily home to the M. tuberculosis (Mtbc)-infected lung parenchyma and mediate superior protection. Importantly, however, we have also recently shown that Mtbc infection itself drives Th1 cells toward terminal differentiation and a non-protective state. This complicates vaccine approaches in regions of the world in which TB is endemic because most individuals who benefit from immunization have already been exposed to Mtbc or are persistently infected. The goal of this proposal is to elucidate the mechanisms that enable protective CD4 T cell populations to enter and be maintained within the lung parenchyma and to evaluate the influence of pre-existing Mtbc infection on these parameters during vaccination in both animal models and human clinical trials. In Aim 1, we will determine the factors that govern homing and localization of CD4 T cells into the parenchyma, with the prime hypothesis that T cell KRG1 binds N-cadherin onto vascular endothelial cells, thus preventing entry. In addition, the role of key cytokines and glycolipid interactions in granuloma-associated high endothelial venules will also be explored. In Aim 2, we will assess whether vaccine-dependent CD103 expression serves to retain protective CD4 T cells within the parenchyma, and if so, whether this is beneficial or detrimental to immunity. Finally, in Aim 3, we will determine the impact of vaccine dose on priming protective, lung-homing CD4 T cell responses and how previous Mtbc exposure influences this priming. The access to clinical samples from dose escalation studies of infected individuals provides a unique opportunity to compare human immune profiles to those observed in mice and to translatore murine findings into testable hypotheses for human TB. The proposed experiments will provide a framework for the future design of novel vaccine delivery strategies with the ability to induce and maintain lung-localizing T cells even in TB endemic regions.</td>
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<td>WALKER, WILLIAM OTIS</td>
<td>Center for Child Health, Behavior and Development (CHBD)</td>
<td>Seattle Children's Urologic Management to Preserve Initial Renal Function Protocol for Young Children with Spina Bifida (UMPIRE Protocol) (Component C) No abstract available.</td>
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<td>YI-FRAZIER, JOYCE P</td>
<td>Center for Clinical and Translational Research (CCTR)</td>
<td>The Promoting Resilience in Stress Management PROJECT SUMMARY Adolescents with type 1 diabetes (T1D) are at high risk for elevated diabetes distress, which greatly impacts their adherence, glycemic control (A1C), and overall quality of life (QOL). A potential barrier to improving these experiences may be that adolescents have few opportunities to develop the personal resources needed to handle adversity and manage stress. The “Promoting Resilience in Stress Management” (PRISM) intervention is a manualized, brief, skills-based intervention delivered in 2, 45-60 minute one-on-one sessions, followed by a family meeting and supplemented by booster sessions and a digital app. PRISM was developed from 5R01DK121224-02</td>
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**Intervention: a multisite randomized control trial in adolescents with Type 1 Diabetes**

Stress and Coping theory and targets skills in stress-management and mindfulness, goal-setting, positive reframing, and meaning-making. All of these skills are associated with improved patient outcomes in diverse groups of adolescent populations with chronic/serious illness, and findings from a feasibility trial in adolescents with T1D showed PRISM to be highly feasible and desirable in this population. Further, a recent pilot randomized controlled trial among adolescents with cancer suggest PRISM is associated with improved perceptions of resilience, lower psychological distress, and higher QOL. This application proposes to build on our prior experience and fill three critical knowledge gaps: (1) PRISM’s impact on A1C among adolescents with T1D; (2) PRISM’s impact on diabetes distress, self-reported adherence, and other patient-reported outcomes including resilience and QOL; and (3) the cost-effectiveness of PRISM compared to usual care in a prospective economic analysis. This funding opportunity seeks to test interventions targeting diabetes distress for impact on glycemic control. Thus, we propose a multi-site randomized controlled trial among N=120 adolescents (n=60 PRISM, n=60 Usual Care; ages 13-18) with the primary trial outcome of glycemic control 6-months post-enrollment. Time-in-range will be evaluated for participants on continuous glucose monitors as an exploratory aim. Secondary outcomes will include diabetes distress, and patient-reported adherence, resilience, and quality of life. Cost-effectiveness will also be assessed to address the potential for sustainability and dissemination. We hypothesize PRISM will promote better glycemic control, improved diabetes distress, and be more cost-effective than usual care. This application offers an opportunity to expand the body of knowledge regarding methodologically rigorous and evidence-based interventions for adolescents with T1D. Ultimately, this research has the potential to offer a practical, skills-based curriculum designed to improve outcomes for this high-risk group.