- **Mark Kaplan, Ph.D.** – 2023 Awardee

  Professor and Chair, Department of Microbiology and Immunology
  Indiana University

  "Inhibitors Targeting Peanut-Specific Allergic Reactions"

Allergies are a result of allergen proteins cross-linking allergen-specific IgE (sIgE) on the surface of mast cells and basophils. The diversity and complexity of allergen epitopes, and high-affinity of the sIgE–allergen interaction are roadblocks in the development of allergen-specific inhibitors of allergic responses. This study presents the design of food allergen-specific sIgE inhibitors termed covalent heterobivalent inhibitors (cHBIs) that selectively form covalent bonds to only sIgEs, thereby permanently inhibiting them. We have developed peanut-specific inhibitors that have demonstrated efficacy and specificity in blocking mast cell or basophil degranulation and anaphylaxis using in vitro assays, ex vivo samples from peanut-allergic patients, and humanized mouse models. The next critical steps in moving these inhibitors to testing in humans are to manufacture GMP/GLP inhibitors for testing (Aim 1) and to perform more detailed dosing and toxicity studies in non-human primates (Aim 2). Together, these Aims will provide the critical data required to move forward with IND approval and preparation for phase I clinical trials.

- **Jonathan Kurtis, M.D., Ph.D.** – 2023 Awardee

  Full Professor Department of Pathology & Laboratory Medicine
  Rhode Island Hospital

  "APOPTOSIS-INDUCING ANTI-MALARIA DRUGS TARGETING PFGARP"

The overall aim of this application is to discover novel therapeutics for Plasmodium falciparum malaria. P. falciparum is a leading cause of morbidity and mortality in developing countries, infecting hundreds of millions of individuals and killing over 300,000 children each year. The spread of parasites resistant to the artemisinin family of compounds threatens recent progress achieved by antimalarial campaigns and underscores the urgent need to identify new antimalarial drugs. In previous work, we discovered PfGARP, a previously unrecognized vaccine candidate found only in P. falciparum.

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

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

- **Reshmi Parameswaran, Ph.D.** – 2023 Awardee

  Assistant Professor, Department of Medicine
  Case Western Reserve University

  "Allogeneic "off the shelf" therapy for B cell malignancies using BAFF CAR-NK cells."

CAR-T immunotherapies have produced remarkable clinical responses, but several challenges remain: disease relapse due to antigen escape, serious side effects (Cytokine Release Syndrome/neurotoxicity) and long lead times with high manufacturing costs. To address these challenges, we developed B cell activating factor (BAFF) ligand-based CAR-NK cells to target three antigens expressed by B cell cancers: BCMA, TACI and BAFF-R. With three antigen targeting, we anticipate mitigating antigen escape. Under the Catalyst award, I developed 15 new BAFF CAR designs, optimized for NK cells, and selected the CAR construct exhibiting maximum tumor killing. Preliminary data demonstrates the BAFF CAR-NK cells efficiently kill B cell cancers and secrete significantly low amounts of cytokines compared to CAR-T cells. This is expected to minimize CRS in patients without compromising the tumor killing potential. NK cells from random donors can be used to generate BAFF CAR-NK cells, enabling an "off-the-shelf" product. Manufacturing costs are significantly reduced as patient cell harvesting is eliminated. Under the Transformational Award, I will perform IND enabling efficacy and safety studies with humanized mouse models of different B cell cancers; and define the manufacturing process. I anticipate initiating clinical trials at the end of the 3-year period.

- **Richard Pomerantz, Ph.D.** – 2023 Awardee

  Associate Professor of Biochemistry and Molecular Biology
  Jefferson Medical College of Thomas Jefferson University

  "PROTACs for Targeting BRCA-Deficient Cancer"

DNA polymerase q (Polq) is a promising new synthetic lethal drug target in BRCA1/2 (BRCA) mutant breast/ovarian cancers. Polq promotes DNA repair via microhomology-mediated end-joining (MMEJ). Polq is essential for BRCA-deficient cells, but is dispensable for normal cells and Polq null mice show no phenotypes. Thus, Polq is an ideal drug target in BRCA-deficient cancers. Consistent with this, recently published Polq inhibitors (Polqi) selectively kill BRCA-deficient cells, while showing no effects in BRCA-proficient cells. Yet, whether these early stage Polqi will lead to clinically effective drugs is unknown. Towards the goal of developing a clinically effective Polqi, we have taken a novel approach for targeting Polq: to develop a Proteolysis Targeted Chimera (PROTAC) Polq degrader to completely abolish all Polq activities in cancer cells. Because Polq null mice exhibit no major phenotypes, complete degradation of Polq is expected to be a safe and effective therapeutic method. Based on this rationale, we plan to optimize the drug-like properties of our leading Polq PROTAC (developed in the Catalyst Award) and evaluate its anti-tumor activity against BRCA-deficient xenografts and ability to overcome PARPi resistance. Our leading PROTAC selectively kills BRCA-deficient cells and overcomes PARPi resistance, demonstrating its strong potential as a pre-clinical drug-lead.

- **Hongwei Yu, M.D.** – 2023 Awardee

  Professor of Anesthesiology, Research Division
  Medical College of Wisconsin

  "Initial Translational Development of Sensory Neuron-Specific Inhibition of Sodium Channels for Pain Therapy"

Our long-term objective is to develop a novel AAV-mediated peripheral sensory neurons (PSNs)-targeted approach for chronic pain treatment that is highly effective and selectively targets PSN sodium channels (NaVs), is safe, flexible (applicable in many settings), has minimal side-effects and without abuse liability, and is low-cost (since a single AAV injection has lasting genetic efficacy). We identified a novel NaV inhibitory peptide aptamer, named NaViPA1, derived from a regulatory sequence of NaV1.7. Dorsal root ganglia (DRG) injection of AAV-NaViPA1 significantly reduces pain behaviors in rat models of chronic pain, demonstrating NaViPA1 as a promising analgesic lead that, combined with AAV-mediated PSN-specific block of NaVs, has capability for peripherally targeted analgesic. Specific aims and milestones to achieve for the transformational phase studies include: 1) Develop a translationally suitable AAV (a double strand dsAAV without GFP for efficient in vivo expression of NaViPA1), 2) Validate long-term analgesic efficacy and safety of dsAAV-NaViPA1 administrated by DRG delivery in male and female rats with neuropathic and osteoarthritis pain, and 3) Determine NaViPA1 efficacy and molecular engagement in human induced pluripotent stem cells (iPSC)-derived sensory neurons. Completion of these studies will provide valuable and solid data for moving this novel pain-treatment approach to translational-level investigation.

Hermine Brunner, M.D., M.B.A., M.Sc. – 2022 Awardee

Professor of Pediatrics
Children's Hospital Medical Center dba Cincinnati Children's Hospital Medical

"Lupus Nephritis Biomarkers for Introducing Personalized Therapies and Improving Clinical Trials"

Our objective is to bring much needed non-invasive predictive urine biomarkers, i.e., the Renal Activity Index in Lupus (RAIL) that reflect kidney inflammation and activity with lupus nephritis (LN), into the hands of clinicians, researchers, and industry to improve LN care. Funded by the FALK Catalyst award, a multiplex assay (RAIL-MPA) was developed using cutting-edge FirePlex® technology. We also developed a RAIL-MPA using Luminex®xMAP® technology.
Methods/Approach: Collaborating with pharmaceutical companies that provide clinical data and urine samples from LN clinical trials, we will pursue generation of Laboratory Developed Tests (LDT) for the RAIL-MPAs using (1) FirePlex® technology and (2) Luminex®xMAP® technology. We will disseminate information about the value of the RAIL-MPAs among stakeholders.
Expected Results/Deliverables:
(1) To offer an LDT for the RAIL-MPA using FirePlex® performed by the CLIA-, CAP-, and GCLP-certified Nephrology Clinical Lab at Cincinnati Children's Hospital (CCHMC) in partnership with Ethos R&D
(2) To offer an LDT for the RAIL-MPA using Luminex®xMAP® performed by Ethos R&D
(3) Commercialization and dissemination of information about the RAIL-MPA and their impact on patient care and clinical trial design in collaboration with Ethos R&D, CCHMC Innovation Ventures, The Lupus Foundation of America and the Lupus Research Alliance.

Nikki Johnston, Ph.D. – 2022 Awardee

Associate Professor of Otolaryngology and Communication Sciences, and Microbiology and Immunology
Medical College of Wisconsin

"Inhaled Fosamprenavir for the Treatment of Laryngopharyngeal Reflux (LPR)"

More than 20% of the US population suffer from laryngopharyngeal reflux (LPR) with no effective medical therapy. We have identified a well-tolerated drug, which holds promise for the treatment of LPR. While the FDA has approved a proof-of-concept clinical trial using the high dose oral formulation, the long-term objective is to develop a dry powder inhaler for local delivery. Local administration of a low dose to the laryngopharynx can achieve the same therapeutically effective drug level as the high dose, oral formulation. It follows that a lower dose will be associated with fewer and/or less severe side effects. In the Falk Catalyst study, we demonstrated efficacy and safety of inhaled fosamprenavir in a mouse model. The next logical step is to perform IND-enabling inhalation toxicology studies required by the FDA for a phase I clinical trial. We request consideration of a Transformational Award to:
Specific Aim 1: Perform a 1-hour inhalation, single dose safety study of fosamprenavir.
Specific Aim 2: Conduct a 28-day, GLP, regulatory decision-making, inhalation toxicological assessment of fosamprenavir.
Novel delivery of fosamprenavir by dry powder inhaler will foster an industrial partnership to provide an effective treatment for the millions of people that suffer from LPR.

Jeannette Messer, D.V.M., Ph.D. – 2022 Awardee

Assistant Staff
The Cleveland Clinic

"Crafting New Weapons for the Fight Against Infectious Diseases"

Background: We have identified a conserved amino acid motif (CAMo-1) with an associated molecular feature in adherence proteins from a range of microbes. In our preliminary work, we identified this novel drug target and validated it experimentally by showing that a protein binding to this structure blocks microbial adherence to human cells. Overall hypothesis: Therapeutic agents binding to CAMo-1 will block microbial adhesion for treatment or prevention of microbial disease. Aims: 1) Identify a lead agent with optimal CAMo-1 binding and adhesion inhibition characteristics; 2) Validate the lead agent for activity against microbes isolated from patients; and 3) Characterize biological activity of the lead agent in an animal model of microbe-induced disease. Methods: We will use a set of antibodies derived from our lead hit molecule and assays designed to determine its functionality against bacterial type strains, patient-derived bacteria, and microbes in an animal model of disease. Broad, long-term objective: Develop a completely new type of antimicrobial drug designed to target dangerous microbial behaviors, regardless of the microorganism. This would be a paradigm shift in how these diseases are conceptualized and treated and a major advance toward cures for infectious diseases in which no cures currently exist.

Lonnie Shea, Ph.D. – 2022 Awardee

William and Valerie Hall Chair, Biomedical Engineering Steven A. Goldstein Collegiate Professor
University of Michigan

"A cell capture device to predict transplant rejection"

Over 36,000 solid organ transplants are conducted annually in the US, costing $30B. As there is no assay to predict rejection, clinicians rely on biopsy and one-size-fits-all immunosuppression, which increases toxicities and infections. Graft biopsy is flawed, as histological rejection inherently lags behind molecular biomarkers, and blood-based assays also measure lagging indicators of graft damage.
A novel method with is urgently needed to predict risk of transplant rejection. We have developed a subcutaneous biomaterial implant ("scaffold") that remotely collects biomarkers of graft health to preserve function while reducing the need for biopsy and aggressive immune suppression. We derived a 28-gene scaffold biomarker signature to predict the onset of mouse skin or heart rejection. In this work, we will perform a First-In-Human safety study of these scaffold implants in kidney transplant recipients. We will simultaneously enhance our scaffold-derived biomarkers of graft rejection in the context of clinical immune suppression and identify rejection during respiratory infection in mice. We hypothesize that these scaffolds will prove safe as long-term implants in humans and that the predictive power of the scaffold device will enable preventative immune modulation. This immune cell-capturing scaffold could prove transformative in personalizing immunosuppression for transplant recipients.

Sarah Slavoff, Ph.D. – 2022 Awardee

Assistant Professor of Chemistry
Yale School of Medicine

"Targeted degradation of a melanoma transcription factor"

Invasive melanoma kills thousands of Americans each year because nearly half of patients fail to respond to, become resistant to, or experience adverse events as a result of current treatments. Novel therapeutic strategies are therefore desperately needed. Innovative new technologies have enabled access to previously "undruggable" cancer-driver proteins, including the development of cell-permeable bicyclic peptide binders and proteolysis targeting chimeras, or "PROTACs", that promote target protein degradation. In our preliminary studies, we have developed a cell-permeable bicyclic peptide that binds to a melanoma transcription factor, and we will leverage this molecule to select efficacious PROTACs from a focused library of candidates to enable degradation of this transcription factor and inhibit proliferation in patient-derived melanoma cells. We will subsequently establish the efficacy of our molecules for transcription factor degradation and xenograft tumor inhibition in vivo, alone and in combination with existing and developmental melanoma therapies. If successful, our work will afford a first-in-class melanoma transcription factor degrader and demonstrate a novel therapeutic approach.

Allan Brasier, M.D. - 2021 Awardee

Institute for Clinical and Translational Research
University of Wisconsin-Madison

"Smart Nanoparticles Targeting the Myofibroblast Epigenome for First-In-Class Treatment of Idiopathic Pulmonary Fibrosis"

Idiopathic Pulmonary Fibrosis (IPF) affects over 3 M people. Triggered by smoke and other injuries, IPF is an unrelenting process of airway destruction and repair leading to death by asphyxiation within 3-4 years. No effective treatments are available. Here, structural changes are produced by bromodomain containing protein 4 (BRD4)-activated myofibroblasts that secrete extracellular matrix (ECM), such as fibronectin (FN). Advances from our Falk Catalyst Award showed that highly selective BRD4 inhibitors (BRD4i) improves reduces lung fibrosis in a mouse model. This is the first demonstration of an epigenetic therapy that reverses myofibroblast activation and fibrosis. We further have encapsulated BRD4i into core nanoparticles, that localize to alveolar spaces and reduce ECM production. We propose the following specific aims:

1. Optimize the formulation of BRD4 inhibitor-encapsulated FN-targeting DMs (FBP-DMs) for delivery to myofibroblasts in lung.

2. Demonstrate efficacy and safety of BRD4i-encapsulated FBP-DMs in a human-relevant rodent model of irreversible fibrosis.

3. Scale up BRD4i-FBP-DM synthesis and formulation for aerosol delivery.

Our Community engagement plan involves incorporating patients and Pharma input in project development. Our Commercialization plan takes advantage of a streamlined regulatory approval process for orphan disease approval and addresses a substantial unmet need in IPF therapy.

Chaitan Khosla, Ph.D. - 2021 Awardee

Chemistry
Stanford University

"A Novel Therapeutic Approach for Major Depressive Disorders"

We seek to prototype a small molecule agent with fast antidepressant activity for the treatment of major depressive disorders (MDD) based on the ability of acetyl-carnitine to epigenetically regulate neuronal plasticity. Acetyl-carnitine is known for its role in fatty acid oxidation. In rodent models of MDD, it induces faster antidepressant activity than well-known agents. In MDD patients, plasma acetyl-carnitine is lower than in controls; these changes correlate with increased symptom severity. Over the past year, our Catalyst Award facilitated the: (i) design, synthesis and testing of acetyl-carnitine analogs; (ii) development of assays to aid lead optimization efforts; and (iii) identification of a lead compound with superior activity to acetyl-carnitine at a lower dose in our most advanced mouse model of MDD. With the support of a Transformational Award, we will further modify our lead compound to increase post-translational acetylation required to alter gene expression and achieve fast antidepressant action. Specific Aims include: (1) further lead optimization; (ii) testing promising analogs in our mouse model of MDD; and (iii) identifying clinically relevant molecular pathways regulated by acetyl-carnitine using single-cell RNAseq. To accelerate entry of the new antidepressant candidate into the clinic, ketamine and fluoxetine will be used as reference compounds.

Takanori Takebe, M.D., Ph.D. - 2021 Awardee

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

"Developing Human Liver Organoid Therapy"

The foundation of this proposal lies in the recognition that breakthrough discoveries cannot change the world if they never leave the laboratory. Our team's long-standing commitment to basic science research has enabled key discoveries in organ development and function, resulting in cutting-edge iPSC (induced pluripotent stem cell) technology and team science supporting the generation of human organoids from human stem cells.

Through the Catalyst Award using lab-scale manufacturing protocol, we demonstrate the remarkable therapeutic potential of human liver organoid (HLO) to rescue urea cycle disease in a rodent model, the most common inborn error of hepatic metabolism. The data highlights the immense transformative potential of HLOs that supports the overarching vision of ORGANOID therapy - life saving treatments and therapies that can exponentially increase the survival rate of people suffering from liver disease, especially infants. The first step towards this goal is to focus on IND-enabling studies for first-in-human use of iPSC-derived organoids to replace damaged and diseased liver (focused on an ornithine transcarbamylase deficiency, or OTCD). We will develop clinical-grade and large-scale manufacturing protocols and preclinically identify risk/benefit profiles of mass-produced HLO. This work will establish a preclinical proof-of-concept for cell therapy approach by bringing ORGANOID CURE concept, allowing us to extend the therapeutic potential to other pediatric liver disease conditions that currently have limited treatment options.

Shirit Einav, M.D. - 2020 Awardee

Medicine (Infectious Diseases) and of Microbiology & Immunology
Stanford University

"Towards Predicting and Preventing the Development of Severe Dengue"

Dengue virus (DENV) is a threat to global and child health for which no effective vaccines or approved antivirals exist. 5-20% of symptomatic patients progress to severe dengue (SD), associated with morbidity and mortality. There are no accurate means to predict which patients will progress to SD. This project's goals are to: i. elucidate the cellular and molecular factors contributing to SD and decipher why children have worse disease outcome; ii. translate this knowledge into prognostic tools to identify patients at risk for progression to SD and countermeasures to prevent or treat SD.

We collected blood samples from 288 children and 163 adults prior to progression to SD in Colombia. Moreover, we developed a virus-inclusive, single-cell transcriptomic (viscRNA-seq) platform and a multi-cohort analysis to monitor host gene expression dynamics in the course of natural infection, while capturing tissue and real-world heterogeneity, respectively. Using these transformative approaches, we recently discovered the cell populations carrying DENV RNA, candidate biomarkers for SD prognosis, and a new class of potent broadly neutralizing antibodies. Ready for translation is the groundbreaking discovery of 8- and 20-gene sets predictive of SD that are generalizable across countries and ages. Lastly, we demonstrated a proof-of-concept for the utility of host-targeted approaches to combat DENV.

Aim 1 will use an integrative, single-cell immunological (viscRNA-Seq and mass cytometry (CyTOF)) approach to profile expression of host genes and proteins across multiple DENV-infected and bystander immune cell populations in uncomplicated and severe dengue children relative to adults. It will define the phenotype of DENV-harboring cells and age-dependent determinants of SD pathogenesis, and validate candidate biomarkers and antiviral targets. Aim 2 will compare the predictive power of the 8- and 20-gene sets, develop a sample-to-answer diagnostic/prognostic assay, and validate it in our cohort. The roles of prioritized factors in SD pathogenesis will be studied.

This bold, interdisciplinary proposal will further transformative single-cell and bioinformatics technologies and provide insights into SD pathogenesis with an unprecedented resolution. It will also yield host functions as candidate biomarkers and targets for preventive or therapeutic strategies and the first molecular diagnostic/prognostic assay for dengue, thereby potentially improving human health.

Nicholas Leeper, M.D. - 2020 Awardee

Surgery
Stanford University

"Precision Nanotherapies for Cardiovascular Disease"

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

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

Accordingly, we used the Falk Catalyst award to partner with experts in nanomedicine, bioengineering, and immune cell biology during to develop a 'precision' nanoparticle that could specifically target the diseased blood vessel. We generated an exciting new 'Trojan horse' therapy that homed to the inflamed macrophage, reactivated phagocytosis in the plaque, and potently prevented atherosclerosis without any off-target toxicity in mouse models.

Having achieved each of the milestones set forth in our Catalyst application, we now have the broad, long-term objective of fully translating these insights from bench-to-bedside. During the Transformational phase, we aim to test whether our nanoparticle retains its exquisite cell-specificity in explanted human arteries (Specific Aim 1- human), and validate its safety and efficacy in a large animal model of established CVD (Specific Aim 2– pig). Building upon robust preliminary data, we believe these final proof-of-principle studies will allow us to translate our discovery out of academia and into a drug-development program designed to prevent or even cure the leading killer in the United States.

Anita Shukla, Ph.D. - 2020 Awardee

School of Engineering
Brown University

"Advancing Bacteria-Triggered Hydrogel Therapeutics to Combat Antibiotic Resistance"

Antibiotic resistance is a global public health threat. With the lack of government and private investment, this threat is more pressing than ever before. Stimuli-responsive antibacterial drug delivery systems have the potential to provide effective treatments while reducing susceptibility to resistance development; yet most respond to non-specific stimuli, thus limiting translation to clinical use. Through the Catalyst Award, we have developed a bacteria-triggered drug delivery platform technology with tremendous promise for clinical translation. We synthesized beta-lactam containing biomaterial building blocks that are selectively cleaved by beta-lactamases (enzymes produced only by bacteria that inactivate beta-lactam antibiotics and cause antibiotic resistance). Upon molecular hydrolysis of the backbone, a macroscale degradation of the biomaterial enables bacteria-triggered release of encapsulated antibacterial agents. We have applied this technology to fabricate hydrogels due to their versatility as biomaterials and their many advantages when used as antibacterial wound dressings. We have developed and optimized beta-lactamase degradable poly(ethylene glycol) hydrogels and demonstrated the ability to control the release of model polymeric nanoparticles in the presence of beta-lactamase producing bacteria, including several of the ESKAPE pathogens, which are of particular interest due to the high rate of multidrug resistance in these bacteria. Through the Transformational Award, we propose to further advance this technology by investigating the antibacterial potential of these hydrogels. In the first aim, we will synthesize several model antibacterial nanoparticles, examine their efficacy against ESKAPE pathogens, and encapsulate those that display optimal antibacterial activity within our hydrogels. The stability of these hydrogels will be examined in conditions simulating future product packaging. The antibacterial efficacy in vitro and ex vivo will be investigated, along with resistance development, compared to conditions simulating uncontrolled release of antibiotics. In the second aim, an in vivo murine superficial skin wound infection model will be established and hydrogel efficacy in eliminating these infections will be examined. The data obtained in this project will provide critical information needed for future commercial and clinical translation of our bacteria-triggered drug delivery technology. We expect these materials will greatly improve treatment options for difficult to treat wounds prone to infection (e.g., diabetic ulcers and burns).

Mark Kay, M.D., Ph.D. - 2019 Awardee
Pediatrics
Stanford University

"Enhanced rAAV Mediated Genome Editing Using Ribonucleotide Reductase Inhibitors"

The goal is to safely co-deliver an FDA approved ribonucleotide(RNR) reductase small molecule inhibitor and a recombinant adeno-associated viral vector (rAAV)- that is designed to mediate enhanced levels (≥5-fold) of homologous recombination-(HR) levels such that a single-dose administration can permanently treat individuals suffering from life-threatening metabolic disorders.

Even though classical rAAV vectors show early promise in the clinic, limitations remain: (1) Inability to get life-long persistence in neonates/infants/children from any growing tissue such as the liver. (2)rAAV-vector administered into neonatal mice results in high rates (>50%) of hepatocellular carcinoma because rare integration events activated a proto-oncogenic because of the strong promoter used to drive the transgene.

Additionally, nuclease-mediated rAAV approaches are being considered for in vivo genome editing. This presents several problems: (1) Multiple rAAVs are required to transduce the same cell. (2) No means to limit expression or integration of the vector containing the promoter driven nuclease. (3) Off-target cutting, immunogenicity and/or insertional mutagenic risks remain.

We developed a new universal plug and play technology for AAV-mediated homologous recombination (AAV-HR) that overcomes the problems cited. Our nuclease-free, promoterless AAV-HR approach (called GeneRideTM), uses a vector containing a ribosome skipping sequence and therapeutic protein coding sequence flanked by homology arms to an endogenous gene. After AAV-HR, transcription from the endogenous gene locus produces a chimeric mRNA producing both the endogenous and therapeutic protein. This technique has been used to treat three mouse models of human genetic liver diseases. This has the potential for single administration lifelong cure in neonates, children, or adults, and mitigates current concerns for vector-induced cancers. Nonetheless, to truly make this a universal approach for many potentially treatable diseases, higher rates of AAV-HR will be required. Our previous funding via the Falk Catalyst award allowed us to discover that transient administration of a specific FDA-approved ribonucleotide reductase inhibitor enhances AAV-HR by 5-fold in cultured cells and mouse liver.

The plan is to further the path to the clinic. To do this we propose 3 aims.
(1) Optimize the AAV-HR/RNR inhibitor approach for the enhancement of genome editing in mice.
(2) Develop novel nucleoside analogs that we predict will have more specificity and hence enhanced potency and/or an improved safety profiles.
(3) Perform AAV-HR/RNR inhibitor studies in mice with chimeric human/murine livers as a model for human therapeutics.

Ari Melnick, M.D. - 2019 Awardee
Medicine/Hematology & Medical Oncology
Weill Medical College of Cornell University

"SIRT3 Targeted Therapy for B-Cell Lymphomas"

Our goal is to develop curative therapeutic regimens for the most aggressive forms of B-cell lymphoma, without unacceptable toxicity and in a manner that is widely applicable to patients regardless of access to the highest complexity health care. We propose that SIRT3 targeted therapy is important step to achieve this goal. Our preliminary data show that i) DLBCLs are broadly dependent on SIRT3 to maintain their survival regardless of genetic backgrounds and subtype, ii) SIRT3 expression is linked to inferior clinical outcome in DLBCL patients, iii) SIRT3 is required for lymphomagenesis in vivo, yet dispensable for normal B-cells. Mechanistically we showed that SIRT3 is the master regulator of anaplerotic metabolism in DLBCL, required to drive production of metabolic precursors through the TCA cycle to support the massive biosynthetic needs of lymphoma cells. Loss of SIRT3 function causes a precipitous drop in production of metabolic precursors in DLBCL cells, forcing them to engage in destructive autophagy which in turn triggers apoptosis.
The Falk Catalyst award enabled us to a) show that SIRT3 mediates its lymphoma effects through deacetylation of specific lysine residues in the glutamine dehydrogenase (GDH) enzyme, which drives its catalytic activity towards conversion of Glu to α-Ketoglutarate (αKG); and b) identify metabolic feedback mechanisms through which lymphoma cells might eventually develop resistance to SIRT3 inhibitors and which can guide development of combinatorial therapies. Most importantly, the Falk Catalyst allowed us to develop and prove the efficacy of YC8-02, the first SIRT3 selective inhibitor with potent ex vivo and in vivo anti-tumor efficacy.
Based on these data we propose this Falk Transformative Award to i) perform YC8-02 medicinal chemistry optimization, define the pharmacokinetic (PK) properties, and identify pharmacodynamic biomarkers to support clinical translation, and ii) facilitate and support the translation of SIRT3 inhibitors to the clinic by developing rational combination therapies, identifying and validating synthetic lethality and resistance mechanisms, and iii) identifying clinical predictive biomarkers. This collaborative project between leading experts in lymphoma biology and experimental therapeutics (Melnick) and in sirtuin biochemistry and medicinal chemistry (Lin), will deliver an entirely novel class of therapeutic agents for the treatment of the most aggressive and resistant lymphomas.

Mark Mitton-Fry, Ph.D. - 2019 Awardee
Medicinal Chemistry and Pharmacognosy
The Ohio State University

"Combating Antibiotic Resistance with Novel Bacterial Topoisomerase Inhibitors"

Methicillin-resistant Staphylococcus aureus (MRSA) is the leading cause of fatal infections from multidrug-resistant bacteria in the US. The Centers for Disease Control and Prevention (CDC) has identified MRSA as a serious threat. The dangers posed by antimicrobial resistance globally are even more striking: 10 million people per year will die from antimicrobial-resistant infections ("superbugs") by 2050 unless novel therapeutics are developed.

Our drug discovery/development program targets the inhibition of ubiquitous bacterial Type II Topoisomerase (TopoII) enzymes. These structurally unique compounds, known as Novel Bacterial Topoisomerase Inhibitors (NBTIs), have potential to circumvent/prevent multidrug resistance across diverse bacteria, including MRSA. Our vision is to deliver an NBTI into clinical practice for the treatment of multidrug-resistant bacterial infections, especially MRSA.

During the Catalyst Award period, we developed several lead compounds meeting the milestones/goals proposed (summarized below), effectively setting the stage for our current proposal to further develop/optimize candidate compounds for forward clinical development.

Milestones/Goals/Accomplishments

1) Dual Enzyme-Targeting: We tailored molecular properties of new NBTIs to simultaneously target the TopoII enzymes, DNA gyrase and TopoIV. We discovered several leads that potently inhibit both enzymes and one candidate which demonstrated a >10-fold reduction in the rate of resistance emergence in S. aureus. We also developed new computational tools to facilitate the optimization of dual-targeting NBTIs.
2) Cardiovascular Safety: One potent lead compound especially has little effect on hERG and other cardiac ion channels most closely tied to cardiac arrhythmias, a key limitation of earlier generation NBTIs in clinical development.
3) Antibacterial Spectrum: We exploited the ubiquity of bacterial TopoIIs to discover NBTIs with potency against not only MRSA but also a number of other pathogenic bacteria, such as vancomycin-resistant Enterococcus faecium (VRE), penicillin-resistant Streptococcus pneumoniae, and Acinetobacter baumannii, all of which are serious threats according to the CDC. We envision generation of an NBTI with therapeutic potential that extends well beyond MRSA.

We now propose to further develop NBTIs with in vitro/in vivo efficacy appropriate for preclinical development. Our integrated multidisciplinary research team will synthesize and prioritize candidates based on mechanism of action, low frequency of resistance, and cardiovascular safety. We will advance structural and computational tools to be used for iterative design and optimization of bacterial topoisomerase inhibition. Building on collaborations established during the Catalyst Award, NBTIs will be rigorously evaluated for pharmacokinetics, safety, and in vivo efficacy. Candidate NBTIs will be positioned for preclinical toxicology studies in anticipation of human clinical trials.

Xin Qi, Ph.D. - 2019 Awardee
Physiology & Biophysics
Case Western Reserve University

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

Mitochondrial dysfunction is an early prominent feature in patients with neurodegenerative diseases such as Alzheimer's (AD), Parkinson's (PD) and Huntington's disease (HD). Significantly, we recently reported in vitro and in vivo proof of concept that suppression of mitochondrial impairment is therapeutically effective in various models of these diseases. We used rationally designed peptides to demonstrate that improving either impaired mitochondrial dynamics or aberrant mitophagy was protective both in neurons derived from patient induced pluripotent stem cells (iPSCs) and in mouse models of these diseases, in particular HD. Because peptides often face challenges during drug development, we identified small molecules, including CHIR99021, that increase mitochondrial function as a new therapeutic approach. During the Catalyst Award phase, we characterized the mechanism of our lead molecule and validated its in vivo efficacy. The objective of this Transformative proposal is to generate and characterize optimized analogs of our lead molecule. If successful, our proposal will enable further drug development efforts to evaluate whether enhancing mitochondrial efficacy represents a novel therapeutic strategy in HD and other neurodegenerative diseases.

The Milestones of the Catalyst Award phase have been met. First, we have identified a key signaling mechanism by which CHIR99021 functions in HD cells. We found that CHIR99021 provided mitochondrial and neuronal protection in HD models via increasing Calpastatin protein level, which led to suppression of the calpain-CDK5-Drp1 pathway known to promote mitochondrial dysfunction in HD. While the direct cellular target of CHIR99021 in HD cells remains obscure, we have proposed new experimental approaches to address this question during the Transformative phase. Additionally, we have demonstrated that CHIR99021 is neuroprotective in a variety of HD models in vitro and in vivo. We established that CHIR99021 treatment reduced mitochondrial damage and neuronal death in neurons derived from HD patient iPS cells. Additionally, treatment with CHIR99021 reduced neuronal pathology and behavioral deficits in both the R6/2 and YAC128 mouse models of HD. These studies completed during the Catalyst Award period provide the mechanistic foundation and in vivo validation needed to support medicinal chemistry optimization of our validated lead. The goal of this Transformative Award proposal is to generate optimized derivatives of CHIR99021, confirm their cellular mechanism-of-action, and establish their efficacy in in vivo models of HD.

We anticipate that the successful completion of our studies provide optimized molecules and targets that will propel the field toward novel therapeutics for HD and other neurological disorders marked by dysfunctional mitochondria.

Markus Müschen, M.D., Ph.D. - 2018 Awardee

Department of Systems Biology
Beckman Research Institute of City of Hope

"CD25 as a Therapeutic Target in Refractory B-cell Malignancies"

Studying gene expression and clinical outcome data from 136 clinical trials for patients with cancer (~21,000 patients with 26 cancer types), we found CD25 as one of the strongest predictors of poor clinical outcome in patients with B-cell malignancies, but not in other cancer types. In addition, re-analyzing data from a genome-scale vulnerability screen (Tsherniak 2017), we identified CD25 as a specific dependency in B-cell tumors (B-ALL and DLBCL; n=11) but not in the other 490 cancer cell lines. This was unexpected because CD25 is known as one of three chains of the IL2 receptor on T-cells and NK-cells (Mier 1980, Siegel 1987, Noguchi 1993). In this Transformational Project, we validate two complementary strategies to target CD25-mediated feedback regulation of BCR signaling in refractory human B-cell malignancies.

CD25 is known as one of three chains of the IL2 receptor on T-cells and NK-cells. Based on genetic mouse models and engineered patient-derived B-cell leukemia and lymphoma xenografts, experiments during the Catalyst phase revealed that CD25 expressed on B-cells is not an IL2 receptor chain, but in fact binds the B-cell receptor (BCR) to regulate its activity. We identified CD25 as essential feedback regulator of BCR-signaling and oncogenic BCR-mimics in B-cell tumors.

CD25-function was regulated by cell-membrane translocation, which required phosphorylation of its cytoplasmic tail at S268. In a family with monogenic autoimmunity, a mutation immediately preceding S268 compromised CD25-surface translocation, which was restored by homology-directed repair of the S268 motif. CD25-interactome analyses identified PKC-delta as critical effector molecule downstream of CD25 to activate inhibitory phosphatases (e.g. SHIP1) and calibrate oncogenic BCR signaling in B-cell tumors. Owing to imbalances of oncogenic BCR-signaling and p53-checkpoint activation, Cd25-/- B-cell leukemia failed to initiate fatal disease in transplant recipients.

We propose three Aims to validate CD25-dependent feedback as new drug target and to develop strategies for therapeutic intervention: (1) test CD25 as a biomarker of oncogenic BCR-signaling, (2) validate CD25 as a therapeutic target in refractory B-cell tumors and (3) perform preclinical proof-of concept studies to validate safety and efficacy of strategies to target CD25 for the treatment of drug-resistant B-cell tumors. These strategies are based on a CD25 antibody-drug conjugate (ADC) and autologous T-cells that are engineered with newly generated chimeric antigen receptors against CD25 (CD25-CAR).

Harikrishna Nakshatri, Ph.D., B.V.Sc. - 2018 Awardee

Surgery
Indiana University Purdue

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

Preclinical studies of primary cancer cells are done after cells are removed from patients or animals at ambient atmospheric oxygen (O2, ~21%) yet, O2 concentrations in organs are in the ~3-10% range, with most tumors in hypoxic environment in vivo. While effects of O2 tension on tumor cell characteristics in vitro have been studied, typically at 1% O2, it is only after the cells were first collected in ambient air. Dr. Broxmeyer's lab showed that hematopoietic stem cells exposed to ambient air within minutes undergo irreversible differentiation through a phenomenon termed extra physiologic oxygen shock/stress (EPHOSS). Studies conducted during our catalyst award collaboratively by Drs. Broxmeyer and Nakshatri showed that EPHOSS affects cancer stem cell differentiation through diminished expression of stemness-associated genes. In addition, drug screening done at ambient air provided misleading information on sensitivity of cancer cells to targeted therapies. Cancer cells collected/processed/propagated at physiologic 3-5% O2 compared to ambient air were less sensitive to epidermal growth factor receptor antagonist erlotinib and the PI3K inhibitor BYL719. Additionally, EPHOSS could have an impact on selection of patients for immunotherapy as expression level of PD-L1, a clinical biomarker of immunotherapy response, was higher in cancer cells at 3-5% O2 compared to ambient air. Therefore, lack of consideration to EPHOSS during tissue collection could explain the limited translatability of preclinical models, particularly drug sensitivity/resistance studies, and poor clinical trials success rates. This proposal will further validate these initial observations in different contexts. Aim 1: we will determine sensitivity of mammary tumor and human ovarian cancer cells to 219 FDA-approved anti-cancer drugs collected/processed at 3-5% O2 vs. air. Syngeneic and xengraft models will validate therapeutic efficacy of drugs in vivo. Unbiased transcriptome analyses of untreated and drug treated cells will reveal potential mechanisms of intrinsic resistance and identify new combination therapies. Aim 2: we will use functional proteomics to identify signaling molecules that determine sensitivity or resistance to identified/validated drugs of aim 1 at 3-5% O2 and ambient air to allow a gene/protein expression signature of intrinsic drug resistance under physiologic O2. Aim 3: we will use preclinical models to investigate how/whether EPHOSS affects results of immunotherapy preclinical models and need to change tissue collection procedures to measure biomarkers of immunotherapy. Positive outcomes from these studies will have transformational impact on future drug screening strategies and changes in tissue processing for accurate selection of patients for chemotherapy and immunotherapy.

Stephen Strittmatter, M.D., Ph.D. - 2018 Awardee

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"Fyn Kinase Inhibition for Tauopathy in Dementia and Glaucoma"

Neurodegenerative diseases produce diverse symptoms, but share molecular mechanisms. Pathological misfolding, phosphorylation and accumulation of Tau protein are observed in Alzheimer's, Fronto-Temporal Dementia (FTLD-Tau), Chronic Traumatic Encephalopathy (CTE) and glaucoma. Our work has mapped a pathway in Alzheimer's from Amyloid-beta oligomers to synapse loss through Fyn kinase, and Fyn inhibitors are being tested in Phase 2a. It is known that Fyn associates with Tau and that Fyn inhibition reduces Tauopathy in Alzheimer's mice. We propose that Fyn inhibition may provide effective treatment for other Tauopathies, including CTE and glaucoma.

During the Catalyst phase, we treated FTLD-Tau and CTE mice with the Fyn inhibitor, AZD0530. In both models, kinase inhibition rescues cognitive deficits. Early prophylactic treatment fully prevents later deficits in transgenic FTLD-Tau mice, but delaying treatment after impairment occurs is ineffective. Here, we focus on CTE, in which the timing of tauopathy is clinically more discrete. Our novel CTE model uses repeated mild closed head injury coupled with chronic variable stress. Importantly, in this CTE model, the initiation of Fyn inhibition after the two-week induction fully reverses learning and memory deficits as well as aggregation of phospho-Tau. Because AZD0530 has a narrow therapeutic window in Alzheimer's studies, we will define the dose and time window for this exciting benefit in preclinical CTE as a precursor to clinical testing.

Vision loss in glaucoma is driven by increased intraocular pressure (IOP), and reducing IOP is the mainstay of therapy, though benefit is partial. There is a recognized unmet need for neuroprotective therapy to prevent progressive vision loss. It has been shown that glaucomatous retina exhibits Tauopathy, and silencing Tau expression eliminates ganglion cell loss. We propose that Fyn inhibition will reduce Tauopathy in glaucoma, preserving function and cell number separately from IOP lowering. Importantly, our Catalyst Preliminary Data reveal that retinal pattern ERG physiology, as well as retinal ganglion cell number, are maintained by systemic Fyn inhibition in a glaucoma model. In order to ensure a broad safety window for this successful glaucoma intervention, we have now developed an ophthalmic drop formulation of AZD0530, and pharmacokinetics studies show robust local delivery with limited systemic exposure. We will assess whether the ophthalmic AZD0530 formulation preserves retinal ganglion cell numbers and function in the glaucoma model. We will also generate IND-enabling toxicology data for this formulation. This work will allow clinical testing of Fyn inhibition as the first successful neuroprotective therapy for glaucoma.