

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 (α KG); 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).

“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 xenograft 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

CNNR, BCMM 436295 Congress Avenue
Yale School of Medicine

“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, Frontotemporal 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.

Andrew Aplin, Ph.D. - 2017 Awardee

*Sidney Kimmel Cancer Center
Thomas Jefferson University*

“Targeted Inhibitor Strategies in Uveal Melanoma”

Uveal melanoma (UM) is the most common intraocular malignant tumor in adults. Even after treatment of the primary tumor, up to 50% of patients succumb to metastatic disease. There are no FDA-approved therapies for metastatic UM and patient survival is poor. Our long-term objective is to inform effective treatment options for advanced stage UM. UM frequently (90%) harbors mutations within alpha subunits of heterotrimeric G proteins, GNAQ or GNA11. Mutant G alpha q/11 activate the MEK-ERK1/2 signaling pathway. Additionally, mutations in BAP1 are associated with aggressive disease and higher likelihood of metastasis. In many patients, macro-metastases do not develop for years-decades indicating early tumor dissemination and subsequent dormancy of disseminated tumor cells. When macro-metastases develop, the response to targeted therapies is low. In clinical trials, MEK inhibitors gave low response rates and marginally improved median progression-free survival compared to standard chemotherapy. How mutant forms of G alpha q/11 and BAP1 promote tumor initiation and progression in UM is poorly characterized and mechanisms of dormancy and therapeutic resistance are not known. In the Catalyst award, we generated new models of metastatic UM and utilized them to show that mutant GNAQ displays altered trafficking and to characterize an inhibitor of mutant G alpha q/11. Furthermore, we have engineered these models to alter BAP1 expression, to identify dormant cells, and to express proliferation reporters. We demonstrated synergy through multiple publications and the collaborative generation of new data. In the Transformational award, we will examine the effect of mutant G alpha q/11 localization on signaling and UM proliferation, identify the pathways regulated by mutant G alpha q/11, and dissect the role of these pathways in UM cell proliferation and migration. Second, we aim to identify dormancy-inducing signals and test dormancy-inducing epigenetic therapies to reprogram malignant UM cells into long-term dormancy. Third, we aim to optimize MEK inhibitor therapeutic strategies for late-stage UM by combining with CDK4/6 inhibitors in in vivo UM reporter models and patient-derived tumors. The multi-investigator application brings together expertise in signal transduction, mechanistic basis of dormancy and clinically-relevant targeted inhibitors. The team is complemented by access to large numbers of UM patients at Thomas Jefferson University. This synergy is expected to advance the understanding of the late development of overt metastatic disease in UM, shift the paradigm of metastasis treatment and address unmet clinical needs.

Samira Azarin, Ph.D. - 2017 Awardee

*Chemical Engineering and Materials Science
University of Minnesota*

“Scaffold-Based Vaccination Strategy to Target Metastatic Disease”

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

During the Catalyst Award, novel tools were developed to track in vitro and in vivo T cell activation. A comparison of heat, cryotherapy and irreversible electroporation (IRE) indicated that IRE holds the most promise in generating an immune response when a small number of tumor cells are destroyed, as will be critical for clinical application of this approach to disseminating cancer cells. In addition, IRE is an emerging technology with advantages over conventional focal therapies including lower cost, easy implementation, and preservation of the tissue architecture, which enables repeated treatment of the site.

This Transformational Award proposal utilizes a clinically-relevant, immunologically robust mouse melanoma model system to validate the technology needed for clinical translation of this novel vaccination approach through the following Specific Aims: (1) Optimizing IRE parameters to push the lower limit for the number of tumor cells needed to generate a robust CTL response, (2) Demonstrating that application of IRE to tumor cells captured in novel responsive biomaterials can lead to a clinically relevant reduction in metastatic burden when coupled with local and systemic immunomodulation. IRE field strength, pulse duration, frequency, and number of pulses will be varied to determine optimal conditions for activation of tumor-specific CTLs in vitro and in vivo when applied to small numbers of tumor cells. Biomaterials will be modified for uniform application of IRE and release of chemokines upon IRE treatment to investigate local and systemic immunomodulation in combination with IRE to enhance the immune response. This approach is patient-specific yet does not require large-scale ex vivo expansion of cells, and thus it has the potential to be broadly applicable to many types of cancer.

Paul Bollyky, M.D., Ph.D. - 2017 Awardee

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

“Targeting Bacteriophage to Treat Biofilm Infections”

The bacterium *Pseudomonas aeruginosa* is a major human pathogen responsible for chronic infections in diabetic wounds, Cystic Fibrosis (CF), and other settings. Because of increasing antibiotic resistance, the World Health Organization recently categorized *P.aeruginosa* as a “priority pathogen” of the greatest risk to human health.

P.aeruginosa is deadly in part due to its propensity to form robust biofilms - slimy layers of polymers and bacteria that promote adherence, antibiotic tolerance, and immune evasion. Bacterial pathways involved in biofilm formation are therefore tempting targets for novel therapies.

We recently discovered that *P.aeruginosa* biofilms have the physical properties of a liquid crystal as a result of entropic interactions between polymers and a bacteriophage (a virus) called Pf phage that is produced by *P.aeruginosa*. This crystalline architecture endows *P.aeruginosa* biofilms with impressive structural stability, making them tenacious and difficult to disrupt. We reported that Pf phage enhance biofilm adherence, antibiotic tolerance, and immune evasion.

The identification of a critical role for Pf phage in *P.aeruginosa* biofilm pathogenesis creates exciting opportunities for novel therapies. Supported by Catalyst Award funding, we created two novel, complimentary therapies against *P.aeruginosa* infection. Both harness the power of antibodies to provide specific immune protection against Pf phage.

First, we made a vaccine that prevents *P.aeruginosa* wound infections in mice. Our vision is that this vaccine could be administered to individuals newly diagnosed with diabetes or CF patients before they become infected with *P.aeruginosa* resulting in the generation of protective antibodies against Pf phage.

Second, we also created novel monoclonal antibodies (mAb) against Pf phage that promote clearance of existing *P.aeruginosa* infections. Because *P.aeruginosa* typically infects individuals with impaired immunity, these mAb may have particular benefit in hospitalized patients and the elderly who are unable to efficiently respond to vaccines.

In our Falk Transformative Award application we propose key pre-clinical studies that will position these therapies for evaluation in human clinical trials. In particular, we will develop assays for quantifying anti-Pf antibody levels in serum, we will define the affinity, specificity, and specificity of these therapies for *P.aeruginosa*, and we will evaluate these therapies in physiologically relevant wound models, including in a pig wound infection model and in an innovative human skin organ culture model that uses discarded surgical tissues. If successful, these efforts will yield novel classes of therapies against *P.aeruginosa*.

This program represents a bold and radically unconventional approach to treating *P.aeruginosa* biofilm infections and improving human health.

John Imig, Ph.D. - 2017 Awardee

Pharmacology & Toxicology
Medical College of Wisconsin

“Novel Therapy of Kidney Disease”

Renal disease in cancer patients and those being treated for cancer is an emerging and rapidly growing healthcare issue. Cancer therapies have increased cure rate and survival time, but anti-cancer drugs, such as vascular endothelial growth factor receptor tyrosine kinase inhibitors (VEGFRI) cause severe renal injury that significantly compromises their effective and safe use. This healthcare issue has led to the development, evolution, and emergence of a subspecialty, Onco-Nephrology. Current treatments only slow the loss of kidney function, or have no benefit at all. New approaches are urgently needed. Our ongoing project is attacking this emerging cancer arena and we are focused on developing epoxyeicosatrienoic acid (EET) analogs to protect the kidney from cancer therapy-associated toxicity. Our team made significant progress during the Catalyst Award funding period to clearly demonstrate that our novel small molecule EET analogs are a therapeutic candidate for kidney diseases associated with cancer therapies. A lead candidate EET analog, EET-C22, was identified and a kidney targeted EET analog was developed to enhance therapeutic potential. We have identified VEGFRI-induced hypertension and nephrotoxicity as an excellent path forward for EET-C22 as a therapeutic for kidney disease associated with cancer therapies. Our Transformational Award goal is to complete identified key gaps in EET-C22 preclinical studies and enhance the portfolio by developing kidney targeted EET analogs that will allow a reduction in the drug level needed to achieve efficacy, decrease side-effects, prevent potential interference with cancer therapeutics, and enhance overall therapeutic potential for kidney disease. The proposed Transformational Award project is innovative, in our opinion, because we have developed novel small molecule EET analogs that demonstrate great potential as a therapeutic approach for kidney diseases. Guided by strong progress during the Catalyst Award project, our Transformational Award goal will be completed by pursuing three specific aims: 1) Test the hypothesis that EET-C22 will treat kidney disease associated with cancer therapies without interfering with the anti-tumor actions; 2) Conduct essential studies with the lead candidate, EET-C22, to fill in identified Pre-IND application gaps; 3) Develop an EET analog that targets the kidney to enhance therapeutic potential. We are quite confident that our team and approach will deliver a first-in-class family of molecules with multi-faceted pharmacological activities that are well suited for treating kidney disease associated with cancer therapies.

Mark Feinberg, M.D. - 2016 Awardee

Medicine

Brigham and Women's Hospital

“MicroRNA-based Therapeutics for Diabetic Wound Healing”

Patients with diabetes are frequently afflicted with impaired wound healing that may progress into chronic, diabetic ulcers, often leading to complications including limb amputation with increased risk of cardiovascular morbidity and mortality. Consequently, innovative medical therapies are desperately needed in the treatment of diabetic wound healing to improve patient suffering and reduce health care-associated economic burden.

Accumulating studies demonstrate that angiogenesis, or the formation of blood vessels from pre-existing ones, may promote wound healing. However, angiogenesis is impaired in diabetic patients and the mechanisms controlling this process are not fully understood. Our published and preliminary studies demonstrate important roles for specific microRNA inhibitors in mouse models of diabetic wound healing. In this proposal, we will translate these findings using novel delivery and targeting strategies in mice, human skin preparations, and larger animal models of diabetic wound healing as IND-enabling therapeutics in an effort to improve outcomes.

MicroRNAs are small, single-stranded, non-coding RNAs that suppress the expression of target genes at the post-transcriptional level and are involved in a range of disease states. By microarray profiling, we originally identified microRNA-26a (miR-26a), and its target gene *Smad1*, among the most differentially expressed under diabetic conditions in endothelial cells (ECs). Using diabetic wound miRNA profiling, our preliminary studies indicate that the miRNA, miR-615-5p, is also differentially expressed in diabetic wounds and functions as an anti-angiogenic miRNA in a cooperative, non-redundant manner with miR-26a. Our preliminary studies show that expression of both miR-26a and miR-615-5p are glucose-responsive miRNAs in ECs. Punch skin biopsy wounding of diabetic db/db mice revealed increased expression of both miR-26a (~2-fold) and miR-615-5p (~4-fold) compared to WT mice. Preliminary studies reveal that administration of new chemically modified inhibitors to miR-26a and miR-615-5p may more effectively promote endothelial cell angiogenic functions. Mechanistically, inhibition of miR-26a and miR-615-5p in ECs increased their respective target genes and signalling pathways, *Smad1/BMP* signalling and *AKT/eNOS* signalling. We therefore hypothesize that miR-26a and miR-615-5p may serve as critical regulators of pathological angiogenesis in diabetes and propose to study the cooperative effect of miR-26a and miR-615-5p neutralization using complementary targeting and delivery approaches on dermal wound healing in mice, pigs, and human skin models as a translatable foundation for bringing these findings closer to the clinic as IND-enabling therapeutics.

Michael Nishimura, Ph.D. - 2016 Awardee

Surgery

Loyola University of Chicago

“TCR Gene Modified T Autologous Cells for Treating RCC Patients”

Renal cell carcinoma (RCC) is responsible for approximately 12,000 deaths every year in the US. When detected early, it is often cured surgically. However, most patients are diagnosed with advanced disease. Despite great progress with new treatments, metastatic RCC is generally lethal. Therefore, more effective therapies are needed for patients with this disease. Recent clinical trials using T cells that are genetically modified to express T cell receptors (TCR) or chimeric antigen receptors (CAR) have shown great promise in treating cancer patients. However, this treatment can only be used for selected malignancies because of the lack of tumor reactive TCR's and CAR's. Dr. Childs at the NHLBI/NIH isolated a HERV-E reactive T cell clone from an allogeneic stem cell transplant RCC patient. HERV-E is encoded by an endogenous defective retrovirus. While silent in normal cells, mutations in the von Hippel--Lindau (VHL) tumor suppressor gene lead to stabilization of the hypoxia-inducible transcription factor HIF-2 α which is a transcription factor leading to expression of HERV-E. Therefore, we believe that targeting HERV-E has tremendous potential to be safe and effective for treating RCC.

Using Falk Foundation Catalyst funding, we identified, cloned, and characterized a TCR that targets HERV-E. T cells engineered to express this HERV-E TCR were found to recognize HERV-E+ RCC in cytokine release and killing assays. A high titer retroviral producer clone was generated and is being GMP qualified for clinical use. Based on these results, this Falk Foundation Transformative Award application proposes to conduct a phase I clinical trial to treat 12 patients with advanced RCC. The GMP compliant T cells will be prepared at Loyola by Dr. Nishimura using the Falk Foundation funding. The patients will be treated by Dr. Childs at the NHLBI/NIH using Clinical Center resources. In addition to the proposed clinical trial, we will clone a different HERV-E TCR which is HLA-A2 restricted. This second TCR will expand the use of HERV-E TCR transduced T cells to more patients.

Stephen Strittmatter, M.D., Ph.D. - 2016 Awardee

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

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

One approach to target identification for AD is to study the biochemical basis for Amyloid-beta oligomer (A-beta-o) toxicity in neurons. We defined an A-beta-o --PrP-C--mGluR5--Fyn cascade that damages synapses in AD models. In this cascade, mGluR5 is a druggable target. Here, we seek to develop specific mGluR5 agents that preserve physiological function while blocking A β o pathophysiology. A lead compound has been effective in reversing memory and synaptic deficits in mouse transgenic models during the Catalyst stage.

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

This Falk Transformational project seeks to advance the success of mGluR5 and Pyk2 agents during the Catalyst phase towards a full drug development program to preserve synapses in AD. We will verify disease-modifying activity, validate with additional models, examine dual benefit for Tau pathology and provide toxicological safety information to advance these novel agents as potential disease-modifying drugs in AD.

Olufunmilayo Olopade, M.D. - 2015 Awardee

Medicine

University of Chicago

“Genomics, Metabolomics and Epigenetic Regulation in Breast Cancer”

This application represents the output from a fully integrated team of scientists, clinicians and community advocates who are committed to finding new cures for the most aggressive types of breast cancer by establishing a new Center for Innovation in Global Health. Global health is an area for study, research, and practice that places a priority on improving health and achieving equity in health for all people worldwide. Breast cancer is a heterogeneous disease and the morbidity and mortality from the disease has no geographic boundaries. The basal-like breast cancer subtype is unique in its aggressive behavior and overrepresentation in young women and women of African ancestry who have been underserved and understudied for too long. The cross-continent comparative studies that we have conducted over the past decade suggest that both genetic factors, which are common among Africans and African Americans, and environmental/lifestyle factors contribute to the aggressiveness of breast cancer in both populations. Our long-term goal is to reduce global disparities in breast cancer outcomes by developing novel strategies for screening, early detection and treatment of basal-like breast cancer in high-risk populations. We leverage our institutional strengths in chemistry, human genetics, systems biology, advanced imaging and experimental therapeutics. These advantages, together with our location in the ethnically diverse south side of Chicago and our exciting new collaborators make this a truly unique Transformative Research Program. Our overall goals are in two interrelated thematic areas with the following specific aims: 1) To personalize risk prediction for prevention and early detection of aggressive basal-like breast cancer. We will deploy tools for cancer risk assessment within a large network of primary care providers and examine whether population-specific polygenic risk scores can be used to stratify women into more meaningful risk categories so that our interventions to reduce risk can be better targeted and be more effective than current standards; 2) To find novel pathways that are dysregulated and potentially druggable in basal-like breast cancer. We will perform genome-wide gene expression, whole genome methylation sequencing and metabolomics profiles in early and late stage breast tumors. By performing chemical screens for drugs that target metabolic pathways, we will test two compounds in vitro and in vivo as potential new treatments for the most lethal subtype of breast cancer.

The University of Chicago has a unique history of organizing around research questions and cross-disciplines, which provides a robust foundation for the transformational program.

Richard Pestell, M.D., Ph.D. - 2015 Awardee

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Baruch S. Blumberg Institute

“Targeting CCR5 In Cancer Treatment”

The repurposing of FDA approved drugs may provide a more rapid path to new treatments for cancer patients. Our objective is to define the role of CCR5 in breast cancer progression and metastasis and to carry out a biomarker-driven clinical trial using a CCR5 inhibitor for high-risk breast cancer patients. To ensure the completion of this objective we have assembled a national collaborative team of experts who have worked successfully together for many years, including the Falk Catalyst Award. For the analysis of human breast cancer molecular mechanisms of therapy resistance (Dr. Pestell, Thomas Jefferson University), for the quantitative analysis of annotated breast cancer patient samples for target protein expression (Dr. Rui, Medical College of Wisconsin), and for breast cancer circulating tumor cells (CTC) and breast cancer clinical trials (Dr. Cristofanilli, Northwestern University).

Metastasis is the primary cause of death in breast cancer patients. No treatments are directed specifically to the metastatic process. We provide strong evidence that CCR5 is expressed in human breast cancer, and that CCR5 inhibitors, previously developed and FDA approved for treatment of HIV, can effectively block breast cancer metastasis in preclinical models. Our studies from the current Falk Catalyst award show >50% of human breast cancer patients express CCR5 in their tumor. Thus a very large number of patients are candidates for CCR5 inhibitor therapy.

Understanding the mechanisms of resistance to current breast cancer chemotherapeutic agents is an urgent matter of broad importance to our patients. Breast tumor initiating cells (BTIC) are resistant to chemo- and radiation-based therapeutics. Our studies from the Falk Catalyst award demonstrate that CCR5 promotes BTIC formation. We show that the CCR5+ cells within the breast cancer are sufficient to drive breast cancer metastasis. Furthermore, we show CCR5 induces DNA repair in breast cancer cells after chemotherapy or irradiation. CCR5 inhibitors reduce the DNA repair response. Combination therapies aimed at reducing the DNA repair response selectively in tumors would be a highly favorable addition to current therapies.

In order to conduct an expedited clinical trial it is advantageous to identify a marker for the therapeutic target in the peripheral blood. During the Falk Catalyst Award we identified CCR5 on the surface of patient's CTC, to identify and monitor the candidate patients for treatment with CCR5 inhibitors. Our proposed studies provide the rational basis for repurposing of CCR5 inhibitors for CCR5+ human breast cancers that are resistant to current therapies.

Menachem Shoham, Ph.D. - 2015 Awardee

Biochemistry

Case Western Reserve University School of Medicine

“Novel Antivirulence Agents against MRSA and other Bacterial Pathogens”

MRSA is the most widespread bacterial pathogen in the developed world. In the US, MRSA caused 75,309 severe infections, resulting in 9,670 deaths in 2012. MRSA is resistant to most antibiotics and strains have emerged that are even resistant to vancomycin, the antibiotic of last resort. Thus, resistance to antibiotics and decline in the development of new antibiotics create an urgent unmet medical need to search for novel unconventional agents to prevent and treat MRSA.

Antivirulence agents present an alternative or an adjuvant to antibiotics. In contrast to antibiotics, antivirulence agents are not bactericidal and not even bacteriostatic. Their mechanism of action is based upon disarming the pathogen of toxins and virulence factors without killing it, thereby decreasing the pressure on the pathogen to develop resistance.

During the Catalyst Award period we have developed small-molecule inhibitors of the *S. aureus* quorum sensing response regulator AgrA, a transcription factor that drives the expression of a series of disease-causing toxins. Lead compounds F12 and F19 promote healing of MRSA-contaminated wounds in mice. F19 reduces bacterial load on kidneys in a murine MRSA model. Remarkably, F12 and F19 sensitize MRSA to beta-lactam and fluoroquinolone antibiotics, to which MRSA is resistant in mono therapy. This finding opens the possibility of reintroducing "old" antibiotics, such as penicillin, into the clinic. Preliminary toxicity studies have established F12 and F19 safety up to 200 micromolar concentration. This data forms the basis for the development of safe and potent novel treatment option against MRSA infections.

We now propose to further develop the preclinical data package of compounds F12 and F19 in vivo by exploring efficacy against an already established infection, extending the applicability to lung infections in wild type and cystic fibrosis mice, exploring protection of implants against infection in a murine MRSA graft model, and broadening the spectrum to other Gram-positive pathogens. Efficacy against Gram-negative pathogens will be explored by combination therapy with antibiotics. Possible emergence of resistance will be probed in vitro and in vivo. Medicinal chemistry will be employed to improve F12 and F19 solubility, stability and spectrum of applicability.

Antivirulence therapy has the potential to revolutionize treatment of MRSA and other bacterial infections.

A Falk Transformational Award will enable us to generate preclinical data for an IND application to the FDA and subsequent clinical trials.

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

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Yale School of Medicine

“Medical Therapy To Promote Neural Repair And Functional Recovery From Spinal Cord Injury”

Devastating and persistent neurological deficits occur after Spinal Cord Injury (SCI), despite survival of nearly all neurons. The primary cause of disability is disconnection of networks by axon transection. Even without full-blown regeneration, studies of electrical stimulation and weight-supported training demonstrate important gains from the few surviving, but otherwise silent, circuits in clinically complete patients. Thus, neuroplasticity and sprouting of axons are as important as regeneration per se, and medications to enhance all forms of axon growth are expected to be strongly synergistic with stimulation and training regimens. Recovery of some movement would be adequate for patients to gain a level of independence in wheel chair transfers, bowel and bladder management, and locomotion. Today, there is no approved medical therapy for the 300,000 to 1,200,000 individuals in the USA with SCI.

Our axonal growth studies included discovery of Nogo and Nogo Receptor (NgR1). We demonstrated their role in preventing axonal sprouting, regeneration and recovery after injury. With Falk Trust support, we have demonstrated that NgR1(310)-Fc is efficacious for recovery from SCI, even when treatment starts months after damage. It is being developed for human SCI trials with CSF administration. A small molecule therapeutic could broaden utility and reduce complications. With exciting preliminary data from the Catalyst period, we will advance a drug-like antagonist to rodent SCI efficacy testing.

While specific factors, such as NgR1, limiting axon regeneration have been identified, they provide an incomplete explanation for poor adult mammalian CNS regeneration. We completed a genome-wide shRNA screen for endogenous genes limiting mammalian CNS axon repair. We also conducted experiments to identify conserved genes that affect axon regeneration in the model organism *C. elegans*. Factors common to both systems are expected to identify fundamental regeneration mechanisms likely to benefit human patients. We aim to study and develop the translational potential of such mechanisms here.

One pathway is bioinformatically the most enriched gene set in the mammalian screen, and also regulates regeneration in *C. elegans*. The relevance of this pathway will be tested in preclinical models of traumatic SCI. Both gene deletion strains and pharmacological inhibition will be studied to provide a validated pathway for future therapeutic development. The findings will have high relevance for the development of novel therapeutics for SCI.

Michael Whitfield, Ph.D. - 2015 Awardee

*Dartmouth Scleroderma Center of Excellence
Geisel School of Medicine at Dartmouth*

“Mining Genomic Data to Identify Novel Therapeutics for Systemic Sclerosis”

Autoimmune diseases have a variable presentation and progressive course. Many are difficult to treat and their root causes are elusive despite the significant health burden they impose worldwide. Among the most difficult to treat is systemic sclerosis (SSc), a disease whose hallmark is skin fibrosis, but internal organ fibrosis and vascular disease are the main causes of death. One in three patients dies within 10 years of diagnosis giving SSc the highest fatality rate of any systemic autoimmune disease. There are no validated diagnostic markers and no curative treatments. SSc represents a significant unmet medical need, making it an ideal target for the Falk Transformational Medical Research Program (FTMRP). Finding effective therapies is critical and is the focus of this proposal.

SSc progresses through multiple molecular states of inflammatory, fibroproliferative and normal-like, that are identifiable by our recently discovered gene expression subsets. The ability to prescribe targeted therapies that modulate the underlying deregulated molecular pathway(s) in each patient is key. We have developed diagnostic strategies that identify underlying aberrant molecular pathways in patient skin. We have also shown that analyses of skin gene expression provide a window into SSc internal organ disease. In our Catalyst grant, we identified the macrophage/dendritic cell axis as being a central cell type in SSc.

The trigger(s) for SSc are unknown, although several studies have suggested the root cause may be immune surveillance of cancer or aberrant immune responses triggered by viral or fungal infections, within the context of a predisposing genetic background. Our hypothesis is that SSc results from an aberrant immune response mediated in part by pro-fibrotic macrophages, triggered by an environmental stimulus.

Our interdisciplinary team will address the four principal focus areas of the FTMRP by providing insight into the basic disease process, identifying biological markers of disease and therapeutic targets, and developing novel methods for therapeutic intervention.

Our aims are:

- Aim 1: Develop biomarkers of disease activity and progression into a marketable diagnostic test.
- Aim 2: Leverage our SSc multi-tissue network to better understand the pathways modulated in SSc drug trials and the critical cell types driving disease; use the network to reposition FDA approved drugs.
- Aim 4: Characterize tissue resident macrophages in SSc skin, lung and esophagus both immuno-phenotypically and using RNA-seq.
- Aim 5: Develop a strategy to therapeutically target alternatively activated profibrotic macrophages using CAR-T cell technology and / or bi-specific antibodies.