

## Dr. Ralph and Marian Falk Medical Research Trust Catalyst Award Program

**Amanda Garner, Ph.D.** - 2016 Awardee

*College of Pharmacy Department of Medicinal Chemistry  
University of Michigan*

“Leveraging the microRNA Interactome for Cancer Drug Discovery”

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MicroRNAs (miRs) comprise a large family of small, ~21–23 nucleotide noncoding RNAs that have emerged as key post-transcriptional regulators of gene expression and act by silencing the translation of target mRNAs. To date, there are ~1,000 predicted human miRs believed to control the activity of >60% of all protein-coding genes. Not surprisingly, these small RNAs have been shown to play crucial roles in nearly all aspects of human biology from development to disease. In cancer, global down-regulation of tumor suppressor miRs (TS-miRs) is commonly observed and has been demonstrated to be a causative feature in tumorigenesis. Loss can stem from genetic mutation or deletion, promoter methylation or dysregulation of miR biogenesis. Of these mechanisms, alteration of global miR biogenesis is receiving increased attention due to recent findings demonstrating the critical role that miR-binding proteins (miR-BPs) play in the inhibition of this process, ultimately stimulating TS-miR degradation and cancer development. Current strategies for restoring TS-miR function rely on replacement therapy, whereby chemically-modified and formulated mature miR mimics are delivered to cells and tissues. Despite the promise of these agents in xenograft mouse models, similar to RNAi-based therapeutics, many liabilities exist. These include the requirement for chemical modifications to enhance in vivo pharmacokinetic/pharmacodynamic properties, hybridization-based off-target effects, toxicity, drug delivery problems and high cost. As such, no miR-targeted drugs have yet been approved for use. To overcome these challenges and provide a druggable and transformative solution for restoring TS-miR levels and conquering cancer, we propose to target miR–miR-BP interactions. Our overall goal is to discover and develop small molecule-based inhibitors of these interactions to prohibit the degradation of specific TS-miRs, thus providing the basis for next-generation miR-targeted therapeutics for cancer treatment. These proposed investigations are based on the hypothesis that regulatory miR-BPs specific for select miRs have been evolved as a quality control mechanism for controlling mature TS-miR levels, and this network is hijacked in cancer. If successful, these studies will catalyze the development of new therapeutics for the treatment of cancer, in addition to the identification of new miR–miR-BP targets for future drug discovery efforts. Our Specific Aims are as follows: (1) To develop a high-throughput screening (HTS) assay of the let-7–Lin28 miR–miR-BP interaction for small molecule inhibitor discovery, and (2) To develop a strategy for the discovery of miR-BPs using chemically-modified pre-miR probes.

**Jeffrey Glenn, M.D., Ph.D.** - 2016 Awardee  
*Department of Medicine*  
*Stanford University*

“Targeting RNA Secondary Structure--Towards a New Class of High Barrier to Resistance Antiviral Agents.”

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Our broad, long-term objectives are to develop a new class of antiviral therapeutics that target essential RNA secondary structures present in viral genomes, and that can be developed for all viruses for which no effective therapies exist.

As proof-of-concept, we will seek to target the influenza virus RNA secondary structure that we have recently discovered by SHAPE (selective 2'-hydroxyl acylation analyzed by primer extension) and demonstrated to be a universal influenza virus packaging signal--absolutely conserved in all viral isolates from 1918 epidemic flu to H1N1 and H5N1 bird flu--that mediates both packaging of the influenza virus genome as well as in vivo disease. In particular, we will test our hypothesis that potent pharmacological inhibitors of the virus' packaging and life cycle can be identified using SHAPE, which offers a dramatic means of overcoming the critical scientific roadblock of conveniently determining viral RNA secondary structure targets, and identifying molecules capable of disrupting those targets. Our specific aims are to: 1) Perform the first SHAPE-based small molecule screen, and identify small molecules capable of disrupting the RNA secondary structure of the influenza virus packaging signal; 2) Identify a candidate lead molecule by determining the anti-influenza virus activity of the most potent hits from the SHAPE screen; 3) Optimize the lead molecule by determining its structure-activity relationships (SAR) using medicinal chemistry to synthesize analogs and assessing their SHAPE and antiviral activity; 4) Nominate a pan-genotypic anti-influenza virus development candidate with the best therapeutic index.

Successful accomplishment of our specific aims will not only result in a novel universal pan-genotypic inhibitor of influenza virus with a high barrier to the development of resistance that can be developed for clinical use; the exact same approach can then also be undertaken to develop an exciting new class of drugs targeting virtually any virus of interest--thereby opening up new avenues for treating and curing a wide range of diseases.

**Zhiwei Hu, M.D., Ph.D.** - 2016 Awardee  
*Surgery/Division of Surgical Oncology*  
*The Ohio State University College of Medicine and Public Health*

“Targeting Tissue Factor as a Novel Oncotarget for Immunotherapy of Triple Negative Breast Cancer Using a Second Generation Icon”

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Triple-negative breast cancer (TNBC), representing ~15% of breast cancer diagnosed across the globe, is considered as incurable malignancy. Due to the lack of surface markers for current targeted therapy, there are currently no molecularly targeted therapies approved for TNBC.

To eliminate the mortality associated with TNBC, there is an urgent need to discover novel surface markers for TNBC and develop corresponding targeted therapy for TNBC. We hypothesize that tissue factor (TF) is a novel surface marker and oncotarget in TNBC and that TF-targeted immunotherapy is effective for the treatment of TNBC. The rationale behind the hypothesis is that (i) our preliminary studies demonstrate that TF is over-expressed on TNBC cancer cells as well as the tumor neovasculature in over 85% of TNBC patients (n=14) when using standard paraffin-embedded tumor tissues or in nearly 60% of TNBC patients (n=157) when employing tissue microarray slides. Importantly, TF is not expressed on normal breast gland and quiescent endothelial cells of normal vasculature. Thus, TF is a novel surface marker in TNBC and targeting TF may constitute a novel targeted therapy for a majority of TNBC patients. (ii) Dr. Hu's prior work demonstrated that TF-targeted therapies are effective and safe in preclinical animal studies and clinical studies. He has co-invented and patented a TF-targeting Immuno-Conjugate agent named ICON that consists of factor VII (fVII, the natural ligand to TF) fused to the Fc region of human IgG1. As a neovascular-targeting agent, ICON has shown therapeutic efficacy and safety in preclinical animal models against solid cancers, macular degeneration (AMD) and endometriosis and in a Phase I trial for AMD patients. (iii) Dr. Hu has improved ICON by reducing 50% of molecular weight and completely eliminating its pro-coagulant effects yet with intact binding to TNBC, as a second-generation TF-targeting immunotherapy agent, called L-ICON. The major goal and specific aims of this proposal are to evaluate the novel L-ICON as single agent therapy and combination therapy with current immune modulatory agents (such as immune checkpoint inhibitors) and natural killer (NK) cells for TNBC. To ultimately translate the findings into clinic for TNBC patients, Dr. Hu is collaborating with breast cancer expert and clinical trialist, GMP manufacturing NK cell expert, pathologist and biostatistician on this proposal. If successful, the significance and impact of this project is that it will provide novel targeted approaches for the treatment of TNBC and hopefully to eliminate the mortality associated with this disease.

**Joseph Leventhal, M.D., Ph.D.** - 2016 Awardee  
*Comprehensive Transplant Center*  
*Northwestern University*

“Clinical Transplant Tolerance Induction by the Use of Ex Vivo Expanded Antigen Specific Regulatory T Cells”

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Organ transplantation currently relies on nonspecific immunosuppressive agents (IS) given life-long to prevent graft rejection. These drugs have significant and cumulative toxicities, including nephrotoxicity and increased rate of infections, malignancy, diabetes, and hypertension. The elusive goal since organs were first transplanted in humans is to establish donor-specific immunologic tolerance, a state where a donated organ is accepted as "self," eliminating the need for IS. Regulatory CD4+CD25+FOXP3+ T cells (Tregs) have been shown to induce tolerance in transplantation models and to be elevated in tolerant human recipients. However, there have been limited efforts to harness their therapeutic potential in the clinic.

Our long-term goal is to develop tolerogenic protocols that will lead to controlled IS minimization or withdrawal after organ transplantation. As a step in that direction, the overarching goal of this proposal is to develop a recipient derived, DONOR-specific expanded regulatory T cell "product" for use in kidney transplant recipients, to be pursued under two specific aims.

Specific Aim 1: To further define and optimize the conditions for laboratory-based expansion of allospecific Tregs

(A) Our approach will be to verify if greater expansion without the loss of efficacy can be obtained by a non-specific restimulation with anti-CD3/CD28-beads on day 14, following the initial donor-specific stimulation with "donor" irradiated B cells on Days 0 and 7. (B) After this optimization, we will make two more small-scale Tregs expansions in the laboratory and then test these for their in vivo safety and efficacy (below).

Specific Aim 2: To optimize the conditions for large scale (i.e., clinically applicable) expansion of allospecific Tregs. The technology optimized in the laboratory will be transferred to the GMP Facility where two large-scale expansions will be carried out.

The Tregs expanded in both the laboratory (n=2) and the GMP Facility (n=2) will be tested for their (a) in vivo safety, testing whether they would lose their suppressive properties following infusion and instead cause rejection of human skin grafts on NSG mice, and (b) in vivo efficacy, determining if they protect human skin grafts on humanized NSG mice from rejection. Completion of these studies will enable us to file for an IND from the FDA to perform a future phase I clinical safety trial during the Transformational Award Phase, and then to a future phase II efficacy trial. The significance of this proposal lies in its broad applicability for induction of tolerance in organ and tissue transplantation.

**Richard Lieber, Ph.D.** - 2016 Awardee  
*Physical Medicine & Rehabilitation*  
*Rehabilitation Institute of Chicago*

“Breakthrough Technology to Measure Human Muscle Function”

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Here at the Rehabilitation Institute of Chicago, we treat over 55,000 patients per year with a variety of diagnoses ranging from traumatic brain injury, spinal cord injury and stroke to Parkinson's disease and cerebral palsy. Virtually all of these patients suffer from movement disorders and weakness. Currently, the gold standard for diagnosis of neuromuscular problems involves painful and sometimes impossible muscle biopsies or very qualitative clinical exams. We believe the device described in this proposal to measure muscle properties will revolutionize treatment of these problems. Using this device, patient muscle function can be diagnosed and objectively characterized in a matter of seconds. This would have dramatic impact on the course of patient care and clinical decisions since muscle structure and function are so central to patient well being. While a few tools are available to collect in vivo muscle data, none can measure sarcomere properties noninvasively or with this degree of resolution. Necessary attributes are: simultaneous sampling across millimeters of muscle tissue, real-time sampling, compatibility with gross movement, inducing no or minimal tissue damage, and resolving nanometer-scale sarcomere length changes.

To solve this challenge, we are developing a new method termed resonant reflection spectroscopy (RRS). RRS is an optical method that relates reflected spectra to the muscle sarcomere structure. Unlike current methods, RRS operates through a minimally invasive optical probe, collects large sample sizes of sarcomere lengths, and is compatible with muscle movement and activity. In the catalyst stage, our goal is to develop RRS so that it can be routinely used in clinical settings. Specific aim 1 will develop a new optical source so RRS can be applied across general muscle architectures and geometries. Specific aim 2 will result in a robust optical probe designed for use in the clinical setting. Specific aim 3 will result in a packaged RRS system that can be used in a future transformational award across clinical settings. Our ultimate goal is to revolutionize the way that our patients with neuromuscular disorders are diagnosed and treated.

**David Masopust, Ph.D.** - 2016 Awardee

*Microbiology and Immunology*

*University of Minnesota*

“Ablating Tumors with Resident Memory T Cells”

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This proposal will address major limitations in the clinical effectiveness of emerging cancer immunotherapy regimens. Adoptive cell therapies and administration of checkpoint blockade antibodies, which depend on CD8 T cells of the patient's immune system to specifically kill tumor cells, are predicted to revolutionize how cancer is treated. This proposal will exploit tissue resident memory CD8+ T cells (TRM) to overcome two major limitations of cancer immunotherapy efficacy: 1) tumors represent a strongly immuno-suppressive environment, which antagonizes immune-based therapies, and 2) therapies need to localize to tumors but current approaches are systemic and not anatomically targeted. TRM are a recently defined lineage of T cells that comprise the most abundant immunological surveyors of non-lymphoid tissues in mice and humans. TRM are established throughout the body as a consequence of natural infections and vaccines. TRM reactivation, achieved via topical application of cognate peptide, rapidly licenses these cells to direct the activation of all arms of the immune system, triggering a highly immune-stimulatory environment. This response, referred to as the 'sensing and alarm' function, occurs locally and recruits adaptive immune cells and serum antibody to the site of TRM reactivation. Reactivated TRM also potently activate local innate responses, dendritic cell priming, and natural killer cell activation. We hypothesize that TRM reactivation can reverse the immunosuppressive tumor microenvironment and can be exploited to enhance existing adoptive cell transfer and checkpoint blockade therapies. This proposal will answer two major questions. 1) Can TRM reactivation be exploited to promote an immune-stimulatory environment within solid tumors and mediate tumor regression? 2) Does TRM reactivation recruit engineered anti-cancer chimeric antigen receptor (CAR) T cells and checkpoint blockade antibodies to solid tumors? These questions will be addressed in autochthonous inducible tumors of the female reproductive tract (uterine and/or ovarian cancer) and skin (melanoma) in mice. Successful outcome will provide proof-of-principle demonstration of a human translatable strategy for improving upon cancer immunotherapies by modifying tumor microenvironments and focusing therapies to tumor sites. Future pursuits would interrogate TRM biology, specificity, and function within human tumors and will define regimens for therapeutic application.

**Steven McMahon, Ph.D.** - 2016 Awardee

*Cancer Biology*

*Thomas Jefferson University*

“Modulation of the Anti-tumor Immune Response by the Myc Oncoprotein, Implications for Cancer Therapy”

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Perhaps the most common hallmark of cancer is activation of the MYC oncogene. Nearly all tumor cells are strictly dependent on high MYC levels in order to drive malignant phenotypes like rapid proliferation, elevated invasion and migration, reprogrammed metabolism, and survival in the presence of cellular stresses like hypoxia and genetic damage. Despite this central role in the biology of cancer, strategies for directly targeting MYC have not been successful. We report here evidence suggesting that MYC may regulate an additional hallmark of cancer, i.e. immune evasion. At the molecular level, MYC drives expression of the TNF receptor family member CD30/TNFRSF8. While CD30 expression is known to be important in rare forms of lymphoma (e.g. Cutaneous T Cell Lymphoma/CTCL and Anaplastic Large Cell Lymphoma/ALCL), we demonstrate that more common lymphoid malignancies, and also common epithelial tumors, express CD30 as a surface antigen. Remarkably, CD30 levels are controlled by MYC in all tumor types we examined. Confirming the functional relevance of these observations, we demonstrate that blocking CD30 expression in either lymphoid or epithelial tumor cells causes rapid cell death. Thus, CD30 may serve as a biomarker and potential therapeutic target in cancers with elevated MYC. Furthermore, based on published findings on CD30 in Hodgkin's Lymphoma, it likely serves as an immune checkpoint molecule to provide immune evasion properties to MYC-driven tumors. The experimental plan outlined in this Catalyst Award proposal will formally test these highly impactful predictions. Successful completion of this proposed research will provide a solid rationale for a Falk Transformational Award that explores methods for exploiting these biomarker, targeted therapy and immune checkpoint blockade strategies.

**Markus Müschen, M.D., Ph.D.** - 2016 Awardee

*Department of Systems Biology*

*Beckman Research Institute of City of Hope*

“CD25 As Therapeutic Target in Refractory B Cell Malignancies”

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B cells critically depend on continuous survival and proliferation signals from a functional B cell receptor (BCR). In >80% of B cell malignancies, the tumor clone is driven by an oncogenic BCR-mimic. Oncogenic BCR-mimics include BCR-ABL1, EBV-encoded oncoproteins, RAS-pathway and NF- $\kappa$ B-pathway activating lesions. While CD25 mediates IL2 signaling on T cells, we recently revealed that CD25 is a critical feedback regulator of B cell receptor (BCR) and oncogenic BCR-mimics in human B cell malignancies. Indeed, we found that CD25 is a reliable biomarker of tumor clones driven by oncogenic BCR-mimics (e.g. BCR-ABL1, LMP2A, BRAF, NF- $\kappa$ B) and genetic experiments demonstrated that CD25 is critical for the initiation of B cell leukemia and lymphoma in transplant recipients. Surface expression is rapidly induced by activity of PKC- $\delta$  and NF- $\kappa$ B downstream of the BCR. CD25 then recruits an inhibitory complex to the surface to reduce kinase signaling downstream of the BCR or its oncogenic mimics. Analysis of three clinical cohorts revealed that high expression levels of CD25 are associated with poor clinical outcome in various B cell malignancies. While CD25 expression is associated with drug-resistance, inhibition of CD25 sensitizes multiple B cell malignancies to conventional drug-treatment.

Based on these and other findings, we propose three Aims to (1) elucidate mechanisms of CD25 regulation, (2) explore usefulness of pharmacological suppression of CD25 and (3) CD25 as target for immunotherapy in B cell malignancies.

**Aim 1: Mechanisms of CD25 regulation in B cell malignancies: Which mechanisms control transcriptional regulation (e.g. Rel-A/NF- $\kappa$ B) and surface expression (phosphorylation of cytoplasmic tail by PKC- $\delta$ ) of CD25 and how are these events related to oncogenic mimicry of BCR signaling?**

**Aim 2: Pharmacological suppression of CD25 in human B cell malignancies.** For suppression of CD25 at the transcriptional level, we will test the Rel-A small molecule inhibitor IT-901 (genetic validation based on RelA<sup>fl/fl</sup>, Nfkb1<sup>fl/fl</sup> leukemia and lymphoma cells). To inhibit translocation to the cell membrane, we will inhibit PKC- $\delta$  function by Enzastaurin and CID2858522 (genetic validation based on leukemia and lymphoma cells from Prdkcfl/fl and analog-sensitive Prkcd mice).

**Aim 3: CD25 as a target for immunotherapy of B cell malignancies.** We will test the efficacy and specificity of antibody-based targeting of CD25<sup>+</sup> B cell malignancies in humanized NSG-KitW41 mice. In two experimental series, antibody-drug conjugate (ADC) against CD25 (ADCT-301) will be studied.

**Tommi Raij, M.D., Ph.D.** - 2016 Awardee  
*Center for Brain Stimulation*  
*Rehabilitation Institute of Chicago*

“Re-Wiring the Human Brain”

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Re-wiring the human brain represents a critical frontier in clinical neuroscience. Behaviors and mental function are emergent properties of large-scale neuronal networks where the connectivity strengths between nodes define the network. Correspondingly, many neurological and psychiatric symptoms arise from network-level derangements where the structural and/or functional connectivities between separate brain areas have been altered by the disease process (e.g., sub-cortical stroke, traumatic brain injury) and/or secondary maladaptive network changes. While the adult brain has little capacity to re-grow damaged long-range axons, it might be possible to restore functional connectivity by training the brain to use an alternate route connecting the areas, with the ultimate goal of improving motor/behavioral/cognitive function. However, currently there are no clinical treatments available for restoration of functional connectivity between specific brain areas.

Here, we propose to develop a non-invasive brain stimulation/recording technique to functionally re-wire the human brain. Inspired by our previous animal studies and existing literature in healthy humans using paired associative stimulation (PAS), the instrument system utilizes two transcranial magnetic stimulator (TMS) channels to activate two cortical regions in precisely orchestrated temporal patterns to selectively up- or down-regulate functional connectivity between them. To this aim, we will develop new stimulation sequences and monitor the ensuing interregional connectivity changes with source-resolved electroencephalography (EEG). To improve the navigation and targeting accuracy of brain stimulation, we will use the newest-generation small but powerful cooled TMS coils, combined with our published TMS E-field computations with individual magnetic resonance images (MRIs) and a TMS navigator device. The TMS-EEG-MRI data will be correlated with connectivity estimates from high-angular resolution diffusion imaging (HARDI) diffusion MRI tractography and resting-state functional MRI (rs-fMRI), along with behavioral measures.

In summary, this proposal develops non-invasive techniques to transform our capability to manipulate brain interregional functional connectivity in humans, therefore laying the foundation for a new class of network-level therapies in disorders that involve white matter lesions or aberrant functional connectivity due to other reasons. We build the techniques within studies in healthy human subjects and stroke/TBI patients, but the findings will be applicable to a broad range of neurological and psychiatric disorders. If the Catalyst phase of the award is successful, the techniques may be applied in a controlled clinical trial during the Transformational phase. The TMS, EEG, and MRI devices are noninvasive, safe, and already FDA-approved, and the experiments can be conducted in their allotted time frames.

**Stephen Waggoner, Ph.D.** - 2016 Awardee  
*Center for Autoimmune Genomics*  
*Cincinnati Children's Hospital Medical Center*

*"Harnessing Natural Killer Cells to Treat Autoimmune Disease"*

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Systemic lupus erythematosus (SLE) is a devastating autoimmune disease in which autoantibodies drive potentially fatal tissue damage in hundreds of thousands of individuals in the U.S. Tragically, the disease frequently affects ethnic minorities, children, and women of child bearing age. There is no cure for lupus and the current standard of care for lupus relies heavily upon the symptom-suppressing use of immunosuppressive drugs that are associated with harmful side effects. To achieve remission in lupus, new treatments must be developed to restrain the pathogenic production of autoantibodies by B cells.

The laboratory of Dr. Stephen Waggoner at Cincinnati Children's has discovered a novel and critical role for natural killer (NK) cells in suppression of antibody production by B cells. His lab is currently funded to pursue means of inhibiting this regulatory function of NK cells to improve immune responses during vaccination and chronic infection. In this proposal, an innovative alternative strategy aimed at harnessing NK cells to eliminate follicular helper T cells (TFH) and suppress B-cell production of autoantibodies is proposed as a novel means to improve treatment and potentially cure lupus. There is strong precedence for the clinical use of NK cells in therapy of cancer. Investigators at our institution are developing chimeric antigen receptors (CARs) that can be expressed in NK cells to increase their ability to eliminate patient tumors. The premise of the current proposal, supported by Dr. Waggoner's preliminary studies, is that CAR-expressing NK cells can also be used to kill normal, non-cancerous cells that are central to the pathogenesis of autoimmune diseases like lupus.

This Catalyst Award will enable development of a novel CAR construct and transduction of primary human NK cells isolated from the blood of lupus patients. The subsequent experiments supported by this award will test the hypothesis that this CAR construct endows patient NK cells with the ability to specifically recognize and kill TFH cells. Furthermore, the ability of these CAR-expressing NK cells to suppress antibody production by B cells from lupus patients in vitro and in vivo will be evaluated.

This proposal represents an important paradigm shift in NK-cell biology and highlights a transformative use of NK cells in clinical therapy of lupus. Our broad, long-term objective is to introduce CAR-expressing NK cells into patients in order to reduce autoantibody secretion and promote remission of lupus and other TFH-mediated autoimmune diseases (e.g. rheumatoid arthritis) for which we have no cure.

**Andrew Aplin, Ph.D.** - 2015 Awardee  
*Sidney Kimmel Cancer Center*  
*Thomas Jefferson University*

“Targeted Inhibitor Strategies in Uveal Melanoma”

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Uveal melanoma (UM) is the most common intraocular malignant tumor in adults. Even after treatment of the primary tumor, 20-50% 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, either GNAQ or GNA11. Mutant GNAQ/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, even decades, indicating early tumor dissemination and subsequent dormancy of disseminated tumor cells. When macro-metastases develop, the response to targeted therapies is low. In phase II and III trials, the MEK inhibitor, selumetinib, had a <15% partial response rate and marginally improved median progression-free survival compared to standard chemotherapy. How mutant forms of GNAQ, GNA11 and BAP1 promote tumor initiation and progression in UM is poorly characterized and mechanisms of dormancy and therapeutic resistance are not known. We propose to create novel cell lines expressing fluorescently tagged forms of mutant G alpha q and 11 to test for altered subcellular localization and trafficking of mutant versus wild type forms of G alpha q and 11. We will also characterize the ability of novel inhibitors to block activated GNAQ and growth of UM cells. To characterize dormancy, we will generate inducible UM models to modulate BAP1 and test effects on response to dormancy inducing agents. Finally, we will develop novel reporter models to monitor signaling pathways and response to targeted inhibitors in UM cells in vivo. This multi-PI application brings together expertise in signal transduction, mechanistic basis of dormancy, novel UM models and clinically relevant targeted inhibitors. The synergy between team members 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.** - 2015 Awardee  
*Chemical Engineering and Materials Science*  
*University of Minnesota*

“Scaffold-Based Vaccination Strategy To Target Metastatic Disease”

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Novel treatments targeting metastatic cancer are urgently needed, as cancer mortality typically occurs upon the disrupted function of vital organs due to the growth of metastatic cancer cells. While focal ablation technologies such as application of heat, cold or electrical pulses to destroy tumor cells have shown promise in treatment of some solid tumors, they have not been successfully applied to disseminated disease. It has recently been shown that polymer-based biomaterial scaffolds can capture metastasizing cells in vivo, which provides 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 and release tumor antigens that will promote robust CD8<sup>+</sup> cytotoxic T-cell (CTL)-mediated destruction of disseminated cancer cells.

This proposal utilizes a clinically-relevant, immunologically robust mouse melanoma model system to demonstrate proof-of-concept of this novel therapeutic approach through completion of the following Specific Aims: (1) Utilizing polymeric scaffolds to attract metastatic melanoma cells, (2) Determining the optimal focal therapy strategy for release of tumor-specific antigens, and (3) Evaluating the immune response following focal therapy applied within implanted polymeric scaffolds in vivo. Poly(caprolactone) scaffolds will be engineered to recruit B16 melanoma cells. In parallel, heat, cryotherapy, and irreversible electroporation (IRE) will be applied to B16 cells in vitro to determine which focal therapy releases the most tumor-specific antigens upon cell lysis, and these lysates will then be loaded onto scaffolds containing immunostimulatory signals and implanted in mice to determine if the lysates can activate CTLs in vivo and promote an anti-tumor response following secondary challenge by tumor inoculation in the groin. The addition of immune checkpoint blockade (i.e. anti-CTLA-4 treatment) will also be evaluated to enhance the CTL response to the lysates. Finally, the focal therapy will be applied to disseminated cells captured by immunostimulatory biomaterials scaffolds in vivo in combination with immune checkpoint blockade, and the ability of the CTL-mediated anti-tumor response to inhibit growth of lung metastases will be evaluated. 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.** - 2015 Awardee  
*Medicine (Infectious Diseases)*  
*Stanford University School of Medicine*

“Targeting Bacteriophage to Treat Biofilm Infections”

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The bacteria *Pseudomonas aeruginosa* is a major human pathogen responsible for much of the morbidity and mortality associated with diabetic wounds, cystic fibrosis, and nosocomial infections. This massive disease burden is caused by biofilms - slimy coats of bacteria and polymers that allow *P.aeruginosa* to stick to catheters and other surfaces and resist both antibiotics and the host immune response.

We have discovered a surprising and unprecedented role for bacteriophage (viruses produced by bacteria) in biofilm formation. We have learned that the extracellular matrix produced by *P.aeruginosa* self-assembles into a liquid crystal through entropic interactions between polymers and Pf phage, a bacteriophage that is ubiquitous among clinical isolates of *P.aeruginosa*. This liquid crystalline structure enhances biofilm function and bacterial fitness by increasing adhesion, resistance to desiccation, and antibiotic tolerance.

These findings are groundbreaking in several ways. First, they indicate that Pf phage have a unique, symbiotic relationship with their bacterial hosts. Second, they implicate Pf phage in human biofilm infections. Third, the identification of a critical role for Pf phage in biofilm formation creates exciting opportunities for novel classes of anti-microbial agents to treat *P.aeruginosa* infections.

In light of these findings, we propose to generate innovative therapeutics that will treat biofilm infections by targeting Pf phage. We will focus on two therapeutic strategies in year 1 of this program with a plan to make the agent that shows the greatest potential our sole focus in years 2-3.

In Aim 1, we will develop anti-viral compounds effective against *P.aeruginosa* biofilms. Our hypothesis is that we can prevent biofilm formation by suppressing bacterial production of Pf phage. We will first develop a high-throughput reporter system to quantify Pf phage production by *P.aeruginosa*. Then, we will screen a 300,000 molecule compound library for candidates that inhibit Pf phage production. Finally, we will test the efficacy of these anti-viral molecules against *P.aeruginosa* biofilms and clinical isolates.

In Aim 2, we will develop antibodies that prevent and treat *P.aeruginosa* infections by targeting Pf phage. Our preliminary data suggest such antibodies indeed inhibit biofilm formation. First, we will screen a panel of monoclonal antibodies for efficacy against biofilm formation in vitro. Then we will develop a *P.aeruginosa* wound infection model and use this to test the efficacy of anti-phage antibody treatments in vivo.

Together, these aims represent a bold and radically unconventional approach to treating *P.aeruginosa* Biofilm infections and improving human health.

**Steven Dowdy, Ph.D.** - 2015 Awardee  
*Cellular and Molecular Medicine*  
*University of California San Diego*

“Development of Next-Generation Antibody-RNAi Conjugates (ARC) to Treat AML”

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Due to chromosomal translocations, Acute Myeloid Leukemia (AML) cells that express the Mixed Lineage Leukemia (MLL) gene fused to the AF9 gene (MLL-AF9) are devastatingly aggressive, difficult to treat malignancies. Unfortunately, the current standard of care remains at 40% disease-free survival (DFS) at five years for children and <10% for adults over 60. Consequently, there is a great need to develop precision medicines that can selectively kill AML cancer cells and their clonogenic cancer stem cells, without harming normal cells.

siRNA-induced RNAi responses can selectively target each and every MLL fusion oncogene, including MLL-AF9. Unfortunately, despite RNAi's promising therapeutic features, siRNA drugs are unstable in serum and due to 40 negatively charged phosphates, siRNAs cannot enter cells. To address these issues, we spent 8 years synthesizing a next-generation RNAi trigger, called RiboNucleic Neutral (siRNN) prodrugs. siRNNs are synthesized with neutral bioreversible phosphotriester groups that mask the negative charge and result in many drug-like properties of extreme stability and deliverability. Once inside cells, ubiquitous cytoplasmic thioesterase enzymes unmask siRNNs and convert them into negatively charged, active siRNAs that induce robust RNAi responses. Importantly, unlike all other anti-cancer therapeutics, RNAi therapeutics can evolve at pace with the AML cancer cell's mutation rate to treat recurrent disease. In contrast, all current anti-cancer therapeutics are static molecules where unfortunately, the cancer cells often become resistant after a relatively short period of time.

The goal of this transformative high risk/high gain Catalyst Award is to develop anti-CD33 Antibody-RNAi Conjugates (ARC) using our next-generation siRNN RNAi triggers to target MLL-AF9 fusion oncogenes and selectively kill AML cancer cells.

**Aim 1. Develop anti-CD33 MLL-AF9 ARC and Optimize Delivery into AML Cells.**  
siRNNs bridging the MLL-AF9 t(9;11) fusion site will be conjugated to the C-terminus of anti-CD33 humanized monoclonal antibodies. We will optimize ARC delivery and induction of RNAi responses in AML cells.

**Aim 2. In Vivo Optimization of anti-CD33 MLL-AF9 ARC Delivery into AML Tumors.**  
We will optimize anti-CD33 MLL-AF9 ARC delivery and induction of RNAi responses in preclinical mouse models of AML.

Successful development of MLL-AF9 ARC here will be a springboard for extensive preclinical optimization using patient-derived (PDX) AML samples in a Transformational Award application with the ultimate goal to declare a MLL-AF9 ARC clinical candidate for IND enabling studies and eventual clinical trials. siRNN ARC technology has the potential to fundamentally transform how we treat human disease, from cancer to pandemic flu to heart disease.

**Nickolai Dulin, Ph.D.** - 2015 Awardee  
*Department of Medicine*  
*University of Chicago Pritzker School of Medicine*

“Development of a Novel Small Molecule for Treatment of Fibrotic Diseases”

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Fibrosis represents a critical mechanism in the pathogenesis of a wide range of important diseases that remain poorly treated; salient examples include: congestive heart failure, secondary or idiopathic pulmonary fibrosis, advanced kidney disease, scleroderma, and liver cirrhosis. There is a huge clinical need for new treatments that prevent fibrosis. Across these various fibrotic diseases, there is a unifying cellular mechanism that leads to scar deposition: the transformation of tissue fibroblasts into an activated phenotype called "myofibroblasts". Myofibroblasts are the principal cells that deposit extracellular matrix proteins and are responsible for scar formation during fibrosis. Therefore, disrupting cellular mechanisms which induce and/or maintain myofibroblast differentiation may be a potential strategy to attenuate fibrosis of various organs.

We have discovered a small molecule ("C085") that inhibits myofibroblast differentiation in vitro and inhibits experimental pulmonary fibrosis in living mice, without obvious immediate toxicity during 2 weeks of daily administration. Our collaborators have synthesized a new (and patentable) synthetic analog ("C308") that also inhibits myofibroblast differentiation in vitro. Now, we propose to confirm the anti-fibrotic efficacy of C308 in vivo in three models of organ fibrosis -- heart, lung, and kidney -- and to define the molecular mechanisms by which C308 inhibits myofibroblast differentiation. To achieve our goal, we propose two specific aims: (i) determine whether C308 inhibits organ fibrosis in heart, lung, and kidney, using two models of fibrosis for each organ; and (ii) determine the mechanisms by which C308 inhibits myofibroblast differentiation and function.

This research matches perfectly the high risk, high reward intent of the Falk Catalyst Award, for if successful it could reveal a new strategy for treating fibrotic diseases and would position us to apply for Transformational Research Award in order to achieve our final goal -- move C308 to clinical trials as antifibrotic medicine.

**John Imig, Ph.D.** - 2015 Awardee

*Pharmacology & Toxicology*

*Medical College of Wisconsin*

“Novel Therapy for Kidney Disease”

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Renal disease in cancer patients and those being treated for cancer is an emerging and rapidly growing healthcare issue. Newer cancer therapies have increased cure rate and survival time, but therapy-associated fluid and electrolyte abnormalities and acute and chronic kidney injury are common. This healthcare issue has led to the development, evolution, and emergence of a subspecialty, Onco-Nephrology. This project will tackle this emerging cancer arena and focus on novel therapeutics to protect the kidney from cancer therapy-associated toxicity. Kidney disease afflicts 33 million in the United States and chronic kidney disease (CKD) accounted for over \$60 billion in Medicare costs. Cancer treatments and radiation exposure represent a lesser-known cause of CKD. Thus there is a significant clinical need for novel therapies to effectively treat and stop CKD in cancer patients. We have identified EET analogs/mimetics with anti-hypertensive, anti-inflammatory, and anti-oxidative activities that will be particularly beneficial for hypertensive patients and patients undergoing chemotherapy and radiation exposure. Our central hypothesis is that EET analogs are a novel therapeutic for CKD caused by hypertension, chemotherapy, and radiotherapy. The rationale that underlies the research proposed is that EET analogs are a novel chemical entity (NCE) with multi-faceted pharmacological activities well suited for the treatment of CKD. Our long-term goal is to complete preclinical requirements for an Investigational New Drug (IND) application for EET analogs for the treatment of CKD in cancer patients and as a consequence of chemotherapy or radiotherapy. The overall objective of this application is to identify a lead candidate EET analog that prevents or arrests the progression CKD in cancer patients. We will also optimize the EET analog chemical scaffold and develop kidney-targeted EET analogs. Our preliminary studies strongly suggest that the experimental studies and outcomes will have an important positive impact and these will make a significant advance towards fulfilling the preclinical IND filing requirements for an EET analog as a CKD therapy. Guided by strong preliminary data, our central hypothesis will be tested by pursuing two specific aims: 1) Test the hypothesis that EET analogs will prevent or mitigate the development of kidney disease associated with cancer therapies; 2) Identify a lead EET surrogate drug candidate and develop EET analogs that target the kidney to enhance their therapeutic potential. The proposed research is original and innovative, because the novel small molecule EET analogs demonstrate great potential as a therapeutic approach improving outcomes in cancer patients.

**Mark Rosenblatt, M.D., Ph.D.** - 2015 Awardee  
*Ophthalmology & Visual Sciences*  
*University of Illinois College of Medicine at Chicago*

“Nanoengineered Silk Biomaterials for Ocular Regenerative Medicine”

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Damage to the ocular surface is a common endpoint for an array of corneal conditions including trauma, infection, and inflammation. In many cases the regeneration of the ocular surface epithelial barrier is impeded by the underlying pathology leading to persistent epithelial defects, or encroachment of con-corneal epithelium onto the ocular surface with concomitant risk of corneal ulceration and loss of vision. In these cases, reconstruction of the ocular surface may be necessary, and the use of biomaterials (such as amniotic membrane) may be required. We have characterized the ability of transparent and biocompatible silk films with modified surface topography to modulate the properties of corneal epithelium. We hypothesize that silk film biomaterials with engineered surface topographies modulate corneal epithelial cell behavior and gene expression, and can be optimized for ocular surface reconstruction. Specific aim 1 will investigate the role of surface topography in regulating the expression of genes responsible for cell differentiation in vitro. Specific aim 2 will investigate the role of surface topography in regulating regenerative potential in vitro. Specific aim 3 will investigate the role of surface topography in the differentiation of epithelial cells on silk film biomaterials in vivo.

**Stephen Strittmatter, M.D., Ph.D.** - 2015 Awardee  
*Cellular Neuroscience, Neurodegeneration & Repair*  
*Yale School of Medicine*

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

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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 validate the preclinical efficacy of specific mGluR5 agents that preserve physiological function while blocking A $\beta$ o pathophysiology in mouse transgenic models.

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 in this project is to validate Pyk2 inhibition as a target for disease modification in AD. We will use both genetic and pharmacological tools to assess this potential target.

This Falk Catalyst project seeks to validate two targets with high potential for effective disease modification of Alzheimer's. This is the first step in advancing one or both approaches into a full drug development program during a transformational phase.

**Su-Chun Zhang, M.D., Ph.D.** - 2015 Awardee

*Waisman Center*

*University of Wisconsin-Madison*

“Refine Cell Therapy Outcomes by Remote Regulation of Neural Circuitry in Non-Human Primates”

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Stem cell therapy holds promise for some of the most debilitating diseases, such as Parkinson's disease (PD). Its prospect is raised substantially with the development of human induced pluripotent stem cells (iPSCs) and our ability to guide iPSCs to functionally specialized cells. However, transplanted neurons do not always integrate into a correct circuit, which may result in unwanted outcomes. In PD, a degenerative disorder resulting from loss of dopamine (DA) neurons in the midbrain and insufficient DA release in the striatum, transplantation of human fetal ventral midbrain tissue into the striatum can restore DA release and ameliorate symptoms. However, uncontrolled movements, named graft-induced dyskinesia (GID), are present in some patients, partly due to unregulated release of DA from grafted cells. Hence, an ability to tune up or down the activity of grafted cells will not only overcome side effects but also refine therapeutic outcomes.

We intend to tackle this fundamental issue by building a functional "switch" into grafted neurons. For the present (one-year) catalyst project, we propose to engineer monkey iPSCs to express both the active and inhibitory forms of DREADDs (designer receptor exclusively activated by designer drug) that are regulated by respective designer drugs. The iPSCs with the "bidirectional switch" will be differentiated to dopamine neurons, which will be transplanted to the brain of a monkey model of PD. This will mimic potential future clinical application. We will then determine if the activity of and transmitter release from the differentiated neurons are up or down regulated by peripheral (remote) application of the designer drugs after transplantation into the monkey brain as well as in vitro. Successful completion of the study will lay down a solid foundation to evaluate the feasibility of refining the therapeutic outcomes in a preclinical nonhuman primate model of PD.