HARVARD CATALYST

THE HARVARD CLINICAL AND TRANSLATIONAL SCIENCE CENTER

Target Inhibition and Silencing: "Hits to Lead" Advances in Clinical and Translational Research

Funded Projects

In this initiative of the Harvard Catalyst Reactor Program, in partnership with the ICCB-Longwood Screening Facility, the community was invited to develop proposals that apply the high-throughput laboratory automation, or small molecule or siRNA library screening capabilities of ICCB-Longwood to problems of clinical and translational importance as they pertain to human health. Applications were required to focus on human specimens or human disease in areas such as infectious disease or biodefense, therapeutic targets, emerging human disease pathways, gene knockdown and functional genomics, or target silencing and inhibition.

This funding opportunity was only open to investigators who attended a training/educational event or met with ICCB-Longwood personnel.

Eight pilot grants were awarded in amounts of up to $50,000 for each one-year project.

Funding decisions for the Target Inhibition and Silencing pilot grants were announced in December 2014.

Discovery of Small Molecules to Inhibit Human Cytomegalovirus Nuclear Egress

Principal Investigator: Donald Coen, PhD, Harvard Medical School

Co-Investigator: Ming Lye, PhD, Harvard Medical School

Human cytomegalovirus (HCMV) is a ubiquitous herpesvirus that causes severe disease in immunocompromised individuals such as transplant and chemotherapy recipients and patients with HIV. HCMV is also a major cause of birth defects, with deafness and mental retardation frequent outcomes. Current anti-HCMV treatments are limited by toxicities, drug resistance, and/or poor oral bioavailability. Our proposal addresses the need for new anti-HCMV drugs by focusing on the HCMV nuclear egress complex (NEC) as a novel target for drug discovery. The NEC, which is unique to herpesviruses, orchestrates the process of nuclear egress in which viral nucleocapsids transit from the nucleus into the cytoplasm. The NEC is composed of a single-span transmembrane protein, UL50, and a nucleoplasmic protein, UL53. Our on-going structural and biochemical studies on the complex indicate that UL50 has a novel protein fold that includes a groove that serves as a binding site for UL53. The groove should also be a ready binding site for small drug-like molecules. Single substitutions of residues in this groove ablate subunit interactions and viral replication. We therefore hypothesize that we can identify small molecules that would eliminate UL50 and UL53 interactions and thus have selective antiviral activity. We have developed an assay suitable for high throughput screening. We aim to utilize this assay and the small molecule libraries available at ICCB-Longwood to screen for inhibitors against the NEC. We will conduct follow-up assays to identify compounds with selective anti-HCMV activity. These compounds can serve as starting points for new, much-needed anti-HCMV drugs.

Novel Gene Discovery in *Plasmodium falciparum* Malaria

Principal Investigator: Jeffrey D. Dvorin, MD, PhD, Boston Children's Hospital

Co-Investigator: Karin Blomqvist, MD, PhD, Boston Children's Hospital

Human malaria is a leading cause of death and disease worldwide. The most severe forms of malaria result from infection by the *Plasmodium falciparum* parasite. Resistance to existing anti-malarial medications is an emerging hurdle to the effective treatment of malaria. A molecular understanding of the fundamental biological process of *P. falciparum* replication will provide the necessary tools to develop new anti-malarial therapeutics. Although the genome of *P. falciparum* has been fully sequenced, the function of more than half of the 5,300 genes in the parasite remains unknown. Many of the genes with unknown function have little or no homology with characterized genes from other organisms. Therefore, existing molecular genetic and bioinformatics techniques cannot be used to efficiently determine the function of many of the genes in the parasite. Our goal is to discover essential genes in *P. falciparum*. We have generated a transgenic parasite strain that readily allows high throughput analysis of the growth and replication of individual parasite clones. Utilizing the automated liquid handling facilities at ICCB-Longwood, we are able to perform a forward-genetic screen for essential genes in *P. falciparum*. The long-term objectives of these studies are to identify novel targets for new anti-malarial therapeutics.

Targeted Inhibition of BRD-NUT Oncoproteins

Principal Investigator: Christopher A. French, MD, Brigham and Women's Hospital

Co-Principal Investigator: Erica M. Walsh, PhD, Brigham and Women’s Hospital

We propose to develop a new drug to improve treatment of an incurable cancer, NUT midline carcinoma (NMC). NMC affects people of all ages with nearly zero survival. NMC is defined by mutation of the NUT gene, which is abnormally fused to a gene called BRD4 in most cases, forming a chimeric BRD4-NUT fusion gene that is the cause of this lethal entity. Despite its rarity, NMC has sparked great interest because it was in this cancer that a new class of anti-cancer therapy was discovered that target the BRD4 aspect of the BRD4-NUT gene. While this new class of drug effectively inhibits NMC tumor growth, it also inhibits normal BRD4 function, which is required for the health and maintenance of normal cells; thus, the BRD4 inhibitors are toxic at therapeutic doses. Moreover, resistance to BRD4 inhibitors has already been seen in both NMC and other cancers. Thus, new drugs to inhibit only the BRD4-NUT cancer protein are needed to more effectively treat this disease while minimizing toxicity. Here, we propose to inhibit the NUT protein, which is only expressed in NMC and in human testes, thus minimizing toxicity. We have found that inhibition of the binding of the NUT protein to another protein completely inhibits the function of the BRD4-NUT protein and the growth of NMC cells. Here, we propose to use the AlphaScreen to identify small molecules that inhibit the interaction of NUT with this other protein, which will then be developed into drugs to treat NMC patients.

Screening for Inhibitors of the Proteolytic Complex ClpXP1P2 from *M. tuberculosis*

Principal Investigator: Alfred Goldberg, PhD, Harvard Medical School

Tuberculosis remains one of the leading causes of death from infectious diseases worldwide. *Mycobacterium tuberculosis* (Mtb) has become increasingly resistant to available antibiotics. Therefore, identifying new drug targets and developing selective inhibitors are critical. ClpP1 and ClpP2 are serine proteases that are essential for growth and infectivity of Mtb. Because similar enzymes are not present in the mammalian cytosol, they are attractive drug targets. We have shown that the ClpP1 and ClpP2 proteases function together in Mtb (unlike ClpP proteases in other cells) and have characterized the active 14-subunit ClpP1P2 complex. We have found certain dipeptide derivatives that dramatically stimulate complex formation and enzyme activity against short fluorescent substrates (even in the absence of their associated ATPases). We have also characterized the essential Mtb ATPase complexes, ClpC1 and ClpX, which facilitate protein degradation by ClpP1P2 and are also attractive drug targets. However, no inhibitors of ClpX have been found, nor has any screen for ClpP1P2 inhibitors been reported. Inhibitors of the ATP-dependent protease complex, ClpXP1P2, should be selectively toxic to Mtb, and we have a fluorescent assay appropriate for High Throughput Screens. We now propose to screen for such inhibitors in the small molecule library at HMS's Institute of Chemistry and Cell Biology. When Hits are obtained, we have secondary assays to identify their exact targets in the ClpXP1P2 complex and to test for inhibition of ClpXP1P2 function in bacterial cells and their viability. Hopefully, this work may identify lead compounds for the development of novel treatments for tuberculosis.

Metabolic Targeting of Tumor Cells with Hyperactive TORC1

Principal Investigator: Elizabeth Henske, MD, Brigham and Women's Hospital

Lymphangioleiomyomatosis (LAM) is an often-fatal destructive lung disease of young, nonsmoking women. LAM cells carry mutations in the tuberous sclerosis complex (TSC) genes, resulting in activation of mammalian target of Rapamycin (mTOR) complex 1 (TORC1). TORC1 is a “master regulator” of protein translation, cellular metabolism and autophagy. Treatment of tumor cells in TSC or LAM patients with mTORC1 inhibitors (Rapalogs) results in a cytostatic response and the tumor cells regrow when the drug is discontinued. Therefore, prolonged therapy, with accompanying toxicity, is required. There is a significant unmet medical need for treatments that induce a cytocidal response in mTORC1 hyperactive tumor cells in TSC, LAM, and sporadic human tumors with hyperactivation of mTORC1, including kidney cancer and bladder cancer.

We propose a completely novel strategy for tumors with hyperactive TORC1: targeting TORC1-dependent metabolic vulnerabilities, and not directly targeting TORC1 itself. We previously screened 6,600 compounds at the ICCB in TSC2-deficient, patient-derived cells for agents that induce cell death selectively in the setting of TORC1 hyperactivation. Here, we will complement this small molecule screen to identify molecular targets that selectively induce death in TSC2-deficient cells.

Novel strategies targeting the metabolic vulnerabilities of cells with hyperactive mTORC1, leading to durable remissions and thereby preventing progression to end-stage lung disease and death in LAM and making life-long use of Rapalogs unnecessary in children with TSC. Thus, our project will have high clinical impact for LAM, TSC, and cancer patients whose tumors have mutations in the PI3K/Akt/TSC/mTOR signaling network.

Plasmid Eviction to Restore Susceptibility in Carbapenem-Resistant Enterobacteriaceae

Principal Investigator James E. Kirby, MD, Beth Israel Deaconess Medical Center

The abstract for this grant award has not been published at the request of the Principal Investigator. Interested parties should refer to the Principal Investigator's website for general information.

High Throughput Screen for siRNA Modulators of Non-homologous End Joining

Principal Investigator: Ralph Scully, MB BS, PhD, Beth Israel Deaconess Medical Center

The repair of double strand breaks (DSBs) plays a crucial role in cancer predisposition and cancer therapy. Transient inhibition of DSB repair might be useful as a method for sensitizing tumors to radiotherapy ("radiosensitization"), allowing the dose of radiation to the patient to be minimized. This could benefit specific cancer patients of all ages, including a subset of children with curable cancers. DSB repair entails two major pathways: homologous recombination (HR) and non-homologous end joining (NHEJ). Of these, the most potent radiosensitization comes from inhibition of NHEJ. NHEJ inhibition might have additional value for cancer treatment independent of radiation therapy. Germ line mutations in NHEJ genes can also cause rare recessive human immunodeficiency syndromes. Although the "core" human NHEJ genes are known, we believe that additional NHEJ genes remain to be discovered, some of which may be human disease genes. Thus, there is a compelling rationale for identifying the full spectrum of human genes that mediate NHEJ. Our goal here is to work with the ICCB-Longwood screening facility to conduct a high-throughput screen for small interfering RNAs that modulate NHEJ in human cells, with the aim of identifying new human NHEJ genes.

High-throughput Screening to Identify Inhibitors of Dengue Virus (DV) Entry

Principal Investigator: Priscilla Yang, PhD, Harvard Medical School

Co-Investigator: Amal Rameh, PhD, Harvard Medical School

Dengue virus (DV) is the most widespread mosquito-borne viral disease affecting humans today. DV causes a broad spectrum of disease ranging from the asymptomatic to classical dengue fever and can progress to more severe and life-threatening dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS). As with other viral hemorrhagic fevers, such as those caused by Ebola virus, there is no vaccine and the only treatment for DHF/DSS is supportive care. An estimated 500,000 cases of DHF/DSS occur annually with estimated 2.5% fatality although fatality rates can exceed 20% if untreated. The diversity of DV serotypes and propensity of non-neutralizing antibodies to exacerbate disease have limited success in vaccine development. Efforts to develop antivirals targeting conventional viral polymerase and protease targets have also been unsuccessful. We have discovered compounds that potently inhibit DV in cell culture by binding to the envelope protein, E, on the virion surface and blocking membrane fusion during viral entry. Despite considerable medicinal chemistry efforts, we have thus far been unable to improve our existing lead compounds sufficiently for *in vivo* validation studies. Here we propose a one year HTS effort to identify alternative scaffolds against this target. Our long-term plan is to develop DV entry inhibitors that can be advanced as preclinical candidates. We have developed a screening platform and infrastructure (i.e., medicinal chemistry, virology, structural biology, PK/PD, ADME/toxicity, and *in vivo* efficacy testing) to enable this goal.