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Principal Investigator	Principal Investigator's Organization	Project Title	General Audience Abstract
Eric Haura, M.D.	H. Lee Moffitt Cancer Center and Research Institute	Signaling-associated Protein Complexes for the Molecular Annotation of Therapeutic Vulnerabilities, Resistance-associated Signaling & Tumor Heterogeneity in Lung Cancer	This research will study ways to identify and overcome drug resistance in lung cancer. In recent years, it has become standard of care to identify altered genes in lung cancer patients as identification of these genes can predict response to pill based therapy. However, resistance to treatment is universal, and this precludes the cure of patients with advanced lung cancer. One major driver of resistance is the activation of other proteins that bypass the utility of the pill-based therapy. This can occur through new changes in the tumor cell or can be driven by noncancer cells in the tumor. Importantly, genes, encoded by DNA, do not function in isolation but rather as part of larger molecular machines. Our research is focusing on the importance of these machines in affecting drug resistance. We will use new technology to identify and create systems to read out these machines in cancer tissues from patients. This project will expand our research capacity in Florida and will improve the treatment of patients with lung cancer. The work can ultimately enhance enrollment on clinical trials by developing new tools to optimize treatment decisions for patients and their physicians.

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Principal Investigator	Principal Investigator's Organization	Project Title	General Audience Abstract
Anthony Capobianco, Ph.D.	University of Miami	Lead Optimization and Preclinical Evaluation of Small Molecule Inhibitors of Notch Transcriptional Activation	In many human cancers, deregulation of the Notch pathway has been shown to play a role in tumorigenesis. Aberrant Notch activity also plays a central role in the maintenance and survival of cancer stem cells, which may underlie a role in metastasis and resistance to therapy. Since Notch plays an important and diverse role in cancer, it has become an exceedingly attractive target for cancer therapeutics. However, the full range of potential targets in the pathway have been under explored. To date, there are no small molecule inhibitors that directly target the intracellular Notch pathway. Notch mediates the formation of a core transcriptional activation complex, termed the Notch Ternary Complex (NTC), thus initiating and maintaining a transcriptional cascade. The NTC comprises the DNA binding protein CSL, the intracellular domain of Notch (NICD) and the co-activator protein Mastermind (Maml1). The overarching hypothesis of this proposal is that compounds that prevent the recruitment of Maml1 by targeting NICD would be potent inhibitors of the Notch pathway. We have identified a lead compound (1- 134-83) that is a bona fide inhibitor of the NTC that uncouples the Notch-mediated transcriptional cascade and inhibits tumor growth in patient derived mouse models of cancer. Therefore, the overall goal of this project is to optimize the scaffold of the lead compound (1-134-83) to identify clinical candidates that inhibit NTC assembly in order to develop novel potent drug-like small molecule inhibitors of Notch-mediated transcription. To this end, we will use an innovative approach that combines current state-of-the-art computational, biochemical and biophysical techniques. Successful completion of this study will fulfill an unmet need in terms of therapeutic agents targeting the Notch signaling pathway, providing specific inhibition of the Notch transcriptional activation complex, which could complement and/or offer an alternative to current therapeutic approaches. We will achieve

			the goals of this proposal through the following specific aims: (I) Lead optimization through structure-activity relationship studies and scaffold hopping, (II) Biochemical and biological assessment of lead analogs, (III) Preclinical evaluation of efficacy of lead clinical candidates. Our recent identification and validation of a small molecule inhibitor of the NTC (1-134-83) demonstrates proof of concept for the proposed research.

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Principal Investigator	Principal Investigator's Organization	Project Title	General Audience Abstract
David D. Tran, M.D., Ph.D.	University of Florida	Novel Strategies to Target Disseminated Tumor Cells in Triple Negative Breast Cancer	Patients with locally advanced triple negative breast cancer (TNBC) who have persistent disease after chemoradiation are at a significantly increased risk of developing lethal metastasis within two years after diagnosis. Currently no known therapy can prevent this development. A major cause of this high metastatic risk is the presence of cancer cells residing in distant organs after having spread there from the primary tumor well before the tumor is treated and surgically removed. Some of these metastatic cells, known as disseminated tumor cells (DTC), are thought to represent cancer stem cells that are dividing slowly and therefore highly resistant to treatment. These low-proliferative DTCs (lpDTCs) can persist in distant organs for an extended period of time before becoming reactivated to form metastasis. Attempts at eliminating lpDTCs have not been successful due to a poor understanding of their biology and a lack of therapeutic targets. To this end, we recently identified a critical signaling pathway present in lpDTCs that is responsible for their quiescence and treatment resistance. This pathway consists of a circular signaling loop involving the p38MAPK and TWIST1 proteins, both of which have been found to regulate breast cancer metastasis. In cultured breast cancer cells and mouse models of breast cancer, we demonstrated that lpDTCs could be forced out of quiescence simply by inhibiting their p38 pathway. More importantly, once reactivated, lpDTCs became exquisitely sensitive to chemotherapy, indicating that p38 is attractive therapeutic target. In this proposal, we will test the innovative therapeutic concept that lpDTCs in TNBC can be eradicated when they are induced to divide again while being exposed to chemotherapy. We shall achieve this goal by using PH797804, a highly selective, potent, well-tolerated p38 inhibitor, to reactivate lpDTCs, followed by treatment with carboplatin chemotherapy in patients with TNBC who have persistent disease and lpDTCs after chemoradiation. First, we will establish that the combination is safe and effective at reactivating lpDTCs, initially using mouse models of aggressive breast cancer, then followed by a phase I trial enrolling patients who have advanced metastatic breast cancer. The goal of the phase 1 study is to determine the maximal tolerated dose (MTD) of PH797804 plus carboplatin that is effective at reactivating lpDTCs. The MTD will be used for a phase 2 study testing whether the combination of PH797804 plus carboplatin results in improved progression-

			free survival at 2 years and eventually lead to better overall survival. If successful, this innovative therapeutic concept has the potential to change clinical practice not only in aggressive breast cancer but also in many other aggressive cancers. In the last and equally exciting aim, we will perform comprehensive genomic analysis of samples collected at different stages of metastasis and couple it with ARACNe, a powerful computational platform, to identify genetic driver mutations and changes in signaling pathways relevant to transitions between these stages. The goals are to 1) provide further insights into the mechanism of how lpDTCs are generated and maintained; and 2) yield additional therapeutic targets to eliminate lpDTCs and improve this novel treatment approach.

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Principal Investigator	Principal Investigator's Organization	Project Title	General Audience Abstract
Aubrey Thompson, Ph.D.	Mayo Clinic, Jacksonville	Predictive Markers of HER2-Targeted Therapy	The use of humanized monoclonal antibodies such as trastuzumab (Herceptin®) has dramatically improved outcome in breast cancer patients who overexpress the HER2 receptor (HER2+ tumors). Treatment of early stage HER+ tumors with trastuzumab results in long term disease-free survival in ~75% in patients with HER2+ breast cancer. However, 25% of patients with early stage HER2+ disease relapse after trastuzumab. The prognosis for patients who relapse is much worse. This is a very important point: patients who respond to first line therapy are effectively cured; whereas patients who relapse after first line therapy are at grave risk. The clinical challenge is to identify the patients who are at high risk of relapse after first line therapy with trastuzumab. We have recently completed genomic analysis of patients from a very large clinical trial of early stage HER2+ tumors treated with trastuzumab (NCCTG/Alliance N9831, Edith Perez, principal investigator). We used these genomic data to identify a set of immune function genes that are linked to favorable outcome, and we built a "first draft" model that predicts outcome in early stage HER2+ tumors treated with trastuzumab. This model identified a cohort of patients who were depleted of immune function genes and who derived little or no benefit from trastuzumab. The ability to predict response to trastuzumab has great clinical significance. Patients who are unlikely to benefit can be spared the expense (~$40K) of trastuzumab, as well as the risk of adverse heart events associated with this therapy. Patients who are more likely to relapse can be evaluated as candidates for some of the newer, and even more costly, anti-HER2 therapies that are currently being approved or are being tested in clinical trials. Third, these are likely to be the patients who should be enrolled in clinical trials to test newly emerging therapies to activate the patient's immune system against the tumor. Our predictive model therefore has great potential for rapid translation into clinical medicine, and we have been approached by several companies (Nanostring, Agendia, Illumina) who are interested in licensing this assay. However, before the assay can be licensed, several key steps are necessary. We must carry out analytical validation of the assay using a platform that is adaptable to routine clinical analysis. Once that

			has been accomplished, we must demonstrate clinical validity using an independent sample cohort to show that our model works for patients other than those used to develop the model. Once these parameters have been locked down, commercialization of the assay is essentially assured. These objectives therefore define the aims of this technology validation and transfer proposal.

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Principal Investigator	Principal Investigator's Organization	Project Title	General Audience Abstract
Michael Antoni, Ph.D.	University of Miami	Stress Management Effects on Affective Status and Influenza Vaccine Response in Older Breast Cancer Patients	Women undergoing breast cancer (BCa) treatment face many stressors and experience increased negative affective/mood states (depressed mood, anxiety) and decreased positive affect (happiness, contentment). These not only compromise their quality of life but, as we have shown, also contribute to inflammation and negative health effects, including on their immune system. This application brings together our research and intervention strengths for psychosocial intervention and the aging immune system for BCa patients. It is well established that systemic inflammation increases with aging, negative affect and cancer treatment and our hypothesis is that older women (60yrs+) who confront the challenges of BCa treatment have less coping resources than younger women, resulting in greater negative affect and depressive symptoms. The proposed studies will address these issues and contribute to the health, well-being, and longevity of BCa patients. Our studies identified immune and psychological biomarkers for optimal humoral immune response in older humans as measured by the antibody response to the influenza vaccine and decreased inflammation. We found poorer Affective Status (greater negative and less positive affect) is associated with lower immune response. Little is known about the impact of Affective status and inflammation on IR in particularly vulnerable older populations, such as women undergoing BCa treatment. Our prior studies have shown 1) poorer Affective status (depression) associates with greater levels of inflammatory cytokines in the weeks after BCa surgery, 2) behavioral intervention (cognitive behavioral stress management (CBSM)) improves Affective Status (decreases negative affect and depressive symptoms and increases positive affect), and 3) CBSM also reduces leukocyte pro-inflammatory gene expression during BCa treatment and also in older BCa patients in particular. Our hypothesis is that CBSM will decrease negative affect and inflammation and improve immune response, in the target sample of older BCa patients who report elevated levels of distress. We propose to recruit older BCa patients (60yrs +) with elevated distress levels up to 8 weeks after surgery, measure Immune Status (inflammation and immune function) and Affective Status as well as other potential confounders. They will then be randomly assigned to either 10 weeks of Remotely Delivered (via tablet with broadband connection) group-based CBSM commencing just after T0, or a WaitList control (WLC) condition that will receive an identical 10 week Remotely Delivered group-based CBSM after study completion. Similar measures will be done and at 6 month follow-up and 7 days and 28 days after the seasonal influenza vaccine (IV). We will examine intervention group differences in the serum response to IV at as the primary outcome. We will also test the effects of CBSM on changes in affective status, inflammatory measures (e.g., serum cytokines), and

			immune measures and associate these with the changes in serum response to IV. This innovative approach will 1) use remote technology, designed to reach a wide range of patients, to 2) improve the overall immune response in older women with BCa, both supporting a strong public health significance. This approach would support future delivery of psychosocial interventions during treatment for other cancers in underserved groups.

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Principal Investigator	Principal Investigator's Organization	Project Title	General Audience Abstract
Michael P. Kladde, Ph.D.	University of Florida	Temporal Epigenetic Mechanisms in Breast Cancer Oncogenesis	The permanent inactivation of critical genes that protect cells against cancers, termed tumor suppressor genes (TSGs), is a well-established event in cancer progression. TSG inactivation is often caused by changes or mutations in the sequence of the A, C, G, and T bases or rungs on the ladder that make up the DNA double helix, destroying the function of the protein specified by a TSG. Recent scientific advances have also recognized that TSGs in tumors frequently have normal, non-mutated DNA sequences, although the TSG is not expressed to produce protein as it is inactivated by alterations in molecular characteristics that regulate gene expression, referred to as epigenetic regulation. Much has been learned about this mode of regulation with regard to the types and gross placement of epigenetic changes that occur in cancer cell DNA compared with normal cells; however, we have a limited understanding about the order and precise location of the initial or primary epigenetic alterations in TSGs that drive their silencing in cancer. Based on our preliminary findings, we hypothesize that DNA sequences that promote expression of TSGs become more tightly packed in particles called nucleosomes, and this increased packaging precedes the accumulation of repressive chemical changes in DNA, i.e., DNA methylation, which reinforces an aberrant epigenetic environment that silences TSG expression. To test this hypothesis, we will use a progression model of breast cancer formation; specifically, introducing a copy of an oncogene (Ha-ras) into non-cancerous human mammary epithelial cells (HMEC) to drive oncogenic transformation and new (de novo) epigenetic silencing. Using this approach, we will then monitor the temporal sequence of molecular events that accompany silencing of select loci with pinpoint accuracy. These studies will employ innovative, integrative single-molecule assays that we have developed to directly relate changes in the presence and positions of nucleosomes and DNA methylation on TSG promoter sequences to gene expression, i.e., synthesis of RNA transcripts that encode for TSG proteins. Elucidating epigenetic mechanisms driving cellular transformation is crucial to a better understanding of cancer etiology and will aid the identification of early diagnostic markers and novel targets for therapeutic intervention.

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Principal Investigator	Principal Investigator's Organization	Project Title	General Audience Abstract
John L. Cleveland, Ph.D.	H. Lee Moffitt Cancer Center and Research Institute	Epigenetic Regulation of Androgen Receptor in Castration Resistant Prostate Cancer	For over half a century, prostate cancer research has focused on the protein expressed in male reproductive system including in prostate, Androgen receptor (AR). AR binds to androgen or testosterone and gets activated to perform its function as transcriptional co-activator. However, cancer cells hijack AR's transcriptional activity and thus in cancer cells, AR is not only necessary for initiation and growth of the disease, but also plays a crucial role in its progression to the highly metastatic stage, commonly referred to as castration resistant prostate cancer or CRPC. Due to absolute dependence on AR, anti-androgens were common therapeutic modality for patient with this disease, wherein AR was deprived of its biological ligand, androgen. This resulted in loss of AR transcriptional activity leading to suppression of tumor growth. Although effective initially, antiandrogen therapies soon lost its effectiveness; these patients rapidly developed drug-resistance and progressed to CRPC stage. Interestingly, CRPC tumors maintained high AR levels even when prostate cancer cells were exposed to protracted androgen deprivation therapy. Presence of elevated AR levels in spite of prolonged AR antagonist treatment in CRPCs is a paradox that has mystified researchers. Over the years this has emerged to be the topic of intensive research due to obvious therapeutic benefits that could be drawn if understanding of the mechanism were to be obtained. We uncovered a novel mechanism of auto-regulation of AR transcription wherein AR protein coordinated functionally with another protein called ACK1. AR when complexed with ACK1 performed a new task- they facilitated modification of a DNA binding protein called histone H4. Significantly, they not only modified the histone, but also deposited these abundant proteins specifically near AR gene, causing AR expression even when androgen was absent. Significantly, these data have exposed an Achilles' heel for targeting new treatment strategies in CRPCs-disruption of ACK1/AR interaction could sensitize CRPC tumors. To achieve therapeutic benefit from this new signaling event, we generated a novel ACK1 small molecule inhibitor (R)- 9bMS, which not only inhibited ACK1 activity and AR expression but also suppressed CRPC tumor growth in mice. Overall, our preliminary data indicates that disruption of

			ACK1-AR interaction by novel ACK1 inhibitor (R)-9bMS opens up a new therapeutic option for this essentially incurable malignancy. In this proposal will perform detailed mechanistic & in vivo studies to test efficacy of (R)-9bMS as a new class of prostate cancer inhibitor. Successful completion of this proposal could lead to availability of a potent inhibitor of metastatic prostate cancer, a desperate need for aging American male population.

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Principal Investigator	Principal Investigator's Organization	Project Title	General Audience Abstract
Walter O'Dell, Ph.D.	University of Florida	Early Markers of Subclinical Pulmonary Vascular Radiation Toxicity in Breast Cancer	Over 200,000 women each year are diagnosed with breast cancer. With improved early detection and treatment more breast cancer patients experience long-term survival. There are over 2.8 million breast cancer survivors in the US and Florida alone contributes roughly 9,000 additions breast cancer survivors annually. Most patients are treated with radiation therapy (RT) to the affected breast and chest wall to minimize the risk for recurrence. However, the lung is highly susceptible to radiation and even with our best methods for minimizing exposure of the lung, 14% of breast cancer patients treated with radiation develop clinical lung toxicity (evidenced by pain and/or reduced breathing capacity), with 4% overall experiencing high-grade clinical toxicity. The use of protons rather than X-rays for radiation treatment holds tremendous promise for reducing exposure of the lung during breast RT, but until now it has been difficult to quantify its actual benefit in human subjects. Our team has recently developed and demonstrated tools to characterize radiation-induced vascular injury in the lungs of cancer patients using only conventional 3D X-ray computed tomography (CT) chest images. In the first part of this project we will take repeat CT scans in patients receiving conventional radiation or proton therapy to study the development and extent of lung vessel damage following treatment and compare the two treatment approaches. Our goal is to provide direct evidence to support/refute the predictions that using protons for treatment will reduce the amount of damage to the lung. In the second part of this study, we will look at a select group of proteins that are released into the bloodstream to try to better understand which of these contribute to the long-term damage in the lung and other organs. This task builds upon many years of experience our team has in studying radiation in animal models. In part 3, we will use our mathematical modeling skills to tie the newly found data from parts 1 and 2 to existing models of how lung tissue responds to radiation. We will use the new data to extend our scientific models to give us a better understanding of the factors that cause radiation toxicity. With these improved models, we will then be better able to identify which patients will experience high-grade clinical toxicity before they undergo treatment so that we can change the method of treatment or make available to them medications that can protect normal tissue from the radiation. Together, the vessel analysis and modeling tools will help in the future to test whether new medications or treatment approaches are effective and safe

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Principal Investigator	Principal Investigator's Organization	Project Title	General Audience Abstract
Anthony Capobianco, Ph.D.	University of Miami	Development of Small Molecule Inhibitors of NACK as Novel Cancer Therapeutic Agents Targeting the Notch Pathway	Aberrant Notch signaling is linked to many human cancers. Notch signaling has been demonstrated to play a vital role in the initiation and maintenance of the neoplastic phenotype as well as in cancer stem cell self-renewal, which may underlie a role in metastasis and resistance to chemotherapy. In this regard, Notch has become an exceedingly attractive therapeutic target in cancer. However, full range of potential inhibitors targeting the pathway has not been well explored. Notch signaling mediates its effects by forming a core transcriptional scaffold, termed as the Notch Ternary Complex (NTC), which is comprised of Notch intracellular domain (NICD), Mastermind (Maml) and DNA binding protein CSL. There is a great interest in designing small molecule inhibitors to directly target the Notch transcription complex, either by blocking the assembly of Notch transcriptional activation complex or by inhibiting the activation of the Notch pathway. Previously, we reported the identification and characterization of NACK, which acts as a Notch transcriptional co-activator and an essential regulator of Notch-mediated tumorigenesis and development. Furthermore, NACK functions in an ATP dependent manner to bind to the Notch transcription complex and to activate Notch-mediated transcription. Given the critical role of NACK in Notch pathway, we hypothesize small molecule inhibitors of NACK activity will function as specific Notch transcriptional activity inhibitors, and therefore be effective as anti-neoplastic agents for Notch-dependent tumors. We have identified a lead inhibitor of NACK (iNACK, Z271-0326), a bona fide inhibitor of NACK, which can interrupt NACK recruitment to the Notch transcription complex that inhibits Notch-mediated transcriptional cascade and suppresses tumor growth in patient derived xenograft (PDX) cancer mouse models. The overall goal of this project is to develop and validate additional lead candidates from the scaffold of the lead compound (iNACK, Z271-0326) to develop novel potent drug-like small molecule inhibitors of NACK as clinical candidates. Our current discovery of iNACK (Z271-0326) demonstrated the proof of concept for the proposed research. We will use a sophisticated approach, which combines the cutting-edge computational, biochemical and biophysical

			techniques, to discover small molecule inhibitors of NACK targeting the Notch transcription complex. Successful completion of this proposal will provide specific and direct inhibition of the Notch transcriptional activation complex, which will open avenues for the development of new therapies for the Notch-dependent cancers. We will achieve this goal through the following three specific aims: (1) Lead optimization of NACK inhibitor Z271-0326 by iterative computational design and chemical synthesis of the predicted best compounds, (2) Identification and validation of lead analogs of iNACK through biochemical and biological assessment, (3) Preclinical evaluation of lead clinical candidates.

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Principal Investigator	Principal Investigator's Organization	Project Title	General Audience Abstract
Andrew Judge, Ph.D.	University of Florida	Initiating Mechanisms of Cancer Cachexia	Cachexia is a devastating condition that affects up to 80% of cancer patients and is characterized and defined by progressive skeletal muscle wasting and body weight loss. This loss of muscle mass contributes to significant muscle weakness and diminished physical function and quality of life and is associated with reduced tolerance to chemotherapy and increased complications from surgical and radiotherapeutic treatments. Consequently, cachexia decreases survival time in cancer patients and cachexia itself is estimated to be responsible for up to 30% of all cancer related deaths. However, unfortunately there are currently no medical therapies to counter cancer-induced muscle wasting which is due, in part, to a lack of understanding of the initiating mechanisms. This proposal was developed to identify novel mechanisms which initiate limb and respiratory muscle wasting in response to cancers of the lung, colon and pancreas, which is critical to the development of therapeutic strategies to enhance the quality of life and survival of cancer patients. Specifically, our proposal will focus on the role that two specific proteins, called interleukin 8 and CXCL1, play in the initiation of cancer-induced muscle wasting. Both of these proteins are increased in the serum of cancer patients and their receptors are increased in the muscle of cancer patients. We will therefore study the biological importance of these proteins and their receptors as they relate to cancer cachexia.

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Principal Investigator	Principal Investigator's Organization	Project Title	General Audience Abstract
Emmanuel Thomas, M.D., Ph.D.	University of Miami	Identifying Infection and Molecular Determinants of Health Disparities in HCV Infected Minority Populations for the Prevention and Early Detection of HCC	HCV infection is the most common blood-borne infection in the U.S. with estimates of 4 million HCV infected individuals in the U.S. and 170 million worldwide. About 30% of individuals with persistent infection develop chronic liver disease including cirrhosis and hepatocellular carcinoma (HCC). HCC is directly linked to obesity and it is one of the few cancers whose frequency is increasing in the U.S. mainly due to the aging HCV infected population. Given the increasing incidence of obesity throughout the U.S., HCC will become increasingly important unless current trends are dampened through intervention. In South Florida, where the incidence of HCV infection is highest among minority populations, the endemic high prevalence predisposes this population to the development of HCC. This cancer is frequently diagnosed in the later stages and it has a median survival of 6-20 months, resulting in 250,000-1,000,000 deaths/year. Major gaps exist in our understanding of the progression from HCV infection to severe clinical outcomes such as the development of HCC. In addition, HCV infection is more prevalent among African Americans than among persons of any other racial group in the United States. Furthermore, patients of European ancestry have a significantly higher probability of spontaneously clearing the virus than patients of African ancestry. Fortunately, very potent antiviral agents, utilizing shortened treatment durations, are now FDA approved. Consequently, a unique opportunity now exists to minimize health disparities resulting from this virus infection. However, it is significant that if a patient has evidence of liver disease, they are still at risk for the development of liver cancer/HCC even after cure of HCV. Our research aims to prevent HCC and promote screening and early identification through efforts to monitor patients at increased risk. We will identify those most susceptible to poor clinical outcomes including the development of liver cancer. Because HCV is the leading cause of liver cancer, these efforts may prevent the development of hepatic tumors that would otherwise arise over the next decade.

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Principal Investigator	Principal Investigator's Organization	Project Title	General Audience Abstract
Clement K. Gwede, Ph.D., M.P.H., R.N.	H. Lee Moffitt Cancer Center and Research Institute	Community CARES: A Multilevel Intervention to Increase Colorectal Cancer Screening Adherence in Community Clinics	A leading cause of death in the U.S. is colorectal cancer (CRC). It is a significant health concern that affects both men and women and one that our local community has identified as important. Many adults do not get screened for colorectal cancer for reasons such as limited access to screening tests, information that is difficult to understand, as well as other sociocultural and environmental factors. Community involvement is needed for sustainable solutions. The proposed study called Community CARES (Colorectal Cancer Awareness, Research, Education and Screening) or C-CARES for short, tests a promising intervention delivered in Federally Qualified Health Centers (FQHCs). It builds on the work of a well-established community partnership network (the Tampa Bay Community Cancer Network), that was formed over a decade ago to address health disparities through education, outreach and research. C-CARES is also fueled by a new generation of high sensitivity and high specificity fecal immunochemical test (FIT) that can be widely delivered at a lower cost (compared with colonoscopy), and done conveniently in the privacy of one's home. We recently completed an intervention study in clinics called CARES that was guided by community members, and which tested low-literacy materials (i.e., photonovella+DVD) + FIT. In this study, 80% of participants got screened with FIT, a rate that exceeds Healthy People 2020 CRC screening goal of 70.5% and the national goal to reach 80% by 2018. Although highly beneficial, the CARES study emphasized initial vs. repeat annual screening behaviors to help increase effectiveness of FIT. The study also did not provide follow-up intervention on 20% of patients who did not respond to the initial intervention. C-CARES extends this foundational work by collaborating with community clinics. It seeks to implement a multicomponent, dual-language (English/Spanish), theory-driven educational intervention to promote long-term annual FIT. In Phase I - the Preparatory Phase (months 0-6), the team activates its Community Advisory Board, completes packaging of additional C-CARES components, and finalizes procedures to utilize existing electronic medical record systems at the FQHCs—an important tool for identifying eligible patients for screening, delivering patient reminders, and documenting CRC screening completion. In Phase II - the Intervention Phase (months 7-60), a two-arm randomized comparative design will be used to examine whether C-CARES Plus versus C-CARES improves annual FIT screening among 328 individuals, 50-75 years of age, who are not up to date with CRC screening. In the C-CARES group, participants are given CARES materials + FIT kit. In the C-CARES Plus group, a stepped approach is used: participants are given CARES materials + FIT kit plus added personalized components that include one-on-one education, mailed or text message reminders, and booster education and/or coach. We think that C-CARES Plus will result in greater screening rates at 3, 15, and 27 months. This sets the stage for future statewide dissemination for improved community health. The study will also help to impact health disparities in colorectal cancer.

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Principal Investigator	Principal Investigator's Organization	Project Title	General Audience Abstract
Keiran Smalley, PhD	H. Lee Moffitt Cancer Center and Research Institute	Defining and Targeting Epigenetic Deregulation in Uveal Melanoma	Uveal melanoma is the most common primary cancer of the eye. It arises from melanocytes that reside in the uveal tract of the eye and tends to be most common in individuals who are at risk for skin melanoma (e.g. blue eyes, blonde hair). Although most patients with uveal melanoma present with local disease only, half will eventually succumb to distant metastases – even when the primary tumor is treated successfully. At this time there are no effective treatments for disseminated uveal melanoma, and even treatments that have proven effective for skin melanoma such as immunotherapy seem ineffective in uveal melanoma. Work from our team has shown that uveal melanomas present as having either a high risk or a low risk of metastasis and that this risk can be determined on the basis of gene expression. We have further observed that it is possible to convert the high-risk subset of tumors to low risk through use of drugs that regulate tumor cell plasticity called HDAC (histone deacetylase) inhibitors. We have also found that specific HDAC inhibitors can sensitize uveal melanoma cells to other experimental drugs that are being evaluated in the clinic, such as MEK inhibitors. The goal of this proposal is to determine the mechanisms that push some uveal melanomas into the high-risk category and to characterize whether this presents new therapeutic opportunities. Ultimately, we wish to design new therapies that target high risk uveal melanoma with the expectation of evaluating these clinically in the near future. This proposal represents a true collaborative effort between three investigators with extensive, complimentary expertise in uveal melanoma (Dr. Harbour), melanoma signaling and therapy (Dr. Smalley) and the mechanisms of tumor cell plasticity (Dr. Licht).

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Principal Investigator	Principal Investigator's Organization	Project Title	General Audience Abstract
Amy E. Wright, PhD	Florida Atlantic University	Discovery of Marine Natural Product Antagonists of Survivin as Novel Cancer Therapeutics	The HBOI marine natural products chemical library represents a diverse library of genetically encoded small molecules that have actively co-evolved with cellular targets involved in both cell survival and death. The nodal protein survivin has been identified as an important target for intervention in a number of cancers including colon, lung and breast cancers. It plays key roles in many cancer supporting processes including: inhibiting apoptosis; supporting mitosis and metastasis; conveying drug and radiation resistance through changes in the DNA repair response; inducing angiogenesis, and maintaining stem cell populations. Survivin has been demonstrated to play a role in the aggressiveness of many cancers and its expression correlates to poor prognosis. A number of approaches to antagonize survivin's multiple functions have been explored including vaccination, use of single amino acid mutants, ribozymes, siRNA and small molecule inhibition. Even with these successes, many have significant clinical drawbacks and there remains a need for additional small molecules that antagonize the activity of survivin. We hypothesize that screening the HBOI library for compounds that reduce the levels of surviving will identify novel inhibitors with the potential to be useful as new treatments for cancer or as tool molecules to address the remaining questions in survivin biology. We will specifically focus on discovery of natural products that reduce the levels of activated survivin in colon, lung and breast cancer cell lines bearing activating Ras mutations, where survivin function is especially important. We will use high content imaging (HCI) to rapidly screen chemically diverse materials from the library for their ability to reduce levels of survivin in cancer cell lines. Active compounds will be further profiled for their effects on survivin related processes. Discovery of additional small molecule antagonists will advance this field in both our understanding of basic biology of surviving and in clinical practice.

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Principal Investigator	Principal Investigator's Organization	Project Title	General Audience Abstract
Shari Pilon-Thomas, Ph.D.	H. Lee Moffitt Cancer Center and Research Institute	Lymphodepletion generated Myeloid Derived Suppressor Cells Decrease the Efficacy of Adoptive Tcell Therapy for Melanoma	Melanoma is a leading cause of cancer mortality in the United States. Patients with melanoma and other cancers have immune cells (T cells) that are capable of recognizing and killing tumor cells. These T cells are ineffective due to suppressive factors in the cancer patient that allows tumors to "escape" from recognition by T cells. These factors include myeloid derived suppressor cells (MDSC) that actively shut off T cell responses. One strategy to improve immune responses against tumors is adoptive cell therapy (ACT) using tumor-specific T cells. In this strategy, T cells are isolated from patient tumors and expanded in the laboratory to high numbers. This process allows the T cells to become re-activated and capable of mediating tumor killing. The expanded T cells are transferred back to the patient. ACT with tumor-specific T cells has emerged as one of the most powerful therapies resulting in a 50% response rate in patients with unresectable metastatic melanoma. In order for this therapy to be effective, the patient must be treated with drugs that induce lymphopenia (depletion of circulating white blood cells). Induction of lymphopenia is important as it creates extra space for the transferred T cells to survive and proliferate. In addition, suppressive factors including MDSC are reduced during lymphopenia, allowing for maximum activity of transferred T cells. Lymphopenia is a temporary state and white blood cells will begin to repopulate the blood within a week after T cell transfer. Our preliminary results show that MDSC recover quickly after the induction of lymphopenia and are even more suppressive than prior to induction of lymphopenia. This rapid repopulation of highly suppressive MDSC may decrease the effectiveness of ACT by shutting off T cells and preventing complete tumor regressions. The research proposed in this application will improve the understanding of MDSC expansion and suppressive functions in the setting of lymphopenia and determine the effects of blocking MDSC expansion and function on T cell responses after ACT. The goals of this

			proposed research are threefold: 1. To evaluate the role of MDSC populations at the tumor site after induction of lymphopenia; 2. To define the factors that contribute to the rapid expansion and function of MDSC populations in the setting of lymphopenia; and 3. To examine the reconstitution and function of MDSC populations in melanoma patients enrolled in ongoing ACT clinical trials. Achievement of these goals will determine whether eliminating MDSC in the setting of lymphopenia is feasible to improve the activity of tumor-specific T cells. As a consequence of these studies, novel approaches based on MDSC blockade and ACT may result and improve therapies for patients with advanced cancers.

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Principal Investigator	Principal Investigator's Organization	Project Title	General Audience Abstract
John Copland, PhD	Mayo Clinic, Jacksonville	Novel Metabolic Target Induces Immunogenicity and Antitumor Synergy with Immune Checkpoint Inhibitor Leading to Survival Benefit	Recent studies have implicated lipid or fatty acid (FA) biosynthesis and desaturation as a requirement for tumorigenesis, survival and progression. A key mediator of FA biosynthesis, stearoyl CoA desaturase one (SCD1) is rate-limiting in the conversion of saturated fatty acids (SFA), such as oleic and palmitic acid, to monounsaturated fatty acids (MUFAs), palmitoleate and oleate, which are preferentially transformed into triglycerides for storage or phospholipids for membrane formation. SCD1 mRNA and protein are overexpressed in most aggressive cancers. Specifically, high SCD1 levels correlated with poor patient survival in breast cancer. Our published cell culture and animal model data demonstrated endoplasmic reticulum (ER) stress induced cell death as a mechanism of action for antitumor activity alone and in synergistic combination therapy. From these promising results, we developed four novel SCD1 inhibitors. Two lead SCD1 inhibitors bind SCD1 with EC50s of 1.9 and 29 nM with similar proliferation IC50 values. SSI-4 induced apoptotic cell death via ER stress across a wide range of cancer histotypes. The results led to a patent filing of novel composition of matter. We now show for the first time that inhibition of SCD1 increases the immunogenicity of poorly immunogenic tumors. The enhanced immune activation is accompanied by upregulated ER stress. Inhibition of SCD1 increased both recruitment and activation of immune cells in vivo, which when combined with PD-1 blockade resulted in potent and durable anti-tumor T cell responses in models of HER2 breast cancer. In the TUBO model, tumors were completely insensitive to anti-PD1 therapy but when combined with SSI-4, 80% of mice were cured. Thus, we discovered that aberrant de novo lipogenesis is linked to tumor immunogenicity, SCD1 inhibitors are immune-sensitizing agents and SSI-4 may be used as an adjuvant therapy with other immunotherapies including checkpoint blockade. Together, our results indicate that inhibition of tumorigenic de novo lipogenesis represents a novel approach to enhance T cell-based cancer immunotherapy. We propose to further develop SSI4 combination therapy with anti-PD1 and anti-PD-L1 immune checkpoint inhibitors using mouse models of breast and colon cancers and melanoma leading to a patent filing to protect and enhance commercialization potential of our SCD1 inhibitors. We also intend to file an investigation of new drug (IND) with the Federal Drug Administration (FDA) for SSI4 leading to clinical trials testing blockade of SCD1 and immune checkpoint as a therapeutic strategy to enhance survival in cancer patients. To reach these goals, we propose three aims: (1) demonstrate antitumor synergy and survival benefit of SSI-4 in combination with anti-PD1 and anti-PD-L1 antibody in triple negative and HER2+ breast cancers as well as

			colon cancer and melanoma, (2) examine mechanisms of action whereby SSI-4 sensitizes tumors to checkpoint inhibitors, (3) write a clinical trial. In summary, we are developing novel SCD1 inhibitors which are currently not in development for the treatment of cancer. We predict that these inhibitors will find broad applicability and benefit patient survival, especially in cancer populations where immune checkpoint inhibitors are not effective.

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Principal Investigator	Principal Investigator's Organization	Project Title	General Audience Abstract
Brian Ruffell, Ph.D.	H. Lee Moffitt Cancer Center and Research Institute	Regulation of Dendritic Cell Function and Tumor Immunity by TIM-3	Tumor immunity is predicated upon the de novo activation and expansion of antigen-specific cytotoxic T lymphocytes. However, to impact tumor growth these T cells must also infiltrate into tumors, overcome a suppressive environment, and avoid becoming exhausted in the presence of persistent antigen, barriers that are thought to be major impediments to immunotherapy. Conventional dendritic cells are well established as the central inducers of the adaptive immune response, but emerging evidence suggests they may also play in supporting T cell activity within peripheral tissues, including tumors. In support of this, we have found in preliminary studies that TIM-3 (T-cell immunoglobulin and mucin domain containing-3) is highly expressed by tumor dendritic cells, and that TIM-3 blockade induces expression of the chemokine CXCL9 in vitro and in vivo, thereby promoting T cell cytotoxic effector function in models of mammary (breast) carcinoma. Here we propose to identify the dendritic cell activation pathways altered by TIM-3, determine if non-migratory dendritic cells maintain T cell function within tumors, and determine the role of CXCL9 expression by dendritic cells in tumor immunity. These studies will delineate a putative dendritic cell regulatory pathway and improve our understanding of the role of dendritic cells within tumors, both factors that may have important implications for the design of combinatorial immunotherapies in breast cancer.

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Principal Investigator	Principal Investigator's Organization	Project Title	General Audience Abstract
Keiran Smalley, Ph.D.	H. Lee Moffitt Cancer Center and Research Institute	Defining and Therapeutically Targeting HDAC8-driven Reprogramming in Melanoma Brain Metastasis Development	The long-term goals of this research program are to develop strategies that improve the survival of patients with advanced melanoma, the deadliest form of skin cancer. Among all tumor types, melanoma has a high likelihood of spreading to the brain. Brain metastasis occurs in ~30% of melanoma patients (as high as 75% at autopsy), and the brain is often the major site of treatment failure for patients who otherwise responded well to current FDA-approved melanoma therapies. A new therapy that prevented melanoma cells from growing in the brain would allow our patients to live for longer and have a better quality of life. We currently do not understand how melanoma cells spread to the brain and this limits our ability to develop new therapies for this devastating complication of advanced melanoma. New research will help address this gap in our understanding. In order to spread to the brain, melanoma cells escape from the primary tumor and enter the blood (which is typically a hostile environment for cancer cells) and then survive for long enough to reach the brain. Once in the blood supply of the brain, the melanoma cells stick to the blood vessels and then crawl through small gaps in the vessel walls to enter the brain. We believe that the melanoma cells that can do this have special molecular properties, which may also make them vulnerable to new drugs. In preliminary studies, we uncovered a "molecular switch" called HDAC8 that reprogrammed the melanoma cells to survive for longer in the circulation (the veins and arteries) by increasing their physical toughness and allowing them to move more fficiently through the blood vessels into the brain. We believe that this new cellular "program" we identified could be the key to preventing patients from developing new melanomas in their brains. Our proposal has three key goals. 1) To map out the special cellular "program" that makes the melanoma cells more tough, this will allow us to identify new therapeutic targets in these cells 2) determine how this cellular program changes the behavior of melanoma cells so they can survive in the blood vessels and then crawl through the blood vessels and enter the brain 3) test new drugs that kill the melanoma cells with the "brain homing" properties and determine whether the new therapy can be used in combination with established melanoma therapies that either kill the melanoma cells directly or stimulate the immune system to kill melanoma cells.

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Principal Investigator	Principal Investigator's Organization	Project Title	General Audience Abstract
Robert J. Gillies, Ph.D.	H. Lee Moffitt Cancer Center and Research Institute	Targeting the Lipogenic Phenotype Induced by Extracellular Acidosis in Breast Cancer	Solid tumors are unequivocally acidic due to elevated rates of glucose fermentation coupled with poor perfusion. Tumor microenvironmental acidity has been shown to promote local invasion, metastasis, and resistance to immune surveillance. It is axiomatic that adaptation to this acidic microenvironment is essential for tumor cells to survive and thrive and further, to out-compete the stroma into which they invade. In prior work, published in Cancer Research and Nature Communications, we have shown that acid adaptation is associated with chronic activation of autophagy and redistribution of the lysosomal proteins to the plasma membrane. These interrelated processes are key survival mechanisms adopted by tumor cells under acidic conditions. In subsequent studies (manuscript in preparation), we have also observed that acid adaptation is accompanied by a robust and dramatic increase in the accumulation of cytoplasmic lipid droplets ("adiposomes"). We hypothesize that adiposomes are coupled to autophagy and lysosomal redistribution and, hence, adiposome formation is a rapid readout for these other processes that together form an acid adaptation network. This has been observed in prostate, melanoma, lung, cervical, and breast cancer cells, as well as normal fibroblasts. Adiposomes are known in other organ systems (e.g. NASH and FLD in liver) to be induced under metabolic stress. They are dynamic organelles that store neutral lipids surrounded by a shell of proteins (perilipins) and a phospholipid monolayer. Notably, perilipin expression is a negative prognostic indicator in breast and ovarian cancers. Although a lipogenic phenotype is frequently observed in cancer, little is known about why they accumulate in acidic conditions or how acid signal perceived at the cell surface results in accumulation of lipid droplets. To identify if plasma membrane acid sensors are involved in transducing the signal, we used CRISPR/Cas9-mediated depletion of major acid-sensing G-protein coupled receptors (GPCRs) in breast cancer: TDAG8 and OGR1. In both MCF7 and T47D cells, we observed that OGR1 (but not TDAG8) depletion inhibited acid-induced adiposome accumulation. In this proposal we will explore signaling downstream of OGR1 and functionally characterize OGR1 knockout cells to unravel the entire signaling cascade. We have also shown that acid-induced accumulation of lipid droplets persists even when cells were in de-lipidated serum, indicating de novo synthesis. Indeed, 13C tracer studies indicate that ketogenic amino acids resulting from autophagic protein degradation are the primary source of carbons for de novo synthesis in adiposomes. Paradoxically, fatty acid synthesis appears to occur contemporaneously with lipid β oxidation (βox), indicting a high turnover of adiposomes. Notably, inhibition of fatty acid synthesis or βox selectively killed cells under acidic, compared to neutral, conditions hence, this has identified a novel therapeutic vulnerability. We propose to further characterize the mechanisms controlling these

			metabolic pathways using gene editing in combination with 13C tracer metabolite analyses of fatty acid synthesis and degradation. Finally, we will assess if adiposomes are associated with acidosis and/or aggressiveness using mouse models and human tissue micro arrays. We believe that the proposed studies will shed light on novel aspects of cancer biology and identify further new therapeutic opportunities.

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Principal Investigator	Principal Investigator's Organization	Project Title	General Audience Abstract
Otto Phanstiel, PhD	University of Central Florida	Developing Polyamine Transport Inhibitors for the Treatment of Human Cancers	The long-term objective of this project is to develop new cancer therapeutics. We are focused on pancreatic cancer because it is the fourth-leading cause of cancer-related death and has a shockingly-low five-year survival rate of <8%. As such new medicines are desperately needed. The most common pancreatic cancer is pancreatic ductal adenocarcinoma (PDAC). We have discovered a unique protein signature which indicates which PDAC tumors have high polyamine transport activity. We hypothesize that these tumors are driven to maintain high levels of intracellular polyamines by the genetic mutation in the Kras gene which occurs in the vast majority of PDAC cases. This makes sense because polyamines are important growth factors for cells and play critical roles in translation, transcription and chromatin remodeling. The high polyamine levels are maintained via increased polyamine biosynthesis and up-regulated polyamine import. The danger to the tumor is polyamine overload as the native polyamines can become toxic to the cell at high concentrations. The tumor avoids this danger by exporting polyamines into the surrounding tumor microenvironment. This has consequences. For example, secreted polyamines like spermine can inhibit immune cells from mounting a proper immune response. We speculate that polyamine exchange between tumor and stroma creates a zone of high polyamine concentration near the tumor. This polyamine 'shield' provides immune privilege because the approaching immune cells are compromised, when they import spermine. Indeed, this is a fetal strategy because spermine is present at high levels in amniotic fluid to protect the developing fetus from maternal immune cells. We believe that PDAC uses this fetal strategy, a spermine shield, to temper the immune response. There is solid evidence which suggests that immune cells (e.g., T cells, NK cells, macrophages) with high polyamine content have dramatically reduced ability to fight the tumor. In short, the tumors' polyamine trash is used to inhibit the immune response and is also recycled by the stroma and used to cross feed the tumor. This creates a zone of immune privilege which the tumor uses to survive and grow. Our approach is to develop new medicines (polyamine transport inhibitors, PTIs) which block the import of polyamines into cancer cells. These compounds when used with a polyamine biosynthesis inhibitor will starve the tumor of its polyamine growth factors and eventually shunt the tumor to programmed cell death (apoptosis). The project will also explore how polyamine transport genes are regulated and respond to external polyamine stimuli. An increased understanding of these transport response processes could also lead to new drug targets. Lastly, we will evaluate the new medicine in two mouse models of pancreatic cancer to show that this therapeutic approach works in vivo as well as on ex vivo human PDAC samples. The PTIs are owned by UCF and there are no impediments in terms of intellectual property rights and commercialization. A success here will provide a new combination therapy for pancreatic cancer.

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Principal Investigator	Principal Investigator's Organization	Project Title	General Audience Abstract
Barry Hudson, Ph.D.	University of Miami	Therapeutic Targeting of RAGE in Breast Cancer Progression and Metastasis	The metastatic spread of breast cancer cells is the leading cause of cancer death, and therefore identifying new ways to treat metastatic breast and other carcinomas is imperative. We have shown that the Receptor for Advanced Glycation End-products (RAGE) and its ligand are a critical pathway underlying breast cancer pathogenesis. Human studies have revealed RAGE protein levels are increased in aggressive breast cancers, and higher levels of RAGE are predictive of worse breast cancer outcomes. Our preliminary and published data suggest RAGE drives key molecular processes leading to tumor invasion and metastasis. Further, in both xenograft and syngeneic mouse models of breast cancer, we have shown RAGE signaling drives malignancy through effects on cells of both the tumor and its surrounding stroma. Most importantly, we show for the first time that novel small molecule inhibitors of RAGE powerfully suppress breast cancer metastasis in mouse xenograft models. Therefore, further preclinical testing and validation of RAGE inhibitors are critical before translation to people with breast cancer. The current Technology Transfer Feasibility application aims to perform extensive testing and validation in animal models of breast cancer, in order to advance our novel RAGE inhibitors to clinical trials. Further the data generate from these studies will greatly improve the commercial viability of RAGE inhibitors. We will use multiple animal models and extensive ex vivo analysis to assess the efficacy of RAGE inhibitors. These will include patient derived breast cancer xenograft models, syngeneic mouse models (in different mouse strains; C57BL6 and BALBc), and spontaneous mammary mouse tumor models. Successful completion of this study will not only help understand how breast and other cancers metastasize, validate RAGE inhibitors in preclinical models, but also help in the translation to human clinical trials by improving their commercial value. Thus, the proposed research is highly relevant to the Florida BRAC mission and research priority areas which pertain to the understanding of the causes of breast cancers, the development of novel treatments, and their ultimate translation to clinical practice.

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Principal Investigator	Principal Investigator's Organization	Project Title	General Audience Abstract
Ashok Saluja, Ph.D.	University of Miami	Role of Microbiome in Modulating Liver Metastases in Colon Cancer	Metastases or the consequence of their treatment are the biggest contributor to death from cancer. Colorectal cancer is no different. In 2017 about 140,000 people will be diagnosed with colon and rectal cancer in United States. Out of which about 50% will be diagnosed with metastases, either at presentation or during the course of their disease. Thus, there is an urgent need to better understand the process of metastases and develop novel treatment strategies. Unfortunately, our understanding of the process of metastases is still rudimentary. For instance, it is unclear why certain organs are more prone to metastases as compared to others. While mechanical factors such as blood flow and lymphatic drainage pattern are certainly at play, cancer cells demonstrate tropism to certain organs for metastatic growth. In this regard liver is the most common site of liver metastases from colon and rectal cancer. Better understanding of why liver is such a favorable organ for metastases will lead to development of targeted therapies. Liver is believed to be an immune-privileged organ which favors the induction of tolerance than induction of immunity. Whether, this immune-tolerant phenotype contributes to the preponderance of metastases in liver is unknown. Furthermore, the reason why liver is an immunotolerant organ is unclear. Our preliminary data suggest that gut microbiome is responsible for creating an immunosuppressive environment in the liver. In our studies depletion of gut-microbiome with antibiotics prevents growth of liver metastases. We have also observed that depletion of microbiome is unable to inhibit liver tumor growth in a mouse lacking adaptive immunity suggesting that adaptive immune system is required for modulation of liver metastases by microbiome. Furthermore, T cells obtained from the animals after gut microbiome depletion are very effective in killing cancer cells. Based on this and other literature we have hypothesized that exposure to gut microbial antigens causes immunotolerances and creates a permissive environment for the metastatic colon cancer cells to grow. In the current grant-proposal, we will test this novel hypothesis. In aim 1 using models of colon cancer liver metastases and using antibiotics induced microbiome depletion, use of germ-free mice and use of probiotics we will establish that microbiome modulates liver metastases. In aim 2, using immune profiling and animal experiments we will establish that the reduced liver metastases growth observed on depletion of microbiome is dependent on T cells. And finally, in aim 3, we will evaluate the mechanism by which gut microbiome modulates immune cells in the liver to create an immunosuppressive environment. These innovative studies will provide potential therapeutic breakthrough in treating colon cancer liver metastases by modulating entero-hepatic axis by routine antibiotics, probiotics or by targeting novel pathways identified in this research.

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Principal Investigator	Principal Investigator's Organization	Project Title	General Audience Abstract
David Robbins, PhD	University of Miami	Targeting Wnt dependent colorectal cancer	Constitutive activation of WNT signaling drives the growth of a broad array of human tumors, including nearly all colorectal cancers (CRCs). Despite this prominence, no Wnt inhibitors are currently approved for clinical use. The major reasons for this lack of inhibitors are the paucity of druggable WNT pathway components and the on-target gastrointestinal (GI) toxicity observed in animal models with many candidate inhibitors. Our results show that reduced expression of Casein Kinase 1α(CK1α), a negative regulator of Wnt signaling, is associated with decreased survival of CRC patients. These findings validate CK1α as a druggable target in CRC. We therefore characterized a novel, small molecule activator of CK1α, SSTC3, with pharmacokinetic properties that would allow us to target CK1α in vivo. SSTC3 attenuated CRC growth in vitro and in vivo, prolonging the survival of a mouse colorectal tumor model and inhibiting the growth of CRC xenografts. Importantly, SSTC3 did not exhibit significant GI toxicity. Thus, CK1α is a bona fide druggable target in CRC, activation of which inhibits tumorigenesis without inducing the GI toxicity that has hampered the clinical development of Wnt inhibitors. Despite this promise, many mechanistic questions remain regarding this novel class of Wnt inhibitors, which we will begin to address here. Specifically, we propose to i) determine how this class of small-molecules functions to activate CK1α activity, ii) identify the CK1α substrates that drive its efficacy but limit its effect on normal GI homeostasis, and iii) develop biomarkers that could be used to identify those CRC patients most likely to respond favorably to such inhibitors.

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Principal Investigator	Principal Investigator's Organization	Project Title	General Audience Abstract
Eric Wieder, PhD	University of Miami	Multiplex Imaging Resource for Florida State	In order to develop new treatments for cancer, and to better understand which cancer patients will benefit from a specific treatment, more sophisticated tools are continually being invented. These new technologies allow doctors and scientists to gain increasingly complex information on each person's cancer and will ultimately allow us to customize therapy to best benefit each patient and to provide the best possible outcomes. One such tool is the ability to look at tumor samples under the microscope and determine which cell types are in a tumor, what unique markers are on those cells and how other cell types within the tumor are interacting with it. This is done by taking a slice of tumor (biopsy), and staining it on a slide and then taking a magnified picture of it using a microscope. There are various staining methods which allow pathology labs to identify various characteristics of tumors, but a more sophisticated way uses antibodies tagged with colors to be able to distinguish different markers on cells within the tumor. In most labs, it is typical to be able to look at 1-4 markers at the same time, although there is specialized equipment that can look at 10-12 at a time. A recently developed technology uses metal atoms instead of colors to tag and identify each marker, which has increased the number of markers that can be studied simultaneously to 50 markers or more. This technology was commercialized to look at single cells, but not tumor biopsies, within the last decade. More recently, this tool was modified to allow it to work to image cells in a tumor biopsy. Although there are over 60 installations of the recently developed single cell technology at academic and government research centers across the USA, and 30 installations of the new tumor imaging technology across the world, there are none for either single cells or tumors in all of Florida. This disruptive technology has begun to be used by scientists all over the world and results are beginning to be published. If we purchase this new equipment, it will be able to be used both for tissue samples and for cells in a suspension. We have numerous labs at University of Miami and at Moffitt Cancer Center which have identified this technology as useful for their research. Creating this Imaging Center, which will be open to all cancer investigators in Florida, will greatly enhance our ability to stay competitive in the developing areas of cancer research since soon, it will be required that these complex measurements will be included in any study that involves either heterogeneity of tumors (differences within them), or immune therapy.

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Principal Investigator	Principal Investigator's Organization	Project Title	General Audience Abstract
Shanta Dhar, PhD	University of Miami	Multifunctional Nanoparticle for Targeted Combination Therapy of Prostate Cancer	Prostate cancer is the second leading cause of death in American male population. Prostate cancer at an early stage may be cured by surgery and/or radiation therapy. However, the advanced castration resistant prostate cancer is difficult to treat with currently available therapies. The use of a single therapeutic modality has limited success since several factors, inflammation, resistance, bone metastases, and participation of metabolically altered cancer stem cells (CSCs) play integral roles for progression and spread of this disease. We have developed a multifunctional polymer-based nanoparticle (NP) technology which has the ability to deliver a predefined stoichiometric combination of chemotherapy, anti-inflammatory dose, and an inhibitor of bone metastasis in a spatio-temporal and targeted manner to prostate cancer. More recently, we found that low-dose irradiation further sensitizes the activity of this targeted multifunctional platform towards prostate specific membrane antigen (PSMA) expressing advanced prostate cancer cells. Under ionizing radiation condition, this NP system was able to modulate mitochondrial metabolism and fatty acid oxidation-based respiration of PSMA expressing prostate cancer cells. Based on these results, we now formulated the current project combining several unique strengths offered by a highly integrated and interdisciplinary team and strong preliminary data to provide a platform with ability of loading multiple drugs with a predefined stoichiometric ratio for targeted co-delivery of chemotherapeutics, anti-inflammatory agents, and inhibitors of bone resorption to metastatic prostate cancer attacking PSMA expressing cancer cells, tumor associated inflammation and simultaneously reducing bone metastasis and inhibiting mitochondrial respiration, ATP production in CSCs forcing this population to undergo apoptosis, and evaluating this platform in patient derived prostate cancer preclinical model. Successful completion of the proposed aims will allow us to discover a therapeutic modality for treating metastatic prostate cancer, a major unmet clinical need.

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Principal Investigator	Principal Investigator's Organization	Project Title	General Audience Abstract
Esther Guzman, PhD	Florida Atlantic University	Discovery of Marine Natural Products Active Against Triple Negative Breast Cancers Using 3D spheroid Cultures; an In Vivo Relevant Assay Platform	Among women in Florida, breast cancer is the most commonly diagnosed cancer. Breast cancer ranks second as a cause of cancer death in women in the US (and in Florida). Triple negative breast cancers (TNBC) in particular have limited treatment options, as they are resistant to targeted therapies. The current project seeks to discover marine natural products from the Harbor Branch Oceanographic Institute (HBOI) natural products library with the ability to induce programmed cell death in triple negative breast cancer cells grown as spheroids. Triple negative breast cancers represent about 12% of breast cancers diagnosed in the US. TNBCs can be very aggressive spreading to other organs, particularly the brain and the lungs, and are more likely to recur than other breast cancers. They are also classified as high-grade tumors because of the minimal resemblance these cancer cells have to normal cells. Finding targeted therapies against TNBC remains an elusive goal for researchers. Over 50% of cancer drugs currently used originated from natural products. The oceans, which cover over 70% of the earth's surface, are a rich source of bioactive natural products. The uniqueness, chemical diversity and structural complexity of marine natural products represent an unexploited supply of potential new drugs, lead compounds for medicinal chemistry or biological probes to allow for better understanding of diseases. The Marine Biomedical and Biotechnology Research Program at HBOI has developed a unique library of pure and highly enriched fractions (peak) derived from marine organisms that will be used in this project. Many of the fractions/compounds are derived from deep water marine invertebrates that are not readily available outside of HBOI. We hypothesize that compounds with the novel activity of inducing programmed cell death in triple negative breast cancer cell spheroids can be found among secondary metabolites from marine organisms and that these compounds will have the potential to be novel therapeutics for the treatment of triple negative breast cancer or as probes to further our understanding of this kind of cancer. Cancer cells

			grown in spheroid conditions (3Dcultures) allow the cells to interact with each other and the extracellular matrix providing a better representation of the in vivo environment than 2 dimensional cultures. This proposal seeks to screen the HBOI library of marine natural compounds using TNBC cells grown as spheroids to identify compounds expected to be clinically active to address the overarching challenge of revolutionizing treatment regimens by replacing them with ones that are more effective, have less side effects, and impact survival. A multiparametric cell based assay that uses high content imaging will be used to measure cell number, induction of programmed cell death (apoptosis), and viability in two TNBC cell lines grown as spheroids. The assay was validated by screening a small subset of about 150 fractions from the HBOI enriched fraction library as well as a small subset of pure compounds. Lead compounds will be subjected to advanced genomic and proteomic differential profiling to increase our understanding of their cellular effects and potential mode of action. Successful completion of the proposed research should lead to the identification of a verified hit set, and chemical & biological characterization of the top active compounds.

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Principal Investigator	Principal Investigator's Organization	Project Title	General Audience Abstract
Michael Lu, PhD	Florida Atlantic University	PAK6 in Advance Prostate Cancer	Despite clinical evidence indicating that androgen promotes the metastatic progression of prostate cancer cells, the underlying mechanism remains unclear. The current proposal aims at studying a novel signal molecule PAK6 that functions as a hormone-regulated dominant factor in contributes to 'treatment-induced drug resistance' in advanced prostate cancer. The identification of PAK6 activation as an androgen-stimulated AR-mediated event suggests a potential target for intervention of hormone-regulated prostate cancer metastasis. We determined PAK6 is pivotal to the development of treatment-induced drug resistance in neuroendocrine (NEPC) advanced prostate cancer. Our data indicate PAK6 expression in advanced prostate cancer promotes drug resistance by downregulating Hippo pathway and up regulating Wnt signaling. The characterization of PAK6 as a therapeutic target to mitigate the treatment-induced drug resistant in prostate cancer will pave the way to a novel modality in combating this dreadful disease.

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Principal Investigator	Principal Investigator's Organization	Project Title	General Audience Abstract
Jennifer Steiner, PhD	Florida State University	Impact of Alcohol on Cancer Comorbidities	Colorectal cancer is among the most prevalent cancers and is the second leading cause of cancer related death in the United States. Frequently drinking moderate (>14 drinks/day) and high (≥4 drinks/day) levels of alcohol increases colon cancer risk. In Florida, drinking prevalence was above the national average as 24% of people 65 years and older reported higher levels of alcohol intake classified as 10+ drinks/week for men or 7+ drinks/week for women. This is concerning as cancer prevalence in Florida is the second highest in the nation and colon cancer risk increases with age. While the health implications of a cancer diagnosis are obvious, accompanying comorbidities, like cancer cachexia, lead to additional health concerns. Cancer cachexia occurs in ~50% of colon cancer patients and is characterized by the loss of skeletal muscle and fat mass directly contributing to decreased muscle strength, quality of life, and treatment compliance and efficacy, as well as increased mortality. Cachexia can develop at any point in the disease process, but typically worsens either as the tumor burden increases or during chemotherapy. Lifestyle may also influence cachexia risk and development. Alcohol for example, was recently shown to worsen cachexia and increase mortality in a mouse model of melanoma. Much remains to be learned about how alcohol contributes to cachexia especially in relation to the loss of skeletal muscle. Therefore, the main objectives of this work include: 1. Determination of the molecular factors enhancing the loss of muscle at each stage of cachexia when alcohol is consumed before and/or during colon cancer; 2. Test whether alcohol worsens cachexia during chemotherapy treatment with 5flurouracil (5FU); and 3. Assess the effectiveness of using muscular exercise to prevent or delay cachexia associated with alcohol and/or chemotherapy. These research questions will be addressed using a mouse model in which implanted colon cancer cells readily lead to cachexia. Two different paradigms of alcohol consumption will be used to determine whether the cachectic effects differ if the patient stops drinking alcohol before tumor growth or chemotherapy treatment versus continuing. Lastly, because exercise in the form of electrically stimulated muscle contraction has been shown to attenuate or prevent the loss of skeletal muscle caused by cancer, its efficacy in the presence of alcohol and/or chemotherapy treatment will be tested. The primary outcome of each experiment will be muscle size (i.e. cachexia development). Other variables will include measurement of molecular factors regulating muscle size including those in muscle growth pathways and

			those involved in muscle breakdown. A variety of functional tests will also be used to determine the incidence and severity of fatigue as well as any changes in muscular strength or function. This series of experiments will provide immediately translatable information to clinicians and patients about potential lifestyle changes that can be made to reduce their risk of cancer cachexia. Scientifically, this work will provide novel, foundational data pertaining to how alcohol increases skeletal muscle loss in the presence of cancer and will lead to the future identification of therapeutic targets or therapies to mitigate cachexia risk in those that have drunk alcohol or continue to drink alcohol even after a cancer diagnosis.

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Principal Investigator	Principal Investigator's Organization	Project Title	General Audience Abstract
George Rust, MD	Florida State University	Modeling Paths to Cancer Health Equity	While the quest to cure cancer continues, the greatest opportunity to dramatically reduce cancer deaths in the next decade will be to optimize utilization of existing innovations in effective screening and treatment across all segments of the population. Breast and colorectal cancers are two of the most screenable and treatable cancers, yet both still rank in the top five for cancer deaths. While death rates for each are declining in the U.S., the benefits of advances in early detection and treatment are disparate and not equitably shared. In fact, racial disparities in breast and colorectal cancer deaths have paradoxically widened in spite of myriad screening and treatment innovations. A major reason for this is that the benefits of these cancer innovations in early diagnosis and lifesaving treatments diffuse less quickly to disadvantaged segments of the population (racial-ethnic, socio-economic, rural-urban, and insurance sub-groups). These subgroup variations in uptake of new innovations are a preventable, yet major contributor to health disparities. Additionally, racial-ethnic disparities in optimal application of these innovations varies at the local level from community to community. Racial-ethnic minority persons in one community might have easy access to screening tests, but poor access to cutting edge treatments, or vice versa. Traditional prevention research and public health interventions have sought to reduce racial-ethnic disparities by testing the same intervention across many communities. Unfortunately, the intervention might be highly relevant to one community but not to another. For example, mobile mammography or cellphone text reminders for women to get screened might be very useful in a community that still has a low rate of breast cancer screening in the African American community, but it might have no impact on a nearby community with high screening rates in all racial-ethnic groups. Missing, yet desperately needed is a public health surveillance system and strategic decision support system that helps each community to understand where to target local interventions to achieve the most strategic impact on cancer outcomes. Our research program will create a method for using available data to define precisely the levels at which cancer disparities are being generated and amplified in each local community. We will provide rapid throughput computer models of what the most strategic leverage points are in each local community to achieve the most optimal and equitable cancer outcomes possible. Application of these models will provide a measure of "likely impact" for

			interventions at specific levels through common measures that define the relationship between screening rates, early diagnosis, and survivorship, leading to reduced disparities. Ultimately such mathematical and computer modeling will allow us to develop user-friendly web portals or even smartphone apps in which community health leaders could enter (from public health data sources) specific input parameters from their own community and manipulate variables to see what levels of interventions would be most impactful in improving cancer outcomes and eliminating cancer disparities in their communities. Additionally, this would be a powerful tool for enhancing communications and community buy-in for these interventions, as well as assuring that investments made to improve cancer outcomes would actually have their intended impact.

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Principal Investigator	Principal Investigator's Organization	Project Title	General Audience Abstract
Katarzyna Rejniak, PhD	H. Lee Moffitt Cancer Center and Research Institute	Metabolic Reprogramming to Improve Immunotherapy in Melanoma	Melanomas are generally aggressive tumors that are resistant to radiation and chemotherapy. Tumor-specific T cells are present in the peripheral blood of melanoma patients and infiltrate their tumors. However, these T cells generally fail to provoke tumor regression, and immunotherapeutic approaches to expand and activate tumor-specific T cells rarely result in cures. Immunotherapies to improve antitumor T cell activity (CTLA4, PD1 checkpoint blockade or adoptive T cell therapies) have led to durable antitumor responses in melanoma patients where conventional therapies have failed. However, the response rates remain low which suggests that additional immunosuppressive pathways may be active. We hypothesize that specific metabolic conditions in the melanoma microenvironment may play such an immunosuppressive role. It has been observed that the extracellular pH levels in some areas of melanoma tumors are very acidic, and tumors contain regions of profound hypoxia. Both low pH and low oxygen levels can lead to metabolic changes in tumor--infiltrating lymphocytes and the functional suppression of immune cells *in vitro*. Therefore, careful manipulations of these factors in the tumor microenvironment using pharmacologic interventions may enhance the function of tumor-specific T cells and improve responsiveness to immunotherapy. Our overarching hypothesis is that carefully planned manipulation of the tumor microenvironment can result in improved melanoma response to immunotherapy. Our methods include computational simulation studies informed by experimental data, to direct in vitro and in vivo experiments using defined T cells in a murine model of melanoma. We previously characterized changes in the properties of T cells when they are exposed to acidic pH and to low levels of oxygen in vitro. We also developed a computational model capable of simulating dynamical interactions between immune cell, tumor cells and tumor microenvironment. In this Bankhead Coley Bridge grant we employ our integrated experimental and computational techniques to predict the most optimal immunotherapeutic interventions at physiologically relevant pH (Aim 1) and O2 (Aim 2) that will lead to antitumor immunity. Subsequently, we will manipulate the tumor microenvironment in vivo in order to modulate its pH or hypoxia by pharmacologic interventions predicted by in silico simulations in order to increase the effects of immunotherapy. This will allow us to generate preliminary data to make our future applications for federal funding more competitive. This cross--disciplinary team science approach will lead to improvements in melanoma therapy by providing novel methods to enhance antitumor immune responses. The approaches developed in this proposal have high translational potential and may be used to design novel clinical trials.

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Paulo Rodriguez, PhD	H. Lee Moffitt Cancer Center and Research Institute	Functional reprogramming of tumor-MDSC through antibody-based therapies targeting Notch ligands	Solid tumors create a highly immunosuppressive microenvironment that impairs the development of protective immunity and limits the therapeutic efficacy of promising cancer immunotherapies. Myeloid-derived suppressor cells (MDSC) are primary components of the tumor milieu and have emerged as major drivers of T cell dysfunction in cancer. Despite their undeniable relevance in tumor-induced immune suppression, there are no current approaches to effectively block the immunosuppressive activity of MDSC in patients with cancer. Thus, novel therapeutic strategies to inhibit MDSC are urgently needed. Throughout the proposed research, we aim to determine the mechanisms by which the antibody-based blockade of the Notch ligands Jagged12 in tumor-bearing hosts functionally transforms MDSC into myeloid subsets that prime antitumor T cell responses. This is supported by crucial supporting results showing that treatment of tumor-bearing hosts with a humanized blocking mAb that recognizes the human and murine Jagged12 (CTX014) significantly delayed tumor growth and transformed MDSC into populations that promoted the infiltration of reactive CD8+ T cells into tumors and enhanced the efficacy of T cell-based immunotherapy. Therefore, we *hypothesize* that: 1) The expression of Jagged12 in cancer cells and/or tumor-infiltrating MDSC plays a central role in the suppression of protective T cell immunity in tumors; 2) Treatment of tumor--bearing mice with CTX014 functionally reprograms MDSC, overcomes tumor-related T cell suppression, and increases the efficacy of promising cancer immunotherapies. To test these postulates, we proposed the following Specific Aims: 1) Determine the role of cancer cell-Jagged12 in the immunosuppressive activity induced by MDSC in tumor-bearing hosts; 2) Elucidate the mechanisms leading to the upregulation of Jagged ligands in tumor MDSC and understand the endogenous effects of MDSC-expressed Jagged1 in tumor-induced tolerance; 3) Test the prediction that combined inhibition of Jagged in cancer cells and MDSC overcomes tumor-induced T cell suppression and enhances the efficacy of various forms of immunotherapy. Completion of this highly innovative and translational research will elucidate the role of Jagged1 and 2 as primary mediators for the T cell dysfunction occurring in tumors and pave the way for the development of a novel therapeutic approach to functionally reprogram MDSC in patients with cancer, which is expected to prevent and/or reverse tumor--induced T cell tolerance and boost the efficacy of promising forms of cancer immunotherapy.

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Gina DeNicoola, PhD	H. Lee Moffitt Cancer Center and Research Institute	Therapeutic Strategies for KEAP1/NRF2 Mutant Lung Cancer	Targeting the overactivation of cellular growth signaling is now a standard of care for subtypes of advanced non-small cell lung cancer (NSCLC), where adenocarcinomas driven by the EGFR or ALK genes can be successfully treated with selective inhibitors. Unfortunately, many patients do not respond to these treatments or relapse following an initial response. Further, despite the presence of overactive growth signals due to alterations in the FGFR, DDR2 or PIK3CA genes, such targeted agents have yet to demonstrate a clear benefit in patients with squamous cell lung cancer (SCC). Thus, there is dire need to develop new strategies for these patients. Notably, mutations in NRF2 and KEAP1 genes have been identified in 29% of SCC tumors and in 18% of adenocarcinomas. These loss of function mutations in KEAP1 and gain of function mutations NRF2 lead to constitutive NRF2 activity. Preclinical studies have suggested mutations in this pathway are associated with poor overall survival and modify the responses to targeted agents, chemotherapy, and radiation therapy. We submit that new therapeutics specifically designed against NRF2/KEAP1 will benefit lung cancer patients having mutations in this pathway and that this circuit may play broad roles in treatment responses to all standard treatment modalities used by clinicians. Despite the prevalence of NRF2/KEAP1 mutations, this pathway is under-investigated in lung cancer. Constitutive NRF2 activity promotes lung cancer growth and the resistance to therapy, but targeted therapies for patients with tumors having NRF2/KEAP1 mutations are lacking. Due to its function within tumor cells, it is difficult to directly inhibit NRF2 directly. Our work has shown that NRF2 alters the metabolism of cancer cells and confers druggable metabolic vulnerabilities. We will use two approaches to selectively target the metabolism of lung cancer cells with high NRF2 activity. The first exploits the addiction of these tumors to NRF2regulated metabolic processes, whereas the second relies on increased levels of select NRF2 targets that can be exploited to provoke cell death. We submit these studies will lay a foundation towards a cure for NRF2/KEAP1mutant NSCLC, which represents a significant patient pool. Further, we have developed model systems harboring NRF2/KEAP1 mutations and are uniquely positioned to use these as platforms to test the response of NRF2/KEAP1 mutant tumors to new therapies. Finally, we will leverage the Oncology Research Information Exchange Network (ORIEN), which integrates "big data" and directs data sharing for cancer research and care, to assess how NRF2 affects patient treatment responses. Collectively, our research team will evaluate the feasibility and efficacy of targeting tumor metabolism for treating NRF2/KEAP1 mutant lung cancer and will assess if NRF2/KEAP1 mutation status can be used as a predictor of treatment response and can guide treatment strategy.

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Nelli Bejanyan, MD	H. Lee Moffitt Cancer Center and Research Institute	Donor γδ T-cell Infusion for Treatment of High-Risk Leukemia	Stem cell transplantation (SCT) is the only curative treatment for many patients with acute myeloid leukemia (AML), which is the most common acute leukemia in adults. SCT, however, only cures some of the patients with AML. If leukemia cells remain detectable in the bone marrow after the initial chemotherapy, SCT may clear the leukemia for some time, but recurrence occurs in up to 65% of the patients and survival without leukemia recurrence drops to 25% only at 1 year after SCT. Immunotherapy with donor lymphocytes administered after SCT have increased cures and prolonged survival of patients with residual AML detected at the time of SCT. However, some donor lymphocytes, called aβ T cells, can attack the patient healthy tissues and result in life threatening graft-versus-host disease (GVHD). removal of donor aβ T cells can eliminate the risk of GVHD but preserve the potent antileukemia effect of the γδ T cells. Selecting γδ T cells from donor lymphocytes holds promise to increase the cures of AML patients who receive SCT for residual leukemia after chemotherapy. γδ T cells are rare in the blood. Promising results have shown that γδ Tcells circulating in patient blood can be expanded and used to treat various cancers. However, there is little to no experience with γδ T cell immunotherapy for AML. We have engineered artificial antigen presenting cells (AAPC) that activate γδ T cell in the laboratory and achieved up to 1000fold expansion of healthy donor blood γδ T cells. We hypothesize that donor γδ T cells expanded with AAPC and infused to patients with post-chemotherapy residual AML can prevent leukemia recurrence after SCT without causing GVHD and improve survival without leukemia recurrence from 25% to 50%. The purpose of this application is to obtain funding support to test the safety and effectiveness of expanded donor γδ T cells for treatment of AML after SCT and to assess its impact on leukemia recurrence, GVHD and patient survival after SCT. Specific Aims: (1) Determine safety and effectiveness of AAPC expanded donor γδ Tcells infused after SCT in patients with evidence of AML just before SCT in a first-in-human phase I/II relapse prevention trial. *Hypothesis: Donor γδ T cells infused after SCT will reduce leukemia recurrence without increasing the risk of GVHD.* Infusion of donor γδ T cells expanded in the laboratory for the prevention of leukemia recurrence after SCT is highly innovative. (2) Study the leukemia cell killing activity of chimeric antigen receptor (CAR)engineered γδ T cells in a laboratory setting. *Hypothesis: γδ T cells engineered with a synthetic receptor developed in our laboratory will be more effective in killing AML leukemia cells than nonengineered γδ T cells.* These experiments are innovative and will direct future development of synthetic CAR γδ T cells for immunotherapy of AML.

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Principal Investigator	Principal Investigator's Organization	Project Title	General Audience Abstract
Ernst Schönbrunn, PhD	H. Lee Moffitt Cancer Center and Research Institute	Development of novel TAF1 inhibitors	The bromodomain-containing protein TAF1 (Transcription initiation factor TFIID subunit 1) is important for the placement of RNA polymerase II during transcription initiation. TAF1, a 250 kDa protein, is the largest subunit of the general transcription factor TFIID and is comprised of an N-terminal kinase domain, a histone acetyltransferase (HAT) domain, an ubiquitination domain, a tandem bromodomain and a C-terminal kinase domain. Recent reports established a role of TAF1 in diverse cancers. A genomics landscape study identified TAF1 as one of five significantly mutated genes in uterine serous carcinoma, and TAF1 overexpression has been associated with the high mitotic activity of human lung and breast carcinoma cells. Furthermore, TAF1 has been reported to promote Mdm2mediated degradation of the tumor suppressor p53 while downregulation of TAF1 decreased levels of the protooncogene c-MYC. Our long-term goal is to develop cancer therapeutics using structure-based drug design. We hypothesize that inhibition of the histone-binding modules of the multifunctional protein TAF1 by small molecules may present a viable strategy to attenuate the transcription machinery of cancer cells through an epigenetic mechanism of action. To date, three small molecule inhibitors of TAF1 have been reported, two of which are nonselective pan--bromodomain inhibitors, while compound BAY299 showed high potency for TAF1 but also against other bromodomains including BRPF2 (involved in embryonic stem cell differentiation). No TAF1 inhibitor has reached the clinic yet. Recently, we discovered that the kinase inhibitor AZD6738, a specific inhibitor of the Ataxia telangiectasia and Rad3related protein (ATR) which is in clinical trials for various solid and liquid tumors, also potently inhibits the second bromodomain of TAF1. While we and others previously identified a number of kinase inhibitors as inhibitors of BET bromodomains (dual BET-kinase inhibitors), AZD6738 is the first kinase inhibitor discovered which acts on a bomodomain outside the BET family. In preliminary studies we determined high resolution cocrystal structures of TAF1 liganded with AZD6738 and close analogues, revealing large conformational changes of the tandem bromodomain upon inhibitor binding. The knowledge of the precise binding pattern of AZD6738 in the acetyllysine binding site provides a new structural framework for the design of inhibitors with high potency and selectivity for TAF1 with or without the ability to concurrently inhibit ATR. Here, we propose an interdisciplinary approach towards the development and in-depth characterization of novel TAF1 inhibitors using diverse cell and animal models of lung and breast cancer.

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Peter Storz, PhD	Mayo Clinic, Jacksonville	Role of ICAM1 in development and progression of pancreatic cancer	Pancreatic ductal adenocarcinoma (PDA) carries a dismal prognosis. Understanding the mechanisms that lead to the development and progression of PDA in order to identify novel methods of intervention is the greatest hope for prevention and treatment. Animal models have shown that the development of pancreatic cancer is driven by two events, the acquisition of an oncogenic mutation in KRas and pancreatic inflammation. Our previous work demonstrated that oncogenic KRas upregulates a soluble form of ICAM1 (sICAM1), which acts as chemo-attractant for inflammatory macrophages (M1) to initiate the formation of pancreatic lesions. We also have shown that pancreatic lesions can crosstalk with M1 macrophage populations in order to induce their polarization to an alternatively-activated phenotype (M2) that is tumor promoting. This proposal focusses on understanding the mechanism of how macrophages are attracted by precancerous lesions, but also on how their conversion into tumor-associated macrophages can be prevented. Successful completion of our project will demonstrate the importance of KRas-induced expression and processing of ICAM1 for the development and progression of pancreatic cancer, but also lead to novel strategies to keep macrophages absent, and thus halt desmoplasia, lesion progression and metastasis.

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Izidore Lossos, MD	University of Miami	Identify the mechanisms of LMO2-mediated inhibition of homologous recombination and establish PARP-targeted synthetic lethality as a new therapy for DLBCL	Diffuse large B-cell lymphoma (DLBCL) is the most common subtype of non-Hodgkin lymphoma (NHL), with ~25,000 new cases yearly. Despite marked improvement in therapy, about half of these patients succumb to their disease. Therefore, there is a strong need for new therapeutic approaches to improve DLBCL patients' survival. Here we show that in DLBCL cells the LIM domain-only 2 (LMO2) protein inhibits DNA double-strand break (DSB) repair via homologous recombination (HR), resulting in HR-dysfunction. This HR-dysfunction phenocopies BRCA1/2 mutations in breast, ovarian and castration-resistant prostate cancers. Accordingly, we show that LMO2 predisposes DLBCL cells to synthetic lethality upon treatment with Poly(adenosine diphosphate ribose) polymerase 1 and 2 (PARP1/2) inhibitors. The *long-term goal* is to demonstrate that PARPi activity may improve outcome of patients with LMO2 expressing DLBCL. The *overall objectives* of this proposal are to determine the mechanisms by which LMO2 inhibits the repair of DNA breaks via HR and whether LMO2 expression levels can be exploited as a biomarker for sensitivity of DLBCL to PARP1/2 inhibitors. The *central hypothesis* is that inhibition of DNA repair via HR induced by LMO2 will sensitize DLBCL tumors to PARP1/2 inhibitors. The *rationale* for this project is that deficiency in HR and failure to repair DSBs produced during replication can lead to genomic instability and/or cell death. Indeed, PARP1/2 inhibitors that cause the accumulation of toxic DSBs during replication, had been exploited for the treatment of HR-deficient solid tumors. Our preliminary data showed that in DLBCL cells LMO2 inhibits the HR pathway. Thus, we propose that inhibition of HR by LMO2 will sensitize DLBCL tumors to PARP1/2 inhibitors. In order to test the central hypotesis and determine the mechanism by which LMO2 controls DNA repair we propose three *specific aims*: *1)* Identify mechanism(s) of LMO2 mediated inhibition of HR in DLBCL; *2)* Determine how LMO2 affects immunoglobulin class switch recombination in normal B-cells; and *3)* Demonstrate that DLBCL expressing LMO2 are sensitive to PARP1/2 inhibition. The proposed research is *innovative* because it represents a substantive departure from the current status quo by demonstrating that expression of LMO2 protein predicts therapeutic activity of PARP1/2 inhibitors in DLBCL and represents an effective new therapeutic strategy that will broaden the existing arsenal against this lymphoma. The research proposed is *significant* because it is expected to provide strong scientific justification for the development of a novel therapeutic approach for DLBCL based in PARP1/2 inhibitors that could potentially change the current treatment of DLBCL patients and improve their outcome. Our studies will generate evidence for the rationale to use PARPi alone and in combination with DNA-damaging chemotherapy for clinical trials in DLBCL.

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Anthony Capobianco, PhD	University of Miami	Development of Small Molecule Inhibitors of Wnt/β-catenin Transcriptional Activation	Deregulation of the Wnt/βcatenin signaling pathway has been demonstrated to play a role in tumorigenesis, as evident by its involvement in multiple malignant tumors in humans. This pathway is also implicated in maintenance and survival of cancer stem cells, which may confer resistance to chemotherapy. The important role played by Wnt/βcatenin signaling in cancer makes it an exceedingly attractive target for cancer therapeutics. However, the full range of potential targets in the pathway have been underexplored. To date, there are no small molecule inhibitors that successfully target the intracellular Wnt/βcatenin signaling pathway. Once βcatenin translocates to the nucleus, it is involved in the formation of a core transcriptional activation complex, where it binds to TCF/LEF, BCL9/BCL92 and other cofactors, which then recruit additional members of the transcriptional machinery. This βcatenin nuclear complex initiates and maintains transcriptional activation of Wnt target genes. The overarching hypothesis of this proposal is that compounds that prevent the formation of the βcatenin nuclear complex by targeting βcatenin and BCL9/BCL92 interface would be potent inhibitors of the Wnt/βcatenin pathway. We have used a combination of computational and biochemical studies and identified a lead compound that inhibits Wnt/βcatenin signaling. Therefore, the overall goal of this project is to optimize the scaffold of this lead compound to identify clinical candidates that inhibit assembly of the βcatenin nuclear complex in order to develop novel potent druglike small molecule inhibitors of Wnt/βcatenin mediated transcription. To this end, we will use an innovative approach that combines current state of the art computational, biochemical and biophysical techniques. Successful completion of this study will fulfill an unmet need in terms of therapeutic agents targeting the Wnt/βcatenin signaling pathway that specifically target the βcatenin nuclear complex, therefore providing specific inhibition of Wnt/βcatenin transcriptional activation complex, which could complement and/or offer an alternative to current therapeutic approaches. We will achieve the goals of this proposal through the following specific aims: (I) Lead optimization through structure activity relationship studies (II) Biochemical and biological assessment of lead analogs, (III) Preclinical evaluation of efficacy of lead clinical candidates.

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Kerry Burnstein, PhD	University of Miami	Data-Driven Identification of Novel Precision Drug Combination Therapies for Prostate Cancer	Advanced prostate cancer (PC) is particularly challenging to treat, because tumors almost always develop the ability to evade drugs, leading to uncontrollable and incurable cancer growth. Tumors acquire "resistance" to drugs by a number of strategies that vary in different men and can even differ between tumors growing in the same patient. Race and ethnicity also contribute to differences in PC tumors and their response to drugs. Thus, there is no "one size fits all" therapeutic regimen for aggressive forms of PC. To combat currently incurable stages of PC most effectively, a precision medicine approach is needed, that is, one in which treatment is tailored specifically to the features of an individual patient's tumor. Fortunately, researchers have huge amounts of available molecular, genetic and clinical information on PC from a broad variety of different patients. With advanced computer-aided (computational) methods, researchers are beginning to identify distinct patterns or "signatures" that occur in drug-resistant tumors. The challenge is to exploit these massive amounts of data (also called "big data") in an efficient and logical manner to identify and prioritize new drugs for treating PC. Such data-driven approaches, based on disease signatures, have already led to "drug repurposing" in which drugs that are commonly used for one type of disease or condition can be prescribed for treating a different disease including PC. Also, combining two different drugs has proven highly promising for prostate and other cancers, because this approach often results in beneficial clinical responses that are greater than the sum of the two individual drugs (termed synergy). This proposal will leverage the distinct and complementary expertise of two principal investigators: a prostate cancer researcher with a long track record of identifying and testing new experimental PC therapies and a chemist / data scientist who is pioneering the use of big data to identify new drugs and drug combinations (as well as entirely novel methods) to block cancer growth. The proposed study will integrate collections of big data, including disease and gene signatures that are specific to and representative of a large variety of prostate tumors, with the known responses of over 50 human cancer cells (including PC) to over 1,500 FDA-approved drugs and compounds in clinical trials plus thousands of additional druglike molecules. A computational algorithm will identify drugs that have a known effect on PC-specific gene signatures and are therefore predicted to block growth of tumors with distinct features. The goal is to make highly-informed choices about

			which new drug regimens are appropriate to use on particular tumors. A novel computational platform developed by one of the principal investigators employs computer-aided evaluation of tens of millions of different combinations of drugs to predict the precise combinations that will block the growth of different PC tumors. The highest ranked predicted drug combinations will then be tested in mice bearing different human tumors and mimicking the different stages that occur in men with PC, e.g., recurrent tumors, metastatic tumors. Successful treatment of cancer depends on initiating the right therapy as early as possible. Therefore, the proposed study aims to improve PC treatment outcomes by identifying patient-specific targeted therapies customized to the exact tumor type and the exact stage of tumor development.

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Rays Jiang, PhD	University of South Florida	Targeting Heme Dependency in Leukemia	Red blood cells (RBCs) are the most numerous cells in the human body and are constantly replenished at the astonishing rate of 106per second. When the red blood cell production process, called erythropoiesis, becomes malignant, patients develop erythroid neoplasia (EN), often with poor prognosis. Identification and targeting EN progenitors are key to stamp the enormous flow of cancer cell formation for effective EN treatment. Currently, identifying different hematopoietic progenitor cells involved in cancer formation, at distinct developmental steps, is technically challenging. In this proposal, we will use our single-cell genomics expertise to construct the first single-cell EN development map. Particularly, we hypothesize that erythroid malignancy is inextricably linked with heme overdrive, the high level of dependence of heme biosynthesis in cancer progenitor cells. We will track down the origin of malignant erythroid lineages and identify potential diagnostic and therapeutic targets, including those responsible for heme overdrive. Of relevance, recent studies involving CRISPRCas9 genome editing technology demonstrated that increased expression of the gene for housekeeping 5aminolevulinate synthase, the enzyme responsible for the rate-determining step of heme biosynthesis, correlates with enhanced heme production in thirteen different cancer cell lines. The ubiquity of heme synthesis extends the significance of our proposed work to other types of cancer and to the general postulate that lineage determines the disease type. By providing and validating the first single-cell EN differentiation map, results from this proposed project will allow investigators to track the differences between cells in not only EN but also other heterogeneous tumors, which could guide and lead to treatment.

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Minjung Kim, PhD	University of South Florida	Testing the value of PTPN11 as a novel therapeutic target in BRAF wild-type melanomas	Melanoma is the deadliest form of skin cancer. Analysis of melanoma tumors has shown that abnormally activated Ras signaling pathways, resulting in the activation of RAF, MEK and ERK proteins, frequently contribute to melanoma formation. Inhibitors targeting BRAF or MEK have been approved to treat a subset of melanoma patients with BRAF V600E/K mutation, but no effective targeted therapies are available for the treatment of *BRAF* wild-type melanoma (40~60%). Therefore, identifying additional proteins that can be therapeutically targeted is required for the improved treatment of melanoma patients. Several previous studies have shown that mice and humans share several genetic events in the development of cancer and that these events may point to functionally important and evolutionary conserved alterations. Recently, we identified an activating mutation in *PTPN11* (Tyrosine Protein Phosphatase Non-Receptor Type 11) in melanomas developed in mice, which were previously seen in human cancer patients. Our preliminary studies uncovered the frequent activation of PTPN11 in melanoma patients and the tumor promoting roles of PTPN11 in *BRAF* wildtype melanoma melanomas by activating Ras signaling pathways. Supporting its therapeutic value, inhibition of PTPN11 caused regression of tumors, which was associated with decreased growth and increased death of melanoma cells as well as increased recruitment of immune cells into the tumors. This proposed study aims to 1) identify downstream effectors of PTPN11 and the resistance mechanisms to PTPN11 inhibition, 2) develop a rational combined therapy based on PTPN11 function in order to enhance the response rates in melanoma patients, and 3) assess the effect of PTPN11 inhibition on tumor and immune cells utilizing an activated PTPN11 mouse melanoma model we generated. Recently, a new class of drugs targeting PTPN11 has been developed and is currently being tested in clinical setting for safety. This proposed study will allow us to understand the molecular mechanisms underlying the response of melanoma to PTPN11 inhibition and will identify a better way to predict a patient's likelihood of responding to PTPN11 inhibition allowing for novel therapeutic opportunities for melanoma patients. In addition, this study will validate PTPN11 as a novel therapeutic target in melanoma providing a path to the clinic.