Cancer Research Coordinating Committee

Abstracts for 2018 Awards

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Outcomes in Stage IV Cancer Patients with Bowel Obstruction

Host Campus: Davis
Lead Investigator: Robert Canter
Start Date: 1/1/2018 *End Date*: 12/31/2018 *Amount*: $55,000

Abstract:
Although patients and clinicians consider oncologic outcome and survival the pre-eminent goals of cancer therapy, quality of life (QOL) and avoidance of therapeutic morbidity, particularly among patients with stage IV cancer, are receiving increasing attention as important goals of care. Consequently, prolonged hospitalizations, intensive care stays, emergency room visits, hospital readmissions, and aggressive therapies, such as chemotherapy and surgery, have come under scrutiny given the increasing emphasis on improved palliative care and QOL for patients near their end of life. These issues create a dilemma for surgeons, as patients with disseminated malignancy (DMa) commonly present with acute surgical conditions, such as malignant bowel obstructions (MBO), for which surgery has historically been the standard of care. The goal of this proposal is to examine the morbidity, mortality and surrogate endpoints for QOL among patients with DMA who present with MBO and are treated medically versus surgically. We hypothesize that surgical management will lead to higher rates of these morbidity/adverse QOL outcomes with correspondingly negligible differences in overall survival. We will test our hypothesis through the following specific aims: Aim 1: To demonstrate that rates of morbidity and associated endpoints (e.g. prolonged hospitalizations) are higher for surgically-managed versus medically-managed DMa patients with MBO. Aim 2: To compare overall survival between the surgically and medically managed cohorts. We will test this hypothesis using the California Office of Statewide Health Planning and Development database, specifically consisting of patients with the diagnosis of DMa and MBO admitted to a California licensed hospital from 2005 to 2010. We will obtain inpatient and emergency visit data to evaluate differences in endpoints (i.e. morbidity, prolonged hospitalizations, ICU stays, readmission, emergency room visits and disposition to nursing facilities) for surgically versus medically-managed patients. In addition, we will use linked death data to examine differences in survival among cohorts. These data will have important implications for patients and surgeons as the data will provide a population-based assessment of the impact of medical versus surgical management on morbidity and survival as well as important metrics of QOL. This research is critical to shared patient and surgeon decision-making for this increasingly common and high risk patient population.

Analysis of cellular transformation during tissue renewal

Host Campus: Merced
Lead Investigator: Nestor Oviedo
Start Date: 1/1/2018 *End Date*: 12/31/2018 *Amount*: $55,000

Abstract:
Approximately 90% of the cancer related deaths originate in tissues undergoing constant cellular turnover (i.e. epithelia). Continuous cellular division that is essential for the renewal of adult tissues, also provides recurrent opportunities for cancer development. However, identifying the molecular connections between systemic tissue renewal and cancer formation remains as one of the most puzzling problems in modern biology. The process of cellular turnover relies on resident stem cells (SCs) that are instructed to proliferate, migrate, and differentiate in order to replace senescing and damaged cells throughout an organism's lifespan. We propose a novel approach to track cell proliferation during tissue renewal and in the complexity of the whole adult organism. We capitalize on a unique model system, the planarian flatworm, which constantly renews tissues through many years. Tissue renewal in planarians relies on SCs (neoblasts) that divide to attend high rates of cellular turnover and regeneration. Recently, we developed a planarian cancer model based on functional disruption of PTEN, one of the most commonly mutated genes in human cancers. PTEN disruption in planarians is characterized by neoblast hyperproliferation and tissue colonization by abnormal cells, which rapidly kill the animal. We catalogued the process of neoblast transformation during tissue renewal and defined early and late stages of the PTEN phenotype. Transcriptomic analysis comparing tissue renewal versus different time points of cancer development revealed candidate genes and signaling pathways that are altered prior to the onset of hyperproliferation. Intriguingly, inhibition of PTEN leads to differential response among neoblast progenitors and some neoblasts appear more susceptible to DNA damage before overproliferation. Our results suggest interplay between DNA damage response mechanisms and susceptibility among resident SC that may have important effects on the establishment and evolution of genomic instability during tissue renewal. We hypothesize that early manifestation of cellular transformation in planarians involve deregulation of PTEN and DNA repair mechanisms, which facilitates DNA replication stress and genomic instability in a particular neoblast subtype. We expect to identify crucial markers of cancer initiation and distinctive molecular features in SCs that drive cancer during tissue renewal.

Recurrent GLI mutations in drug-resistant skin cancer

Host Campus: Irvine
Lead Investigator: Scott Atwood
Start Date: 1/1/2018 *End Date*: 12/31/2018 *Amount*: $55,000

Abstract:
Basal cell carcinoma (BCC) are locally invasive skin cancers that affect over 4 million patients a year and are solely driven by activating mutations in the Hedgehog (HH) pathway. Inappropriate HH pathway activation also drives growth of a variety of cancers including brain, pancreatic, prostate, and small cell lung cancer that account for up to 25% of all human cancer deaths. The GLI1 and GLI2 transcription factors drive HH transcriptional output, with current therapies for advanced or metastatic BCCs limited to HH pathway antagonists that target proteins that lie upstream of the GLI transcription factors. Although effective, over 50% of advanced tumors display inherent drug resistance and 20% of tumors that do respond acquire drug resistance, indicating a critical need to understand the nature of drug resistance and to find the next generation of therapeutics. Towards this goal, we have found 110 mutations in GLI1 and GLI2 that may drive drug resistance by mining for recurrent mutations from our drug-resistant BCC patient tumor samples and cross referencing them to previously published tumor datasets across all sequenced cancers in the Catalogue of Somatic Mutations in Cancer (COSMIC) database. We have generated all 110 mutations and plan to stably express all variants in several HH responsive cell lines that include BCC lines ASZ001 and BSZ. We will characterize how each GLI mutation alters HH signaling, cell growth, and protein stability with a goal to identify specific clinically observed mutations that drive pathway activation. Positive hits that increase two out of the three criteria will be assayed for DNA binding, transcriptional activity, tumor growth, and drug resistance to understand how each mutation alters GLI function. So far, we have identified a cluster of mutations that disrupt interaction with the negative regulators PKA and SUFU, which significantly increases GLI activity levels. These results will provide insight on how GLI1 and GLI2 are regulated during HH pathway activation, how this regulation is altered during tumor growth and drug resistance, and will be invaluable in the discovery of future treatments for HH-dependent cancers.

Studying breast cancer initiation in single cell resolution

Host Campus: Irvine
Lead Investigator: Kai Kessenbrock
Start Date: 1/1/2018 *End Date*: 12/31/2018 *Amount*: $55,000

Abstract:
Breast cancer is one of the most prevalent forms of cancer in women worldwide. Despite recent advances in understanding the genetic mutations driving breast cancerogenesis, prognosis still remains poor especially due to late diagnosis and subsequent high mortality from metastatic tumor formation. One major scientific roadblock is that most of our scientific knowledge in cancer research is based on averaged ensemble analyses, although heterogeneity within the cell population is a striking feature of many tumors and plays a critical role in driving disease progression and therapy resistance. BRCA1+ carriers have a high risk of developing triple negative basal-type breast cancer, and thus commonly undergo prophylactic radical mastectomy. Studying these tissue samples from BRCA1+ carriers at pre-neoplastic and neoplastic stages offers a unique opportunity to study cancer initiation and progression in a primary human and clinically relevant setting. We hypothesize BRCA1-driven breast cancer leads to the disruption of the normal breast epithelial cell hierarchy and distinct systems-level changes in gene expression signatures not only within the subset of transformed tumor initiating cells, but also within other epithelial cell populations and non-epithelial microenvironmental components. We have established an interdisciplinary research approach utilizing comprehensive single cell RNAseq in combination with cutting edge bioinformatics pipelines to study tumor heterogeneity and to build a cell atlas delineating cancer initiation and progression in single cell resolution. By creating a cell atlas of the human breast in single cell resolution, and interrogating how the system goes awry during tumor initiation, we will identify disease promoting subpopulations, discover novel biomarkers and testable gene signatures to improve cancer early detection, and reveal novel therapeutic targets to prevent breast cancer from progressing into a life threatening condition. Ultimately, this project has the potential to revolutionize cancer genomics and precision medicine by introducing single cell genomics to translational breast cancer research, and thereby providing a first impetus towards the generation of a Single Cell Cancer Genome Atlas (SCCGA).

Towards in vivo imaging of immune cells for cancer therapy

Host Campus: Los Angeles
Lead Investigator: Shimon Weiss
Start Date: 1/1/2018 *End Date*: 12/31/2018 *Amount*: $50,000

Abstract:
Cancer immunotherapy is in need of simple and cost-effective tools to monitor and quantify the presence of CD8+ T cells and other immune cell types (e.g. CD4 T cells, macrophages, NK cells) in their target organs. The Wu group at UCLA has developed anti-CD4 and anti-CD8 radio-labeled cys-diabodies allowing non-invasive and quantitative assessment of endogenous T cell and post hematopoietic stem cell injection in lethally irradiated mice. While this technique allows detection and quantification of T cell populations using microPET/CT, it uses radionuclides and does not allow the distinction between different cell populations (PET does not allow for multiplexed imaging). To overcome this limitation, we propose to use our expertise in ultrasensitive fluorescence detection to develop a new approach based on the combination of near-infrared fluorescence lifetime imaging (NIR FLIM) and a novel photon-counting camera developed by our group. FLIM has many advantages over standard fluorescence by providing a concentration- and intensity-independent measure of local molecular properties. In collaboration with the Intes group at RPI, we have demonstrated a fast and simple in vivo NIR FLIM technique called phasor analysis. We believe that we can improve the performance of this approach by several orders of magnitudes using our detector instead of the camera used by the Intes group. In particular, its increased sensitivity should allow detection of low T cell concentrations such as encountered in cancer immunotherapy studies. Importantly, using lifetime imaging, two different fluorescent probes can be distinguished simultaneously, enabling simultaneous detection of two different immune cell subsets. The proposed project will establish the feasibility of NIR FLIM in mice using a pulsed laser and our photon counting camera, by demonstrating simple proof-of-principle imaging of NIR dyes in animal models (phantoms and animal carcasses with artificial fluorescent inclusions). The work of a post-doctoral associate in our laboratory, under the direction of Dr. Michalet, and in collaboration with the Wu lab, will generate preliminary data for a follow-up proposal, which will focus on developing a dedicated preclinical imaging instrument using near-infrared fluorescent probes and phasor-based FLIM analysis, capable of both 2D reflectance imaging and 3D tomography.

Chlamydia, HPV and centrosome dysregulation

Host Campus: Irvine
Lead Investigator: Ming Tan
Start Date: 1/1/2018 *End Date*: 12/31/2018 *Amount*: $55,000

Abstract:
Cervical cancer is caused by human papillomavirus (HPV) but only develops in a subset of women infected with HPV. Chlamydia trachomatis has been proposed as a co-factor in cervical cancer development based on sero-epidemiologic studies. This intracellular bacterium is an extremely common cause of sexually transmitted infection. HPV and Chlamydia could have a cooperative or synergistic effect on oncogenesis because they both cause centrosome dysregulation in infected cells (Duensing et al., 2000; Grieshaber et al., 2006). Centrosome abnormalities are common in many cancer cells and cause chromosome segregation defects and genetic instability, and enhance metastasis. HPV causes formation of supernumerary centrosomes via its E7 oncoprotein, which hyperactivates Cdk2 via negative effects on cyclin-dependent kinase inhibitors (Duensing et al., 2007; Funk et al., 1997). HPV E6 oncoprotein also causes centrosome amplification, although this effect may be indirect (Duensing et al., 2001). Chlamydia induces centrosome amplification at much higher rates than HPV by mechanisms that have not been fully elucidated. We propose to investigate if Chlamydia and HPV cause greater effects on the centrosome than either pathogen alone. We will use methods that we have developed to quantify centrosome abnormalities in Chlamydia-infected cells grown in cell culture (Johnson et al., 2009). We will compare cells infected with C. trachomatis, cells transiently expressing HPV E6 and/or E7 from high-risk HPV type 16 (kindly provided by Dr. Hans-Ulrich Bernard), and cells that are infected with Chlamydia and express HPV oncoproteins. Specifically, we will measure the proportion of cells with supernumerary centrosomes, the average number of centrosomes per cell and morphologic changes detected by immunofluorescence and transmission electron microscopy. We will assay for defects in the ability of supernumerary centrosomes to nucleate and organize microtubules and to form multipolar spindles in mitosis, and will detect chromosomal segregation errors by FISH analysis. We will assay for malignant transformation by the ability of cells to grow in the absence of adhesion in a soft agar transformation assay. These studies of the combined effects of Chlamydia and HPV on the centrosome may provide a molecular mechanism for Chlamydia as a co-factor for HPV in the development of cervical cancer.

Vanadocene Phosphates: ROS Generation and Cancer Therapeutics

Host Campus: Santa Barbara
Lead Investigator: Gabriel Menard
Start Date: 1/1/2018 *End Date*: 12/31/2018 *Amount*: $55,000

Abstract:
Cytotoxic anticancer metallodrugs, particularly those with reduced side-effects relative to cisplatin, are of increasing interest as potential cancer therapeutics. Bis(cyclopentadienyl)vanadium "vanadocene" dichloride (VDC) has gained much attention recently due to its anti-tumor and anti-proliferative properties (both in vitro and in vivo) primarily via oxidative damage. The cytotoxicity of certain VDC derivatives further surpasses cisplatin's, with some in phase II clinical trials. Certain vanadium metallodrugs have exhibited cytotoxic effects against cancer cell lines, with an observed increase in reactive oxygen species (ROS) concentration as a possible pathway to cell apoptosis. It is well established that while modest levels of ROS are needed for cell survival, high levels trigger apoptotic mechanisms. As part of this seed grant project, and with our expertise in inorganic synthesis, we will build on our initial library of new vanadocene phosphate complexes in both the +3 and +4 oxidation states (VP3 and VP4, respectively). The substituted phosphate-type ligands were chosen due to their structural diversity and increased solubility in polar media, such as blood plasma, likely increasing the VP bioavailability. Moreover, ligand substitution of the chlorides in VDC has been shown to increase their cytotoxic properties. As part of our electrochemical studies into our initial candidates, particularly VP3 derivatives, we have discovered that tuning redox potentials, by coordination of Lewis acidic components, allows for controlled generation of superoxide or peroxide species (both ROS) from molecular oxygen. The reaction products were unambiguously confirmed by x-ray diffraction (XRD) studies. Building on these results, we will attempt to shed light on the mechanism of vanadium-mediated increase in cellular ROS content described above. Finally, in concert with the synthesis and electrochemical studies, we will probe the fate of VP3 and VP4 in aqueous media, as well as the potential role of V oxidation states in the redox chemistry of ROS. A suite of tools, such as MS, NMR, EPR, and magnetommetry will be used to determine the behavior of our new complexes. Lastly, we have established a collaboration with a cancer expert who will screen these new compounds against appropriate cancer cell lines.

Computer vision approaches to detect cardiotoxicity

Host Campus: San Francisco
Lead Investigator: Rahul Deo
Start Date: 1/1/2018 *End Date*: 12/31/2018 *Amount*: $53,904

Abstract:
Despite the availability of an unprecedented number of chemotherapeutics, oncologists have had to face a difficult truth: some of the most promising anti-neoplastic agents can cause severe and potentially irreversible cardiac toxicity. Anthracyclines and inhibitors of a surprisingly diverse number of gene targets, including HER2, MEK, VEGF and the proteasome, all damage the myocardium, leading to heart failure and even death. Since these toxicities are unpredictable, physicians must monitor their patients through surveillance echocardiography (echo), a strategy which, in turn, has exposed a failing of this modality: echo often fails to detect the earliest signs of cardiac dysfunction. Although this may represent a flaw of the technology, it more likely represents a limitation of interpretation, as readers of echo's only make a few measurements by hand for each study, which must be used to detect change from prior examinations. Given that a typical echo study includes up to 5,000 individual frames, physicians are discarding nearly all of the collected information, a decision partially driven by expediency (UCSF performs nearly 18,000 echo's per year), but markedly hampering the ability to detect early disease. This proposal is based on the central hypothesis that computer vision methods, developed over the past two decades, and used widely for object recognition and tracking, can be applied to echo studies to detect subtle drug-specific cardiac toxicities at an individual patient level. My laboratory has recently developed a suite of approaches for raw two-dimensional echo analysis including image de-identification, automated chamber segmentation, and quantitative analysis of structure and function, taking advantage of computer vision algorithms used for facial recognition and particle tracking. These approaches can process thousands of images for a single patient. We can then use machine learning algorithms to synthesize information across multiple views to detect early dysfunction. The primary input for this proposal will be previously obtained serial echocardiographic studies for 150 breast cancer patients treated with HER2 inhibitors, although approaches can be readily extended to emerging drug classes. The output will be a validated computational approach to identify early evidence of cardiac toxicity in patients who go on to develop severe cardiac involvement.

Mechanisms of anti-estrogen resistance in breast cancer

Host Campus: Santa Cruz
Lead Investigator: Lindsay Hinck
Start Date: 1/1/2018 *End Date*: 12/31/2018 *Amount*: $55,000

Abstract:
Endocrine therapy has proved an effective treatment for estrogen receptor-positive (ER+) breast tumors. Many of these tumors, however, become refractory to anti-estrogen treatment, especially in a metastatic setting. The cancer stem cell (CSC) model posits that tumors are driven by a small fraction of cells with self-renewing and differentiative capacity that give rise to hierarchically organized tumors of varied phenotypes. Mounting evidence suggests that CSCs increase in ER+ breast tumors following anti-estrogen treatment. The goal of this application is to understand how extracellular factors, SLITs, influence the development of endocrine resistance by signaling through their ROBO receptors. Our preliminary data show that knockdown of Robo1 in ER+ breast cancer cell lines results in increased levels of nuclear NOTCH4 and increased CSC frequency. Recently, it was shown that CSCs develop resistance to anti-estrogen therapies through the activation of NOTCH4 signaling, but mechanisms regulating NOTCH4 activity in these cancerous cells are not fully known. Here, we propose studies to determine how loss of Robo1 activates NOTCH4, and whether the subsequent increase in CSCs drives endocrine resistance. Successful completion of the proposed studies will increase our understanding of signaling pathways contributing to endocrine resistant breast cancer by identifying a new mechanism that increases CSC frequency by stimulating NOTCH signaling in a ligand-independent manner. Positive outcomes from the proposed experiments will provide key preliminary data necessary for a successful application to the NIH, exploring these discoveries in vivo in xenograft studies using breast cancer cell lines and patient-derived tumor tissue.

Identifying Functional and Regulatory Domains in FLT3

Host Campus: San Francisco
Lead Investigator: Catherine Smith
Start Date: 1/1/2018 *End Date*: 12/31/2018 *Amount*: $55,000

Abstract:
Fms-Like Tyrosine Kinase-3 (FLT3) is a receptor tyrosine kinase that is mutated in 30% of acute myeloid leukemia (AML). FLT3 is the most frequently mutated gene in AML, with mutations commonly occurring in the form of activating in-frame tandem duplication (ITD) mutations in the juxtamembrane (JM) domain. Patients with FLT3-ITD mutations carry an extremely poor prognosis. While the recent development of clinically effective FLT3 tyrosine kinase inhibitors (TKIs) has extended survival for newly diagnosed FLT3-mutant AML patients and improved response rates for relapsed/refractory FLT3-mutant patients, the majority of patients still relapse and die. Patients who relapse on FLT3 TKI therapy do so due to on-target FLT3 KD mutations, underscoring the central role of FLT3 as an oncogenic driver in this disease. Structural studies suggest that the FLT3 JM domain serves an auto-inhibitory role in kinase regulation and this function is disrupted by ITD mutations. Rare deletion mutations in the FLT3 JM domain have been identified in patients, further supporting the role of this domain in FLT3 regulation. Widespread genomic sequencing of AML and other tumors has revealed mutations in other FLT3 domains of uncertain significance. Mutagenesis studies in mouse models have suggested N-terminally truncated FLT3 protein lacking most of the extracellular domain can cause ligand-independent FLT3 activation and result in leukemic transformation. This observation implicates the FLT3 extracellular domain in FLT3 regulation and autoinhibition. At this time, the functional role of FLT3 protein domains in FLT3 kinase regulation remains incompletely understood. Recently, Shi et al. (Nature Biotech 2015) pioneered the use of exon-targeted CRISPR-Cas9 genome editing to identify functional protein domains, based on the assumption that cells with CRISPR-edited null mutations in critical protein domains undergo disproportionate negative selection over time. We propose to adapt this technique to identify and validate the protein domains of FLT3 relevant to kinase regulation by assaying for FLT3 domain mutations which result in cytokine independent transformation or conversely, negative selection in FLT3-driven cell lines. By understanding the functional domains of FLT3 relevant to FLT3 function and oncogenesis, we hope to identify critical domains that might be targeted therapeutically.

Enhanced Gemcitabine delivery with fluorous polymer micelles

Host Campus: Los Angeles
Lead Investigator: Ellen Sletten
Start Date: 1/1/2018 *End Date*: 12/31/2018 *Amount*: $55,000

Abstract:
Gemcitabine (Gem), 2',2'-difluoro-2'-deoxycytidine, is a clinically-approved chemotherapeutic. The major drawback to Gem is its rapid deamination resulting in a short (~15 min) serum half-life. The rapid metabolism necessitates excessive doses to be administered causing significant side effects and resistance. Efforts to stabilize Gem include the creation of prodrug variants or delivery via nanocarriers. We will use a hybrid approach that involves a novel, stable, chemically-tunable nanocarrier for Gem. Our approach employs a hydrophilic-fluorous block copolymer nanocarrier and fluorous-tagged Gem. Hydrophilic-hydrophobic polymer micelles have been popular delivery vehicles but suffer from low stability when diluted in complex biological systems. Exchanging the hydrophobic block for a fluorous block will overcome these limitations because there are no naturally occurring fluorinated compounds to interfere with the self-assembled fluorous core. Additionally, fluorinated amphiphiles have critical micelle concentrations 2-3 order of magnitude lower than their hydrocarbon analogs. Based on work by Kumar, the fluorous-tags on Gem will expedite its internalization into cells. We will prepare hydrophilic-fluorous block copolymers through the controlled RAFT-polymerization of oligo(ethylene glycol) and fluorous acrylates. The side-chains within the fluorous block will contain less than 14 fluorine atoms to prevent bioaccumulation. Simultaneous to our preparation of the block copolymers, we will append fluorous tags to Gem using self-immolative cleavable linkers. Fluorous-tagged Gem will be combined with the block copolymers. We will characterize the size, stability, loading capacity and biocompatibility of the resulting nanomaterials alongside unmodified Gem, and no cargo controls. We will employ UV/Vis, HPLC, DLS, and cryoTEM for this characterization. Micelles that appear promising will be introduced to a panel of cancer cell lines along with their appropriate controls and cell viability will be assayed.

R-loop Driven Oncogenic Translocations in Prostate Cancer

Host Campus: Davis
Lead Investigator: Frederic Chedin
Start Date: 1/1/2018 *End Date*: 12/31/2018 *Amount*: $53,424

Abstract:
Genomic instability is a hallmark of many cancers. This instability often results in oncogenic translocations such as the well-known MYC-IgH translocation in B cell tumors or TMPRSS2-ERG translocation in prostate cancers. Understanding the mechanisms driving such translocations is of critical importance for future therapies aimed at blocking these events. I hypothesize that co-transcriptional R-loop structures formed upon re-annealing of the nascent mRNA to the DNA template are a critical source of oncogenic translocations in prostate cancer. Building on groundbreaking genomics technologies developed by my laboratory, the first aim of this proposal will be to map sites of R-loop formation in prostate cells and their response to stimulation by androgen signaling. Pilot experiments show that R-loops significantly increase over androgen-responsive genes in response to androgen stimulation. Major common translocation partners such as TMPRSS2, NDGR1 and Kallikrein 3 (KLK3 – also known as prostate-specific antigen, PSA) show particularly strong increases in R-loop formation. The second aim of the proposal will test the hypothesis that co-transcriptional R-loops coincide with double-stranded DNA breaks that often initiate translocations. For this we will leverage the recently published END-seq method to map these breaks in prostate cells undergoing androgen stimulation or not. My group is well-versed in all the genomics techniques necessary for completing this work. We also have strong in-house expertise in computational biology including algorithm development and visualization techniques, necessary to analyze and cross-reference these large datasets. Overall, this proposal offers to leverage key breakthroughs in R-loop mapping developed by my group to the study of cancer initiation mechanisms. This represents a novel research direction for us. Upon completion of this 1-year grant, my goal is to extend this work into a more complete NIH R01 proposal aimed at characterizing mechanisms of genomic instability in prostate cancer.

A study of H2A.Z isoforms in Glioblastoma multiforme

Host Campus: Merced
Lead Investigator: Ramendra Saha
Start Date: 1/1/2018 *End Date*: 12/31/2018 *Amount*: $55,000

Abstract:
Glioblastoma multiforme (GBM) is the most lethal primary brain tumor in adults with a median survival of 12-18 months from the time of initial diagnosis. GBM recurs in most cases and no standard treatment exists for the recurrent disease. Advances in basic understanding of GBM are therefore urgently required. Epigenetic mechanisms –processes that regulate gene expression by altering the epigenome – are one of the major factors contributing to the pathogenesis of cancers, including glioblastoma. Therefore, epigenetic modulators are being exploited as therapeutic drug targets. Histone 2A.Z (H2A.Z) is one such epigenetic modulator. H2A.Z is a highly-conserved histone 2A (H2A) variant that is overexpressed in breast, prostate and bladder cancers. H2A.Z has a complex biology. In mammals, H2A.Z has two paralogs (isoforms) – H2A.Z.1 and H2A.Z.2 – that are products of two non-allelic genes, H2AFZ and H2AFV. Although H2A.Z has been implicated in several forms of cancer, most studies have not clearly distinguished between the isoforms. One recent study reported a unique role for H2A.Z.2, and not H2A.Z.1, as a driver of malignant melanoma. In the current project, we are proposing to test the role of H2A.Z isoforms in GBM. Over the past three years, research on H2A.Z in our laboratory – a laboratory of neurobiology and epigenetics – has identified distinct roles of H2A.Z in neurodevelopment and brain functions. Now, we are proposing to extend our H2A.Z isoform-related tools and expertise to investigate the role of this major epigenetic player in GBM. In this seed project, we will manipulate levels of H2A.Z isoforms and their chaperones (proteins responsible for their nucleosomal inclusion/exclusion) in human and rat GBM cell lines respectively using RNAi and rescue approaches. We will observe effects of such manipulations on gene expression, cell proliferation, and cell survival. Once completed, these novel studies will provide insights into molecular mechanisms of GBM and set up a preliminary platform for subsequent in depth studies of H2A.Z isoform functions in these processes.

"Altered self" neuroblastoma epitopes for immunotherapy

Host Campus: Santa Cruz
Lead Investigator: Nikolaos Sgourakis
Start Date: 1/1/2018 *End Date*: 12/31/2018 *Amount*: $55,000

Abstract:
Immunotherapy harnessing a patient's own T cell responses to selectively eliminate tumor cells is an emerging trend for the treatment of cancer. The therapy uses autologous T cells from the patient and subsequently activated in vitro, that are highly reactive to tumor neoantigens. These are peptides derived from mutated oncogene proteins and displayed on the cell surface, in tight association with the Major Histocompatibility Complex (MHC or HLA in humans). The chief barrier in developing personalized immunotherapy approaches is identifying suitable antigens displayed in a given patient, since minor changes in both the peptide and the HLA sequences can have drastic effects on the stability of the MHC assembly. Addressing the complexity of interactions will eventually allow us to evaluate the potential of a given patient as a candidate for immunotherapy, and to identify recurring targets towards engineering specific T cell responses. The UCSC Genome center has recently developed a tool, ProTECT, to predict therapeutically relevant peptide antigens from tumor sequencing data, and we have used it to predict "hotspot" neoantigens in neuroblastoma patients. In the research proposed here, we will characterize binding of the mutated peptides to the MHC in vitro, using mass spectroscopy, fluorescence polarization anisotropy and differential scanning fluorimetry. In addition, towards obtaining a detailed atomic picture of the interaction, we will elucidate the crystal structures of their respective MHCs. We will then use molecular modeling to investigate the extent to which changes in the HLA sequence can influence binding affinity. We will specifically evaluate mutations that result in a higher MHC stability relative to their parental self-epitope (termed "altered self" mutants), as ideal candidates for immunotherapy due to being less affected by immune tolerance mechanisms. Once we have identified such peptides, we will prepare MHC tetramer reagents for staining T cells from cancer patients, towards assessing the extent of specific responses to the neoantigen. The proposed research is significant because will provide new conceptual advances that directly link the molecular basis of neoantigen/MHC interactions with their immunogenicity. The pilot data obtained from this study will be used to seek additional, long-term funding from the NIH.

Cannabis and Tobacco co-use in Adolescents

Host Campus: San Francisco
Lead Investigator: Mark Rubinstein
Start Date: 1/1/2018 *End Date*: 12/31/2018 *Amount*: $54,895

Abstract:
In the US, tobacco use remains the number one preventable cause of cancer and as more adolescents smoke cannabis, the dual-use with tobacco raises the risk of nicotine dependence. In order to stem the tide of tobacco related cancers, there is a critical need to better understand the neurobiology of how cannabis co-use during adolescence influences vulnerability to tobacco dependence. Objective: This trans-disciplinary pilot project seeks to determine associations between cannabis and tobacco exposure and symptoms of dependence and specific brain network features including the functional and structural reorganization that occurs with exposure to tobacco and cannabis. Specific Aims: Using fMRI we will investigate the potentiating effects from dual use of cannabis and tobacco on cue reactivity in adolescents (AIM 1). We will examine alterations in resting-state functional and structural brain networks in adolescents with dual use of cannabis and tobacco (AIM 2). Study Design: Using a cohort design, this study will examine patterns of brain activation, network characteristics, substance use behaviors and levels of dependence to both tobacco and cannabis in a multiethnic sample of 48 adolescent (aged 13-17 years): 1) tobacco smokers (n=20), 2) dual-users (e.g., users of both cannabis and tobacco; n=20), and a 3) control group of never-users of either drug (n=10). We will use MRI to examine activation and connectivity patterns and measure reported dependence and craving symptoms. We will then examine associations between substance use patterns, levels of dependence and multimodal imaging biomarkers. Significance: The potential for increased harm for adult cancers from adolescent tobacco exposure along with the increasing prevalence and move to legalize cannabis underscore the urgency of studying co-use among this age group. Determining the associations between cannabis and tobacco exposure, symptoms of dependence, use disorders and specific brain imaging features will enhance understanding of the basic bio-behavioral mechanisms involved in the development of tobacco dependence in adolescents, especially dual users. Furthermore, this study will provide the pilot data necessary for performing a larger scale prospective study.

Single-cell analysis of breast cancer metastasis

Host Campus: Irvine
Lead Investigator: devon lawson
Start Date: 1/1/2018 *End Date*: 12/31/2018 *Amount*: $55,000

Abstract:
Individual tumors are remarkably heterogeneous, comprised of subpopulations of tumor cells with different genetic, transcriptomic, proteomic and epigenetic landscapes. This heterogeneity can have immense implications on the basic biology of the tumor's function, as well as the clinical management of the disease, because subclones within tumors may demonstrate differential capacity to survive therapy and initiate metastasis. Clinical and experimental data suggest that metastasis is initiated from rare tumor cells with unique properties. Sequencing efforts to investigate the genetic relationship between primary tumors and metastases in patients present a mixed picture; in some patients the metastases appear very similar, but in most patients there is clear genetic divergence, suggesting either subclonal origin or parallel evolution of the metastases. Using current algorithms, it is not possible to deconvolute sequencing data generated from bulk tumor samples and extrapolate what the genotype of the cell of origin of a metastasis may have been. We hypothesize that there are a limited spectrum of cancer cell genotypes capable of initiating metastasis, and that although metastasis-competent subclones in different patients will not have the exact same genotype, they will share genetic alterations in specific types of genes, which are necessary for effective metastasis. The goal of this pilot study is to optimize a protocol for single-cell whole exome DNA sequencing, and use it to track subclonal relationships between primary and metastatic tumor cells in human Patient-derived xenograft (PDX) models of breast cancer. The two specific aims will be: 1) Catalog subclonal heterogeneity in human PDX models by bulk whole exome sequencing, and 2) Develop a single-cell whole exome sequencing protocol for identification of tumor subclones capable of metastasis initiation and progression. This work will generate the necessary preliminary data for an R01 grant application, which will seek to investigate the biological basis for why particular genotypes may have enhanced fitness for metastasis. This will represent a new project developed in my lab as an independent investigator at UC Irvine.

Leukemia Stem Cells in B-Cell Acute Lymphoblastic Leukemia

Host Campus: Davis
Lead Investigator: Noriko Satake
Start Date: 1/1/2018 *End Date*: 12/31/2018 *Amount*: $55,000

Abstract:
Leukemia stem cells (LSCs) are the root of cancer and are responsible for treatment resistance and disease relapse. However, LSCs have not been identified in acute lymphoblastic leukemia (ALL), the most common cancer in children. Recently, our group discovered a method to identify and isolate LSCs from primary ALL samples. We demonstrated that the LSCs isolated using our marker have in vivo leukemia-initiating capability and distinct transcriptome profiles. We have identified 1,135 genes that are differentially expressed between LSCs and the counterpart of LSCs, non-LSCs ($p < 0.05$). Of these, 315 genes are upregulated in LSCs. The goal of this project is to identify the gene(s) that regulate the "stemness" of LSCs in ALL. In this pilot study, we will focus on B-cell type ALL (B-ALL), the most common ALL in children. We will identify the genes which are important for LSC maintenance using an in vivo shRNA screening method and leukemia xenograft models with cell lines and primary leukemia samples. We hypothesize that one or more genes play a dominant role in regulating stemness and phenotypic properties of LSCs in B-ALL. The specific aims are to determine 1) the key genes associated with stemness in LSCs and 2) the key genes associated with differentiation in non-LSCs. We will investigate the two counterpart populations (LSCs and non-LSCs) using the same method, which should provide complementary results. We expect to identify potential novel genes (and pathways) which regulate the stemness of LSCs in B-ALL. We will pursue this goal using 1) our well-annotated series of patient-derived xenograft mouse models, 2) our novel LSC isolation technique, and 3) in vivo shRNA screening and targeted gene RNA sequencing. If successful, this project could have a significant impact on the most important challenges in cancer treatment: resistance or recurrence of disease.

Tuning metastasis using beta-blockers and biophysics

Host Campus: Los Angeles
Lead Investigator: Amy Rowat
Start Date: 1/1/2018 *End Date*: 12/31/2018 *Amount*: $55,000

Abstract:
New treatment strategies are critically needed to block metastasis, which is the leading cause of cancer deaths. ß-blockers are anti-hypertension drugs that block ßAR signaling and are being clinically evaluated for cancer treatment. While altered tumor stiffness is associated with cancer progression, it is unclear how ß-blockers may alter the physical properties of circulating tumor cells to regulate metastasis. We recently discovered that ßAR signaling causes diverse types of cancer cells to be less deformable and more invasive. To guide translation of ß-blockers to the cancer clinic, it is now critical to investigate the effect of ß-blockers on the deformability and viability of disseminated tumor cells, and how this treatment impacts chemotherapy response. The proposed studies will determine how ß-blockade, together with existing chemotherapy treatments, regulates the deformability and viability of disseminated tumor cells to stop metastasis. We will utilize clinically-used ß-blockers, together with well-characterized ßAR agonists and common chemotherapy drugs to define effects on the deformability and viability of disseminated tumor cells after exposure to shear stresses during circulation. We will use cutting-edge fluidic devices, as well as mouse models of breast cancer and patient samples. Our multidisciplinary team consists of experts in biophysics and bioengineering (Rowat), cancer biology (Sloan), and pathology (Rao). Our Aims are to: 1) Determine the effects of ß-blockers and chemotherapy on the deformability and viability of tumor cells from patient pleural effusions. 2) Define the effects of ß-blockers and chemotherapy on the deformability and viability of circulating tumor cells in vivo. We will also determine how ß-blockers and tumor cell stiffness affects lodging in metastatic target organs. Results will define how ß-blockers may be combined with existing chemotherapy treatments to stop metastasis; such knowledge is essential to guide translation of ß-blockers to the clinic. Our findings will also provide crucial preliminary data for a competitive R01 proposal to NCI Neural Regulation of Cancer funding opportunity announcement (PAR-16-246).

Repurposing a toxin-immunity pair to selectively kill cancer

Host Campus: Irvine
Lead Investigator: Celia Goulding
Start Date: 1/1/2018 *End Date*: 12/31/2018 *Amount*: $55,000

Abstract:
One of the great remaining challenges in cancer therapy is the design of therapeutics that will selectively kill cancer cells, but leave healthy cells unharmed. A prevailing method of achieving selectivity comes from designing therapeutics that will bind to extracellular receptors; however, many of these markers are expressed in normal and germ cell tissues. Intracellular metabolic and gene expression profiles in cancer cells are instead drastically different from normal cells. It would therefore be transformative to develop targeted therapies that can 'sense' this intracellular difference, rather than a cell surface marker. Herein, we aim to develop such an approach; one that senses the intracellular environment of cancer cells, thereby triggering their destruction, by engineering a naturally occurring bacterial toxin-immunity complex to 'sense and kill' cancer cells and not normal healthy cells. We will fuse a known bacterial toxin, which is a potent DNase capable of completely degrading human chromosomal DNA, to its cognate toxin-neutralizing immunity protein. The toxin will be activated only by Cathepsin-L protease (CatL), a gene grossly upregulated in many cancer cells. Thus, the toxin will be liberated from the toxin-immunity fusion protein by CatL cleavage that will result in cancer cells death, whereas in healthy cells, which do not upregulate CatL, the toxin will remain fused to the immunity protein and therefore inactive. We will then test our optimized toxin-immunity fusion protein to ensure activation in human cancer cells and cell death, and that it remains inactivation in normal human cells. We shall also discuss potential delivery methods for this novel therapeutic; however experimental testing will out of the realm of this proposal. The final outcome of this design will be a state-of-the-art cancer-cell selective therapy. This initial CCRC study will generate data that will be used as proof-of-principle data for dual or multi-PI R01 NIH funding for anti-cancer therapeutics.

Macrophage plasticity in oral squamous cell carcinoma

Host Campus: Los Angeles
Lead Investigator: Yi-Ling Lin
Start Date: 1/1/2018 *End Date*: 12/31/2018 *Amount*: $55,000

Abstract:
Oral squamous cell carcinoma (SCC) with jaw bone invasion has a high recurrence rate and poor prognosis. The suppressed immunity in the tumor environment represents the major hurdle for the success of the cancer treatment. During tumor bone invasion, macrophages are recruited as osteoclast precursors toward the tumor-bone interface. The influx of macrophages significantly changes the tumor microenvironment and potentially exacerbates immunosuppression. This proposal focuses on the immune modulation of macrophages during tumor bone invasion. A syngeneic murine model will be used to investigate the impact of macrophages on tumor immunity by SCC jaw bone invasion. In Aim 1, we will characterize the immune status altered by oral SCC bone invasion. The unique properties of macrophages associated with SCC bone invasion in supporting an immunosuppressive environment will be investigated. This will be achieved by examining the impact of tumor bone resorption on the function of macrophages and T cells, expressions of immune checkpoint molecules and cytokine profiles in local tumor and systemic host environments. In Aim 2, we will characterize the incidence of SCC-macrophage fusion during tumor bone invasion and investigate the clinical outcome of such phenomenon. Targeting macrophages has become an attractive approach for immunotherapy because they are essential for all aspects of tumor function and increasing evidence shows that they can be polarized to tumoricidal upon proper stimulation. Our goal is to understand the mechanisms of tumor bone invasion associated immunosuppression by macrophages and to develop highly effective immunotherapies against these tumors.

Evaluating the role of phospholipase D1 in pancreatic cancer

Host Campus: Davis
Lead Investigator: Gerardo Mackenzie
Start Date: 1/1/2018 *End Date*: 12/31/2018 *Amount*: $55,000

Abstract:
Pancreatic ductal adenocarcinoma (PDA) is a complex and lethal cancer with unsatisfactory treatment options. Therefore, the magnitude of this problem mandates the search for new strategies to combat this disease; being the identification of new targets for treatment a critical component. Phospholipase D1 (PLD1), a lipid-signaling enzyme, has been shown to play a role in the progression of various types of cancer. For example, PLD1: a) is increased in abundance or activity in various human cancers; b) is linked with proliferative signaling and resistance to cell death, and c) promotes tumor growth and metastasis. In addition, PLD1 has been recently shown to be elevated in clinical samples of pancreatic cancer, and inversely correlate with patient survival. These findings suggest the potential use of PLD inhibitors as cancer therapeutics. However, to date, there is a dearth of information regarding the exact role played by PLD1 in pancreatic cancer. Preliminary studies in our laboratory have shown in animal models of pancreatic cancer, that inhibiting PLD1 reduces pancreatic tumor growth, extends survival and enhances the chemotherapeutic effect of gemcitabine, the most commonly used drug for treating patients suffering from pancreatic cancer. Based on the above, the long-term goals of this project are to understand the role of PLD1 in PDA, to find out whether PLD1 can be a useful new target for PDA treatment. Specifically, the objective of this proposal is to define the role of PLD1 in PDA growth. We hypothesize that PLD1 is a key enzyme in PDA growth. We will employ, biochemical, molecular, and in vivo studies to evaluate this hypothesis and to advance PLD1 as a novel therapeutic target. Accordingly, we will pursue the following specific aims: 1) Determine the role and mechanism of PLD1 in regulating PDA progression; and 2) Establish and define whether PLD1's role in PDA growth is critical in both tumor cells and in the microenvironment. At the completion of these studies, we expect to have deciphered key functions of PLD1 in pancreatic cancer. These results may allow us to define and establish PLD1 as a novel and heretofore unappreciated major regulator of pancreatic cancer progression. Moreover, we expect to use this CRCC project to obtain key preliminary data to submit a competitive grant proposal for external funding.

Novel cancer metabolite-triggered drug delivery

Host Campus: Irvine
Lead Investigator: Szu-Wen Wang
Start Date: 1/1/2018 *End Date*: 12/31/2018 *Amount*: $55,000

Abstract:
Stimuli-responsive drug delivery strategies are designed to react to changes in conditions, such as pH or temperature, within the microenvironment of tissues or cells. However, often these triggers are not adequately specific, as the conditions can occur at alternate off-target locations, or the differences between diseased vs. normal states are not sufficiently high. This proposed project will develop a novel drug delivery strategy that will target tumors by specifically responding to lactate, a signature metabolite of cancer and a hallmark of the Warburg effect. The Warburg effect has not yet been exploited in a drug release mechanism, so our proposed drug delivery material will introduce a novel means to deliver and release drug cargo to tumor environments with elevated lactate concentrations, and it is likely to be more specific towards cancer than existing approaches. We hypothesize that hydrogels responsive to the Warburg effect can be created by incorporating specifically-engineered lactate-binding proteins within polymeric matrices. The polymer component has been utilized in molecular imprinting, and the unique metabolite "sensor" will be engineered mutants of a protein with natural binding affinity to lactate. A small library of rationally-designed mutants will be created to obtain binding affinities appropriate for response. The protein and its polymerizable inhibitor will be incorporated into the hydrogel polymer, with interactions between the protein and inhibitor serving as reversible cross-linkers. Competitive binding of this material with the lactate in the microenvironment will result in material swelling and drug release. This proposed work will generate proof-of-concept data for future studies in metabolite-responsive drug therapy. Our aims are to: (1) engineer proteins that will competitively bind the lactate metabolite and its monomer inhibitor; (2) fabricate Warburg effect-responsive protein-polymer hydrogels; and (3) examine the hybrid materials' response to the lactate metabolite and the corresponding drug loading/release.

Targeting the SUMOylation Pathway for Cancer Therapy

Host Campus: Riverside
Lead Investigator: John Perry
Start Date: 1/1/2018 *End Date*: 12/31/2018 *Amount*: $55,000

Abstract:
The aim of the New Assistant Professor proposal is the use the latest methods in Structural Biochemistry to develop inhibitors targeting the SUMOylation cascade. Recent research has indicated that certain cancers have 'onco-gene addiction', where the tumor cell is heavily reliant on cellular pathways that are not essential to normal cells. One such pathway is the SUMOylation cascade, where studies by my collaborators and others have revealed that targeting this pathway would likely provide significant therapeutic benefit to currently difficult to treat cancers. Notably, this includes tumors driven by Kras, c-Myc or Notch-1 mutations, as well as the emergence of drug-resistance in acute myeloid leukemia. I propose to leverage the experimental expertise of my laboratory in structural biology and early-stage drug discovery methods, together with my specific knowledge and experience in the SUMOylation pathway, as evidenced by my peer-reviewed, published research. The SUMOylation cascade is a post-translational modification pathway utilized by the cell to add the small ubiquitin-like modifier (SUMO) onto a target protein. SUMO is added through the use of E1, E2 and E3 enzymes, and the pathway can be largely eliminated through inhibition of either the single E1 or single E2 enzyme of the cascade. Preliminary efforts from my laboratory to generate inhibitors include the development of protein crystals of both the E1 and E2 SUMO enzymes, which now enables rational-based drug discovery processes. Here, we will our employ fragment-based ligand discovery and computer aided drug discovery methods, aided by synchrotron-based x-ray crystallography, and our latest methods in x-ray diffuse scattering and small angle x-ray scattering. My laboratory will also leverage recent UC-based collaborations, which include the discovery a hit inhibitor compound from a high-throughput screen and the development of analogues of a natural product that can weakly inhibits the pathway; both of which can be analyzed for potential further development. The expected outcomes for this 1-year of research is the development of high affinity, selective hit compounds. Through further funding, I expect the development of these initial inhibitors into drug-like leads that have the potential of moving from the bench to the clinic.

The Role of p21 Phosphorylation at S123 in Tumor Suppression

Host Campus: Davis
Lead Investigator: Michael Kent
Start Date: 1/1/2018 *End Date*: 12/31/2018 *Amount*: $55,000

Abstract:
The cyclin-dependent kinase (CDK) inhibitor p21, also known as WAF1 and CIP1, is a potent suppressor of cell growth and belongs to the Cip/Kip family of cdk inhibitors. p21 is a target of tumor suppressor p53 and mediates p53-dependent cell cycle arrest in response to DNA damage. Due to its potent role in growth suppression, p21 was originally identified as a tumor suppressor. Interestingly, recent studies also showed that p21 has an oncogenic activity as cytoplasmic localization of p21 promotes cell proliferation. Indeed, accumulation of cytoplasmic p21 is found in several types of cancers and associated with tumor progression and poor prognosis. Together, these studies suggest that depending on its cellular context, p21 could inhibit or promote tumorigenesis. Thus, understanding the mechanism how p21 activity is controlled may open a new avenue to explore p21 as a therapeutic target for cancer treatment. We previously cloned the canine CDKN1A gene and found that like human p21, canine p21 is induced by DNA damage in a p53-dependent manner and modulates p53-dependent cell cycle arrest. Interestingly, canine p21 is expressed as two isoforms due to proline-directed phosphorylation at serine 123 (S123), which can be easily visualized as a slower migrating band than the underphosphorylated canine p21. Interestingly, ectopic expression of mutant canine p21(S123D), in which serine 123 was substituted with phosphomimetic aspartate acid, greatly inhibited cell proliferation as compared to that of canine p21(S123A), in which serine 123 was replaced with non-phosphorylatable alanine. However, the role of serine 123 in p21-mediated growth suppression has not been studied in vivo. Interestingly, our pilot study indicated that the level of S123-phosphorylated p21 was reduced by lithium chloride (LiCl), an inhibitor of glycogen synthase kinase 3 (GSK3). Thus, we hypothesize that posphorylation of serine 123 plays a critical role in p21-mediated growth suppression. To test this, we will determine: (1) whether GSK3 phosphorylates canine p21 at S123; (2) whether S123 phosphorylation enhances canine p21-mediated tumor suppression in vitro and in vivo.

Synthesis and Study of Anacardic Acid Derivatives

Host Campus: Riverside
Lead Investigator: David Martin
Start Date: 1/1/2018 *End Date*: 12/31/2018 *Amount*: $55,000

Abstract:
The goal of this proposal is to develop novel therapeutic leads for the treatment of SUMO-dependent cancers through structure-activity relationship (SAR) and mechanism of action studies. Recently, it has been shown that a functioning SUMOylation pathway is critical to acute myeloid leukemia (AML) cells, and dysregulation of SUMO is associated with chemoresistance to current frontline treatments. Likewise, other chemoresistant cancers have shown a dependence on the SUMOylation pathway, often termed “non-oncogene addiction”. Using organic synthesis techniques, computational modeling and protein crystallography, we will design and synthesize analogs of anacardic acid with increased binding affinity and improved physical properties. Binding studies and inhibition assays will be performed by our collaborator (Prof. Jeff Perry, UC Riverside) to evaluate the success of these efforts (see Letter of Collaboration). The ultimate goal of these studies is to provide drug-like therapeutic leads for the treatment of SUMO-dependent cancers and to discover potent, selective probe molecules for use in further investigations of SUMOylation and its role in disease.

Synthesis of Novel Lankacidin Microtubule Stabilizers

Host Campus: San Francisco
Lead Investigator: Ian Seiple
Start Date: 1/1/2018 *End Date*: 12/31/2018 *Amount*: $55,000

Abstract:
The lankacidin class of natural products was discovered in 1960 and was found to have potent antitumor and antibiotic properties. While the mechanism of antibiotic action of this class is well characterized, the mechanism by which they exhibit in vitro antitumor activity against a variety of models (e.g., L1210 leukemia, B16 melanoma, 6C3 HED/OG lymphosarcoma) was unknown until recently. In October 2016, Ayoub et al. published a study elucidating Lankacidins' ability to stabilize microtubules and to displace taxoids from their binding site (J Med Chem 2016, 59, 9532–9540). Microtubule stabilizers such as paclitaxel remain front-line therapy for a variety of cancers, but are accompanied by myriad side effects, many of which are not directly related to their canonical mechanism of action. The discovery and optimization of new therapeutics that stabilize microtubule formation but minimize deleterious side effects would be a welcome advance in the battle against cancer. Lankacidins have been used as antibiotics in veterinary medicine for decades, lending credence to their safety in mammals. Further structural optimization is required, however, before they could be considered suitable for use as chemotherapeutic agents in humans. The largest barrier to the optimization of the lankacidin class is the dearth of methods to modify their complex chemical architectures. We propose the development of a platform for the synthesis of lankacidins from a pool of simple, readily available chemical building blocks. This platform will enable the first systematic exploration of the structure–activity relationships of this class. Additionally, the proposed research will provide an avenue to modify lankacidins to increase potency, improve chemical stability, optimize pharmacological properties, and decrease toxicity. This project also offers great potential to advance understanding of microtubule stabilization by providing access to novel chemical probes. The proposed work is an ideal candidate for CRCC funding because a) this is a new project related to cancer, and the first such project in my nascent laboratory, b) given seed funding, the proposed research has a high probability of becoming competitive for larger, long-term grants, and c) our platform has the potential to both advance cancer research and to generate new cancer therapeutics.

Novel function of GCN5 in ubiquitination of MYC

Host Campus: Riverside
Lead Investigator: Ernest Martinez
Start Date: 1/1/2018 *End Date*: 12/31/2018 *Amount*: $55,000

Abstract:
The MYC oncoprotein is a DNA-binding transcription factor that is overexpressed in most types of cancer. MYC regulates a large fraction of the genome and its transcription activity is required for its oncogenic function. The molecular mechanisms involved in regulation of MYC-dependent transcription are still poorly understood. MYC recruits a variety of histone acetyltransferases (HATs), including GCN5, p300/CBP, TIP60, other coactivators, and elongation factors to activate genes at the level of transcription initiation and elongation. In addition these HATs also acetylate MYC itself. Although the role of MYC acetylation is largely unclear, MYC acetylation by GCN5 increases its stability. Recently, ubiquitin modification and proteasomal degradation of MYC have been reported to be critical for MYC induction of histone acetylation, and activation of RNA polymerase II transcription elongation of most MYC target genes via still unknown ubiquitin E3-ligase enzymes. Although several ubiquitin E3 ligases can modify MYC and either activate or attenuate MYC-dependent transcription, it has been difficult to assign direct causative roles in MYC regulation due to their multiple other substrates. In addition, when tested, these individual ligases (e.g., SKP2) affect only a limited number of MYC target genes. This suggests the existence of additional MYC-modifying E3 ligases. Recently, we have obtained evidence that the GCN5 HAT strongly stimulates ubiquitination of the MYC oncoprotein in mammalian cells. Here we propose to characterize this novel catalytic function associated with GCN5 and its role in MYC regulation. The specific goals of the project are: (1) To identify the domain(s) of GCN5 responsible for MYC ubiquitination in cultured cells and characterize the type of ubiquitin chain linkage involved (e.g., Ub-K48 or -K63); (2) To determine whether GCN5 has an intrinsic E3 ligase activity that directly ubiquitinates MYC, or whether GCN5 associates with an E3 ligase in a complex; and (3) To define the role of GCN5-dependent ubiquitination in regulation of MYC. This project will uncover a novel pathway for MYC ubiquitination involving the GCN5 HAT and provide the first insight into how transcription activation by MYC is connected to its turnover by the proteasome, which is of high relevance to the cancer problem.

Post-transcriptional regulation of gene expression by ELAVL1

Host Campus: Riverside
Lead Investigator: Fedor Karginov
Start Date: 1/1/2018 *End Date*: 12/18/2018 *Amount*: $55,000

Abstract:
The role of post-transcriptional regulators of gene expression in cancer-relevant molecular processes is increasingly recognized. The mRNA-binding protein HuR (ELAVL1) is overexpressed in a variety of cancers and affects tumor growth and malignancy; studies in various systems have connected HuR to the stress response, cell proliferation, survival and senescence, muscle differentiation, and T cell activation. Reacting to signals in the above processes, HuR carries out its cellular function by binding to hundreds of mRNAs, leading to changes in their translation and stability by poorly understood mechanisms. Genetic knockouts of HuR in a cancer T cell line generated in our lab demonstrate a clear function for HuR in control of the apoptotic program and cytokine production upon activation. In robust agreement with the phenotypes, RNA-seq data in KO cells indicates that HuR controls the levels of hundreds of mRNAs in functionally relevant categories, including apoptotic factors, as well as most cytokines. Furthermore, the data leads us to hypothesize that HuR controls the major transcription factors NFATC1 and NFKB2 that drive activation and proliferation programs. Using this experimental system, we propose to investigate the regulatory mechanisms that link HuR with NFATC1 and NFKB2 and upstream activation, and to determine and validate additional functionally important downstream targets of HuR. Overall, the study will shed light on the mechanisms of post-transcriptional control that enable HuR to mis-regulate the expression programs in cancer cells.

Transdifferentiated dendritic cells as cancer vaccines

Host Campus: San Diego
Lead Investigator: Jack Bui
Start Date: 1/1/2018 *End Date*: 12/31/2018 *Amount*: $50,000

Abstract:
Therapeutic vaccination of cancer holds much theoretical promise but has not achieved practical efficacy. Cancer vaccines require potent antigens representative of the cancer and strong adjuvants that can overcome the immune suppressed environment of a cancer patient. We recently identified a cancer-derived "adjuvant" in the form of the cytokine interleukin 17D (IL-17D), but this cytokine needs to be combined with the appropriate antigenic stimuli to produce an efficacious antitumor immune response. Indeed, the major limitation of current cancer vaccines is in the identification of a suitable vaccine antigen that is expressed in cancer cells but not in normal cells. This limitation can be addressed by sequencing the genome of a cancer cell and identifying mutations, or neoantigens, that are unique to the cancer cell and not expressed in normal cells. Although genome sequencing can identify certain neoantigens, it cannot identify every single one, nor can it predict which neoantigens would be the best ones to include in a vaccine. In this grant, we propose to convert a cancer cell into a dendritic cell (DC). DCs are professional antigen presenting cells that have the natural ability to present antigens to stimulate antitumor T cell responses. In converting a cancer cell into a DC, we generate a novel cell type that has the same genome and therefore expresses the same neoantigens as a cancer cell. Importantly, the DC has professional antigen presentation capabilities and will stimulate immunity. Previous studies have shown that two transcription factors, C/EBPa and PU.1, can convert fibroblasts or lymphoid malignancies into macrophages via a transdifferentiation pathway. The reprogrammed macrophages behave like normal macrophages and can phagocytose and present antigen and importantly do not form tumors in vivo. Building on these findings and our expertise in IL-17D and innate immunity, we propose to convert IL-17D-transfected cancer cells into DCs. This would combine a strong adjuvant (IL-17D), a strong antigen presenting cell (DC), and suitable neoantigen targets (derived from the genome of the cancer cell), thus representing the ideal vaccine for use in patients or in the manufacturing of T cells for adoptive immune therapy.

Insulin-Resistance Polymorphisms, Lifestyle in Breast Cancer

Host Campus: Los Angeles
Lead Investigator: Su Yon Jung
Start Date: 1/1/2018 *End Date*: 12/31/2018 *Amount*: $55,000

Abstract:
This award is to provide me with collaboration through mentored guidance necessary to become an independent academic career as a gene–environmental cancer epidemiologist in the fields of cancer prevention and control research. The overarching goal of my proposed research is to understand the influence of gene–lifestyle interactions on the pathways connecting insulin resistance (IR) and breast cancer among postmenopausal women, a population highly susceptible to breast cancer. Obesity is a well-established risk factor for postmenopausal breast cancer; obesity–insulin connections in particular have gained growing attention over the last decade as potential factors of cancer development. Although IR, obesity status, and the related lifestyle factors for postmenopausal breast cancer are well established, the genetic basis of IR that is associated with breast cancer is largely unknown. In addition, no studies to date have explored the interacting role of obesity status and the lifestyle factors in these pathways among IR-related genetic variants, IR traits (e.g., IR), and postmenopausal breast cancer risk. Further, genome-wide association (GWA) study has become standard for exploring associations between common genetic variants across the genome and cancer. However, the statistical tests applied in GWA studies typically focus on single markers and some generic markers may be missed owing to the strict statistical threshold. I will use a pathway analysis as a complementary approach to identify groups of genes or biologic pathways filled with cancer-related single-nucleotide polymorphisms. Our specific aims are 1) to evaluate the influence of obesity status and lifestyle factors as effect modifiers of the associations among IR genetic variants, IR traits, and breast cancer risk and 2) to perform pathway-based association analysis between IR genes and breast cancer risk. These specific aims will be accomplished by using the existing large cohort data of postmenopausal women from the Women's Health Initiative Database for Genotypes and Phenotypes Study. Elucidating how obesity and correlated lifestyle factors intermingle with pathways of IR, the relevant genes, and breast cancer may yield new insight into the study of gene–lifestyle interactions. This study will also suggest data on potential genetic targets in clinical trials for cancer prevention and intervention strategies.

Context-Dependent Codon Optimality Drives Cell Proliferation

Host Campus: Merced
Lead Investigator: Michael Cleary
Start Date: 1/1/2018 *End Date*: 12/31/2018 *Amount*: $54,947

Abstract:
Transfer RNAs (tRNAs) are often considered uniform building blocks with little variation between cells, yet expression levels of tRNAs can profoundly affect cell properties. Multiple studies suggest that the stability of cancer-related messenger RNAs (mRNAs) is determined by tRNA abundance and codon usage. This relationship is based on “codon optimality”: mRNAs containing a high frequency of codons with abundant cognate tRNAs (optimal codons) are stable while mRNAs containing a high frequency of codons with rare cognate tRNAs (non-optimal codons) are rapidly degraded. We recently discovered a context-dependent role for codon optimality in Drosophila: codon optimality determines mRNA stability in developing tissues but optimal codons do not stabilize transcripts in differentiated neurons. Based on this discovery, we hypothesize that codon optimality balances neural progenitor proliferation versus differentiation during neural development. Context-dependent codon optimality may be due to differential expression of tRNAs in progenitors versus differentiated cells, alterations in the cellular machinery that links translation and mRNA stability, or a combination of these mechanisms. Drosophila neural progenitors are an excellent model for studying the balance between cell proliferation and differentiation, with dramatic brain tumors arising when this balance is altered. We propose to study codon optimality in Drosophila neural progenitors as a way to understand how tRNA abundance and codon usage can contribute to oncogenic transformation. This will be accomplished via two aims. Our first aim will measure mRNA decay, tRNA abundance, and codon optimality in progenitors versus neurons in different Drosophila mutants: a “brain tumor” mutant in which the brain primarily contains proliferating progenitors and a “premature differentiation” mutant in which the brain primarily contains post-mitotic neurons. These data will allow us to predict and test how specific tRNAs alter gene expression. Our second aim will dentify how codon optimality balances cell proliferation and differentiation using experimental manipulation of tRNA expression levels and genetic dissection of the regulatory pathway linking ribosome translocation and mRNA decay. This work will establish an animal model to study the effects of codon optimality on cell proliferation, a necessary step toward understanding codon optimality in cancer.

Oncolytic y-herpesvirus for induction of anti-tumor immunity

Host Campus: Los Angeles
Lead Investigator: Ting-Ting Wu
Start Date: 1/1/2018 *End Date*: 12/31/2018 *Amount*: $54,835

Abstract:
Tumor-selective replicating lytic viruses are an emerging approach in the next generation of cancer therapy. In addition to the ability of viruses to preferentially lyse and kill cancer cells, viral induction of the innate immune responses that alter normally immunosuppressive tumor microenvironments is critical for the lytic virus to mediate tumor rejection. Investigators have designed several classes of viruses to limit viral pathogenicity while enhancing viral immunogenicity. Amgen's oncolytic virus, Imlygic (T-VEC), is a first-in-class viral therapy for tumors. This engineered HSV-1 virus is armed to expresses GM-CSF, stimulating the differentiation of dendritic cells. Phase III clinical trials indicate a 16% durable response rate in Imlygic treated patients (1). The mechanism remains elusive and the contribution of the GM-CSF has yet to be defined. Imlygic cannot be used in immunosuppressed individuals due to the occurrence of herpetic infections in patients and the potential for life threatening disseminated infection. The focus for future viral therapies is to attenuate the oncolytic virus to eliminate virus-associated disease in patients. Herein, we aim to design an attenuated y-herpesvirus that is deficient in counteracting type I interferon responses as a cancer therapeutic agent. A key feature of tumor cells is their disabled type I IFN response. In the absence of the IFN response, the attenuated y-herpesvirus will lytically replicate in cancer cells while in healthy tissue, characterized by a functional IFN response, replication of the attenuated y-herpesvirus is inhibited. This attenuated y-herpesvirus is completely replication-deficient in severe combined immunodeficiency mice, while robustly eliciting inflammatory and immunomodulatory cytokines, including IFNb, IL-1b, TNFa and IL-12. This approach directly addresses the adverse effects related to disseminated herpetic infection, which limit oncolytic viral therapy regimens. Uncontrolled viral replication also presents a risk to immunocompromised patients, precluding them from being eligible for oncolytic viral therapy. Our new oncolytic virus design overcomes this shortcoming and aims to include patients who have previously undergone aggressive immunosuppressive treatments, such as chemotherapy and radiation. 1. Andtbacka, R. H. I. et al. Journal of Clinical Oncology (2015).

Single Cell analysis of drug resistance in cancer cells

Host Campus: Santa Cruz
Lead Investigator: Nader Pourmand
Start Date: 1/1/2018 *End Date*: 12/31/2018 *Amount*: $55,000

Abstract:
We propose to investigate at a single cell level the molecular mechanisms that cause development of drug resistance of breast cancer to Paclitaxel, a first line therapy for this disease. Resistance is a major hindrance in the effective treatment of breast cancer and results from a resetting of the genomic expression of cancer cells to adapt to the toxic actions of anti-cancer drugs. We showed that chronic Paclitaxel treatment of the TNBC cell line MDA-MB-231 kills the vast majority of the cells. Using single cell RNA-seq, we showed that the genomic expression profile of the tolerant cells varies dramatically from drug naïve cancer cells and results in the expression of a unique family of single nucleotide RNA variants (SNVs) in the cancer cells. The molecular changes primarily affected cellular pathways targeted by Paclitaxel to compensate for the drugs actions. Drug resistance is maintained as the cancer cells expand and we propose to use a novel nanopipette/nanoGenomics technology to determine how the genomic changes that direct cancer cells to resist Paclitaxel are transferred from single parent to daughter cells as the resistant cells proliferate. To do this, we will make MDA-MB-231 cells resistant to Paclitaxel, use the nanopipette to tag the few resistant cells with carboxyfluorescene and use fluorescent microscopy to monitor the single labeled cells to identify daughter cells after division. We will then use the nanopipette to extract the RNA from single resistant cells for whole transcriptome analysis (WTA). These studies will determine whether genomic changes induced in the resistant cells are maintained from generation to generation and help to identify those changes most important for drug tolerance. We will use the same technique to identify genomic changes in the cancer cells are they regain sensitivity to Paclitaxel after drug removal to reveal what genomic make up in the cells is critical for Paclitaxel anticancer activity. These studies may provide information that can be used to devise approaches to overcome resistance and reveal a genomic signature to use for diagnosing development of resistance in patients undergoing anticancer therapy.