

## Cancer Research Coordinating Committee

Abstracts for 2019 Awards

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**Cancer Research Coordinating Committee***Abstracts for 2019 Awards*

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## **An astroglial mechanism of radiation-induced brain injury**

*Host Campus:* Irvine

*Lead Investigator:* Munjal Acharya

*Start Date:* 1/1/2019    *End Date:* 12/31/2019    *Amount:* \$75,000

*Abstract:*

Cranial radiation therapy (RT) for the treatment of primary and secondary malignancies of the brain are often associated with severe cognitive dysfunction. This is a particularly critical problem for survivors of childhood cancer who frequently live long post-RT but experience reductions in IQ by as much as 3 points per year that seriously impact quality of life. This situation is confounded by the absence of satisfactory treatments for reducing neurocognitive side effects. RT induces progressive neurodegenerative changes including oxidative stress, reduced neurogenesis, increased inflammation, and astrogliosis. However, many of the molecular mechanisms underlying RT-induced cognitive decline remain to be resolved. Astrocytes form complex astroglial networks by contacting thousands of synapses (memory units) that are actively involved in modulating synaptic plasticity. We have shown that RT-induced cognitive disruption coincides with astrocytic hypertrophy, elevated expression of astrogliosis genes and microglial activation. Therefore, we hypothesize that detrimental changes in astrocyte function contribute significantly to cognitive dysfunction. The complement system is a potent mediator of microglial and astroglial activation, but it also has a range of non-immune functions in the CNS, including synaptic pruning. Complement proteins are produced by neurons, microglia and astrocytes. Particularly, global elevation in the expression of complement protein C1q in the CNS has been reported in neurodegenerative conditions including the Alzheimer's disease or epileptic brain. These proteins play important roles in maintaining cellular viability and synaptic function in the CNS. Our preliminary data indicate that cranial RT leads to a chronic upregulation in the gene expression profile of reactive (harmful) astrocytes, microgliosis with elevated C1q which was co-incident with cognitive impairments. We will characterize cranial RT-induced changes in the astrocytic and microglial complement protein (C1q) and cognitive function after 9 Gy head only irradiation. To better understand the differences in the contribution of astrocyte-mediated and microglia-mediated regulation of the complement (C1q) cascade, we will utilize gene silencing and transgenic mouse approaches to study the CNS-radiation response and its impact on cognitive function.

## **Defining targetable pro-tumor effects of neutrophils in GBM**

*Host Campus:* San Francisco

*Lead Investigator:* Manish Aghi

*Start Date:* 1/1/2019    *End Date:* 12/31/2019    *Amount:* \$75,000

*Abstract:*

The growth and treatment refractoriness of glioblastoma (GBM), a brain tumor with a devastatingly short survival of under two years, derives from the dynamic interplay between tumor cells and cells in the tumor microenvironment. Our lab has demonstrated the previously underappreciated importance of neutrophils in this microenvironment. Neutrophils, the most abundant leukocytes in blood, are the first line of defense during inflammation. Like macrophages, tumor-associated neutrophil (TAN) subtypes have been proposed: N1 neutrophils possessing “anti-tumoral” activity and N2 pro-tumoral neutrophils, with little known about subtype-specific markers. We have found that GBM cells secrete leukotriene B4 (LTB4), a chemoattractant which recruits circulating neutrophils. Using single cell sequencing, we defined two TAN gene expression profiles with functional analysis revealing them to be ant-tumoral N1 vs. pro-tumoral N2 TANs. We also found that tumor-secreted factors promote gene expression changes in recruited circulating neutrophils, driving them towards an N1 state in low-grade gliomas and an N2 state in high-grade gliomas. And we found that pro-tumoral N2 TAN osteopontin secretion increases tumor cell and tumor stem cell proliferation. Here, we will build upon this data by investigating our central hypothesis that GBM cells secrete LTB4 to recruit circulating neutrophils and polarize them into N2 TANs in a TGF- $\beta$ -dependent manner, with N2 TANs exerting osteopontin-driven pro-tumoral effects on GBM cells and the GBM microenvironment. We will investigate this hypothesis through two aims: Aim 1 – Define the mechanisms by which GBM recruits and polarizes neutrophils; and Aim 2 – Define the role of osteopontin in pro-tumoral "N2" TAN effects on GBM cells and the tumor microenvironment in culture and in vivo. To accomplish these goals, we will use single cell-technology, site-directed biopsies, and mouse models. Our studies will discover novel mechanisms by which tumor cells regulate their microenvironment to make it more hospitable for tumor growth. This work will challenge conventional thinking by showing that neutrophils are not bystanders passively accumulating in a growing tumor but active participants in the growth of the tumor, as well as a novel therapeutic target.

## **Unveiling targets for treating malignancies of viral origin**

*Host Campus:* Santa Barbara

*Lead Investigator:* Carolina Arias

*Start Date:* 1/1/2019    *End Date:* 12/31/2019    *Amount:* \$75,000

*Abstract:*

Kaposi's sarcoma-associated herpesvirus (KSHV) is the causative agent of Kaposi's sarcoma (KS) and primary effusion lymphoma (PEL), two malignancies predominantly diagnosed in HIV/AIDS and immunocompromised patients. While the advent of antiretroviral therapy has significantly controlled the HIV/AIDS epidemic and has reduced the rates of AIDS-associated KS, infection with KSHV still prevails and causes serious disease in untreated HIV positive individuals and organ transplant patients. Treatment options for patients with severe KSHV-associated malignancies are limited, often involving exposure to chemotherapeutic agents with a wide range of secondary effects and cumulative toxicity. The development of new therapies for the control of KSHV infection in immunocompromised patients with mild to severe KSHV-related malignancies would expand the options for treatment of acute and chronic disease. An aspect of viral infection that remains to be explored for the development of antiviral agents is the strict dependence of viruses on their hosts. The pharmacological inhibition of cellular factors promoting infection or the activation of host pathways impairing viral replication has the potential to pave new avenues for the treatment of infections. This promising approach requires a deeper understanding of host/pathogen interactions at the molecular level. Here, we propose to identify critical cellular factors that are indispensable for KSHV infection, but dispensable for normal cell function, which could be targeted for therapeutic intervention. We will focus on understanding the regulation of viral protein synthesis in cells infected with KSHV. By dissecting the cellular requirements for the synthesis of functional proteins during viral infection, we will reveal potential host targets for the modulation of productive infection. Importantly, recent work showcases the clinical potential of pharmacological modulation of the protein synthesis and folding machinery for the treatment of cancer and other diseases. Our investigations will help pinpoint important host targets for the control of viral infections, offering the opportunity to explore drug repurposing to treat viral diseases, and providing an alternative for the management of KSHV-related malignancies in immunocompromised patients.

## **Tracing Tumor Heterogeneity in Squamous Cell Carcinoma**

*Host Campus:* Irvine

*Lead Investigator:* Alexander Boiko

*Start Date:* 1/1/2019    *End Date:* 12/31/2019    *Amount:* \$75,000

*Abstract:*

Squamous cell carcinoma (SCC) is a common form of skin cancers which results from mutagenesis caused by UV-Radiation, as a part of sun exposure. Numerous genetic mutations that lead to the appearance of SCCs have been identified and provided basis for the current anti-tumor approaches. However, despite rapid improvements in targeted drug discovery, tumor heterogeneity continues to thwart their translation into improved patient outcomes. This underlines the critical importance of understanding the mechanisms of SCC initiation and progression. Substantial heterogeneity observed within SCC tumors suggests two possible scenarios of its growth 1) tumors develop as the result of multiple independent subclones maintaining its growth as predicted by stochastic model of tumor formation, or 2) tumors are initiated and propagated by defined cellular subclone which gives rise to all the progeny lineages capable of accumulating additional mutations during tumor progression as predicted by hierarchical stem cell model. The goal of this project is to delineate between clonal and stochastic models of SCC formation and to define the critical mutations in the cell of origin for the UV-induced SCCs. To reach this goal my lab has established cellular and genetic mouse tracing model of spontaneous SCC formation in response to UV stimuli. These mice contain CK14\_ACTNCRE\_Brainbow transgene which harbors Cre-dependent four-color reporter construct coding for red, orange, green and blue fluorescent proteins (16 different spectra are possible) in all cells of their adult skin. Our experimental setup uses topical induction of Cre-recombinase activity to make every skin cell and all of its subsequent cellular progeny to randomly express only one color. We then expose these mice to UV protocol treatment to induce spontaneous SCC formation. Each arising SCC, classified by tissue specific markers, will consist either of a single color or a mosaic combination of all colors. Single color points to the existence of a tumorigenic cell population derived from a single stem-like cell/clone, while mosaic distribution of colored cell clones signifies a multi-clonal stochastic origin of this tumor. Single cell sequencing of each particular color containing population will then reveal unique spectrum of driver mutations and degree of their divergence depending on the stage of tumor progression.

## **Landscape of 5' UTR somatic mutations in lung adenocarcinoma**

*Host Campus:* Santa Cruz

*Lead Investigator:* Angela Brooks

*Start Date:* 1/1/2019    *End Date:* 12/31/2019    *Amount:* \$75,000

*Abstract:*

We propose to develop a computational approach to identify cancer mutations from RNA sequencing data with the goal of characterizing mutations that alter gene translation in lung adenocarcinoma (ADC). Somatic alterations in the human genome contribute to oncogenesis. Next-generation sequencing has revealed that half of lung ADC lack a mutation in a known cancer driver mutation, or have mutations in known oncogenes (e.g., KRAS) that cannot be targeted with current therapeutics. While alterations in protein-coding regions have historically been a research focal point, the study of mutations in noncoding regions and their relationships to cancer formation are still largely uncharacterized. The 5' untranslated region (UTR) lies upstream of the initiation codon and regulates translation through ribosome recruitment to the mRNA. 5' UTR mutations have been shown to affect translational efficiency of oncogenes and tumor suppressor genes, making them possible therapeutic targets; however, there has not been a global study of these mutations. 5' UTR mutations have been overlooked due to the limitations of data provided by large-scale cancer genome studies, such as The Cancer Genome Atlas. Somatic mutations were identified through DNA sequencing of tumor and matched normal samples from individual donors. Due to cost, protein-coding DNA was captured from ~600 lung ADC donors for sequencing, leaving out information of noncoding regions. Whole-genome DNA sequencing was performed on ~40 lung ADC donors; however, lung ADC has one of the highest mutation rates of any cancer type making it difficult to distinguish driver versus passenger mutations with such low sample numbers. Fortunately, all tumor samples had their mRNA sequenced (RNA-Seq), which contains the 5' UTR. We propose to take advantage of RNA-Seq data to identify cancer-associated mutations in the 5' UTR. In Aim 1, we will develop a computational pipeline that identifies mutations from mRNA sequencing data from tumors. We will evaluate existing variant callers (computational methods that detect variants from sequencing data), modify these methods, and benchmark the sensitivity and accuracy to detect variants in RNA-Seq. We will then apply our variant caller on RNA-Seq data from lung ADC donors. In Aim 2, we will characterize the functional impact of 5' UTR mutations on features of translational regulation.

## **Critical CpG Sites of MGMT Promoter in Glioblastoma**

*Host Campus:* San Francisco

*Lead Investigator:* Farid Chehab

*Start Date:* 1/1/2019    *End Date:* 12/31/2019    *Amount:* \$75,000

*Abstract:*

Our aim in this proposal is to characterize biomarkers that can be used to increase the efficacy of the antitumor therapy of the drug temozolomide in glioblastoma patients and predict their progression free survival. Our preliminary data demonstrate that a group of 5 CpG sites in the MGMT promoter region are subject to methylation in a large cohort of glioblastoma patients. We will use a CRISPR-based gene editing approach to demonstrate convincingly that these CpG sites are indeed associated with temozolomide sensitivity, result in better clinical outcomes and that they act via reduced expression of the MGMT gene. These studies will pave the way to devise similar gene editing strategies that employ these critical CpG sites and that could be tested first in mice and eventually in humans. Another extension of these studies are applicable to other types of cancers with underlying etiologies similar to the association of MGMT and glioblastoma.

## **Detection of tumor DNA repair defects from liquid biopsies**

*Host Campus:* Irvine

*Lead Investigator:* Olivier Cinquin

*Start Date:* 1/1/2019    *End Date:* 12/31/2019    *Amount:* \$75,000

*Abstract:*

The advent of liquid biopsies holds great promise both for detection of cancer and for precision therapy. Yet means to retrieve actionable information from these biopsies are still limited. Current liquid biopsy assays do not address cell DNA repair capacity (DRC), which varies substantially between healthy and cancerous tissue and between cancer types. DRC shapes drug sensitivity and tumor evolution — by controlling mutation accrual and by affecting interactions with the immune system in multiple ways. Precision DRC assays are thus a natural and exciting avenue to fill the void in methods to identify e.g. patients for whom immune blockade therapy is unlikely to succeed, sparing them potentially devastating side effects, and to identify suitable drugs targeting particular DNA repair mechanisms. We propose to measure DRC from liquid biopsies, leveraging the SIP-HAVA-seq technique we recently developed. Our assay provides a genome-wide view of DRC that substantially improves on other DRC assays. We will test our assay by collecting blood and fresh, surgically-extracted tumor tissue from prostate cancer patients. In the future our assay will make it possible to use liquid biopsies to help predict cancer aggressiveness — a problem of particular importance in the context of prostate cancer, for which watchful waiting is often but not always the best approach — as well as the most suitable therapy for each individual patient.

## **18FDOPA PET for the early evaluation of treatment responses**

*Host Campus:* Los Angeles

*Lead Investigator:* Peter Clark

*Start Date:* 1/1/2019    *End Date:* 12/31/2019    *Amount:* \$75,000

*Abstract:*

Early pharmacodynamic biomarkers of therapeutic responses in cancer are critical to limit the amount of time clinicians and patients waste on ineffective therapies. This is especially true for glioblastoma, where the median survival time is 16 months and repeat biopsies are not possible. The use of 18F-FDG PET as an early pharmacodynamic biomarker of treatment responses is remarkably effective across various cancers and therapies, including for Imatinib in GIST and Erlotinib in lung cancer. However, unlike in most cancers where 18F-FDG PET can selectively differentiate tumor from normal tissue, glioblastoma cannot be easily distinguished from surrounding brain tissue with 18F-FDG PET. Instead the radiotracer 18F-FDOPA is used to visualize glioblastoma. 18F-FDOPA is a fluorinated analogue of the amino acid L-DOPA, and 18F-FDOPA accumulation measures L-DOPA consumption. We hypothesize that early changes in tumor 18F-FDOPA consumption in response to a therapy can predict a therapeutic response. We will test this in patient-derived gliomasphere models in culture. There is no published protocol for imaging patient-derived orthotopic xenograft models in mice with 18F-FDOPA PET to use in testing in vitro results in vivo. We propose to develop such a protocol and to test it with patient-derived xenografts. Successful completion of the proposal would lay the groundwork for a new approach to treating glioblastoma patients. The response of a tumor to a therapy would be evaluated early in the course of that therapy and patients would no longer be forced to endure ineffective therapies. In preliminary studies, we have (1) measured 18F-FDOPA consumption in culture in patient-derived gliomasphere models, (2) evidence that effective EGFR inhibition increases 18F-FDOPA consumption, and (3) experience 18F-FDOPA PET imaging subcutaneous glioblastoma models. We propose: (I) To test in culture, across 16 genetically diverse patient-derived gliomasphere models, whether changes in 18F-FDOPA consumption 4 or 24 hours post-treatment with an EGFR or PI3K inhibitor (1) correlate with drug – target engagement at those time points or (2) can predict therapeutic efficacy 72 hours post-treatment. (II) To develop a protocol for imaging patient-derived orthotopic glioblastoma xenograft models with 18F-FDOPA and use it to quantify 18F-FDOPA consumption in 6 models.

## **O-Glycosylation and Immunotherapy for Cancer**

*Host Campus:* Irvine

*Lead Investigator:* Michael Demetriou

*Start Date:* 1/1/2019    *End Date:* 12/31/2019    *Amount:* \$75,000

*Abstract:*

The emergence of targeted anti-cancer drug therapies called Immunotherapies, which stimulate the body's own immune system to fight cancer, hold great promise for cancer treatment over the traditional modalities of surgery, chemotherapy and radiation. These include checkpoint inhibitors that block T cell suppression (eg anti-PD-1), bi-specific antibodies that cross-link T cells to cancer cells and T cells engineered to express antigen receptors specific to cancer cells (eg Chimeric Antigen Receptor T cells - CAR T). Of these, bi-specific antibodies and CAR T cells have shown the greatest activity, with complete response rates as high as ~90%. However, widespread development is limited by the small number of known cell-surface proteins that are sufficiently specific to cancer to safely allow targeting by antibodies. This is particularly true for solid cancers, where unlike hematopoietic malignancies, loss of healthy cells cannot be readily replenished by stem cell progenitors. A solution to this issue is to target cancer specific glycan antigens rather than protein antigens. Indeed, altered glycosylation is a near universal feature of cancer and represent the most abundant and widely expressed cell surface cancer antigens known, while also having limited or no expression in normal tissue. However, generation of monoclonal antibodies specific to complex carbohydrates has proven to be very challenging, greatly limiting their usefulness as targets for cancer immunotherapy. However, evolution has produced 100's of sugar binding proteins known as lectins that bind carbohydrate antigens with very high specificity and sensitivity. Utilizing a lectin rather than an antibody to target cancer cells allows us to generate bi-specific proteins or CAR T cells with high specificity for cancer-associated glycans. Here we propose to use lectins to generate a bi-specific protein and/or CAR T cell specific to carbohydrate antigens that are common to the vast majority of solid > hematopoietic cancers but not expressed in normal cells.

## **Studying tumor heterogeneity using single-cell epigenomics**

*Host Campus:* Santa Barbara

*Lead Investigator:* Siddharth Dey

*Start Date:* 1/1/2019    *End Date:* 12/31/2019    *Amount:* \$75,000

*Abstract:*

While mutations and copy number variations in the genome are known drivers of cancer, there is increasing evidence that dysregulation in epigenetic marks such as DNA methylation (5-methylcytosine or 5mC) and disruption of the 3-dimensional organization of chromosomes within the nucleus of a cell play a critical role in the progression of tumors. In addition to these complex genome-wide transformations, tumors are also characterized by dramatic cellular heterogeneity that remain one of the major challenges in the effective treatment of cancer. However, it remains unclear how the epigenome influences tumor heterogeneity. This is because current measurements are typically made from a bulk population that fail to capture the cell-to-cell variability in 5mC or genome organization and the resulting gene expression heterogeneity. Further, while bulk studies in tumor cells have shown that large blocks of hypomethylation in 5mC appear to correlate with regions of the genome that interact with the nuclear periphery (known as lamina-associated domains or LAD), these experiments cannot distinguish if these profiles occur in the same cell or unrelated cells. Therefore, it remains unknown if a causal relationship exists between 5mC and genome organization, and how dynamic changes in such epigenetic features regulate cellular phenotypes. To overcome the technical challenges in addressing these questions and to understand how dysregulation in 5mC and genome organization together alter gene expression in tumor cells, we propose the following aims: (1) Develop a novel single-cell sequencing technology to simultaneously quantify 5mC, LAD organization and mRNA from the same cell. Integrated measurements of both the epigenetic features and the transcriptome will allow us to directly correlate 5mC to LAD structure and how they combine to regulate gene expression in a single cell. (2) Employ a recently developed model of tumor progression in intestinal organoids to study how simultaneous reprogramming of 5mC and LAD organization directly influences the dynamics of aberrant gene expression by the sequential introduction of mutations in APC, P53, KRAS and SMAD4 genes. This seed grant will lay the foundation for further systematic exploration into the potential mechanisms that mediate the cross-talk between 5mC and genome organization and its influence on gene regulatory networks.

## **Single-cell profiling glioma evolution under therapy**

*Host Campus:* San Francisco

*Lead Investigator:* Aaron Diaz

*Start Date:* 1/1/2019    *End Date:* 12/31/2019    *Amount:* \$75,000

*Abstract:*

Malignant gliomas are the most common primary tumors of the adult brain. Despite treatment, gliomas are rarely curable. Precision oncology approaches for glioma have focused on identifying and targeting driver mutations. However, clinical trials of targeted therapeutics have proven disappointing for glioma. This is partially due to their high intra-tumor heterogeneity. Moreover, increasing evidence disputes a direct linear relationship between tumor genotype and tumor phenotype, and supports a lack of dependency upon any one mutation for malignant progression. Although recurrent genomic alterations in glioma have been extensively characterized, surprisingly little is known about the existence of recurrent cellular phenotypes. There is a critical need to identify the fundamental cell types and states in glioma, and how they evolve under therapy. Until this gap in our knowledge is addressed, precision oncology will be restricted to targeting genomic alterations that may not truly "drive" the tumor, glioma cellular-phenotypic heterogeneity will remain poorly understood and will limit therapy development. Our long-term goal is the rational design of precision therapies, based on an understanding of glioma's cellular architecture. The overall objective of this project is to characterize the recurrent cell types in glioma and determine how glioma composition changes under standard-of-care therapy. Our central hypothesis is that there are durable glioma cell-types, which are recurrent in all gliomas regardless of that glioma's private mutations. Our hypothesis has been formulated based on our preliminary data, in which we have performed single-cell RNA-sequencing of over 30,000 cells from resections from 20 glioma patients. Our data demonstrate patient-independent, recurrent cell-types found in all gliomas. The rationale for the proposed research is that by learning the cellular architecture of human glioma and how it evolves under therapy we will move beyond a paradigm of precision oncology that focuses exclusively on driver mutations and toward a more encompassing notion of a precision target that includes specific malignant cell-types.

## **Risk stratification in prostate cancer using pH imaging**

*Host Campus:* San Francisco

*Lead Investigator:* Robert Flavell

*Start Date:* 1/1/2019    *End Date:* 12/31/2019    *Amount:* \$75,000

*Abstract:*

The central goal of this application is to validate and optimize new method we have developed for imaging of tissue pH using magnetic resonance imaging (MRI) in preparation for future clinical studies. Prostate cancer presents with a broad clinical spectrum ranging from indolent to aggressive disease, and the development of biomarkers for improved discrimination between these phenotypes is an unmet clinical need and active area of research. One potential biomarker for aggressive disease is reduced interstitial (extracellular) pH, which is associated with local invasion, metastasis, and survival in murine models of cancer. We have recently developed a high resolution, clinically translatable method of imaging extracellular pH using hyperpolarized <sup>13</sup>C magnetic resonance imaging using <sup>13</sup>C sodium bicarbonate (HP-BiC-MRI). Briefly, this method relies on administration of a sodium bicarbonate contrast agent which can be imaged on a clinical MRI scanner, and provides millimeter scale resolution imaging of extracellular tissue pH in living animals. We have performed initial validation studies in vitro and in mouse models of prostate cancer, and have strong preliminary data suggesting that decreased pH measured using our HP-MRI method is correlated with aggressive disease as determined through standard histologic analysis. The goal of this application is to complete a preliminary analysis linking decreased tissue pH with high grade disease in the TRAMP mouse model, and to optimize the production and analysis of the hyperpolarized contrast agent in preparation for submission of an investigational new drug (IND) application to the United States Food and Drug Administration. Importantly, since our contrast agent is sodium bicarbonate, which is already routinely administered to patients as part of clinical care, it is highly unlikely that toxicity of the contrast agent will limit development. If accomplished, the experiments outlined in this proposal would validate our new HP-BiC-MRI imaging method as a predictive biomarker for the presence of aggressive, potentially lethal disease, and lay the groundwork for a subsequent first in man application of the technique.

## **Impact of Diet on Myeloproliferative Neoplasm**

*Host Campus:* Irvine

*Lead Investigator:* Angela Fleischman

*Start Date:* 1/1/2019    *End Date:* 12/31/2019    *Amount:* \$75,000

*Abstract:*

Myeloproliferative neoplasm (MPN) is a hematologic malignancy characterized by the clonal outgrowth of hematopoietic cells with a somatically acquired mutation most commonly in JAK2 (JAK2V617F). This mutation endows upon myeloid progenitors cytokine independent growth and consequently leads to excessive production of myeloid lineage cells. Chronic inflammation is a characteristic feature of MPN, inflammation contributes to disease initiation, progression, transformation, thrombosis, and symptomatology. The traditional approach to therapy in MPN is to reduce the risk of thrombosis, manage symptoms, and observe for progression of the disease. This leaves many early stage MPN patients to be managed with aspirin alone and simply observed for progression. Currently there are no therapeutic options that delay or halt disease progression. There is a critical need for interventions in early stage patients that impact MPN disease progression, particularly interventions with minimal risk given that MPN clinical course may range from indolent to aggressive. Additionally, disease interventions that allow patients to self-manage their MPN symptoms are lacking. Nutrition modification is a well-accepted intervention to reduce thrombosis and improve symptoms in cardiovascular disease. To date no interventions evaluating nutrition have been evaluated in the MPN population. We hypothesize that targeting inflammation early during the MPN disease course may relieve symptom burden and retard progression of MPN. Our overarching goal is to develop a low-risk nutritional intervention among MPN patients that may ultimately lead to better-controlled disease by reducing inflammatory cytokines. To test the effect of altering the inflammatory milieu via dietary intervention we will expose a JAK2V617F mutated mouse model to a high inflammatory (high fat) or low inflammatory (Mediterranean) diet and measure the impact on the MPN phenotype. We will also prospectively test the feasibility of a Mediterranean diet intervention which emphasizes foods rich in anti-inflammatory properties in MPN patients and determine the impact on plasma inflammatory cytokines, MPN symptom burden, and peripheral blood counts.

## **Role of LMO1/MYCN in the initiation of human neuroblastoma**

*Host Campus:* Riverside

*Lead Investigator:* Martin Garcia-Castro

*Start Date:* 1/1/2019    *End Date:* 12/31/2019    *Amount:* \$75,000

*Abstract:*

Neural crest (NC) cells are an embryonic stem cell population that gives rise to multiple derivatives, including neurons and glia of the peripheral nervous system. Aberrant NC development leads to aggressive cancers such as neuroblastoma (NB), which accounts for up to 15% of pediatric cancer-related deaths and is highly metastatic. About 25% of NB expresses high levels of the MYC proto-oncogene, MYCN. More recently, the transcriptional regulator LIM-domain-only 1 (LMO1), has been identified as a NB oncogene, yet the precise mechanism by which it promotes metastatic NB is unknown. We propose to precisely address the molecular mechanisms by which LMO1/MYCN contribute to the initiation of human NB using a novel dual inducible model of human NC cell-derived neuroblastoma. We have developed a rapid and robust model of human embryonic stem cell-derived NC, which closely recapitulates different stages of NC development as reported in animal model systems. These hNC cells have migratory potential and the capacity to differentiate into NC derivatives including peripheral neurons (1 week), glia (3 week) and sympathoadrenal cells (5 days) in an efficient manner. Our transcriptomic analysis of hNC development identified LMO1 and MYCN expression during early stages of NC development, yet their role in NC development is also not known. It is well-established that cancer often arises as the result of reactivating developmental pathways. Therefore, we hypothesize that LMO1/MYCN activation in NB triggers early neural crest behaviors that enables cancer. To determine the role of LMO1/MYCN during formation of NC-derived NB, we propose to use CRISPR/Cas9 genome editing to generate a dual inducible hESC line that overexpresses LMO1/MYCN. This will serve, to the best of our knowledge, as the first system modeling early human NB initiation. We will assess the effects of LMO1/MYCN overexpression in hNC cells differentiated towards the sympathoadrenal fate by assaying for cell proliferation, survival, migration, and formation of neuroblastoma phenotype. Finally, we will address the effects of LMO1/MYCN overexpression on gene expression changes during the formation of NC-derived neuroblastoma using RNA-seq analysis. Our study will unveil the function of LMO1/MYCN in the initiation of human NB, aiding in the identification of novel targets for early detection and therapy.

## **Synthesis of Strictosidine, a Precursor to Vinblastine**

*Host Campus:* Los Angeles

*Lead Investigator:* Neil Garg

*Start Date:* 1/1/2019    *End Date:* 12/31/2019    *Amount:* \$75,000

*Abstract:*

Cancer remains the 2nd leading cause of death in the United States. In 2018, it is estimated that over 1.7 million Americans will be diagnosed with cancer, and over 600,000 will die from the malicious disease. More than 50% of cancer patients rely on costly chemotherapeutics to treat cancer effectively. Vinblastine is a frontline cancer therapeutic used to treat multiple forms of the disease. The industrial production still relies on isolation from its natural source, *Catharanthus roseus*, which renders the process inefficient and source-dependent. Although this approach accesses the drug, vinblastine remains one of the most expensive small molecule, off-patent chemotherapeutics. Vinblastine could be made affordable with an efficient method of production; however, typical approaches most commonly used in drug manufacturing have failed. For example, an efficient chemical synthesis of vinblastine remains elusive due to the compound's structural complexity. Further complicating matters is the fact that numerous details of vinblastine's biosynthesis remain unresolved, thus making a fully bioengineered synthesis impossible at present. The overarching aim of this project is to develop a chemoenzymatic means to access vinblastine. Toward this long-term goal, we propose to first develop an efficient synthesis of strictosidine, an important biosynthetic precursor to vinblastine. The highly functionalized six-membered ring of secologanin will be accessed via an asymmetric inverse-electron-demand hetero-Diels–Alder reaction. After further elaboration, an enzyme-mediated Pictet–Spengler reaction will be used to arrive at strictosidine. It is envisioned that these studies will lay the foundation for future studies directed toward the conversion of strictosidine to vinblastine using enzymatic machinery. Thus, the proposed studies would represent a significant milestone toward a scalable and affordable means for the chemoenzymatic production of an important chemotherapeutic.

## Oncogenic potential of an alternate mRNA cap-binding protein

*Host Campus:* Berkeley

*Lead Investigator:* Nicholas Ingolia

*Start Date:* 1/1/2019    *End Date:* 12/31/2019    *Amount:* \$75,000

*Abstract:*

Dysregulated translation promotes tumorigenesis, cancer progression and metastasis. Key oncogenic pathways such as PI3K/Akt/mTOR and MAPK/Mnk control protein synthesis by regulating eukaryotic translation initiation factor 4E1 (eIF4E1). This protein is encoded by a potent oncogene that is misregulated in up to 30% of human tumors and malignancies (1), and heterozygous loss of Eif4e1 in mice (Eif4e1+/-) increases resistance to cancer (2,3). The eIF4E1 protein directly binds the 5'-methylguanosine cap found at the 5' end of all cellular mRNAs as a component of the eIF4F complex. While eIF4E1 is thought to bind all mRNAs, oncogenic transcripts with highly structured 5' UTRs such as c-myc, VEGF, and cyclin D1 are very sensitive to eIF4E1 activity. Taken together, these results argue strongly that eIF4E1 plays a key role in cancer. Vertebrate genomes also encode a poorly-understood eIF4E1 homologue, eIF4E3, which has been suggested to act a tumor suppressor by competing with eIF4E1 and inhibiting the translation of cancer specific transcripts (4,5). In other cell culture models, eIF4E3 has been reported to support tumor growth by virtue of being refractory to regulatory signals impinging on eIF4E1 (6). We propose to investigate how eIF4E3 impacts translation and cancer cell survival in vivo. We will address this in the context of a natural tumor microenvironment by xenografting tumors with increased or reduced eIF4E3 and monitoring the progression of those tumors as well as profiling the translation occurring in the xenograft. Our experiments will test the hypothesis that eIF4E3, like eIF4E1, may serve as an effective target for anti-cancer therapy. 1 Volpon, Laurent, et al. Cell Cycle. 12(8), 1159–1160 (2013). 2 Mamane Yaël, et al. Oncogene 23(18) 3172–3179 (2004) 3 Truitt, Morgan L., et al. Cell. 162, 59–71 (2015). 4 Osborne, Michael J., et al. PNAS. 110(10), 3877-3882 (2013). 5 Joshi, Bhavesh, et al Eur. J. Biochem. 271, 2189-2203 (2004) 6. Landon, Ari, et al. Nature Comm. 5, 5413 (2014).

## **Photoacoustic Ultrasound to Detect/Monitor Ovarian Cancer**

*Host Campus:* San Diego

*Lead Investigator:* Jesse Jokerst

*Start Date:* 1/1/2019    *End Date:* 12/31/2019    *Amount:* \$75,000

*Abstract:*

Screening for ovarian cancer in high risk women is rational because the disease is treatable in early stages, but very deadly in late stages. CA125 testing and transvaginal ultrasound imaging have encouraging metrics including sensitivity and specificity of 89.4% and 99.8%, but have positive predictive values below 50% and are insufficient for use in the general population. Folate binding proteins are highly expressed in ovarian cancer; hence, these targets can be used to increase the specificity and sensitivity of ovarian cancer screening. My central hypothesis is that nanoparticles targeted to the folate receptor will increase tumor signal and contrast above background greater than non-targeted nanoparticles—this will increase the sensitivity and specificity of screening. Because folate receptors are expressed on the cell surface, it is critical to use a contrast agent that leaves the vasculature. My expertise is in nanoparticle imaging agents that do just that—these contrast agents are built from the clinically approved indocyanine green and poly-lactic-co-glutamic acid. I will now use them for ultrasound-based molecular imaging of ovarian cancer. I will image folate binding proteins with both conventional ultrasound and novel photoacoustic ultrasound that combines the high contrast of optical imaging with the resolution of ultrasound. I will evaluate the performance metrics of both through the following steps. Step 1. We will make the nanoparticles via an emulsification process. Step 2. We will add a cloaking polymer and folate. Step 3. We will validate the contrast agent with cell culture experiments. Step 4. We will image an orthotopic model of human ovarian cancer. Ultrasound already provides excellent anatomical information, and the small size of nanoparticles facilitates imaging the sensitive and specific biomarkers present on the cell surface. Although deployed here for ovarian cancer, this imaging agent will have broad utility across many cancer types. This work is also easily reconfigurable to other imaging targets as they emerge in the literature. I will use this pilot data to seek additional funds from the NIH at the conclusion of this project, e.g. PAR-13-185 and PAR-13-189.

## **Control of cell growth in normal and transformed cells**

*Host Campus:* Santa Cruz

*Lead Investigator:* Douglas Kellogg

*Start Date:* 1/1/2019    *End Date:* 12/31/2019    *Amount:* \$66,094

*Abstract:*

Cancer cells show severe defects in control of cell growth and size, yet the underlying causes are unknown. The long-term goal of our work is to discover how control of cell growth and size works in normal cells, and how it goes wrong in cancer cells. With this knowledge, we hope to identify novel vulnerabilities of cancer cells that can be exploited to improve therapies. Our work thus far has focused on budding yeast, since it provides a simple and powerful system in which to discover fundamental mechanisms of cell size control. In our recent work, we discovered that a highly conserved signaling network that surrounds TOR kinase complex 2 (TORC2) controls both cell growth and cell size. The network includes tumor suppressors, as well as numerous kinases directly involved in critical oncogenic signaling pathways. Our discovery that cell growth and size are controlled by a conserved signaling network that is known to be disrupted in cancer suggests that we are close to solving the mystery of why cancer cells show such severe defects in control of cell growth and size. We are now poised to translate our discoveries into vertebrate cells. We will test the hypothesis that key functions of the TORC2 network that we have discovered in yeast are conserved in vertebrates, and that they play important roles in oncogenic signaling. Successful completion of the Aims will provide fundamental new insights into the functions of important oncogenic signaling proteins, as well as insights into the poorly understood functions of the vertebrate TORC2 network.

## **Improving outcome of cancer chemotherapy with CO**

*Host Campus:* Santa Cruz

*Lead Investigator:* Pradip Mascharak

*Start Date:* 1/1/2019    *End Date:* 12/31/2019    *Amount:* \$75,000

*Abstract:*

Recent studies have indicated that moderate doses (>250 ppm) of CO cause rapid reduction of some cancer cells (but not normal cell) through cell apoptosis. In addition, CO appears to sensitize cancer cells to chemotherapy. We have recently shown that that small doses of CO from designed CO-releasing molecules (photoCORMs) can be conveniently used to induce apoptosis in human breast cancer cells in a dose-dependent manner through controlled CO release. We now plan to determine whether such co-administration of exogenous CO increases the efficacy of chemotoxic drugs in the treatment of solid cancers (consequently minimizing treatment-related adverse events). We will utilize in-vitro and in-vivo models of breast and ovarian cancer for our investigation towards assessing the effects of exogenous CO applications. (1) We will determine the optimal concentrations of photoCORMs in diminishing cell proliferation of breast and ovarian cancer cells in-vitro and in xenograft models, in the presence of various doses of commonly used chemotoxic drugs, and (2) We will investigate the detailed mechanism(s) of CO-mediated inhibition of antioxidant pathways in breast and ovarian cancer cells in-vitro and in xenograft models. In both aims, use of photoCORMs will allow delivery of precise doses of CO and study its effects under very controlled conditions. In a recent paper [1], we have shown that CO delivery from our photoCORMs selectively inhibits cystathionine  $\beta$ -synthase (CBS, a heme protein) and attenuates the antioxidant capacity of human breast cancer cells. In cancer, CBS plays a significant role in drug resistance; silencing CBS expression could sensitize cancer cells to chemotherapeutics. Our results also demonstrated that exogenous CO delivery significantly increased the chemosensitivity of human breast cancer cells toward both Doxorubicin and Paclitaxel. We therefore plan to further explore the mechanism of CO-induced enhancement of chemotoxicity in both breast and ovarian cancer cells (especially cisplatin-resistant ovarian cancer cells). We believe that along with CBS, there could be other pathways also involved, for example metallothionein (MT) expression. This is a new venture in my research group and we plan to write a RO1 grant once we have more initial results to support our hypothesis. 1. J. Med. Chem. 2017, 60, 8000-8010.

## **Enhanced immunotherapy against breast cancer with biguanides**

*Host Campus:* Los Angeles

*Lead Investigator:* Jennifer Murphy

*Start Date:* 1/1/2019    *End Date:* 12/31/2019    *Amount:* \$75,000

*Abstract:*

Triple-negative breast cancer (TNBC) occurs in 10-15% of patients, yet it accounts for almost half of all breast cancer (BC) deaths. Although initially responsive to chemotherapy, TNBCs tend to relapse and metastasize early, leading to poor survival. An emerging approach to promote immune responses against several cancers is blockade of immune checkpoints. While these treatments show promising responses for a fraction of TNBC patients, significant progress is needed to achieve more durable responses. Evidence from epidemiologic, preclinical and early clinical trial studies suggests that the metformin, a biguanide drug widely used to treat type 2 diabetes, exerts anticancer activity in breast cancer. Despite modest antitumor effects as a single agent in the clinic, metformin exhibits immune-mediated effects in the tumor microenvironment by inducing increased trafficking of cytotoxic tumor-infiltrating lymphocytes (TIL) to tumors and protecting TILs from programmed cell death. We recently developed novel biguanides that exert more potent antitumor effects in vivo at lower doses than metformin and minimal non-target toxicity. Evidence that CD8+ TILs are targets of metformin and that its analogue, phenformin, inhibits myeloid-derived suppressor cell (MDSC) expansion and activity provide a rationale to study the potentially synergistic effects of these potent biguanides when combined with established immune therapeutics. We will evaluate our novel biguanides (metformin analogues) for enhanced immune cell trafficking and inhibition of MDSC expansion and activity using a TNBC syngeneic mouse model. We will assess if the biguanides amplify responses to current immunotherapeutics. Further, a molecular imaging tool to detect changes in systemic and tumor-infiltrating CD8 expression and non-invasively evaluate immune responses throughout the course of treatment could have profound implications in patient selection and stratification. Bivalent antibody fragments, cys-diabodies (cDb), that exhibit high target uptake and rapid, controlled systemic clearance provide a versatile platform for targeting CD8-expressing T cells, major components of immune response. We will prepare <sup>89</sup>Zr-labeled CD8-specific cDb to monitor the immune system in vivo via antibody-based positron emission tomography (immunoPET) imaging studies.

## **Dynamics of translational dysregulation in cancer.**

*Host Campus:* Riverside

*Lead Investigator:* Sean O'Leary

*Start Date:* 1/1/2019    *End Date:* 12/31/2019    *Amount:* \$75,000

*Abstract:*

Dysregulated translation is a hallmark of aberrant gene expression in cancer. Selective translation of oncogenes is promoted by hyper-efficient recognition of their mRNAs by the translation machinery. mRNA recognition is mediated by the eIF4F complex, which binds the mRNA 5' m7GpppN cap and facilitates loading of the mRNA onto the ribosome. eIF4F is a heterotrimer that scaffolds cap binding, mRNA structural remodeling, and ribosome recruitment; its activity is considered to limit the overall rate of translation for many mRNAs. Overexpression of eIF4E, the cap-binding subunit of eIF4F, by even two- to three-fold, leads to malignant transformation in a range of cells. Conversely, suppression of eIF4E expression to 60% of its initial level reverses malignant phenotypes. eIF4F is thus an attractive target of ongoing interest for therapeutics development. These efforts have been hindered by lack of key mechanistic information on translation: the eIF4F-mRNA complex and ribosome recruitment are highly dynamic, involving dramatic changes in molecular composition and conformation that resist analysis by most experimental techniques. We therefore still do not understand how eIF4F activities are kinetically coordinated on mRNA, or how differential coordination leads to differential ribosome recruitment and translation. Here we propose a biophysical and biochemical approach to define eIF4F dynamics that mediate oncogenesis. Our approach is focused on single-molecule fluorescence microscopy, which is uniquely suited to observing the real-time recognition of mRNA by human eIF4F, and key subsequent translation initiation steps. Building on preliminary studies with human eIF4E, we will first define and contrast eIF4F dynamics on oncogenic and non-oncogenic mRNAs. We will then establish the effects of molecular regulatory mechanisms on these dynamics, and how they are perturbed by eIF4F-targeted therapeutic leads. We will complement these data with biochemistry and enzymology to develop an integrated, quantitative mechanistic framework for regulated and dysregulated mRNA recognition. Our results will be a springboard toward our long-term goals of developing a detailed molecular model of translational (dys)regulation in cancer, bridging the gap between in vivo data and structural biology, and informing the design and characterization of therapeutics.

## **The role of host lactate in promoting *H. pylori* colonization**

*Host Campus:* Santa Cruz

*Lead Investigator:* Karen Ottemann

*Start Date:* 1/1/2019    *End Date:* 12/31/2019    *Amount:* \$75,000

*Abstract:*

*Helicobacter pylori* is a chronically colonizing bacterium that is a significant risk factor for gastric cancer. To chronically colonize, *H. pylori* must gain nutrients and avoid the immune response. Our preliminary work suggests *H. pylori* may do both by taking up lactate. *H. pylori* uses lactate as both a carbon/energy source, and our preliminary data suggests it promotes *H. pylori*'s resistance to the innate immune molecule complement. We became interested in lactate because it's one of a handful of molecules that *H. pylori* seeks out using chemotaxis. In unpublished work, our lab and others also found that lactate is one of the most preferred carbon sources for *H. pylori*. Interestingly, lactate is produced to high amounts by cancer cells. They redirect their metabolism from aerobic respiration to aerobic glycolysis, the so-called Warburg effect. This metabolism results in the formation and excretion of high amounts of lactate. Indeed, studies have shown that gastric cancer resulted in elevated stomach lactate, as compared to normal stomachs. Altogether, these results suggest a model whereby *H. pylori* benefits at least in part from gastric carcinogenesis because of the elevated lactate. There are significant gaps in our understanding of how *H. pylori* metabolizes lactate. Our HYPOTHESIS is that *H. pylori* actively seeks, metabolizes, and uses lactate for both growth and for complement-based immune evasion. IN AIM 1, we will identify the *H. pylori* proteins required for lactate uptake and metabolism using genetic knockouts and phenotypic characterization. We will test these mutants in mouse and complement resistance models, to gain insight into the role of lactate utilization in these processes. In AIM 2, we will characterize whether *H. pylori* infection leads to an increase in lactate production in gastric cell and intact stomach models, and furthermore, whether lactate creation or utilization genes are correlated with cancerous outcomes. Upon completion of this work, we will have a strong understanding of the pathways of *H. pylori* lactate uptake, and how it is metabolized for both growth and complement resistance. This work will allow us to develop biomarkers of lactate consumption that may also be good antibiotic targets, and may provide insight into the long-standing mystery of why *H. pylori* triggers gastric carcinogenesis.

## **Rapid, Label-free Detection of Kidney Cancer by Urinalysis**

*Host Campus:* Irvine

*Lead Investigator:* Reginald Penner

*Start Date:* 1/1/2019    *End Date:* 12/31/2019    *Amount:* \$75,000

*Abstract:*

The incidence of renal cell carcinoma (RCC), the most lethal urologic malignancy, is increasing and has more than doubled since 1975. RCC is generally discovered by the incidental observation of renal masses in abdominal images – no urine test or other screening modality for RCC is currently in use. In 2010, Morrissey and coworkers at WashU St. Louis identified two proteins – aquaporin-1 (AQP1) and perilipin-2 (PLIN2) – that are reliable urine-borne markers for RCC. Subsequent work from this group revealed that these two markers provide redundant information on the presence of RCC, including a correlation of the marker concentration (in the low nM range) with size of the tumor mass. This one-year CRCC project has three goals: 1) Adapt an existing electrochemical biosensor to detect AQP1 and PLIN2 in urine, 2) Compare biosensor performance using two types of receptors: monoclonal antibodies (commercially available) and engineered M13 virus particles (prepared in the G. Weiss group at UCI), 3) Using the receptor deemed to be superior, optimize the biosensor to blanket the diagnostic range required to correlate AQP1 and PLIN2 in urine with RCC. A new biosensor architecture called the Impedance Transduced Bioresistor or ITBR will be exploited in this study. The ITBR is a chemiresistor that consists of an electronically conductive polymer channel, 200-300 nm in thickness and 2 mm in length, into which are embedded receptors that can be either antibodies or M13 virus particles. Two electrical contacts to this channel allow its electrical impedance to be measured across 4 orders of magnitude in frequency, from 1 Hz to 10 kHz. When these receptors recognize and bind a target protein, an increase in the electrical impedance of the ITBR channel is measured and this “signal” can be correlated with the concentration of target with a high degree of precision (< 10% CoV, sensor to sensor). ITBRs using virus receptors are capable of measuring human serum albumin (HSA) in buffer or urine across the concentration range from 7 nM to 900 nM. The ITBR requires no "sandwich assay" or other means of signal amplification in order to function. A stable impedance signal suitable for quantitative analysis of the target is produced within 1-2 min. of exposure to a urine sample.

## **Cas adaptor proteins in cell migration and invasiveness**

*Host Campus:* Riverside

*Lead Investigator:* Martin Riccomagno

*Start Date:* 1/1/2019    *End Date:* 12/31/2019    *Amount:* \$63,750

*Abstract:*

We seek to determine the role of the Cas (Crk-Associated Substrate) family of cytosolic adaptor proteins during migration. Cas family members represent a signaling nexus on which integrin and growth factor receptor signaling converge. As a consequence, this family is implicated in key events in cancer cell biology such as initiation, progression and invasiveness. In particular, accumulating evidence suggests that Cas proteins play critical roles in migration and metastasis. Cas adaptors interact with various classes of signaling proteins and upon phosphorylation Cas proteins can provide docking sites for SH2-containing effectors including Crk, stimulating Rac1-mediated actin remodeling. Although Cas family members have well-characterized in vitro roles in adhesion and cell motility, the in vivo function of Cas proteins in cell migration remains poorly understood. Cortical lamination and stratification provide an exciting and highly tractable in vivo model to investigate the function of Cas proteins during cell migration. Along their radial migratory path, cortical neurons are guided by attractive and repulsive cues while simultaneously interacting with the extracellular matrix (ECM). This process involves the precise and timely regulation of adhesion and detachment of neurons from their substrate. Our Preliminary Data identify Cas adaptor proteins as central players of cortical lamination and migration. We recently found that Cas gene ablation results in severe cortical phenotypes that resemble cobblestone lissencephaly. This phenotype is characterized by ectopic clusters of neurons that migrate beyond the marginal zone and invade the subarachnoid space. We will use this model of aberrant migration to uncover the molecular mechanisms that regulate Cas-mediated cortical neuron migration and invasiveness. Our driving hypothesis is that modulation of Cas function plays essential roles during cortical migration by regulating adhesion. Aim 1 will identify protein residues necessary for Cas function during radial migration. Since modulation of Cas phosphorylation is known to be essential for focal adhesion turnover, Aim 2 will test whether regulation of Cas phosphorylation is required for cortical migration in vivo. The proposed studies will provide comprehensive mechanistic understanding of cell migration in vivo, as this pertains to invasiveness and metastasis.

## Cancer Research Coordinating Committee

*Abstracts for 2018 Awards*

### **Think Biology: Healthy Teen Lifestyles and Cancer Prevention**

*Host Campus:* Santa Barbara

*Lead Investigator:* Laura Romo

*Start Date:* 1/1/2019    *End Date:* 12/31/2019    *Amount:* \$66,941

*Abstract:*

Adolescence is an important life stage during which habits formed may shape trajectories of cancer risk later in life. Negative lifestyle behaviors such as smoking, drinking, use of other drugs, and risky sexual behavior start or peak during these years. Success in helping adolescents engage in self-protective health behaviors that reduces cancer risk depends on the availability of quality instructional materials. The overall goal of this study is to test the efficacy of a novel intervention program on high school adolescents' ability to attain and maintain healthy lifestyle behaviors that reduce cancer risk. We will create a program that utilizes theory-driven teaching practices in the field of science education. The Science of Learning approach posits that accumulated factual knowledge alone is insufficient to have a deep understanding of an area of inquiry. Science facts need to be understood in the context of a contextual framework organized around important core concepts to enable learners to construct explanations about bodily processes. Our curriculum will include a discussion of healthy lifestyles, bodily processes, the biology of cancer, and why certain health behaviors can increase the risk of cancer in adulthood. We will employ a group randomized-controlled-trial design to examine the effects of the newly developed curriculum against a control group of adolescents who are exposed to information about behaviors and cancer risk through standard pamphlets. Pamphlets tend to leave out information about the biology of cancer, bodily process, and their link to behaviors. Summative and formative assessments will be utilized to assess student learning. Misconceptions will be identified. Outcome measures will focus on knowledge gains, intentions, and engagements. The data gathered from this study will be utilized to apply for funding for a large-scale assessment program to develop materials that can be incorporated in high school biology courses. Current collaboration with medical professionals at Cottage Hospital. UCSB faculty collaborations: TBD

## **Centrosome abnormalities, Chlamydia and HPV**

*Host Campus:* Irvine

*Lead Investigator:* Christine Suetterlin

*Start Date:* 1/1/2019    *End Date:* 12/31/2019    *Amount:* \$75,000

*Abstract:*

Cervical cancer is caused by human papillomavirus (HPV), but only develops in a subset of women infected with HPV. Chlamydia trachomatis, the most common cause of bacterial sexually transmitted infection, is proposed as a co-factor for HPV, but the mechanism is not understood. In this application, we will examine if Chlamydia contributes to oncogenesis by enhancing HPV-induced centrosome abnormalities. Overexpression of HPV oncoproteins E6 and E7 cause centrosome amplification indirectly or through effects on Cdk2. Work from my lab and another group showed that *C. trachomatis* also induces centrosome abnormalities. This finding provides biologic plausibility for Chlamydia's role in cancer development because centrosome abnormalities cause genomic instability, trigger invasive behavior and are a hallmark of most human cancers. We hypothesize that co-infection with HPV and Chlamydia may promote oncogenesis by causing cooperative or synergistic dysregulation of centrosome number and organization. In parallel experiments, we will compare centrosome dysregulation caused by HPV and Chlamydia alone and in combination. We will induce HPV-associated centrosome abnormalities by transiently expressing HPV E6 and/or E7 from high-risk HPV type 16 in HPV-negative cervical cell lines. We will also infect such cells with *C. trachomatis* in the presence and absence of HPV oncoproteins. We will use methods that my lab has developed to quantify centrosome abnormalities in Chlamydia-infected cells. For example, we will measure the proportion of cells with supernumerary centrosomes, average centrosome number per cell and morphologic changes using immunofluorescence and transmission electron microscopy. We will assay for defects in the ability of supernumerary centrosomes to nucleate and organize microtubules and to form a bipolar spindle in mitosis, and will detect chromosomal segregation errors by FISH analysis. We will also measure the activity of the small GTPase Rac1, which has been shown to promote invasive behavior of cells with amplified centrosomes. Finally, we will assay for malignant transformation by testing if cells can grow in the absence of adhesion in a soft agar transformation assay. These studies of Chlamydia and HPV's combined effects on the centrosome may provide a molecular mechanism for Chlamydia as a co-factor for HPV in the development of cervical cancer.

## **Enzyme triggered drug delivery for colorectal cancer**

*Host Campus:* Santa Barbara

*Lead Investigator:* Luke Theogarajan

*Start Date:* 1/1/2019    *End Date:* 12/31/2019    *Amount:* \$75,000

*Abstract:*

Colorectal cancer is the third most common cancer diagnosed in the U.S. population. Chemotherapy is often prescribed to patients diagnosed with advanced stage colorectal cancers when aggressive and sustained treatment is required. Site-specific drug delivery to tumors improve the local drug concentration, increasing chemotherapy efficacy. We propose to create site specific drug delivery by leveraging the distinctive biochemical flora create by the dense bacterial microflora in colon. Specifically, we propose to use the microflora secreted azo-reductase enzyme to trigger the drug release from a nanoparticle at the targeted tumor site in the colon. The clinical use of silicone-based implants has paved the path for silicone-based materials for their use in drug delivery. However, these materials have not been studied for stimuli-responsive drug delivery to cancerous tumors. We will synthesize stimuli-responsive silicone-containing block copolymers to form drug encapsulating nanoparticles. These nanoparticles will be designed to undergo reduction in the presence of azo-reductase enzyme to effectively deliver the entrapped drug in the colon. In this study we aim to - 1. Synthesize a stimuli-responsive amphiphilic block copolymer of PDMS- azobenzene - PMOXA which can be chemically reduced by azo-reductases, 2. Prepare the stimuli-responsive polymeric nanoparticles to encapsulate chemotherapeutic agent 5-fluorouracil, 3. Investigate the drug release profiles of these nanoparticles in the presence of colon specific bacterial flora, and 4. Test the viability of cancer cells after the treatment with these nanoparticles in presence of microbial flora. This study will evaluate the potential of silicone-based nanoparticles for future applications in oral drug delivery (by encapsulating in enteric-coated capsule) to colorectal tumors, and for implantable drug delivery devices for chemotherapy via rectal route of administrations. Such localized drug delivery is expected to improve the effectiveness of chemotherapy and reduce the treatment related side effects.

## **4D Dynamic PET of Renal Cell Carcinoma**

*Host Campus:* Davis

*Lead Investigator:* Guobao Wang

*Start Date:* 1/1/2019    *End Date:* 12/31/2019    *Amount:* \$75,000

*Abstract:*

Renal cell carcinoma (RCC) is one of the top ten cancer types in the US. One-third of RCCs are metastatic and associated with a poor 5-year survival rate of 8%. Targeted therapy has become the first-line treatment for metastatic RCC with more than twelve drugs now available. Effective identification of the most appropriate drugs for a patient relies on noninvasive imaging to assess early response to the drugs. However, current practice by anatomical imaging such as computed tomography (CT) or magnetic resonance imaging (MRI) can only assess the response at two months after initialing targeted therapy. Because targeted drugs are commonly expensive and with risks of significant toxicities, the delay in identifying an ineffective treatment can result in high medical costs, harms from drug toxicities, and the delay in switching to other better drugs. We hypothesize that functional perfusion imaging by positron emission tomography (PET) can enable RCC response assessment as early as at two weeks given that RCC is highly related to angiogenesis and most targeted drugs for RCC are antiangiogenic. While dynamic contrast-enhanced CT or MRI can be used for perfusion imaging, their use is limited because 30% of RCC patients have chronic kidney diseases with renal dysfunction and are at higher risk for contrast-induced nephropathy and nephrogenic systemic fibrosis. Existing PET radiotracers (e.g., <sup>15</sup>O-water) for perfusion imaging are short-lived and generally unavailable for clinical use. This project proposes a new PET method for renal perfusion imaging using the widely accessible <sup>18</sup>F-fluorodeoxyglucose (FDG). FDG-PET is conventionally for metabolic imaging and has been rarely used for imaging kidneys because physiological excretion of FDG into renal pelvis contaminates image quality for renal tumor detection. We explore the potential of FDG for imaging renal perfusion by employing four-dimensional (4D: 3D space plus 1D time) dynamic scanning and tracer kinetic modeling to enable parametric imaging of FDG perfusion kinetics without being affected by renal excretion. The focus of this proposal is to conduct a pilot clinical study to demonstrate the method in targeted therapy of RCC. The outcome will support the feasibility of 4D dynamic PET perfusion imaging for early RCC response assessment.

## **Investigate the Hippo pathway in stress response and cancer**

*Host Campus:* Irvine

*Lead Investigator:* Wenqi Wang

*Start Date:* 1/1/2019    *End Date:* 12/31/2019    *Amount:* \$75,000

*Abstract:*

Two decades of studies in multiple model organisms have established the Hippo as a key signaling pathway in organ size control and cancer prevention. Hippo dysregulation exerts a significant impact on tissue homeostasis and cancer development; therefore, further investigation of the functions and regulatory mechanisms of this key signaling pathway will be sure to unveil the mystery of organ size control and more importantly develop novel therapeutic strategies for cancer treatment. The Hippo pathway can sense growth conditions including perceived physical signals from cell microenvironment, growth factors/mitogenic hormones and recently discovered metabolic status, to control cell proliferation and survival by restricting its downstream effector YAP. Excitingly, our preliminary studies have revealed an unexpected role of the Hippo pathway in heavy metal stress response, where inhibition of Hippo signaling significantly protect cells against heavy metal-induced toxicity. Mechanistically, the Hippo pathway can directly target metal-responsive transcription factor 1 (MTF-1), a transcription factor that plays an essential role in heavy metal response. Notably, this Hippo-mediated regulation of MTF-1 is independent of its downstream effector YAP, suggesting a YAP-independent function of Hippo signaling in heavy metal response. Interestingly, the Hippo pathway can be mutually regulated by heavy metals, where targeting metal homeostasis significantly suppresses YAP-dependent oncogenic activities by activating the Hippo pathway. Based on these preliminary findings, we propose to 1) establish the role of Hippo signaling in heavy metal response; 2) elucidate the mechanism underlying the interplay between Hippo and heavy metal homeostasis; and 3) investigate the translational potential by targeting heavy metal homeostasis for YAP-dependent cancer treatment. My current research on the Hippo signaling pathway serves not only as a paradigm but also as a stable platform to pave a way for me to become a leader in the Hippo field. My proposed studies will further inspire my endeavor in the Hippo signaling exploration and allow me to generate solid preliminary data to secure the external funding (e.g., R01, DoD, ACS) in the near future.

## **Structural biology of anthracycline metabolism**

*Host Campus:* Davis

*Lead Investigator:* David Wilson

*Start Date:* 1/1/2019    *End Date:* 12/31/2019    *Amount:* \$70,043

*Abstract:*

Anthracyclines are a large class of natural products which are used in chemotherapy to combat a wide variety of cancers. Despite being decades old, they are still considered to be highly effective and are used to treat a substantial fraction of all cancers so improvements would have wide-ranging effects. Before a significant fraction of the drug is able to exert its effects as a topoisomerase II inhibitor, DNA intercalator or inhibitor of RNA synthesis, the active carbonyl-containing form of the compound is enzymatically reduced to the corresponding alcohol which is much less active and is toxic to cardiac muscle. In humans, two enzymes in the NADPH-utilizing aldo-keto reductase (AKR) superfamily have previously been identified as being responsible for this reaction: aldose reductase (AR) and aldehyde reductase (ALR). Inhibiting the enzymes catalyzing this reaction could therefore enhance the therapeutic effects of these compounds and minimize the severe side effects which limit dosage. Alternatively, finding cytotoxic anthracyclines which are not reduced by AKRs may identify molecules with improved clinical activity. Prior work has shown that AR, ALR and the eight other human AKRs have substantially overlapping substrate specificities when other carbonyl-containing substrates have been examined. Most of these enzymes are expressed in various tumor cell lines and some have in fact been used as biomarkers for certain cancers. To determine which of them are capable of forming the less active and toxic alcohol, we will kinetically quantitate the activity of each versus several clinically relevant anthracyclines such as doxorubicin, daunorubicin and epirubicin. This information will be used to direct later efforts to develop inhibitors which are specific to this set of enzymes using a combinatorial strategy that has allowed us to successfully produce these in the past. After the target enzymes have been defined, crystal structures of dead-end complexes with each enzyme (i.e. AKR/NADP<sup>+</sup>/anthracycline ternary complex) will be determined. This information will then be used to guide attempts to computationally dock the ~2,000 known anthracyclines in an effort to screen for those that are unable to serve as substrates. Once these compounds are identified, assays will be done in the future to determine kinetic and/or binding constants for those deemed most promi

## **Checkpoint blockade and central tolerance in tumor immunity**

*Host Campus:* Berkeley

*Lead Investigator:* Astar Winoto

*Start Date:* 1/1/2019    *End Date:* 12/31/2019    *Amount:* \$75,000

*Abstract:*

Immunotherapy to fight cancer has shown great promise. Antibodies that inhibit T cell checkpoints like anti-CTLA-4 or anti-PD-1 are effective in curing a fraction of patients with advanced melanoma and other types of cancers. These antibodies work by breaking T cell peripheral tolerance. Although autoimmunity may ensue, the treatment allows T cells to attack tumors. However, a significant % of patients with these 'treatable' tumors are nevertheless resistant to the checkpoint inhibitors. Similarly, many other types of tumors are also refractory to this approach. One idea to overcome this is to increase T cell repertoire (thereby increasing tumor-specific T cells) through inhibition of T cell central tolerance, a process where potential autoreactive T cells are removed by apoptosis in the thymus. Recently, two groups showed that T cells from mice with mutation in AIRE, the immune regulator of central tolerance to tissue specific antigens, could synergize with anti-CTLA-4 to eliminate otherwise resistant melanoma. We have been studying apoptosis accompanying central tolerance, which involves the BH3-only Bcl-2 family proteins, Bim and Puma, as well as the Nur77 orphan steroid receptors. The latter may kill through conversion of Bcl-2 into a pro-apoptotic protein. Transgenic mice expressing a Bcl-2-BH3\* mutant protein that blocks all three pathways or Bim-/-/Puma-/- mice exhibit increased T cell repertoire and autoimmunity in old mice. Here, we hypothesize that inhibiting apoptosis accompanying central tolerance can enhance the efficacy of checkpoint inhibitors against many types of tumors. In this proposal, we will test the ability of T cells from Bcl-2-BH3\* transgenic mice to synergize with anti-CTLA-4 or anti-PD1 antibodies for anti-tumor immunity to several syngeneic mouse tumor lines, including melanoma, fibrosarcoma and colon adenocarcinoma. Both Bcl-2-BH3\* mice and purified Bcl-2-BH3\* T cells adoptively transferred into lymphocyte-deficient mice will be used. The kinetics of tumor development, mouse lethality and anti-tumor immune responses will be determined. If successful, future experiments will include testing mice with milder defects in central tolerance and identification of reagents that block central tolerance. The latter could eventually be used in human where active T cell development was found in individuals up to 85 years old.

## **Xestospongins: total synthesis of IP3R inhibitors in cancer**

*Host Campus:* Santa Barbara

*Lead Investigator:* Armen Zakarian

*Start Date:* 1/1/2019    *End Date:* 12/31/2019    *Amount:* \$75,000

*Abstract:*

A gram scale total synthesis of desmethyl xestospongins B (dm Xe B) is proposed. dm Xe B is a leading chemical tool in the study of inositol 1,4,5-triphosphate receptors (IP3Rs), which are ligand-gated calcium channels that control calcium signaling between the endoplasmic reticulum and mitochondria. There is a growing body of evidence that xestospongins through interaction with IP3Rs have a major impact on mitochondrial metabolism in cancer cells, enabling selective destruction of cancer cells with minimal impact on normal cells. Because natural sources of xestospongins are exhausted, a total synthesis of the natural products is necessary to continue research on IP3Rs. We propose a general synthetic platform to access these valuable compounds on scale.

## **Photothermal Therapy of Oral Squamous Cell Carcinoma**

*Host Campus:* Santa Cruz

*Lead Investigator:* Jin Zhang

*Start Date:* 1/1/2019    *End Date:* 12/31/2019    *Amount:* \$75,000

*Abstract:*

Actively targeted photothermal therapy (PTT) is a new and highly promising medical modality for cancer imaging and treatment. Oral cancer is the ninth most common cancer worldwide, and its prognosis remains poor in comparison to other cancer types, representing a continuing challenge in biomedicine. We propose to use a novel photothermal agent based on peptide-conjugated hollow gold nanospheres (P-HGNs) to actively target and treat oral squamous cell carcinoma (OSCC). The P-HGNs are designed with optimal size, shape, strong near infrared (NIR) light absorption, conjugation length, and high photothermal conversion efficiency. Instead of using antibody for targeting, we propose to use short peptides to reduce the distance between the HGNs and cancer cell or tissue to enhance heat transport and thereby PTT efficiency. Moreover, peptides, as recognition elements, are highly specific, yet inexpensive to produce, thus improving the translational potential of our constructs. We will conduct in vitro studies to validate the hypothesis that P-HGNs are highly effective for PTT applications, which lays the foundation for future in vivo studies.

## **Profiling extracellular vesicles by single particle counting**

*Host Campus:* Riverside

*Lead Investigator:* Wenwan Zhong

*Start Date:* 1/1/2019    *End Date:* 12/31/2019    *Amount:* \$75,000

*Abstract:*

Extracellular vesicles (EVs) secreted from tumor cells can promote tumor progression, survival, invasion and angiogenesis. Such EVs could be useful for sensitive and specific cancer diagnosis and prognosis, but their identification requires detailed molecular analysis of the EVs from different sources. Prior to analysis, the EVs are purified from biofluids using ultracentrifugation, chromatography, and polymeric precipitation. These techniques separate EVs based on their physical properties like size and density. However, physical properties are not able to distinguish EVs by their cells of origin or functions; and the EVs purified by the physical separation methods still contain heterogeneous population, enhancing the difficulty in obtaining the information specifically related to EV functions in cancer development. In addition, most physical separation methods are very time-consuming, result in low sample yield, and consume a lot of samples, not affordable by clinical practice. Methods that allow rapid, specific, and sensitive recognition of the EVs related to cancer development are in demand. We propose a simple strategy to enable single vesicle counting and detect multiple EV cargo in individual vesicles. Our proposed work will be focused on the EV class named exosomes, because their sizes are below the diffraction limit and they are difficult to be seen under microscope or in flow cytometer, although the proposed technique will be applicable to all other EV classes. Our long term goal is to reveal the specific exosome population that is closely correlated to cancer development. Such populations will lead to the discovery of more specific biomarkers for development of new diagnosis tools and treatments for cancers. The overall objective of this application is to differentiate individual exosomes based on their molecular signatures and identify the correlation between such signatures and their cell of origin, using conventional instruments deployable in typical clinics. Our central hypothesis is that EVs secreted by cells undergoing different stages of tumor development carry distinct molecular signatures recognizable by the single vesicle counting (SVC) technique to be developed. Thus, profiling the EV populations found in biofluids by SVC can clearly disclose cancer development and provide new understanding on EV functions in cancer development.