

## Cancer Research Coordinating Committee

Abstracts for 2022-2023 Awards

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**Cancer Research Coordinating Committee***Abstracts for 2022-2023 Awards*

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## Investigating G-quadruplex induced instability in B cell lymphomagenesis

*Host Campus:* Davis

*Lead Investigator:* Jacqueline Barlow

*Start Date:* 10/1/2022    *End Date:* 9/30/2023    *Amount:* \$84,996

*Abstract:*

Genome instability arising during DNA replication strongly contributes to the formation of oncogenic mutations and chromosome rearrangements, including B cell lymphomas. Conflicts with the transcriptional machinery are a potent source of replication stress in proliferating cells. In response to antigen stimulation, B cells undergo a massive burst of transcription and proliferation. Recently we identified a set of loci that experience recurrent transcription-replication interactions (TRIs) in activated primary B cells. TRIs accumulate marks of replication stress, colocalize with double-strand DNA breaks, are enriched for deletions, and accumulate mutations in tumors. Together, these results suggest replication-associated damage accumulates at TRI loci in rapidly proliferating B cells. Over 95% of TRI regions are capable of forming G-quadruplex (G4) structures—secondary DNA structures formed in guanine-rich DNA that organize into a planar tetrad. G4s are stable and can impede replication fork progression, inducing DNA damage. Here we propose to investigate the causal relationship of G4 stabilization on genome instability at TRIs. Of particular interest, mutations involving two TRI loci—the oncogenes *Bmi1* and *Bcl2*—are associated with hematopoietic malignancy and chemoresistance. *Bmi1* plays a critical role in stem cell renewal and multipotency while *Bcl2* regulates apoptosis-mediated cell death. Using the G4 stabilizing drug pyridostatin, we will investigate if TRIs exhibit a higher rate of DNA damage and mutation in WT and *Brca1*-deficient primary cells. *Brca1* is a tumor suppressor involved in replication fork stabilization and potentially protects TRI loci from accumulating DNA damage. To investigate the impact of G4 stabilization at TRIs, we will define the frequency of pyridostatin-induced DNA damage at two TRI loci—*Bmi1* and *Bcl2*—and two negative controls in WT and *Brca1*-deficient cells by analyzing unrepaired DNA breaks by fluorescent in situ hybridization (FISH) and micronucleus formation by microscopy. At the same time, we will characterize proliferative index, cell cycle progression and viability. Characterizing how endogenous sources drive the formation of oncogenic mutations and rearrangements is critical to understanding how lymphoid cancers arise and will provide new targets for personalized treatments and cancer prevention.

## **Spatial transcriptomics patterns and correlations with outcomes in head and neck cancer**

*Host Campus:* Davis

*Lead Investigator:* Andrew Birkeland

*Start Date:* 10/1/2022    *End Date:* 9/30/2023    *Amount:* \$75,000

*Abstract:*

Head and neck cancers are the 7th most common cancer worldwide, with over 800,000 annual cases and 400,000 annual deaths. Despite constant efforts for improving standard of care, survival rates remain poor and our ability to predict disease prognosis and behavior are limited. Investigation into spatial expression patterns in head and neck cancers may allow a better understanding of tumor heterogeneity, interactions with the immune microenvironment, and predictions in disease behavior.

We have investigated head and neck cancer tissues for prognostic biomarkers. These include markers associated with the tumor and immune microenvironment, as well as intrinsic tumor alterations and markers. Using this data, we have been able to develop initial stratifiers of patient outcomes and identify prognostic biomarkers. However, there remains a significant need to more accurately investigate prognostic biomarkers, and understanding the role of the tumor-immune microenvironment in head and neck cancer behavior.

Building on our prior work and revisiting biomarker analyses with novel technologies, with improved single cell and spatial analyses, can have immense benefit in identifying unique biomarker expression patterns, investigating potential treatment targets, and developing prognostic models. Spatial transcriptomics of tumors and their surrounding microenvironment are increasingly being investigated in a wide array of cancers.

Here, we propose to utilize new spatial transcriptomic technology to correlate findings with head and neck cancer behavior. We will utilize the Nanostring GeoMx Digital Spatial Profiler in collaboration with the UC Davis Genomics Shared Resource to analyze formalin-fixed paraffin embedded head and neck cancer specimens collected from surgical cases. We will correlate tumor stage, metastatic status, tumor pathologic features, patient demographics and survival with spatial transcriptomic signatures of the primary tumor, surrounding microenvironment, and metastatic lymph nodes. We aim to identify prognostic signatures related to primary tumor and tumor-immune microenvironment expression patterns, with implications to stratify and personalize patient care. We believe this will generate valuable and novel data for publications and subsequent grant submissions.

## **Determining the role of splicing in cancer-related stress adaptation**

*Host Campus:* Santa Cruz

*Lead Investigator:* Angela Brooks

*Start Date:* 10/1/2022    *End Date:* 9/30/2023    *Amount:* \$75,000

*Abstract:*

Recurrent somatic mutations in splicing factors have been identified in multiple forms of cancer, yet how these mutations contribute to cancer, particularly in lung cancer, remains unclear. Cancer cells must mitigate replicative, metabolic, and hypoxic stresses as part of disease advancement and the extent to which splicing plays a role in stress adaptation is unknown. In a cell line model used to examine early genetic changes leading to lung cancer (HBEC), we found that U2AF1S34F, a cancer hot spot mutation in a U2 splicing complex factor, suppresses KRAS-mediated oncogene-induced senescence (a consequence of replicative stress) and upregulates stress response genes. In myeloid lineage cells, U2AF1S34F mutations cause genome-wide augmentation of DNA/RNA hybrids (R-loops) which have also been linked to stress response. Recent studies in leukemic cells treated with splicing inhibitor PladB demonstrate differential R-loop formation with altered stress-response gene expression, also. These studies associating splicing with R-loop formation have been performed in myeloid lineages and in already transformed cancer cells. Our preliminary data in HBEC lines leads to the hypothesis that splicing alterations may contribute to early stages of lung cancer development through stress adaptation. It is unclear if this phenotype would be specific to U2AF1 mutation or through other forms of splicing alteration. We propose to test whether altered splicing by mutation of U2AF1 or treatment with PladB impacts R-loop formation to alter cancer-associated stress tolerance in lung cells. In Aim 1, we will determine the stress-related impact of perturbing splicing in HBEC cells through U2AF1S34F mutation or by PladB. Stress tolerance will be assessed by changes in levels of gene expression, cell proliferation, and senescence levels in response to inducible Kras oncogene expression (replicative stress) and DNA damage by gamma-irradiation (DNA damage stress). To determine whether R-loop formation is mediating the stress adaptation, we will suppress R-loop formation by RNaseH. In Aim 2, we will globally map differences in R-loop formation in U2AF1S34F and PladB-treated cells before and after stress induction. Results from this study will shed light on the role of splicing and R-loops in adapting to stress responses in early cancer development.

## **Targeting LRP1 to Reduce the Incidence of Paclitaxel Induced Painful Peripheral Neuropathy**

*Host Campus:* San Diego

*Lead Investigator:* Wendy Campana

*Start Date:* 10/1/2022    *End Date:* 9/30/2023    *Amount:* \$ 85,000

*Abstract:*

Chemotherapy induced peripheral neuropathy (CIPN) is the most common neurological complication of chemotherapy treatment for cancer patients. Patients with CIPN have sensory nerve impairment accompanied by pain that frequently results in dose reductions and decreases in treatment adherence. Although precision medicine is in development, most cancer patients still receive chemotherapies that jeopardize nerve health. There is a great unmet medical need to identify novel targets and treatments for CIPN associated pain. Paclitaxel is a widely used and highly effective chemotherapy agent that induces painful CIPN. Paclitaxel activates innate immunity including increased Toll-like receptors (TLR) 4 in sensory neurons that have been implicated in persistent pain states. Recent studies have identified the low-density lipoprotein receptor related protein (LRP1), as a receptor highly involved in regulating innate immunity and reducing neuroinflammation. Using innovative activators of LRP1, enzymatically inactive-tissue plasminogen activator (EI-tPA) and a peptide, SP16, derived from  $\alpha$ -1-Antitrypsin (AAT) we demonstrated that activation of LRP1 alleviated pain related behaviors in three distinct pre-clinical pain models (acute, inflammatory and neuropathic). In the neuropathic pain model, LRP1 activation reduced recruitment of inflammatory cells and of TLR4 levels.

This project will be conducted by an established investigator, with expertise in neuropathic pain and LRP1 biology, that is initiating new research in cancer related pain. She has recruited an oncologist, Dr. Jenn Matro, from UCSD Moore's Cancer Center to participate in this preclinical work. The team will investigate effects of LRP1 activators, EI-tPA and SP16 in paclitaxel induced painful neuropathy (PIP). First, we examine effects of LRP1 activation in pain-related behaviors in an established mouse model of PIP. We identify dosing paradigms as both adjuvant therapy and in established pain. Next, we examine changes in innate immunity and neuroinflammation regulated by LRP1 activation in dorsal root ganglia during PIP. Recently, SP16 was safely dosed in a Phase I clinical trial enhancing the potential translational nature of this proposal. Moreover, LRP1 activators do not appear to support breast or oral cancer tumor growth. These studies may identify a novel treatment for patients with PIP.

## **Combinatorial SERS-MOFs platform for ultrasensitive biomarker detection**

*Host Campus:* Davis

*Lead Investigator:* Randy Carney

*Start Date:* 10/1/2022    *End Date:* 9/30/2023    *Amount:* \$75,000

*Abstract:*

Human biofluids contains hundreds of potential biomarkers relevant to early-stage cancer detection and diagnosis, including a subset of oncometabolites amongst the wider grouping of chemicals as volatile organic compounds (VOCs). Common techniques that provide multiplexed and accurate VOC detection, such as mass spectrometry, are bulky and cannot be integrated in future wearable devices for continuous monitoring. State-of-the-art wearable devices are largely based on electrochemical detection that greatly lack specificity, sensitivity, and ease. Besides, multiplexed detection is rare as specific systems need to be developed for each biomarker of interest. To build an effective wearable for multiplexed VOC detection, I propose the integration of two complementary technologies: (1) a multiplexed array of metal organic frameworks (MOFs), computationally designed to capture and enrich different subsets of tumor-associated VOCs, decorated with (2) metal nanostructures that enable ultrasensitive label-free readout via the surface enhanced Raman scattering (SERS) phenomena. Multiplexed detection of relevant metabolites will be used to build advanced cancer models by training on biofluid samples (e.g., plasma, saliva, sweat) collected from cancer patients in clinical care here at UC Davis.. This high-risk/high-reward approach is fundamentally different from competing approaches commonly employed in portable and wearable devices to monitor the concentration levels of a panel of biomarkers and will accelerate clinical diagnosis platforms for a wide range of cancers.

## **VGLL3 Amplification in Sarcoma Development and Growth**

*Host Campus:* Davis

*Lead Investigator:* Janai Carr-Ascher

*Start Date:* 10/1/2022    *End Date:* 9/30/2023    *Amount:* \$75,000

*Abstract:*

Soft tissue sarcomas are a rare and heterogeneous group of more than fifty connective tissue cancers. Patients are often treated aggressively, with surgery, radiation, and chemotherapy. Despite this multimodality approach, approximately 35% of people will develop incurable metastatic disease. Therefore, new treatments are needed for this devastating cancer. To create new therapies, targets must be identified and in the field of sarcoma, this has been met with limited success due to the disease heterogeneity and limited models to study the biology of specific sarcoma subtypes. Our lab has generated a robust and versatile system to study human sarcoma development and growth. In this model, P53 and RB1, tumor suppressors that are commonly mutated or deleted in sarcomas were targeted by CRISPR-Cas9 to generate RB1<sup>-/-</sup>-P53<sup>+/-</sup> mesenchymal stem cells. To these cells, we added a library of genes to identify drivers of sarcoma formation. We have identified four drivers (YAP1, KRAS, CDK4, and PIK3CA) that each result in the development of a distinct sarcoma subtype. Interestingly, in our initial screen that identified these drivers, the transcription cofactor VGLL3 (Vestigial Like Family Member 3) was repeatedly identified as a potential driver yet, the addition of VGLL3 alone to RB1<sup>-/-</sup>-P53<sup>+/-</sup> cells did not lead to the formation of sarcomas. VGLL3 was included in our primary screen because it was shown in the Cancer Genome Atlas (TCGA) data to be amplified across soft tissue sarcomas yet, the significance of this is not known. While VGLL3 is not an independent driver of sarcoma development, we hypothesize that it works in combination with other drivers to facilitate the formation and growth of sarcomas. In this study, we intend to investigate the role of VGLL3 in both the development and subsequent growth of sarcomas.



## **Investigating the Role of Transposable Elements During Cell Fate Commitment**

*Host Campus:* Riverside

*Lead Investigator:* Sihem Cheloufi

*Start Date:* 10/1/2022    *End Date:* 9/30/2023    *Amount:* \$85,000

*Abstract:*

Transposable elements (TEs) are repetitive DNA regions that occupy roughly 50% of mammalian genomes. On the one hand, TEs pose a constant threat to genomic integrity due to their capacity to replicate and insert into new genomic locations. On the other hand, TEs present an important asset for gene evolution and regulation of gene expression. In higher eukaryotes, TEs are activated in the early embryo during reprogramming of the parental genomes, but then rapidly silenced and they normally remain dormant for the rest of the life of an organism. Intriguingly, TEs can become aberrantly reactivated in cancer and their activity can contribute to tumorigenesis. Therefore, it is important to understand how TEs are regulated and how their activation may affect cell fate. We and others have previously shown a critical role of chromatin assembly in silencing of TEs during reprogramming of somatic to pluripotent cells, in embryonic stem cells, and during preimplantation development. However, how chromatin assembly and the associated changes in chromatin accessibility influence TE expression and how TEs in turn impact cell fate control during lineage differentiation has not been explored. To address this problem, we propose to investigate the regulation of TEs and consequences of their reactivation by taking differentiation of the myeloid lineage as a well-defined study paradigm. Our preliminary results reveal discrete, TE-rich heterochromatic regions whose restricted chromatin accessibility is important for maintenance of the myeloid progenitor state. We propose to dissect the mechanisms sustaining the silent status of TEs and their impact on cell fate decisions by (1) defining transcriptional and epigenetic maps of TEs in normal myeloid progenitors, their normal differentiated progeny, and upon genetic perturbation of chromatin accessibility (2) assessing the function of activated TEs through genetic and biochemical approaches. Our studies will shed light on the regulation of TEs during normal cell differentiation and the impact of their misregulation in cancer.

## **Combatting cancer immunosuppression with engineered *Listeria monocytogenes***

*Host Campus:* Berkeley

*Lead Investigator:* Michel DuPage

*Start Date:* 10/1/2022    *End Date:* 9/30/2023    *Amount:* \$83,000

*Abstract:*

Immunotherapy has revolutionized the treatment of cancer. However, our current arsenal of immune-boosting drugs benefits only a fraction of patients, potentially because these strategies do not adequately combat immunosuppression in the tumor microenvironment (TME). Regulatory T cells (Tregs) are a subset of immunosuppressive Foxp3+ CD4+ T cells that infiltrate tumors and dampen anti-tumor immune responses. We have discovered that the facultative intracellular bacteria *Listeria monocytogenes* (Lm) may boost anti-tumor immunity by impeding immunosuppression enforced by Tregs. However, this effect is dependent upon intratumoral injection of Lm. This is highly relevant, as Lm is currently under investigation in several clinical trials where it is only being introduced intravenously and it is thought to function exclusively as an adjuvant to boost anti-tumor CD8+ T cell responses. By exploring the effect of Lm administration via intratumoral (IT) and systemic (intravenous, IV) delivery on tumor progression, we have made several significant initial discoveries. First, Lm can persist selectively in tumor tissues after IV or IT delivery, indicating a proclivity for tumors and/or tumor-mediated resistance to immune clearance. Second, the therapeutic benefit of IV Lm administration is enhanced by an additional IT Lm injection (IV+IT), demonstrating the importance of the presence of Lm in tumors for shaping the immune response. Third, Lm injection in tumors reduced Foxp3 expression and Treg frequencies in tumors in a bacterial lipoprotein/Toll-like Receptor 2 (TLR2)-dependent fashion, revealing a unique role of Lm in controlling Treg biology and potentially immunotherapy. Our proposal aims to uncover: (1) how Lm persists in tumors, (2) the immune cell types critical for mediating therapeutic efficacy with combination IV+IT Lm administration, and (3) the TLR2+ immune cells responsive to bacterial lipoproteins that drive Treg loss in the TME. Ultimately, we aim to use these discoveries to engineer Lm with an enhanced capacity to activate anti-cancer immunity and overcome immunosuppression within cancers.

## **Heparan Sulfate Biomarker for Pancreatic Cancer**

*Host Campus:* San Diego

*Lead Investigator:* Jeffrey Esko

*Start Date:* 10/1/2022    *End Date:* 9/30/2023    *Amount:* \$75,000

*Abstract:*

Pancreatic ductal adenocarcinoma (PDAC) is one of the most fatal types of cancer. This is in part due to difficulties in diagnosing the patients early where intervention would still be effective. Thus, PDAC specific markers that arise early in the disease are in high demand, to develop functional screening methods for early diagnosis. One of the key components to the cancer associated extracellular matrix is a type of carbohydrate, or glycan, called heparan sulfate (HS). HS biology is complex, but it is clear that these molecules accumulate and are structurally altered in many types of cancer. We have found that a unique type of HS, that is found in clinical heparin but rare in healthy tissue, accumulates in early stages of progression to PDAC. This type of HS is called HSAT. Notably, we show that the enzyme that produces HSAT, is elevated in early stages of the disease and correlates with poor disease prognosis. We show preliminary data that HSAT can be detected in PDAC precursor lesions and cancer tissue specimens and that it can be found circulating in the plasma from PDAC patients. We also show preliminary data that HSAT can be used as a target for cancer tracing studies. This project aims to fully investigate the potential of HSAT as a tool for the early diagnosis of PDAC, and potentially pave the way for the investigation of HSAT as a target for directed therapy against PDAC. The discovery of novel markers specific to PDAC has the potential to provide diagnostic and therapeutic strategies to combat this devastating disease, to the benefit of patients worldwide.

## **Targeted manipulation of tumor-associated reactive astrocytes in glioblastoma**

*Host Campus:* Riverside

*Lead Investigator:* Todd Fiocco

*Start Date:* 10/1/2022    *End Date:* 9/30/2023    *Amount:* \$75,000

*Abstract:*

Glioblastoma is the most common and aggressive primary brain cancer, with 3 - 4 cases per 100,000 people in the United States and Europe. Prognosis is poor with median survival rates of 14-16 months, and no cure is currently available. These aggressive tumors are initiated from transformed astrocytes and other glial cells, whose normal role in healthy brain function is trophic and metabolic support of neurons. Evidence suggests that tumor-associated reactive astrocytes (TAAs) feed tumor growth by secreting growth factors and inflammatory mediators. Therefore, development of new strategies to target and reprogram or eliminate TAAs could offer a unique therapeutic strategy to degrade and eliminate these tumors. Unfortunately, currently available tools cannot distinguish between the healthy supportive astrocytes in the rest of the brain from the TAAs feeding the tumor. We have recently developed a new transgenic approach to selectively target and manipulate TAAs, while leaving the healthy astrocytes intact and able to continue their normal supportive functions. Using this new tool, we will first characterize its expression in TAAs in animal models of glioblastoma already on hand in our lab. We will then perform a pilot transcriptome analysis of TAAs using fluorescence cell sorting and bulk sequencing, which will reveal uniquely altered genes in TAAs relative to healthy astrocytes and reactive astrocytes in other disease states (databases which are already available). Transcriptome analysis will reveal unique characteristics of TAAs for further study and novel targets for therapy. Last, we will selectively eliminate TAAs through targeted expression of diphtheria toxin A, removing the supportive environment for tumor growth and allowing the body's natural immune response to attach and degrade the tumor. These studies have the potential to transform the field through development of translational therapies targeting tumor-associated reactive astrocytes.

## **Role of OGDHL in aggressive prostate cancer**

*Host Campus:* Los Angeles

*Lead Investigator:* Andrew Goldstein

*Start Date:* 10/1/2022    *End Date:* 9/30/2023    *Amount:* \$75,000

*Abstract:*

Prostate cancer causes more than 30,000 deaths annually in the United States. When prostate cancer is localized to the prostate, the 5-year survival rate for patients diagnosed with the disease is greater than 99%. However, if the disease spreads beyond the prostate, the 5-year survival rate for patients with metastatic prostate cancer is only 30%. Therefore, understanding the biology that enables prostate cancer progression, and identifying targets to slow or stop disease progression, are critical for improving outcomes for patients with advanced disease. Upon studying models of prostate cancer progression and treatment-resistance in the laboratory, and comparing them to clinical datasets from patients with metastatic prostate cancer, we have identified a new candidate target in aggressive prostate cancer cells called Oxoglutarate Dehydrogenase-Like (OGDHL). OGDHL is found at low levels in early stage prostate cancer but its abundance increases in aggressive treatment-resistant prostate cancer. In preliminary experiments in the laboratory, when we utilize various approaches to interfere with OGDHL, we can alter the growth and aggressive properties of prostate cancer. In this proposal, we aim to better understand how OGDHL contributes to prostate cancer progression and whether it can be targeted as a therapeutic strategy to delay or prevent lethal disease. Most importantly, these experiments will provide the critical preliminary data to compete for external grant funding.

## **Decoding Cancer Acquired Drug Resistance**

*Host Campus:* San Diego

*Lead Investigator:* Matthew Hangauer

*Start Date:* 10/1/2022    *End Date:* 9/30/2023    *Amount:* \$75,000

*Abstract:*

The process by which initially drug sensitive tumor cells acquire drug resistance is poorly understood, but it is widely appreciated that the acquisition of resistance-conferring genetic mutations contributes to acquired cancer drug resistance in patients. The molecular mechanisms underlying the emergence of these mutations are not known. We and others have reported on a subpopulation of cancer cells termed “persister” cells, found within every solid tumor type thus far tested, which enter a quiescent pro-survival state in response to therapy and provide a surviving cancer cell reservoir from which overtly drug resistant tumors can subsequently emerge (e.g. Hangauer MJ et al., Nature 2017, 551, 247). These persister cells initially survive treatment through a reversible, non-genetic mechanism of drug-tolerance which is poorly understood. However, during prolonged treatment, it has been observed that a fraction of persister cells acquire new resistance-conferring mutations which allow for outgrowth of drug resistant cancer cells. It is not understood how persister cells acquire mutations. We recently discovered that during oncogene-targeted therapy treatment, persister cells undergo sublethal apoptotic signaling resulting in activation of apoptotic DNase DFFB which induces DNA damage and mutagenesis. While this exciting discovery points toward a potential new paradigm for how targeted therapy can produce mutations in cancer cells, it is not known whether sublethal apoptotic signaling and DFFB contribute to mutagenesis in other treatment modalities including chemotherapy, antibody treatments and immunotherapy. Here, we address these three distinct treatment modalities. In Aim 1, we will determine whether DFFB is required for chemotherapy- or antibody-induced mutagenesis in persister cells and acquired resistance. In Aim 2, we will determine whether DFFB is required for persister cell antigen loss during acquired resistance to CD8 T cell attack. Together, these proposed experiments will start a new direction of research into the role of DFFB in therapy-induced mutagenesis in persister cells and acquired resistance.

## 12-Lipoxygenase Inhibitors as a Pancreatic Ductal Adenocarcinoma Therapeutic

*Host Campus:* Santa Cruz

*Lead Investigator:* Theodore Holman

*Start Date:* 10/1/2022    *End Date:* 9/30/2023    *Amount:* \$74,997

Abstract:

Goal:

The purpose of this study is to improve the in vitro potency of our human 12-lipoxygenase (12-LOX) inhibitors, confirm their cellular potency and optimize their basic ADME properties, with the ultimate goal of developing an effective therapeutic against pancreatic ductal adenocarcinoma (PDAC).

Background:

PDAC is the fourth-leading cause of cancer-related death in the United States, with a 5-year survival rate of 9%, due to the ineffective surgical and pharmaceutical treatments. Human 12-Lipoxygenase (12-LOX) is proposed to increase PDAC proliferation through its enzymatic product, 12-HETE, which stimulates PDAC growth. This hypothesis is supported by the published observation that higher plasma levels of 12-HETE, the 12-LOX enzymatic product, correlated with a higher incidence of recurrence and metastasis in PDAC patients. In support of this hypothesis, we determined that inhibition of 12-LOX lowered the proliferation of human PDAC cells in cell culture, thus signifying 12-LOX inhibitors as potential therapeutics.

In 2014, we developed a potent/selective inhibitor, ML355 (IC<sub>50</sub> = 350 nM), but recently, we utilized mutagenesis of 12-LOX and computer modeling to improve its potency 7-fold with a next generation inhibitor, named Slug001 (IC<sub>50</sub> = 50 nM). More importantly, Slug001 was shown to be potent against PDAC cellular proliferation in our cellular assay. Based on these encouraging data, we now propose to derivatize Slug001 to optimize its potency/ drug-like properties and then characterize their cellular activity against pancreatic cancer.

Proposal:

We first propose to derivatize Slug001 with a suite of modifications. Slug001 was discovered with computer modeling, so our derivatives will be guided in a similar manner. Our goal is to improve its potency by filling the active site cavity more efficiently, while maintaining its cellular activity. Once potent inhibitors are developed, we will determine their potency in our human pancreatic cancer cell model. Our lead inhibitor will then be subjected to initial ADME investigations to determine its initial drug-like properties. Our ultimate goal is to perform more extensive ADMET studies and generate an IND application to initiate human clinical studies of 12-LOX inhibitors to treat PDAC.

## **Controlling Membrane Rupture and Vibrational Imaging of Repair Dynamics in Cancer Cells**

*Host Campus:* San Diego

*Lead Investigator:* Zeinab Jahed

*Start Date:* 10/1/2022    *End Date:* 9/30/2023    *Amount:* \$ 85,000

*Abstract:*

The plasma membrane (PM) protects the constituents of mammalian cells from the external environment, and its integrity is essential for cell viability and proper function. Since PM rupture is regularly caused in a variety of cellular processes, cells have developed several evolutionarily conserved membrane repair mechanisms. It is now evident that an inadequate PM repair response plays a role in a variety of diseases and conditions such as heart failure, muscular dystrophies, and neurodegeneration. On the other hand an over-activated PM repair response can promote survival of cancer cells as they undergo repeated PM rupture during cancer cell growth and invasion. Therefore, the modulation of PM repair machinery has emerged as a new exciting target for therapeutic purposes. However, the identification of promising therapeutic targets depends on the direct visualization, characterization and mechanistic understanding of PM repair machinery in health and disease, with single cell resolution. This remains challenging due to the spontaneity and rapidness of the PM rupture and repair process. Herein, we aim to utilize advanced nanotechnology techniques to develop and characterize a platform for higher throughput, spatial and temporal control and visualization of membrane rupture and repair for studying mechanisms of repair in various adherent cancer cell types. Our proposed work is innovative from both technical and scientific aspects. From the technical aspect, we introduce a novel platform with the capability to controllably introduce and visualize hundreds of independent PM rupture and repair events in single cells. From a scientific perspective, such a platform will enable high throughput testing, and hence accelerate the identification of various PM-repair related machinery and therapeutic targets. Despite the novelty of the proposed work, I have confidence that this project can be successfully executed our labs. We have already proven the feasibility of fabricating nanoscale platforms with the desired characteristics, for applications other than the one proposed here.



## **Localized translation regulation in a cancer metastasis model**

*Host Campus:* Santa Cruz

*Lead Investigator:* Sarah Loerch

*Start Date:* 10/1/2022    *End Date:* 9/30/2023    *Amount:* \$75,000

*Abstract:*

Metastasis is the main cause of death among cancer patients. Protein synthesis is required to support the high anabolic demand of invasive cells and has emerged as a driver of cancer progression and metastasis. Mesenchymal cell migration, one common mechanism of cell migration in cancer, requires localized translation at the leading edge of the cell. Protrusions are enriched in ribosomes, translation initiation and elongation factors. Yet, how this machinery is recruited and locally regulated remains elusive. With this Cancer Research Coordinating Committee grant we will test two hypotheses:

- 1) We hypothesize that the receptor of activated kinase C (RACK1) anchors ribosomes near the cell membrane via interactions with adhesion complexes. RACK1 is a ribosome-associated protein. RACK1 is also a structural component of early and mature adhesion complexes, which are important for cell migration. We will test the importance of RACK1 interactions with these complexes for mesenchymal cell migration.
- 2) We hypothesize that the metabotropic glutamate receptor 1 (mGluR1) - eukaryotic elongation factor 2 kinase (eEF2K) interaction is important for regulating cancer cell migration by locally affecting translation elongation. Several cancers express mGluR1, a protein typically only expressed in neurons. The intracellular domain of mGluR1 interacts with and regulates eEF2K, the main regulator of translation elongation. Both, eEF2K and mGluR1 upregulation is associated with a poor prognosis, and invasive and metastatic tumors. We will test the importance of the mGluR1 - eEF2K interaction for mesenchymal cell migration.

We will modify an established in vitro migration assay using a human breast cancer cell line that expresses mGluR1, eEF2K, and RACK1. We will leverage overexpression of protein fragments to elicit dominant-negative effects by disrupting specific molecular interactions.

Elongation inhibitors have already emerged as a cancer therapeutic. However, molecules that broadly target protein synthesis are associated with severe side-effects due to the central role of the ribosome in all cells. More targeted approaches are needed. Results from this research will reveal novel therapeutic targets. We further expect that results from this research provide the foundation for further mechanistic studies and allow us to successfully apply for federal funding.

## **Evaluation of ketogenic diet strategies for pancreatic cancer-associated cachexia**

*Host Campus:* Davis

*Lead Investigator:* Gerardo Mackenzie

*Start Date:* 10/1/2022    *End Date:* 9/30/2023    *Amount:* \$85,000

*Abstract:*

Pancreatic cancer (PC) is a deadly disease, with limited effective treatments. At the time of diagnosis, around 80% of PC patients suffer from cachexia, a condition that involves progressive weight loss, nutritional deterioration and loss of muscle mass. Therefore, new strategies to improve patients' survival and quality of life are urgently needed. Our proposed research will study the role of a ketogenic diet (KD) in PC. In particular, we want to know if this particular diet can improve the quality of life and help prevent cachexia. KDs are diets characterized by having a high fat content and almost no carbohydrates (sugars). In preliminary studies, we observed that in mice that have PC, the mice fed a strict KD had significantly improved motor function compared to mice fed a control diet. In addition, the mass of the thigh muscle was higher in mice fed a strict KD, compared to mice that consumed a control diet. These preliminary studies strongly indicate that a KD might be a beneficial dietary treatment for PC-induced cachexia. However, exactly how this happens and whether other KD strategies are useful for PC-associated cachexia remains unknown. Based on the above, in this proposal, we plan to investigate how a KD prevents cachexia progression in a clinically relevant PC mouse model (KPC mice). We will feed KPC mice with a KD along with standard treatment, and evaluate the effect on tumor growth and on indicators of cachexia. We will investigate markers of muscle strength and identify cellular mechanisms that may explain the observed effect. In addition, since strict diets are difficult to maintain throughout cancer treatment, we propose to test if intermittent feeding of a KD (for example feeding a KD for some days a week, instead of every single day), can achieve similar effects as observed with the strict KD. The successful completion of these studies could lead to new treatment strategies to mitigate cancer cachexia.

## **Integrated Model of Cancer, Vasculature, and Immune System**

*Host Campus:* Merced

*Lead Investigator:* Kara McCloskey

*Start Date:* 10/1/2022    *End Date:* 9/30/2023    *Amount:* \$85,000

*Abstract:*

Endothelial cells (ECs) are activated to generate new blood vessels that play a key role in supporting the growth and spread of many cancers. However, treatments shown to be highly effective in mice are proving less robust in humans. Three-dimensional microfluidic chips enable recapitulation of the tumor microenvironment with human cancers, human endothelial cells and human immune cells. Establishing a highly angiogenic tumor vasculature perfused with immune cells is needed to accurately reconstruct the tumor pathology. Our laboratory has identified and characterized a unique highly angiogenic ECs from mouse and human embryonic stem cells (ESC). The proposed studies will examine various cancer spheroids' ability to recruit new blood vessels, undergo metastasis, and examine response to anti-angiogenic drugs for treating growing cancers.

## **Imaging CD40 activation to enable more effective agonists for immunotherapy**

*Host Campus:* Santa Barbara

*Lead Investigator:* Meghan Morrissey

*Start Date:* 10/1/2022    *End Date:* 9/30/2023    *Amount:* \$85,000

*Abstract:*

Immunotherapy has been one of the largest recent advances in cancer therapy. However, current immunotherapy strategies only work in a subset of patients. Blockbuster T cell therapies, like PD-1 and CTLA-4 checkpoint inhibitors, achieve objective responses in ~25% of patients. One reason for this is poor T cell infiltration of solid tumors and T cell exhaustion in the tumor microenvironment. In contrast, macrophages are adept at penetrating solid tumors, where they can either promote tumor progression or execute anti-tumor activities like engulfing cancer cells or secreting cytotoxic cytokines. One emerging immunotherapy strategy is to ‘prime’ macrophages or other myeloid cells to more efficiently stimulate an anti-cancer immune response in T cells. The leading molecular candidate in this area is the TNF superfamily receptor CD40. CD40 is expressed on macrophages, B cells and dendritic cells. CD40 is activated by CD40 ligand on helper T cells, leading to multiple anti-cancer outputs. Despite promising pre-clinical data, CD40 agonists have stumbled in the clinic. A major reason for this is a poor understanding of the molecular details of the signaling pathway, including the essential parameters of effective agonists. Soluble agonist antibody or soluble CD40 ligand is unable to fully activate CD40 compared to cell-bound or oligomerized agonists. The primary goal of this proposal is to test the hypothesis that CD40 clustering is required for full activation of the CD40 signaling pathway. A critical gap in the field is live imaging of CD40 activation, which could convincingly demonstrate CD40 clustering upon CD40 ligand binding. Establishing this technique will also enable future studies examining intracellular signaling complex assembly. In this proposal, I will use high resolution imaging and synthetic biology to visualize CD40 activation in living cells, hypothesizing that effective agonists drive CD40 oligomerization in the plasma membrane. I will also examine how different oligomerization states of CD40 ligand activate downstream signaling molecules to determine why oligomerization is required for some CD40 responses but not all. Overall, these experiments will shed light on this new cancer immunotherapy target, and inform the design of new CD40 agonists.

## **Therapy of Myeloproliferative Neoplasms by JAK2V617F specific Trans-Splicing Ribozymes**

*Host Campus:* San Diego

*Lead Investigator:* Ulrich Muller

*Start Date:* 10/1/2022    *End Date:* 9/30/2023    *Amount:* \$75,000

*Abstract:*

The proposed research will address Philadelphia chromosome negative Myeloproliferative Neoplasms (MPNs), which overproduce terminally differentiated cells of the myeloid lineage. They are associated with a reduced life expectancy and can progress to universally fatal leukemic blast transformation. Currently, the only treatment with curative potential is allogenic hematopoietic stem cell transplantation, which has a severe co-morbidity/mortality and the potential for relapse. About 70% of MPNs harbor the Janus kinase 2 pathogenic variant V617F (JAK2VF) in hematopoietic stem cells, considered the likely driver of the disease.

We propose to optimize engineered, catalytic RNA molecules (ribozymes) that can differentiate between JAK2VF and wild type (WT) JAK2 to specifically kill diseased cells, for a new therapeutic strategy with curative potential for JAK2VF+ MPNs. The approach uses engineered, trans-splicing variants of naturally cis-splicing group I intron ribozymes from the species *Tetrahymena*. These trans-splicing ribozymes can recognize a specific sequence on a target mRNA and replace the 3'-portion of the mRNA with the ribozyme's 3'-exon. If the 3'-exon encodes a cytotoxic peptide and this exon is spliced in-frame into the target mRNA then translation from the target mRNA leads to cell death due to the cytotoxin. Importantly, the V617F mutation can be recognized by the ribozyme to facilitate cell death specifically for JAK2VF+ cells.

The proposed research activities will optimize the specificity of ribozyme trans-splicing on JAK2VF mRNA. A high specificity is essential to the proposed therapy because off-target splicing could kill healthy cells. Together with a collaborator, we have previously shown that a designed, but not optimized ribozyme can indeed splice at the intended target site. To optimize the ribozyme's specificity for JAK2VF mRNA we will generate combinatorial libraries of this ribozyme with partially randomized sequences. These libraries will be exposed to JAK2VF mRNA in a high background of competitor RNA to select for specific splicing on JAK2VF mRNA. Successful ribozyme variants will transfer a unique barcode from their 3'-exon into splice products, where they are identified by High Throughput Sequencing.

## **Dysregulated immune signals in the developing liver promote pediatric hepatoblastoma**

*Host Campus:* San Francisco

*Lead Investigator:* Amar Nijagal

*Start Date:* 10/1/2022    *End Date:* 9/30/2023    *Amount:* \$75,000

*Abstract:*

Development and cancer are two sides of the same coin. Precise regulation of developmental pathways ensures normal cellular differentiation; dysregulation of these same pathways, however, supports unfettered cellular growth and malignant transformation. The immune system plays an important role in both settings by regulating homeostasis in embryonic tissues and by regulating tumor growth. The well-established association between immune dysregulation and preterm delivery, and the 1,200-times higher incidence of hepatoblastoma (HB) among premature infants compared to term newborns indicates that aberrant immune signals during liver development may promote malignant transformation of hepatoblasts. Our analysis of pediatric HB specimens identified a distinct population of tumor-associated macrophages (TAMs) that express several pro-tumorigenic genes, promote HB growth in an in vitro co-culture system, and are most notably distinguished from normal liver macrophages by their attenuated expression of the class A scavenger receptor MARCO. In contrast to the high levels of MARCO on macrophages in human and mouse fetal liver, the expression of MARCO on macrophages is suppressed during perinatal liver inflammation. These data support MARCO as a mechanism by which liver inflammation alters a developmentally conserved immune signaling pathway and promotes hepatoblast transformation. We hypothesize that loss of MARCO on macrophages establishes a tumor-permissive environment and that inflammation-mediated suppression of MARCO on macrophages promotes HB formation. We will use innovative tools to 1) determine whether MARCO deficiency creates a tumor permissive immune environment by enhancing immunosuppressive functions in the neonatal liver, and 2) determine whether suppression of MARCO signaling by perinatal inflammation promotes malignancy in a novel model of HB in immunocompetent neonatal mice. Successful completion of this proposal will establish how alterations to homeostatic immune pathways in the developing liver promote HB. This research will also provide the foundation for broader investigations focused on how immune functions in developing tissues are responsible for maintaining homeostasis and, when dysregulated, contribute to neonatal and pediatric cancers.

## **Defining the molecular mechanism for targeting oxidative phosphorylation in breast cancer**

*Host Campus:* Irvine

*Lead Investigator:* Olga Razorenova

*Start Date:* 10/1/2022    *End Date:* 9/30/2023    *Amount:* \$75,000

*Abstract:*

Triple-negative breast cancer is the only breast cancer subtype that has very limited treatment options. Recently, we and others have identified a metabolic change—oxidative phosphorylation—as a key driver of breast cancer progression and metastasis. My research group established that CUB-domain containing protein 1 (CDCP1) and Src kinase pathway are the main regulators of this metabolic change. While CDCP1 targeting therapeutics are under development, therapeutics targeting Src kinase are in clinical trials for several cancers, with considerable interest to further discover and target Src kinase substrates to reduce normal tissue toxicity. We hypothesize that CDCP1/Src axis regulates oxidative phosphorylation by regulating phosphorylation and activity of the enzymes in the mitochondrial electron transport chain. Some of the Src substrates in electron transport chain have been described (and will be tested in a candidate approach), but there are caveats, including pronounced cell type specificity, lack of information on the exact phospho-sites, lack of CDCP1 dependency studies, lack of the determined outcome of the phosphorylation event in terms of target protein activity. Thus, we propose to implement limited candidate approach, and an unbiased phosphoprotein profiling in cancer and normal cells with manipulated expression of CDCP1 and Src. While my laboratory is well set up to conduct genetic overexpression/downregulation studies, phosphorylation substrate discovery and validation are the new research areas. We have established a connection with UCSD phosphoproteomics facility with cutting edge instrumentation to conduct the study, followed by rigorous validation of the phospho-targets. We have also established a collaboration with Dr. Wang at UCI for in vitro kinase assays. The outcome of the study would be the justification of CDCP1/Src axis targeting in breast cancer knowing the consequences to electron transport chain functioning in cancer and normal cells (with the aim to discover cancer-specific targets), list of potential therapeutic targets in electron transport chain itself, list of targets outside of electron transport chain, indirectly regulating oxidative phosphorylation. This study will help my laboratory to expand to the area of phosphosignaling and facilitate the acquisition of an R01 to rigorously pursue the therapeutic significance.

## **Flexible Robotic Evacuator for Minimally Invasive Brain Tumor Therapy**

*Host Campus:* Riverside

*Lead Investigator:* Jun Sheng

*Start Date:* 10/1/2022    *End Date:* 9/30/2023    *Amount:* \$85,000

*Abstract:*

The goal of this project is to design, fabricate, and test a flexible robotic evacuator that can be deployed by meso-scale steerable robots inside a brain tumor and controlled to evacuate tumor tissue at multiple locations towards conformal tumor evacuation. Glioblastoma multiforme (GBM) is one of the most challenging cancers to cure. Each year, nearly 12,000 new cases of GBM are diagnosed in the US, with the overall median survival being only 12 to 18 months. GBM rarely metastasizes to other organs; however, standard therapies with surgery via a craniotomy combined with adjuvant radiation and FDA-approved drugs can barely prevent the recurrence of GBM. For recurrent GBM (rGBM), although re-surgery can provide the best survival on select patients, most patients cannot afford re-surgery due to their generally poor health conditions. Thus, minimally invasive techniques arise to address this challenge. Nevertheless, existing tools are straight and rigid, and thus are not adequate to remove large and irregularly shaped tumors. Hence, there is an urgent need to develop a steerable robot that can be introduced through a small burr hole and manipulated throughout the tumor to remove the tumor in a minimally invasive manner. This project aims to develop and demonstrate a flexible robotic evacuator that can be integrated with steerable neurosurgical robots. The device will be made of flexible material so that it can be delivered through the curved channel of a steerable robot. In this project, we will: 1) design and fabricate a flexible robotic evacuator with a capability of aspiration and irrigation, 2) Integrate the evacuator with a steerable robot, and 3) evaluate the functionality of the robot system. With the success of this pilot project, we will pursue a NIH RO1 grant to improve the robot design, develop a planning and control system, and perform in vivo studies on normal and GBM pigs.



## **Investigating the role of vascular mimicry in breast cancer metastasis.**

*Host Campus:* Santa Cruz

*Lead Investigator:* Shaheen Sikandar

*Start Date:* 10/1/2022    *End Date:* 9/30/2023    *Amount:* \$75,000

*Abstract:*

The proposed research will explore a new direction in my laboratory of the role of vascular mimicry in tumor initiating cells during breast cancer metastasis. Metastasis accounts for majority of breast cancer related deaths with women and therapeutic strategies that can prevent or reduce metastatic burden are urgently needed. It is now well established that breast tumor cells are heterogeneous and made up of transcriptionally distinct subsets with different tumor initiating potentials. In this model, minority populations of tumor cells referred to as metastasis initiating cells (MICs) display increased cellular plasticity reminiscent of immature stem-like cells in the normal mammary gland, and they have metastatic capabilities.

Leaky vasculature and focal loss of CD31 in tumors has been linked to a phenomenon called vascular mimicry. In vascular mimicry, tumor cells form endothelial-like structures to provide nutrients and oxygen in a hypoxic environment. Increased vascular mimicry in tumors has been associated with immature tumor cells where it is reported that tumor initiating cells (TICs) under hypoxic conditions can differentiate to form endothelial-like structures. Moreover, vascular mimicry breast cancer is higher in more aggressive triple negative breast cancer subtype and is overall correlated with poor outcomes. However, the role of vascular mimicry during metastasis in human breast cancer and the mechanisms underlying this process remain unknown.

Using single cell RNA-sequencing in patient tumors and computational tools that we developed, we recently identified a minority population of immature tumor cells that are THY1+/VEGFA+ and express the T-cell oncogene LMO2. Our preliminary data indicate that LMO2 is critical for metastasis in breast cancer. Surprisingly, we also find that LMO2+ breast cancer cells display vascular features, and co-associate with tumor endothelial cells. We hypothesize that tumor initiating cells in breast cancer utilize vascular mimicry to metastasize and LMO2 is a critical regulator of this process. Specifically, we will test 1. Whether vascular mimicry is required for metastasis in breast cancer? 2. Does expression LMO2 induce a vascular phenotype in breast cancer cells? 2. Does LMO2 regulate vascular leakiness and vascular mimicry in PDX models in vivo to metastasize?

## **The comparative immune-oncology of canine and human brain tumors designed to improve glioma outcomes**

*Host Campus:* Davis

*Lead Investigator:* Christine Toedebusch

*Start Date:* 10/1/2022    *End Date:* 9/30/2023    *Amount:* \$74,950

*Abstract:*

High-grade gliomas (HGGs) are uniformly lethal primary brain tumors of children and adults. Despite their shared aggressiveness, pediatric and adult HGGs are genetically and immunologically distinct tumors, requiring tailored, and likely independent, immunotherapeutic approaches. Anti-tumor immunotherapy has shown promising preclinical efficacy in both pediatric and adult HGG but have failed to translate into increased survival time for human patients. Thus, there is an urgent need for complementary preclinical models which more closely predict therapeutic efficacy in HGG patients. Canine HGGs, which develop de novo in an outbred, immunocompetent host, are increasingly pursued as a therapeutic model for human HGG. Similar to adult HGG, we demonstrated that canine HGG is highly inflammatory, with a robust GAM infiltrate and increased cytokine and chemokine expression. In contrast, genomic analysis indicated that canine HGG aligns closely with pediatric HGG. This discrepancy presents two challenges for canine HGG as a translational model: 1) Which therapeutic interventions are appropriate to evaluate in canine HGG, and 2) which human patient population(s) is most appropriate to translate these findings? A comprehensive analysis of the immune microenvironment is lacking in canine HGG, precluding optimized therapeutic targeting and application of canine trials to human patients. Single-cell RNA sequencing (scRNA-seq) will provide a powerful tool for a comprehensive analysis of immune cell populations in canine HGG. Based on our laboratory's work, we hypothesize that canine HGG exhibits a heterogeneous and functionally distinct immune cell infiltrate, comprised of inflammatory microglia and immunosuppressed macrophages and lymphocytes. Our proposed studies will 1) define the cellular identities and molecular signatures of canine HGG immune cells using scRNA-seq for comparison with human HGG and 2) will inform the use of therapeutic approaches in canine HGG and their subsequent translation to human patients. These data will provide necessary direction and preliminary data for a NIH R01 submission June 2023, while providing a detailed transcriptome of the aged canine CNS immune cell landscape in health and under the influence of glioma that will be made available to the scientific community.

## **Enhancing Online Group Fitness Exercise for Health Improvement for Patients with Cancers**

*Host Campus:* Davis

*Lead Investigator:* Hao-Chuan Wang

*Start Date:* 10/1/2022    *End Date:* 9/30/2023    *Amount:* \$85,000

*Abstract:*

Zumba and similar group-based aerobic exercises have become popular worldwide, engaging approximately 12 million people to attend the fitness dancing parties on a frequent basis. Prior studies showed evidential benefits in mental and physical wellness, as well as quality of life, of such fitness programs among the common public and patients with cancers. Patients with cancers, either on treatment or off therapy, may encounter challenges to attend in-person training due to their illness and mobility constraints, in addition to the current global covid pandemic. Therefore, it's essential to explore online options which offers easy accessibility and inclusiveness of "exercise classes". In terms of personal motivation and social engagement, patients with cancers may also benefit from additional personalization and social support to help them physically catch up with the exercise and socially connect with co-trainees. Our goal in this project is to create a social, accessible and inclusive video-mediated Zumba experience that extends the benefits of group training to the online space (Zumba together!). By enhancing video communication, we will develop a real-time action feedback mechanism and a recommendation mechanism that produces recommendations of training content and co-trainees, both driven by continuous comparison of pose estimations performed by the system using a data-driven machine learning model, with the goal to improve social synchrony and engagement in the virtual program. In this one-year project, we will design and prototype the technological mechanisms and conduct a pilot study with up to 30 health adults to assess the feasibility and benefits of using the enhanced video communication channel for virtual group exercises. The results from this project will be used to apply for an R01 to conduct a multicenter clinical trial targeting young adult cancer survivors and patients undergoing treatment.

## **Optogenetic control of chromosome segregation**

*Host Campus:* San Francisco

*Lead Investigator:* Torsten Wittmann

*Start Date:* 10/1/2022    *End Date:* 9/30/2023    *Amount:* \$75,000

*Abstract:*

During cell division, chromosomes are segregated into two daughter cells by the mitotic spindle. Spindle microtubules pull on chromosomes through kinetochores, large protein complexes that link chromosomes to spindle microtubules. Chromosome segregation errors are one of the hallmarks of cancer. However, how such segregation errors and resulting aneuploidy causally relate to cancer cell proliferation or malignancy has remained largely obscure. A central challenge to understanding this relationship is the paucity of experimental tools to acutely and accurately control chromosome dynamics in dividing cells. Building on our recent advances in optogenetic control of intracellular dynamics in which we, for example, show that acute inhibition of microtubule plus end interactions can change mitotic spindle and chromosome dynamics, I propose to engineer innovative optogenetic tools to control chromosome movement and induce and analyze chromosome missegregation events with high spatial and temporal accuracy in live dividing cells. We will develop and evaluate three alternative approaches to optogenetically modulate chromosome segregation: 1. Design a photosensitive version of the Ndc80 complex, a highly conserved core component of the kinetochore-microtubule attachment machinery in eukaryotic cells; 2. Optogenetically inactivate the spindle assembly checkpoint to allow premature chromosome segregation; and 3. Develop an optogenetic chromosome trap. Once optimized, these optogenetic actuators will be stably integrated by genome editing into normal epithelial cells and cancer models expressing specific oncogenes to control chromosome dynamics globally and locally and ask under which conditions this is sufficient to generate chromosome segregation errors and aneuploid daughter cells. In the long-term, the optogenetic kinetochore toolbox generated in this pilot project will allow us to test hypotheses how aneuploidy causes malignant transformation and/or promotes cancer cell fitness and position us favorably for subsequent extramural grant applications.

## **Developing computational assay for oncogenic MYC/MAX dimerization and search on DNA**

*Host Campus:* Irvine

*Lead Investigator:* Jin Yu

*Start Date:* 10/1/2022    *End Date:* 9/30/2023    *Amount:* \$74,909

*Abstract:*

The MYC transcription factor (TF) regulates cell growth and proliferation in both normal and cancer conditions. Over-expression or deregulation of MYC can lead to many types of human cancer. To function, MYC usually dimerizes with MAX, which controls a network of associated proteins to regulate transcription of thousands of target genes. Either mutation or malfunction of protein associations can contribute to cancer developments. In monomeric forms, the MYC/MAX bHLHLZ domain (DNA binding and dimerization) can be disordered so that targeting on individual protein sites is difficult. In contrast, regulating Myc-Max dimerization can be highly promising for cancer therapeutics. Previous biochemical studies indicate that the MYC and MAX monomers preferentially bind DNA and dimerize on the DNA. Our recently published work suggests that Myc-Max moves along DNA via coordinated inchworm stepping, and the dimer can also dissociate into monomers on DNA. It thus becomes feasible to develop a computational assay to characterize monomer vs dimer propensity of MYC/MAX and related proteins on the DNA from original to mutant or engineered systems. We plan to employ state-of-art modeling and structure-based simulations, at both residue (coarse-grained or CG) and atomic scales, to firstly calibrate Myc-Max dimerization or dissociation into monomers on DNA, based on published experimental results, e.g., on relative association strength of Myc-Max to Max-Max and Mad-Max. Once the dimerization strength tuned in the modeling produces consistent results with existing measurements, molecular dynamics (MD) simulations at both CG and atomic levels can be conducted iteratively revealing structural mechanisms. Via the computational assay, one can analyze dimer vs monomer dynamical equilibrium in the MYC/MAX interaction network under therapeutically relevant conditions, such as incorporating a newly discovered ligand that stabilizes Max-Max dimer, and testing the highly promising mutant Omomyc that competitively dimerizes with Myc as cancer therapeutics. The visualization capability of the assay also helps understanding Myc molecular biology, e.g. revealing how related dimeric TFs associate/dissociate and search along DNA at resolution beyond current experimental technologies can afford, in the original and modified systems, thus further support cancer therapeutics design.