Table of Contents

Click on project titles to see full abstracts

PI First Name	PI Last Name	Title	Host Campus	Start Date
Scott	**Atwood**	Recurrent GLI mutations in drug-resistant skin cancer	Irvine	1/1/2018
Robert	**Canter**	Outcomes in Stage IV Cancer Patients with Bowel Obstruction	Davis	1/1/2018
Frederic	**Chedin**	R-loop Driven Oncogenic Translocations in Prostate Cancer	Davis	1/1/2018
Celia	**Goulding**	Repurposing a toxin-immunity pair to selectively kill cancer	Irvine	1/1/2018
Michael	**Kent**	The Role of p21 Phosphorylation at S123 in Tumor Suppression	Davis	1/1/2018
Kai	**Kessenbrock**	Studying breast cancer initiation in single cell resolution	Irvine	1/1/2018
Noriko	**Satake**	Leukemia Stem Cells in B-Cell Acute Lymphoblastic Leukemia	Davis	1/1/2018
Szu-Wen	**Wang**	Novel cancer metabolite-triggered drug delivery	Irvine	1/1/2018

Recurrent GLI mutations in drug-resistant skin cancer

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

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

Outcomes in Stage IV Cancer Patients with Bowel Obstruction

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

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

R-loop Driven Oncogenic Translocations in Prostate Cancer

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

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

Repurposing a toxin-immunity pair to selectively kill cancer

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

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

The Role of p21 Phosphorylation at S123 in Tumor Suppression

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

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

Studying breast cancer initiation in single cell resolution

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

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

Leukemia Stem Cells in B-Cell Acute Lymphoblastic Leukemia

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

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

Novel cancer metabolite-triggered drug delivery

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

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