PI Name(s) BARCELLOS-HOFF, MARY HELEN

Title Definition of Immune Infiltrate Phenotype and DNA Damage Response Deficits Across Diverse Murine Mammary Carcinomas

Institution UNIVERSITY OF CALIFORNIA, SAN FRANCISCO

Abstract While major strides have been made in cancer treatment, the challenge remains to determine which patients will benefit most from a therapeutic regimen, which is the ultimate goal of personalized medicine. The key to choosing the most effective therapy for a given cancer is understanding the biological basis for differential outcomes and intrinsic sensitivity to cancer therapies. Almost all cancers are treated with cytotoxic chemotherapy and radiation therapy but the emerging clinical success of immunoncology (IO) drugs whose success is predicated on the biology of the immune infiltrate requires new tactics to model optimal combinations. As cytotoxic therapy is a critical component of cancer patient treatment, mammalian models that represent a range of DNA damage response deficits (DDRD), and hence sensitivity to different agents, would improve translational research. Likewise, the multiple mechanisms by which cancer evades the anti- tumor immunity need to be represented in translational research. Lack of diversity in most current preclinical models limits their applicability as a platform for systemic evaluation of these aspects of tumor biology. Given the range of IO approaches and the diversity of DDRD, a critical unmet translational requirement is a model system in which both the tumor DDRD and the immune infiltrate is defined so that combinations can be readily studied. We propose to use murine tumor derived transplants (mTDT) of Trp53 null mammary carcinomas that we have generated and characterized in regard to heterogeneity, relevance to human cancer, and reproducibility to credential the DDR deficits of these syngeneic carcinomas and to evaluate corresponding baseline immune cell infiltrates. We will implement multiplex analysis of tumor and immune features and correlate them with tumor response to radiation, a canonical DNA damaging therapy and arguably the most widely used cytotoxic therapy. The product of these studies is directly responsive to the FOA consisting of a protocol for standardized implementation of this model, comprehensive analysis of tumor types, and repository of specimens, data, and viable tissue. The success of this project will provide a means to conduct mechanistic and translational studies using defined tumor DDRD and immune infiltrate composition for developing patient- specific personalized therapy to IO and cytotoxic therapies.

PI Name(s) BOEHM, JESSE SAMUEL

Title Rapid ex vivo biosensor cultures to assess dependencies in gastroesophageal cancer

Institution BROAD INSTITUTE, INC.

Abstract The ability to predict dependencies given the molecular features of a patient's tumor is central to cancer precision medicine. The systematic use of CRISPR/Cas9 and pharmacologic tools in established cancer models is showing great potential to discover new targets. However, existing model development approaches require long periods of culture time during which evolutionary pressures reduce heterogeneity. And, it remains challenging to create long-term models for certain tumor types and genotypes, making it challenging to use perturbational tools to experimentally map dependencies. To address these challenges, our overarching goal is to develop 'rapid ex vivo tumor biosensors' whereby we would be able to interrogate cancer dependencies in an immediate short-term 'culture' of cancer cells taken from a patient biopsy/surgery/fluid collection as a novel research-grade experimental model of cancer. In doing so, we aim to couple the timing of drug or CRISPR/Cas9 perturbation with the preservation of subcellular heterogeneity. If successful, we hypothesize that this modelling approach will more accurately recapitulate patient tumors and may ultimately serve as a stronger foundation for preclinical therapeutic studies. This work should also substantially expand the fraction of patient samples that can be interrogated. Here, we propose using gastroesophageal adenocarcinoma (GEA) as a test case for this strategy due to our experience as well as the existence of marked intra-tumor heterogeneity. However, once established, this novel modeling platform should enable a wide range of basic and translational questions (both for GEA and other tumors) that require model formats that include heterogeneous cell populations. Our goal will be achieved via two Specific Aims including (1) using patient-derived organoids created on rapid time frames for CRISPR/Cas9 editing to validate emerging GEA dependencies; and (2) developing the ability to directly visualize and perturb single cells from matching patient ascites fluid or disaggregated primary tumors ex vivo using label-free imaging methods. We will benchmark these approaches against each other using the same clinically annotated, serially collected patient samples. In following the instructions for this RFP, we focus on technology-development focused goals as opposed to deeper mechanistic studies. We focus on benchmarking predictions and assessing reproducibility, sensitivity and specificity. This work is innovative, in that it brings together expertise at the intersection of functional genomics, advanced computational approaches for image-analysis and GEA genomics. If successful, this effort could have significant impact by establishing a foundation to expand this approach to other disease (tumor and non-cancer) indications.

PI Name(s) BOURNAZOS, STYLIANOS

Title Novel Transgenic Mouse Models Addressing Outstanding Translational Barriers in Antibody-Based Therapeutics

Institution ROCKEFELLER UNIVERSITY

Abstract Monoclonal antibodies have played a pivotal role in the diagnosis and treatment of cancer for nearly two decades and continue to grow at an exponential pace. Initially developed for their exceptional ability to target tumor antigens and elicit antibody-dependent cellular cytotoxicity (ADCC), they have more recently been used to modulate a patient's immune system for anti-cancer immunotherapy. While the generation and development of antibodies targeting various cell surface proteins has rapidly progressed, appropriate model systems for pre- clinical testing of such therapeutics has lagged. This is because human antibodies i) don't fully engage murine or non-human primate Fc receptors (FcγRs), ii) are foreign proteins that are rapidly rejected in allogeneic hosts and iii) are often inappropriately tested in immunodeficient xenograft models lacking adaptive immune cells or homologous FcγR. Thus, our studies have focused on the generation and testing of clinically relevant models to better understand the in vivo activity of diagnostic and therapeutic antibodies. The current proposal aims to now generate and fully characterize novel murine models that allow better preclinical testing of human antibodies by engineering our previously developed humanized FcγR mouse strains to express human FcRn and IgG1. Expression of human FcRn will allow more accurate pharmacokinetic analysis of human antibodies and assessment of methods aimed at generating antibodies with extended in vivo half-life. By replacing the mouse heavy chain with the constant regions of human IgG1, this model will also allow chronic administration of human IgG-based therapeutics without developing anti-drug antibody responses. By addressing two major hurdles in the field of antibody therapeutics, these models will allow more rapid and efficient pre-clinical toxicology testing and potentially uncover novel mechanisms of Fc-engineered antibodies. Additionally, given the growing interest in immunotherapy, having an immunocompetent model provides an additional advantage over current xenograft models. Finally, as recent data suggest an important role for Fc-FcγR in radiolabeled antibody diagnostics, these models will provide a clinically relevant model to help improve the development and testing of innovative antibody-based molecules for the in vivo detection and localization of neoplasms.

PI Name(s) BRAT, DANIEL J

Title Modeling the Glioblastoma Microenvironment to Uncover Progression Mechanisms and Therapeutic Targets

Institution NORTHWESTERN UNIVERSITY AT CHICAGO

Abstract In nearly all forms of human cancer, the development of necrosis is tightly linked with malignant progression. Whether necrosis accelerates progression or is largely passive remains an open question, yet modeling these events to establish mechanisms and therapeutic vulnerabilities in animals has been challenging. In glioblastoma (GBM; WHO grade IV), the most malignant primary brain tumor, the rapid, radial growth phase that leads quickly to death is consistently preceded by the development of central necrosis. While genetic alterations of GBM are known in great detail, the biological properties that result from their acquisition and lead to this accelerated growth phase require deeper investigation. The tumor microenvironment (TME) changes dramatically following the onset of necrosis, from a sheet-like growth of infiltrating cells with relatively constant growth properties to a highly complex and evolving 3-D microsystem composed of diverse cell types and spatially segregated signaling networks. To better understand the dynamic temporal and spatial changes that promote progression, we propose to advance mouse models that closely parallel these events in human gliomas, since many mouse models of GBM lack necrosis. We developed a novel method to induce focal necrosis within high grade gliomas in vivo and will study TME restructuring and its impact on glioma growth in real time using multiphoton microscopy. As translational applications, we will demonstrate how hypoxia and necrosis promote the enrichment of glioma stem cells (GSCs) in their peri-necrotic niche and lead to the dramatic influx of tumor-associated macrophages (TAMs), which increase in number over 10-fold in the human disease. We propose both genetically characterized patient-derived GBM xenografts grown in mice with humanized immune cells, as well as an immunocompetent RCAS/tv-a model, and will determine how antagonizing these processes impact disease progression and outcomes. Our preliminary data and the literature indicate substantial differences between pre-necrotic and necrotic gliomas with regard to GSC and TAM enrichment and their impact on biological properties, but the mechanisms and evolution have not been studied in depth, in large part due to the absence of a credible animal model. Our model will capture glioma growth dynamics, GSC enrichment, and TAM influx, and facilitate the development of therapies that antagonize these mechanisms to improve outcomes.

PI Name(s) CARLSON, MARK A

Title Development and Application of a Porcine Model of Pancreatic Cancer

Institution UNIVERSITY OF NEBRASKA MEDICAL CENTER

Abstract The long-term goal of this research is to develop a platform on which experimental therapies for pancreatic cancer can be advanced to the clinic in a more efficient manner than achievable with current preclinical pancreatic cancer models. The focused objective of this R01 application is to develop a genetically-defined, autochthonous model of pancreatic adenocarcinoma in immunocompetent pigs, provide some validation data with respect to gross behavior, microscopy, tumor markers, genetic sequence, and transcription, and determine the model's utility for image-guided surgery. Tumors will be induced by local targeting of genes known to be associated with pancreatic cancer (namely, KRAS, p53, SMAD4, and CDKN2A), using a combination of transgenic subjects (the NSRRC Onco-pig), lentiviral-mediated in vivo gene transfer, and tumorigenic ductal cell implantation. Some murine models have failed to reflect human tumor biology because of differences in physiology, anatomy, and genetic sequence with humans. So, the rationale to build a porcine model of pancreatic cancer is that it should be more predictive of human tumor biology and response to therapy than murine models are, because swine have greater genetic and phenotypic homology with humans. The hypothesis of this R01 application is that forced expression of relevant mutant genes or transplanted tumorigenic pancreatic cells within the porcine pancreas will produce pancreatic tumors, and that this tumor model will be clinically relevant and useful. The utility of the model will be demonstrated with a comparative trial of reagents for Fluorescence Image-Guided Surgery, in experiments that would not be possible in the mouse. The work proposed in this R01 application will be accomplished through a collaborative team consisting of a general/oncologic surgeon (PI), a biomedical engineer with experience in molecule design, two molecular/cellular biologists with expertise in pancreatic cancer and gene editing, a medical oncologist who manages patients with pancreatic cancer, a pathologist specializing in pancreatic/GI cancers, sequencing and bioinformatics experts, and a biostatistician. This project is innovative because no large animal model of pancreatic cancer exists. The impact of a validated porcine model of pancreatic cancer would be to enhance, complement, and supplement preclinical data from other tumor models, and also to advance experimental anti-tumor therapies to the clinic in a more efficient manner, with fewer experimental therapies failing in clinical trials. In addition, a porcine model of pancreatic cancer could advance the design and development of minimally invasive catheters and energy sources used to ablate pancreatic tumors, and also to develop and/or improve techniques to detect, image, diagnose, and monitor these tumors.

PI Name(s) CASTRILLON, DIEGO H

Title Polymerase-mediated ultramutagenesis and carcinogenesis in mice

Institution UT SOUTHWESTERN MEDICAL CENTER

Abstract Genetically-engineered mouse models (GEMMs) are essential tools for the study of cancer. However, there is growing concern that GEMMs fail to recapitulate the mutation burden of human carcinomas. GEMMs have startlingly low overall mutation rates, far below what is observed in their human counterparts. This makes such models useful for studies of oncogenic signaling pathways, but greatly restricts their utility for studies of genetic heterogeneity and clonal variation, tumor immunology, or the impact of mutational load/base substitution rates on tumor behavior and response to therapy. The latter has become particularly relevant with the advent of immune checkpoint therapies, given that the best predictor of treatment success is a high incidence of somatic mutations, irrespective of tumor type. The same limitations are likely to be encountered with GEMMs based on newer genome-editing methods, pointing to the need for alternative approaches to optimize with respect to mutational load, which we now know defines so many aspects of tumor biology, clinical behavior and treatment response. In this project, submitted in response to PAR-17-245 “Research Projects to Enhance Applicability of Mammalian Models for Translational Research”, we propose to generate and characterize the first mouse cancer models based on polymerase-driven ultramutation. These approaches will 1) catalyze modelling of any cancer driven by POLE ultramutagenesis and 2) permit efficient “humanization” of any GEMM with respect to mutational load. Our approach represents a new and widely-applicable route to the creation of mouse models that recapitulate the mutational loads inherent to human cancer. These new genetic tools and the diverse animal models they will enable will stimulate a wide range of translational and preclinical investigations for which GEMMs were previously not well-suited, thus fulfilling the goals of PAR-17-245.

PI Name(s) CHAREST, ALAIN; BOUSSIOTIS, VASSILIKI A

Title Advancing treatment outcomes in malignant glioma by integrating immunotherapy and standard of care using genetically engineered mice that recapitulate molecular feature of human glioma

Institution BETH ISRAEL DEACONESS MEDICAL CENTER

Abstract Glioblastoma multiforme (GBM) is a dreadful cancer with a median survival of 14 months due to a lack of effective therapy. Checkpoint blockade immunotherapies have shown promising clinical outcomes for several cancers, and as such there are now many early stage clinical trials for GBM. Trials are designed for both newly diagnosed and recurrent GBMs and in both cases, checkpoint blockade is administered on the background of standard of care (SOC) therapy for GBM, which consists of surgical debulking, followed by fractionated radiation (XRT) with concomitant and adjuvant temozolomide (TMZ) alkylating chemotherapy. In addition, most patients are subjected to steroid use (dexamethasone-Dex)) to alleviate post surgery neurological symptomatic relief. There is a critical deficiency in our understanding on how XRT/TMZ and steroid exposure affect the tumor microenvironment (TME), specifically the immune cells component. Therefore there is a pressing need to understand how the efficacy of checkpoint immunotherapies is affected by XRT/TMZ/Dex and delineate a clinical strategy that will maximize treatment effectiveness. In addition, we demonstrate that the composition and activation status of GBM immune infiltrates is influenced by the driver genotype of the GBM cells. Our proposal will fill a knowledge gap regarding the type and activation status of the immune infiltrate vis-à-vis tumor driver genotypes. The central hypotheses of our proposal are: 1) the immune landscape of GBM is related to the type of driver mutations (genotype) of the tumor and 2) the SOC for GBM will affect its immune component and function, both of which will directly influence the efficacy of PD-1 and CTLA-4 checkpoint blockade immunotherapies. We need to delineate those effects and understand them in order to modify GBM management protocols to take full advantage of the power of immunotherapy. We propose to use EGFR- and PDGFR-driven genetically engineered mouse models, which accurately recapitulate human GBM, to determine the effects of tumor genotype on the immunofauna, to unveil the consequences of SOC on immune function and to relate those findings to clinical practice. Our project will deliver on an effective translational use of genetically cutting edge models of GBM that accurately recapitulate human disease to inform the conduct of clinical trials and to mechanistically interpret their outcomes.

PI Name(s) CHESI, MARTA

Title Credentialing a Genetically Engineered Clinically-Relevant Mouse Model of Multiple Myeloma

Institution MAYO CLINIC ARIZONA

Abstract Thalidomide (Thal) and its derivatives, Lenalidomide (Len) and Pomalidomide (POM), are immune modulatory drugs (IMiDs) used in the treatment of multiple myeloma (MM) and few other hematological malignancies. Although they represent the backbone treatment for both newly diagnosed and relapse/refractory MM patients, their clinical use remains mostly empirical because of lack of suitable in vivo model systems to study their complex mechanisms of action. Murine cells are intrinsically resistant to IMiDs because of different amino acid sequence in the IMiD binding domain of cereblon (CRBN). By building upon our extensively validated Vk*MYC transgenic mouse model of MM, we have generated a novel transgenic mouse, Vk*MYChCRBN, expressing the full human CRBN (hCRBN) gene under the control of its endogenous regulatory elements, rendering it IMiD sensitive. As previously done for the Vk*MYC model, we will extensively characterize the new Vk*MYChCRBN model and will use it to understand the IMiD effects on the tumor and the immune system with the ultimate goal to inform clinical practice.

PI Name(s) CHO, KATHLEEN R

Title Modeling Factors Associated with Risk of High-Grade Serous Carcinoma in Mice

Institution UNIVERSITY OF MICHIGAN AT ANN ARBOR

Abstract High-grade serous carcinoma (HGSC) is the most common and most lethal type of “ovarian” cancer. Most HGSCs are now believed to arise from epithelium in the distal fallopian tube, though a minority of HGSCs lack evidence of tubal origin. Population-based studies have identified several factors that are strongly associated with reduced HGSC risk, including sterilization procedures based on tubal excision, high parity, and oral contraceptive (OC) use. We do not understand how OCs and high parity protect against HGSC or how these protective effects can be maximized. Likewise, the roles of the fallopian tubes and ovaries and their cross-talk in HGSC pathogenesis remain incompletely understood. Intact ovaries could contribute to HGSC development by harboring ectopic tubal epithelium from which non-tubal HGSCs may arise, and/or by exposing the distal fallopian tube epithelium (FTE) to hormones and other factors, including those in follicular fluid released at the time of ovulation. Given the many challenges associated with detecting HGSC precursors and small tubal HGSCs before they have metastasized, and effecting cures for women with widely metastatic HGSC, an enhanced focus on preventing these tumors is warranted. Genetically engineered mouse models (GEMMs) of cancer may provide tractable and relatively rapid systems with which to test cancer prevention strategies and inform cancer prevention trials in humans. To date, no GEMMs have been credentialed for use in studying factors known to alter HGSC risk. We have developed transgenic (Ovgp1-iCreERT2) mice that allow conditional (tamoxifen [TAM]-inducible) activation of Cre recombinase exclusively in the FTE. We have also identified specific combinations of conditional tumor suppressor gene (TSG) alterations, prioritized because they are known to be frequently inactivated in human HGSCs (Brca1, Trp53, Rb1, Nf1 [BPRN] and Brca1,Trp53, Pten [BPP]), that lead to oviductal HGSCs following TAM treatment of Ovgp1-iCreERT2 mice that also carry the conditional TSG alleles. FTE from these mice can be cultured as organoids and transformed in vitro, allowing some risk factors to be tested in parallel with studies in vivo. Our new HGSC GEMMs will be employed to test the impact of factors known to be associated with human HGSC risk, with the goal of credentialing the models as genetically and biologically relevant tools with which to better understand how specific factors reduce HGSC risk, and for future use in testing novel HGSC prevention strategies. Four Aims are proposed: 1) To test whether high parity slows oviductal tumor development and/or progression in our BPRN model of HGSC; 2) To determine whether hormones of the types present in OCs alter the development and/or progression of oviductal HGSCs in BPRN mice; 3) To establish the preventive effects of bilateral risk-reducing salpingectomy (RRS) and salpingo-oophorectomy (RRSO) on the development of ovarian and/or primary peritoneal HGSC in our BPRN and BPP models; and 4) To test effects of pre-ovulatory follicular fluid on FTE in vitro and in vivo.

PI Name(s) DOW, LUKAS EDWARD

Title In Vivo Base Editing for Precision Oncology Models

Institution WEILL MEDICAL COLL OF CORNELL UNIV

Abstract Genetic mutation is the predominant driver of cancer cell growth and therapy resistance. In fact, a major goal of personalized medicine is to identify specific genetic changes in individual tumors with the notion that defining these changes will guide more effective and targeted treatment. While this precision oncology approach shows clinical promise, ongoing tumor sequencing efforts continue to identify potential new disease drivers and new mutations. How these uncharacterized mutant alleles contribute to disease is often not obvious, and requires functional examination. Genetically engineered mouse models (GEMMs) provide an ideal tool to investigate the consequences of genetic changes on tumor biology, yet existing approaches are not fast or precise enough to recreate the spectrum of genetic alterations seen in human cancer. We and others have used CRISPR-based genome editing to accelerate the generation of complex, genetically defined animal models. Yet, while CRISPR systems are fast and simple, the basic tools are imprecise in that they cause insertions and deletions that ablate gene function but cannot mimic the single nucleotide variants most often seen in human cancer. To build in vivo systems that recapitulate specific human cancer-associated mutations, our project exploits new CRISPR tools that couple Cas9 to cytidine deaminase enzymes and enable direct DNA mutagenesis at defined genomic regions. 'Base editing' (BE) technology offers far greater efficiency and flexibility than existing homology directed repair (HDR) approaches by eliminating the need to deliver exogenous DNA templates. We have systematically optimized the expression and activity of BE enzymes to increase the efficiency of genome modification and established a bioinformatic and experimental pipeline to predict and validate BE tools that recreate known and novel cancer mutations. In Aim 1, building from extensively optimized BE enzymes, we will generate a range of knock-in transgenic mice to maximize the number of possible genomic regions that can be mutated using BE, and validate the activity of these mice using a new fluorescence-based reporter system. Further, using a novel sensor assay, we will identify all human and mouse sgRNAs that can target recurrent cancer-associated mutation sites. Together, this work will define the BE efficiency of thousands of independent sgRNAs, and establish the first in vivo somatic base editing platforms. In Aim 2 we will use our in vivo BE tools to generate novel animal models of pancreatic and colorectal cancer, and examine the consequences of distinct cancer-associated mutations in each disease. This work will not only offer a new understanding of key oncogenic mutations, it will provide critical validation of the utility of in vivo BE in multiple cancer settings. By providing an easy and efficient path to capture the diversity of human disease alleles, we believe this new precision editing platform has the potential to fundamentally change the way we design and implement mouse cancer models for translational research.

PI Name(s) FIELDS, RYAN C; FLAVELL, RICHARD A

Title Advancing Precision Oncology in a Humanized, Fully Autologous Mouse Model

Institution WASHINGTON UNIVERSITY

Abstract Progress in the early detection and treatment of cancer requires accurate model systems to further evaluate new, promising discoveries. Small animal, and in particular mouse, model systems are attractive to researchers for numerous reasons, including their ease of use and well-described platforms. Immunotherapy has revolutionized clinical oncology, but lacks pre-clinical models of the human immune system and human cancer to investigate new modalities and limitations/toxicities of treatment regimens. The ability to grow human tumors in immune-deficient mice (so-called patient-derived xenografts, or PDXs) allows researchers to work directly with human cancer tissue in a controlled setting. However, PDX models are limited by their lack of an intact immune system. The broad objective of this proposal is to validate an in vivo model to evaluate human tumors in the context of a complete and intact human immune system in a completely personalized and autologous fashion to study cancer immunotherapy. Herein, we propose to: (1) validate the ability of our humanized system to serve as a model for cancer immunotherapy treatment response and toxicity in patients with melanoma, including immunotherapy checkpoint blockade and vaccine strategies, and to (2) extend our current work in melanoma by validating the ability to establish humanized mice and evaluate tumor growth and leukocyte development in autologous human pancreatic and colorectal tumors established in humanized mice. In each of these areas, we will leverage our multi-institutional team's individual expertise along with our institutional infrastructure to maximize the success of the experimental aims. The results from this project will made widely available to the general research community for future, hypothesis-driven research. Taken together, the studies described in this research proposal will meet multiple goals and address several unmet needs identified in this grant opportunity, thus significantly enhancing the applicability of a fully autologous and immunocompetent precision model system for use in translational oncology research.

PI Name(s) GRIMES, H LEIGHTON; HALENE, STEPHANIE

Title Modeling myelodysplasia

Institution CINCINNATI CHILDRENS HOSP MED CTR

Abstract Myelodysplastic Syndromes (MDS) are a cancer of the hematopoietic stem cell (HSC) on the rise in the aging population and cancer survivors. The only curative treatment for MDS is allogeneic stem cell transplantation with marked limitations in the majority of MDS patients. As a result, standard-of-care focuses on hypomethylating agents (HMA) azacytidine (AZA) and decitabine (DAC), which invariably result in resistance and disease progression. There is a dire need for new therapeutics; however, there are no robust models of MDS to accelerate preclinical testing. We have generated a breakthrough humanized xenograft-recipient mouse model which eliminates conditioning and facilitates engraftment of primary MDS. We will validate the model by single-cell genetic and genomic characterization of diagnostic MDS patient material before therapy and of the same cells engrafted in humanized mice, clearly delineating the transcriptional impact of xenografting. Next, we will establish pharmacodynamic endpoints for AZA within the mouse model and apply the empirically-derived dose of AZA to the model. Human MDS material will be captured for single cell analyses post-AZA therapy from both patients and xenografts. The multi-omics comparative analyses will incisively determine the utility of MISTRG-W41 for MDS preclinical testing, by illustrating the extent to which AZA-affected programs in patients are similarly changed in the xenograft. This deep molecular, genotypic, and phenotypic understanding of HMA effects on subclonal and hierarchical cellular compositions of MDS will build the basis for comparison of novel-targeted-therapeutic agents as alternatives, concurrent, or post-HMA therapeutic approaches.

PI Name(s) HAIGIS, KEVIN

Title Mouse models of Kras-mutant colorectal cancer

Institution DANA-FARBER CANCER INST

Abstract Colorectal cancer (CRC) kills more than 50,000 Americans each year. Fluorouracil-based therapy remains the standard of care and there have been no targeted therapies approved for use in CRC in the past half decade. Mutational activation of the KRAS oncogene – which occurs more than in 40% of cases – is a major source of intrinsic and acquired resistance to both conventional and targeted therapies in CRC. Since there are no effective therapies that directly or indirectly target K-Ras or its downstream effector pathways, KRAS mutation is the single greatest barrier to medical treatment for CRC. Large scale sequencing of cancer genomes has revealed that, among those 40% of CRCs that express mutant K-Ras, the diversity of KRAS alleles is greater than in any other type of cancer. Epidemiological studies demonstrate that survival and response to therapy varies depending on the KRAS genotype of the patient's cancer, suggesting that different mutant forms of the K-Ras oncoprotein could exhibit distinct oncogenic properties. Experimental validation of allele-specific behaviors has never been achieved, however. We will use primary human and mouse organoids and genetically engineered mouse models to address three key questions relating to K-Ras oncogenicity: (1) Are different mutant forms of K-Ras equivalent in their ability to promote colorectal cancer initiation and progression? (2) Are genetic interactions between KRAS and other genes allele-specific? (3) How do mutant forms of K-Ras influence the tumor microenvironment in a non-cell- autonomous manner to promote cancer progression? The ultimate goal of this work is to decipher the “KRAS Allele Code” in order to identify therapeutic strategies that are effective for cancers expressing specific K-Ras mutants. Precision medicine, where a physician tailors a patient's therapy to the genes that are mutated in his/her cancer, requires this level of understanding, especially for mutant oncoprotein that, like K-Ras, cannot be targeted with direct inhibitors.

PI Name(s) HUNG, CHIEN-FU; RODEN, RICHARD BRUCE

Title Mouse modeling of HPV infection

Institution JOHNS HOPKINS UNIVERSITY

Abstract Our overall goal is to create a laboratory mouse-based model of human papillomavirus (HPV) infection and disease to support the development of novel HPV vaccines. Translational research requires animal models that are robust representations of human pathology in which to test questions of clinical importance, and provide reliable information for the development of novel interventions and patient benefit. HPV is the primary etiologic agent of at least 5% of all cancers worldwide, mostly cervical and subsets of other anogential and head and neck cancers and potentially also non-melanoma skin cancers. Unfortunately, there is no specific antiviral therapy, but vaccine-based approaches are very promising. Many groups are bringing candidate therapeutic HPV vaccines to the clinic, including our pNGLV4aCRTE6E7L2 DNA, but to date there has been limited success in treating patients despite promising data with the vaccines in the current standard animal models. This demonstrates the need for more predictive animal models. HPV does not complete its life cycle and produce virions in mice or in cell culture monolayers and so HPV pseudovirions delivering a reporter construct are often used. This system does not fully mimic the assembly and maturation of the viral capsid in E4-expressing differentiating epithelium or provide a disease endpoint. A model that produces disease from virus produced in a papilloma and expressing clinically-relevant HPV sequences is required. Therefore, we propose to transform the utility of Mus musculus papillomavirus type 1 (MmuPV1) by incorporating key HPV sequences and credential it for use as a model for testing novel therapeutic and protective HPV vaccines. SPECIFIC AIM 1: To develop MmuPV1 viruses incorporating HPV sequences. Organ transplant recipients (OTRs) and HIV+ patients exhibit more severe and progressive HPV disease, and dramatically higher rates of HPV-associated malignancies. Non-melanoma skin cancers (NMSC) in immune-compromised patients are associated with a plethora of βHPVs that were initially described in epidermodysplasia verruciformis (EDV) patients. SPECIFIC AIM 2: To develop an MmuPV1-based mouse challenge model of human cohorts at normal and high risk for the development of HPV-associated cancer. To validate these new murine models of HPV infection and disease we propose to examine the efficacy of a licensed HPV vaccine and two of our clinical grade experimental vaccines that will shortly enter early phase testing: RG1-VLP, a single virus-like particle antigen intended to provide broad immunity against diverse HPV types, and the candidate therapeutic DNA vaccine pNGLV4aCRTE6E7L2 administered via electroporation. SPECIFIC AIM 3: To compare the efficacy of the Gardasil 9, pNGLV4aCRTE6E7L2 DNA and RG1-VLP vaccines against disease and viral endpoints in murine models of healthy subjects and those at high risk for HPV-related cancer.

PI Name(s) JACKS, TYLER E

Title Development of novel metastatic mouse models that recapitulate the major immune contexts of human colon cancer

Institution MASSACHUSETTS INSTITUTE OF TECHNOLOGY

Abstract The purpose of this proposal is to develop novel mouse models of colorectal cancer (CRC) with appropriate immune responses. These models have been designed to address limitations present in current models of CRC in order to enhance their suitability for translational research in immunotherapy. Immune checkpoint inhibitors have revolutionized treatment of solid tumors, and brought to light the critical importance of tumor immune context in treatment outcome. CRC with DNA mismatch repair (MMR) deficiency is characterized by a high burden of somatic mutations, increased T cell infiltration, and a favorable response to checkpoint blockade. Unfortunately, the majority of CRC has a lower mutational burden and is refractory to these treatments. Preclinical mouse models are powerful platforms for investigating the factors underlying response to immunotherapy. However, no single model faithfully recapitulates primary tumor development in the colon microenvironment, metastatic dissemination to the liver and lung, and the major immune contexts underlying variability in immunotherapy response in human CRC. To address these significant translational deficiencies, we will use an innovative technique employing colonoscopy-guided sub-mucosal injection of lentivirus or tumor organoids to induce focal autochthonous and orthotopic tumors in the colon that readily metastasize. In Aim 1, we will engineer a model that modulates tumor immunogenicity through inducible expression of a model antigen. We will dissect the features of the induced anti-tumor T cell response and investigate the utility of this model for testing adoptive T cell therapy by transferring antigen-specific T cells. To potentiate adoptive T cell therapy and mirror ongoing clinical trials in humans, we will assess the efficacy of CRISPR-Cas9-mediated deletion of immune checkpoints in T cells prior to transfer. In Aim 2, we will model immunotherapy-responsive CRC by targeting the essential DNA MMR genes Msh2 and Mlh1 and use next-generation sequencing to characterize the mutational landscapes of resulting MMR-deficient versus proficient tumors. In Aim 3, we will perform preclinical trials of immune checkpoint blockade in these models to explore their ability to recapitulate the responses of human CRC patient populations. We will also test a novel combination of immunogenic chemotherapy and checkpoint blockade, based on the hypothesis that immunogenic cell death may sensitize tumors with low mutational burden or minimal pre-existing T cell involvement to immune attack. This strategy is aimed at improving treatment for the majority of CRC patients, whose tumors are non-immunogenic and non- responsive to immunotherapy. The overarching goal of this research plan is to develop and benchmark a set of highly comparable CRC models that will be used to address why only a fraction of patients respond to immunotherapy. The proposed strategy is innovative in that it uses cutting-edge methods in mouse genetic engineering and cancer modeling to capture critical features of human CRC. This research will also include deep characterization of the immune microenvironment in these models and a comparison to humans.

PI Name(s) JAFFE, IRIS Z; LONDON, CHERYL A

Title Credentialing a Cross-Species Platform to Investigate Cancer Therapy-Associated Cardiovascular Toxicity

Institution TUFTS MEDICAL CENTER

Abstract As survival improves with advances in cancer care, cardiovascular (CV) complications associated with treatment have become more prevalent. Effects of traditional chemotherapeutics are generally well known, but incorporation of small molecule inhibitors and immunotherapeutics has led to the emergence of new and unexpected toxicities. The mechanistic drivers underlying many of these have not been well characterized, undermining both appropriate monitoring and effective intervention. This is further complicated by reliance upon models of CV toxicity that do not fully recapitulate the complicated landscape of human cancer. While in vitro studies permit dissection of cellular and molecular alterations in response to drug exposure, they lack context of the whole organism that contributes to pathogenesis. Rodent models have been instrumental in defining fundamental characteristics of treatment induced CV complications, but, there are significant differences in duration of exposure to therapeutics and an absence of co-morbidities that likely influence outcome. Moreover, their small size and short lifespan limit instrumentation, longitudinal analysis, and repeated sampling. Pet dogs with spontaneous cancer are routinely treated with anti-cancer agents known to produce CV toxicity including doxorubicin, tyrosine kinase inhibitors, and more recently immune checkpoint inhibitors and may thus provide an opportunity for mechanistic interrogation in a more clinically relevant context to bridge the gap from cells and mice to humans. Their larger size and longer lifespan permit the use of prospective study designs in the setting of standard cancer treatment that more closely represent the human experience, thereby overcoming some limitations of rodent models. As such, the fundamental premise underlying this proposal is that no single model system of cancer treatment-induced CV toxicity is sufficient to effectively interrogate mechanistic drivers and assess approaches to therapeutic intervention. Instead, a coordinated, integrated effort across the landscape of multiple in vitro and in vivo model systems is required to efficiently identify and validate biomarkers for early intervention, evaluate novel treatments to address complications, and ultimately develop algorithms for predicting potential CV toxicity in the setting of combination therapy. We therefore propose that inclusion of data generated from dogs with spontaneous cancer treated with agents known to induce CV toxicity will permit a more accurate characterization and confirmation of key mechanistic drivers and therapeutic intervention strategies critical for advancing human outcomes. To accomplish this, we created a non-reductionist, multi-species framework for analyzing data generated in the laboratory, mouse models, dogs with spontaneous cancer, and human patients. The studies in this proposal will credential and optimize this novel platform using two established yet unique CV toxicities that constrain effective treatment in cancer patients -anthracycline induced cardiotoxicity and VEGFRI induced hypertension- ultimately creating a blueprint to better address both existing and emergent cancer treatment induced CV toxicities and enhance long-term survivorship.

PI Name(s) JOSHI, NIKHIL

Title Developing translationally-relevant genetically engineered mouse models of lung adenocarcinoma for investigations in cancer immunology

Institution YALE UNIVERSITY

Abstract Immune checkpoint inhibitors (ICIs) are extending the survival of patients with advanced, metastatic cancer, across many cancer types. Remarkably, immunotherapies may be curative, yet, only a fraction of cancer patients have strong durable responses to ICIs. Response rates also differ greatly between cancer types, and some ICIs are only effective against a handful of cancers. The reasons for this remain unclear, underscoring the need for further research. Many ICIs, like antibodies targeting the PD-1/PD-L1 pathway, act on tumor-specific T cells that are already fighting the cancer at the time the patient is diagnosed with disease. The therapies reinvigorate T cells and cause them to attack and sometimes destroy the cancer. Little is known about the T cells that mediate therapeutic responses or the factors that modulate their therapeutic efficacy. Thus, there is great interest in determining how these therapies work and in augmenting them so that response rates increase. Unfortunately, it has not been easy, because few animal models recapitulate the natural biology of human cancer and elicit detectable anti-tumor immune responses. Genetically engineered mouse (GEM) models are widely used for studies in cancer biology because they allow investigators to study developing tumors and to understand how tumors change over the course of disease. Yet, these gold-standard models are not used for cancer immunology studies because tumors do not express neoantigens, which are required for anti-tumor T cell responses. It has been challenging to develop GEM models where tumors express neoantigens. To remedy this problem, we engineered the “NINJA” mouse, and, in this proposal, we will use NINJA to generate “immunogenic” GEM models for cancer (i.e., models that elicit anti-cancer immune responses). We will standardize immunogenic models for lung and pancreatic cancer, investigate how neoantigens alter the immune cell infiltrates into tumors, and confirm their translational potential as faithful mimics of human cancer. Moreover, we will develop cell line and organoid models from these immunogenic GEMs, which will greatly increase the available tools for researchers in lung and pancreatic cancer. These state-of-the-art models will allow scientists to look at lung and pancreatic tumors at early stages (before cancers would be diagnosed in a patient), and to figure out how these early tumors and immune cells interact. Moreover, our studies will validate NINJA as a platform that can be used by other investigators for the generation of immunogenic GEMs for other cancer types. As these models can be used to improve responses of patients to immunotherapy, NINJA will be useful for enhancing the applicability of almost any GEM model for translational research.

PI Name(s) KARIN, MICHAEL

Title Highly penetrant and immunogenic mouse models of non-viral HCC that are suitable for evaluation of immune checkpoint inhibitors

Institution UNIVERSITY OF CALIFORNIA, SAN DIEGO

Abstract The goal of this project is to develop accurate, innovative, and immunogenic mouse models of hepatocellular carcinoma (HCC) that closely mimic the main etiologies of human non-viral HCC in their pathogenic, transcriptomic, and genomic profiles. These etiologies include non-alcoholic (NASH) and alcoholic (ASH) steatohepatitis, and type 2 diabetes (T2D). Although the current major etiologies that underlie HCC development are hepatitis virus B and C (HBV, HCV) infections, non-viral HCC is predicted to become the major form of this aggressive cancer in the US and Europe in the not too distant future. While the incidence of non-viral HCC and its associated mortality continue to grow at an alarming rate, progress in HCC treatment has been disappointingly slow. Recently, however, inhibitors of the PD-1:PD-L1 checkpoint were found to be much more effective in HCC treatment than any other targeted or non-targeted therapeutic used in the past. Nonetheless, with objective response rates around 20%, there is much room for future improvement. Such improvement depends on better understanding of how immune checkpoint inhibition leads to HCC regression and the identification of adjuvants that enhance the response to this new class of drugs. Both objectives are dependent on the availability of immunogenic mouse models of human HCC. We will bank on our recent success in developing an immunogenic mouse model of NASH-driven HCC that is responsive to PD-1/PD-L1 blockade to develop new and improved mouse models of non-viral HCC that show rapid and synchronized tumor development, making them highly useful for translational research. In addition to NASH-driven HCC, we will develop new immunogenic models of ASH-driven HCC. We will also generate a series of mouse HCC-derived cell lines that give rise to synchronized orthotopic tumors, whose growth and response to treatment can be monitored by in vivo imaging. To determine and demonstrate the human relevance of these models, they will be subjected to extensive genomic and transcriptomic characterization and immunoprofiling. The results of these analyses will be compared to the genomic and transcriptomic profiles of human HCC using innovative computational tools. The following Specific Aims will be pursued: 1) Use the MUP- uPA model of NASH driven HCC to compare the carcinogenic efficacy of different NASH-related diets; 2) Use the MUP-uPA mouse to develop new models of ASH-induced HCC that do not involve HFD feeding; 3) Compare mouse and human HCC genomic, transcriptomic, and immune profiles; and 4) Generate cell lines from the different mouse HCC models that will give rise to synchronized orthotopic tumors that are useful for drug testing.

PI Name(s) KARLSSON, ELINOR

Title Transforming family dogs into a powerful and accessible model for human cancer

Institution UNIV OF MASSACHUSETTS MED SCH WORCESTER

Abstract There is an unmet need for novel approaches to cancer research, including improved model systems. Pet dogs are among the most promising natural models for translational cancer research. They share our environment and develop cancers with clear clinical, histological, and genomic similarities to human cancer. We propose to use new genomic technology and a direct-to-dog-owner approach to overcome existing limitations of the canine model. To accomplish this, we will use new liquid biopsy technology, which makes it possible to sequence tumor exomes in circulating cell-free DNA from a blood sample, and thus achieve deeper understanding of tumor genomics without invasive biopsies. The power of these minimally invasive sampling technologies is greatest in application to very large sets of clinical samples. Family dogs, whose environments are shared with humans and for which tumor genomics are similar to human cancers, offer an unparalleled model in which to assemble clinical sets of size sufficient both to confirm the relevance of known genetic pathways, and to identify new ones. We propose to combine the power of cell-free DNA sequencing, the enthusiasm of citizen-scientist pet owners, and the clinical experience of veterinarians. We will create a research portal for collection of information on diagnosis, treatment, and outcome for thousands of dogs with cancer, as well as their environment and lifestyle. We will also develop new computational methodologies to identify genomic similarities between canine and human cancers. Comparison of these canine and human mutational profiles will enable matching of canine cancer subtypes with human cancer subtypes based on genetic pathways, facilitating canine trials to advance human clinical studies. We aim to: Aim 1. Develop software to Identify canine models for human cancers using genomic data and comprehensive, histology-blind analysis approach. Aim 2. Develop and optimize cell-free DNA sampling and sequencing methods in dogs, including ultra-low-pass whole genome sequencing and whole exome sequencing. Aim 3. Implement a direct-to-dog-owner smartphone app to collect and validate detailed clinical, and environmental data, paired with blood samples, for thousands of dogs. By combining the power of genome sequencing and new liquid biopsy technology with the opportunity to collect large sets of samples from a species whose cancers are genomically reflective of those in humans, our project will transform the scale and scope of translational cancer research and precision medicine.

PI Name(s) LANG, FREDERICK F

Title A Novel Adenoviral-Permissive, Immunocompetent Hamster Model to Evaluate Oncolytic Adenoviral Therapy for Glioblastoma

Institution UNIVERSITY OF TX MD ANDERSON CAN CTR

Abstract Despite oncolytic adenoviruses emerging as promising new therapeutics for patients with glioblastoma (GBM), there are no translational mammalian models in an immune competent animal that are permissive to adenoviral infection and that allow for integration of both the oncolytic and immune effects of these viruses. The overall objectives of this proposal are to develop and characterize a Golden hamster model of GBM that addresses the limitations of existing murine models with respect to pre-clinical testing of oncolytic adenoviral therapy. The central hypothesis is that hamster GBM models will provide reliable pre-clinical data to decipher oncolytic adenoviral therapeutic mechanisms and to evaluate preclinical strategies for translation to patients. The rationale for the proposed research is that understanding the interactions between viral oncolysis and the immune system will uncover mechanisms of therapeutic efficacy, identify which patients might respond best, and reveal new combinatorial therapeutic approaches. The central hypothesis will be tested in three specific aims: 1) Develop hamster glioma stem cell (hamGSC) lines that reflect common molecular alteration in human glioma, 2) Characterize the hamster immune response to oncolytic adenovirus, and 3) Evaluate the effects of pharmacological manipulation of the immune system on the efficacy of Delta-24-RGD. In Aim 1, CRISPR gene targeting will be used to create hamGSC lines driven by specific driver alterations to reflect human GBM molecular subtypes. In Aim 2, the immune response to oncolytic adenovirus will be characterized. Using T-cell depletions strategies, the contributions of immune effector cells to oncolytic virus efficacy and long-term immune memory and the impacts of pre-existing exposure to adenovirus will be evaluated. Aim 3 will evaluate the effects of immune function via either corticosteroids or immune checkpoint inhibitors on oncolytic adenovirus therapeutic efficacy. Collectively, the studies proposed in this application will result in the development and characterization of a novel mammalian model for translational evaluation of oncolytic adenovirus in the treatment of GBM. This contribution is significant because it will overcome many limitations of mouse glioma models and for the first time provide a platform for pre-clinical studies to evaluate the efficacy and mechanisms of oncolytic adenoviral therapy against GBM. The proposed research is innovative because this is the first hamster glioma model that is intracranial, immune competent, and adenoviral replication permissive. This represents a distinct advantage over traditional murine models, particularly in the setting of evaluating immune-modulating therapies such as oncolytic adenoviral therapy.

PI Name(s) LINDBLAD-TOH, KERSTIN

Title Enhancing the dog as a model for human cancers: from genome sequence towards clinical trials.

Institution UPPSALA UNIVERSITY

Abstract The domestic dog has become increasingly useful as a comparative spontaneous cancer model to study genetic and environmental risk factors as well as for easing the transition between rodent and human clinical trials for novel cancer therapies. The many similarities between various cancer types affecting humans and dogs and the spontaneous development of these cancers in immune competent canine individuals living in a shared environment with us suggest a common aetiology. The shorter lifespan of dogs and the shorter time to relapse after cancer treatment allows data regarding efficacy, short and long term toxicity and side effects of novel cancer drugs to be generated in years rather than decades as in human clinical trials. However, certain limitations need to be overcome to make full use of the dog model. Slightly different classification systems for common cancers limit translation of data and clinical outcome from dog to human. The canine genome and annotation, especially that of complex immune gene families, could be improved to allow a more careful and correct comparison with the human genome and immune response, a key factor in cancer development and treatment. Using novel long-read sequencing techniques, we will generate a platinum CanFam4.0 genome and improved information of both gene and variant annotation for an old healthy female German shepherd. In addition, we will specifically focus on canine mammary tumors, lymphoma and osteosarcoma where improved models would benefit human studies, and the canine forms are diverse and only partly characterized. Since the molecular sub-classifications used in human cancers are not yet used for these canine cancers, we plan to characterize these tumor types in the dog population using several approaches to meet human standards. We will further set up a Scandinavian-wide veterinary oncology network for research collaboration, increased use of the dog as a model. We will perform repeated blood sampling from dogs with malignant mammary tumors to allow the study of tumor evolution and progression. All of this work will lay the groundwork to enable more useful and efficient future clinical trials.

PI Name(s) LIU, XUEFENG

Title Conditionally Reprogrammed Cell Model for Castration-Resistant Prostate Cancer (CRPC)

Institution GEORGETOWN UNIVERSITY

Abstract Prostate cancer (PCa) is the most frequently diagnosed cancer (180,890 new cases in 2016) in men in the USA. Androgen deprivation therapy (ADT) is an effective first line therapy for locally advanced or metastatic disease. Unfortunately, once PCa recurs, the eventual development of castration-resistant prostate cancer (CRPC) remains an incurable disease and more effective therapies are needed. Currently, a limited number of cancer cell lines (LnCAP, PC3, DU-145, etc.) are available for research and many genetic mutations present in prostate cancer (e.g., SPOP mutation, FOXA1 mutation, TMPRSS2-ERG fusion, CHD1 loss) are not represented in such cells. New patient-derived cancer models are needed. However, patient-derived xenograft (PDX) models are successful at less than 2-5% efficiency with aggressive, high-grade metastatic tumors and organoid cultures only have an efficiency of 20%. However, our preliminary data demonstrate that conditional reprogramming (CR) has nearly a 100% success rate for establishing long-term cultures from either surgical prostate specimens or CT-guided biopsies. In this application, we propose the following specific aims to validate the potential of CR for translational use in human CRPC. We will first establish CR cultures from biopsies of 30 patients with CRPC and will characterize these culture genetically and phenotypically. Second, we compare the patients' drug response to those of corresponding tumor CR cells and their derivative CR- derived xenografts (CDXs). Lastly, we will use CR cultures in an unbiased high-throughput screen to identify new potential therapies for CRPC in collaboration with Dr. Craig Thomas at National Center for Advanced Translational Sciences (NCATS). New “hits” from the screen will be validated by both in vitro cell assays and xenograft models.

PI Name(s) LOWE, SCOTT W

Title Rapid and flexible precision oncology mouse models of epithelial malignancies epithelial malignancies

Institution SLOAN-KETTERING INST CAN RESEARCH

Abstract Genome characterization has enabled the cataloging of genes altered in human tumors and stimulated the development of therapies that exploit these alterations. Still, functional studies are ultimately needed to interpret and exploit the genetic variation that exists in human cancers. Furthermore, it is now apparent that cancer phenotypes and responses to therapy are dramatically influenced by the tissue microenvironment, and hence it is necessary to have in vivo models that accurately recapitulate both the genetics and physiology of cancers in patients. Although existing genetically engineered mouse models (GEMMs) have been instrumental in validating cancer-promoting mutations and developing therapeutic concepts in a physiological and relevant context, these models are simply too slow and expensive to be broadly useful and only recapitulate a minor fraction of the genetic lesions associated with human cancer. Driven by the need for more accurate and facile models, this project combines CRISPR genome engineering and in vivo organ electroporation with the goal of producing the first-in-kind collection of genetically-defined mouse models of three major epithelial malignancies. We refer to these models as electroporation-based genetically engineered mouse models (EPO-GEMMs). EPO-GEMMs have a range of advantages over traditional GEMMs in that they are fast, affordable, modular, highly portable, and avoid the substantial waste associated with GEMMs produced by strain intercrossing. These models are fully somatic, enable focal tumor development and, importantly, enable the study of tumor-host interactions by allowing tumors to be rapidly engineered in hosts of different genetic backgrounds. Based on substantial preliminary data to validate the EPO-GEMM concepts, our project will produce and characterize EPO-GEMMs of stomach, prostate, and pancreatic cancer - three common human cancers for which existing mouse models do not exist or are tedious. We will then perform a series of demonstration projects to evaluate and illustrate the unique potential of the EPO-GEMM approach, ranging from testing the efficacy and toxicity of target inhibition, exploring the effects of specific immune cell types on cancer initiation and progression, and using synchronous cohorts of genetically defined cancer models to test new targeted therapies and immune oncology approaches. Therefore, our project is of direct relevance to the overarching goals of the Oncology Models Forum, as EPO- GEMMs constitute “translational research models that are robust representations of human biology, are appropriate to test questions of clinical importance, and provide reliable information for patients’ benefit”. Each of these models will be credentialed with the Oncology Model Fidelity Score and all reagents will be made available through the NCIP Hub. We believe that the development and detailed characterization of rapid, flexible, and immunocompetent EPO-GEMMs and the adoption of these models for pre-clinical studies will be critical for the functional annotation of genetic variation in human cancer and greatly contribute to the implementation of precision oncology.

PI Name(s) MAITRA, ANIRBAN

Title Translational Applications in an Animal Model of Pancreatic Cystic Neoplasm and Cancer

Institution UNIVERSITY OF TX MD ANDERSON CAN CTR

Abstract Pancreatic ductal adenocarcinoma (PDAC) has a median 5-year survival of only 8%, and early diagnosis of PDAC is an area of highest priority for the NCI. Amongst the best-recognized risk factors for PDAC are mucinous pancreatic cysts, of which the most common subtype is known as intraductal papillary mucinous neoplasm (IPMN). Currently, IPMN patients either undergo surgical resection due to “worrisome” imaging features, or are followed conservatively by serial imaging studies for risk of progression to invasive PDAC. Unfortunately, the imaging criteria reflexing patients to surgery are imperfect, leading to both over- and under-treatment of IPMNs. Further, there are no credentialed blood-based biomarkers with a sensitivity and specificity that warrants reliable therapeutic stratification. Our group has identified oncogenic mutations of KRAS and GNAS as the two most common driver mutations in IPMNs – one or other is present in ~96% of cases. We have now engineered the first animal model of IPMN that harbors the mutational combination (Kras;Gnas) found most commonly in the cognate human disease. Upon doxycycline induction, the Kras;Gnas mice uniformly develop cystic lesions by 6 weeks, with progression to invasive cancer in 25% of mice by 21 weeks, mimicking the multistep progression of human IPMN to PDAC. The objective of this proposal is to enhance the translational applicability of this model by using it as a controlled platform to address key unmet needs in the management of IPMNs in two areas: imaging correlates and circulating biomarkers. In Aim 1, we will use the animal model to investigate two novel imaging platforms – quantitative feature extraction from MRI scans using an indigenously developed algorithm known as “Enhancement Pattern Mapping” (EPM) and second, hyperpolarized MRI (HPMRI), in order to determine imaging correlates that coincide with the transition from low grade IPMN to cancer. In Aim 2, we will use a combination of unbiased mass spectrometry and array-based approaches to identify circulating proteins and autoantibodies, respectively that correlate with progression of murine IPMNs to PDAC. In addition, we will examine the potential of genomic liquid biopsies for cancer prediction, through utilizing an ultrasensitive and quantitative droplet digital PCR (ddPCR) platform for detection of mutant KRAS and GNAS DNA within circulating exosomes. All three classes of blood-based biomarkers (proteins, autoantibodies and exoDNA) will be assessed in matched murine plasma samples, which will allow us to estimate the additive performance for cancer detection using robust statistical paradigms. Both aims will benefit from ready access to imaging scans and biospecimens from IPMN patients for cross-species translational validation studies, through NCI-funded multicenter U01 consortia that are led by the PI. We believe this multidisciplinary proposal has the potential for long-term impact on PDAC mortality through practice changing alterations in the approach towards monitoring cancer progression in IPMNs.

PI Name(s) NATHANSON, DAVID A; GRAEBER, THOMAS G

Title Mammalian models for integrated metabolic and molecular profiling of malignant glioma

Institution UNIVERSITY OF CALIFORNIA LOS ANGELES

Abstract Translational cancer research requires robust preclinical models to most effectively investigate the underlying biology of disease and develop new therapeutics. While all models are imperfect, it is essential to understand the degree by which each model system (e.g., cell culture, xenograft) recapitulates specific molecular and functional characteristics of human tumors. This may be particularly relevant for studying altered metabolism, a hallmark of cancer, as even subtle changes to the environment can greatly impact the metabolic phenotype of a tumor. Moreover, as cancer metabolism is tightly regulated by oncogenic signaling, the diversity of molecular alterations within a given malignancy may elicit unique metabolic characteristics; which, may greatly influence metabolic pathway dependencies for tumor proliferation and growth. To best determine the fidelity of preclinical models in preserving the metabolic features of human cancer, this requires cross-comparing matched patient tissue and preclinical models across tumors with various genetic alterations. However, such a comprehensive investigation has yet to be undertaken. This proposal will perform an integrated metabolic and molecular characterization of matched human tumors, direct-from-patient orthotopic xenografts (GliomaPDOX), and cell lines from patients with glioblastoma (GBM) – one of the most lethal human malignancies that also reside within the unique brain metabolic milieu. In Aim 1, stable isotope-labeled metabolic tracing and liquid chromatography- mass spectrometry (LC-MS) will be used to cross-compare the metabolic phenotypes of prospectively matched GBM patient tumors, GliomaPDOX, and cell lines to determine the metabolic characteristics that are preserved and/or lost from patient to preclinical model. Aim 2 proposes to determine, in genetically diverse preclinical GliomaPDOX models, whether specific metabolic phenotypes align with distinct molecular signatures. Finally, in Aim 3, in vivo genetic knockdown experiments will be performed to assess whether measured metabolic phenotypes represent targetable dependencies for GliomaPDOX growth, invasion, and survival. Collectively, the studies proposed in this application will provide critical insight into the translatability of preclinical GBM models for studying tumor metabolism; which, may ultimately have important implications for developing new therapeutics against metabolic dependencies in GBM, and potentially, other malignancies.

PI Name(s) PADRON, ERIC

Title Developing and credentialing patient-derived xenograft models to advance therapeutic approaches for chronic myelomonocytic leukemia

Institution H. LEE MOFFITT CANCER CTR & RES INST

Abstract Chronic Myelomonocytic Leukemia (CMML) is a lethal subtype of leukemia characterized by cytopenias, marrow dysplasia, monocytosis, and a propensity for transformation to acute myeloid leukemia (AML). It is among the most aggressive chronic myeloid malignancies with a median survival of 34 months. Recurrent mutations in CMML have been identified in over 40 genes affecting chromatin state, mRNA splicing, hematopoietic differentiation, and cytokine signaling pathways. Unfortunately, despite the increasing number of genetic alterations identified in CMML, no therapies have been developed with the potential to improve the poor natural history of CMML. Moreover, attempts to model CMML using genetically engineered mouse models have not recapitulated the unique clinical or histological characteristics of the human disease. Patient-derived xenografts (PDX) of acute leukemias have been created using immunocompromised mouse hosts that accurately model the disease and have been used to credential putative therapeutic targets in vivo. However, until now, there has been limited success with development of PDX models for CMML. Recently, our groups have overcome this limitation and generated highly and genetically accurate PDX models of CMML through the use of several novel modified NOD/SCID IL2R null (NSG) mouse strains expressing human cytokines that uniquely drive CMML. Moreover, we have utilized these PDX models to identify novel therapies targeting aberrant cytokine-signaling characteristics and mRNA splicing in specific genetic subsets of CMML. In this proposal we aim to further define the fidelity of these models to the human condition and test several novel preclinical therapeutic approaches with immediate translatability to CMML patients as follows: In Aim 1 we will rigorously explore the fidelity of our PDX models compared to their respective patients by determining if PDX models recapitulate the entire clinical, transcriptional, and proteomic spectrum of CMML ; Aim 2 will determine whether CMML PDX models can recapitulate patient-specific responses to JAK2 inhibitors using samples from both our completed phase I/II clinical trial of the JAK1/2 inhibitor ruxolitinib in CMML and a prospective phase 2 ruxolitinib CMML clinical study; Aim 3 will determine the efficacy and mechanism of action of spliceosomal modulatory compounds in CMML PDXs based on spliceosomal gene mutation status. The significance of these studies is that they will create genetically and phenotypically accurate models of an aggressive form of cancer that is lacking preclinical models currently. Moreover, the health relatedness is that our studies will identify novel therapies for this condition, which lacks any effective therapies currently.

PI Name(s) ROSI, SUSANNA; GUPTA, NALIN

Title Myeloid cells and radiation-induced memory deficits in rodent glioma model: sex and age effects

Institution UNIVERSITY OF CALIFORNIA, SAN FRANCISCO

Abstract Ionizing irradiation is commonly used to treat both primary and metastatic brain tumors and can cause a number of late effects including progressive cognitive dysfunction. These cognitive changes are particularly severe in individuals who were treated with radiation during childhood. The extent and nature of the resulting cognitive deficits may be influenced by age, treatment and gender. The neurobiological reason for this difference is unknown, and very few experimental studies have addressed this issue. Ionizing radiation in rodents has been consistently shown to activate several neuroinflammatory signaling cascades that can impact multiple neural processes and synaptic transmission, ultimately disrupting hippocampal function. Neuroinflammation, characterized by activation of brain resident microglia and recruitment of peripherally derived monocytes (collectively referred to as `myeloid cells'), has been consistently associated with the loss of cognitive function in mice after radiation. There are still no treatments for preventing or treating radiation-induced cognitive dysfunction. Despite the extensive clinical evidence linking fractionated brain irradiation with cognitive deficits, there are still unanswered gaps in the biologic basis of this observation: the mechanism/s by which activation of the inflammatory response affect cognitive function, and the effect of age and sex. Furthermore, there are no pre-clinical models that recapitulate the features of the most common clinical scenario: patients with central nervous system (CNS) tumors. Our final therapeutic goal is to prevent and treat the cognitive changes observed after fractionated whole-brain irradiation (fWBI) injury. We hypothesize that changes in the composition and function of myeloid cells following brain irradiation can both prevent and rescue cognitive deficits through durable effects on synapses. The translational objective of this proposal is to demonstrate that resetting the immune system by brief microglia depletion prevents the long-term development of memory deficits in a brain tumor model designed to mimic conventional treatment paradigms used in clinical settings. The specific aims in support of our hypothesis are: 1. Establish the effects of fWBI on memory and synaptic composition as a function of age and sex in an immunocompetent mouse glioma model. 2. Determine the role of myeloid cells in the development of fWBI-induced memory deficits. 3. Evaluate the role of myeloid cells as a mechanistic driver of the permanent memory deficits after fWBI. Very little is known in regard to the evolution of radiation induced pathophysiology in the context of peripherally derived macrophage accumulation or inflammation, and how this relates to altered synaptic and cognitive function. Our final therapeutic goal is to modify the cognitive changes observed after radiation injury.

PI Name(s) SESHADRI, MUKUND

Title Radiogenomic Credentialing of Head and Neck Cancer Models

Institution ROSWELL PARK CANCER INSTITUTE CORP

Abstract Head and neck squamous cell carcinomas (HNSCC) are aggressive neoplasms that result in debilitating changes in speech, appearance, and quality of life in humans. Response rates in HNSCC patients have remained relatively unchanged over the years, especially in patients with human papillomavirus (HPV) negative HNSCC highlighting the critical need for novel strategies to meet the therapeutic needs of this patient population. As recognized by PAR-17-245, a critical step in discovering novel therapies for HNSCC patients is the development of tumor models that can reliably recapitulate human disease biology, heterogeneity and therapeutic response. The overall goal of this application is to validate and credential a panel of patient- derived and immunocompetent models of HNSCC. Systematic and in-depth comparison of histopathologic, genomic, and therapeutic response profiles will be performed across multiple preclinical platforms in vitro (organoids) and in vivo (allografts/xenografts). Paired in vitro and in vivo models across these platforms will be used to assess their response to standard of care chemoradiation and immune checkpoint blockade. The models will also be used to screen the activity of novel and FDA-approved agents (‘drug repurposing’) targeting critical pathways implicated in the pathogenesis of HNSCC. The application builds on an existing collaboration between several investigators at Roswell Park Comprehensive Cancer Center with extensive experience and expertise in mammalian models, head and neck cancer, cancer imaging, tumor immunology, genomics, bioinformatics and cancer therapeutics. The project will employ innovative multimodal functional imaging methods to better define and enhance the true translational utility of mammalian models. The proposal will establish a robust panel of credentialed mammalian models of HNSCC and enable development of an integrated preclinical pipeline to assess efficacy of novel therapeutics, identify resistance mechanisms and enable biomarker discovery in HNSCC.

PI Name(s) SMITH, JESSE JOSHUA

Title Expansion of Tumoroid Models for Precise Treatment of the Rectal Cancer Patient

Institution SLOAN-KETTERING INST CAN RESEARCH

Abstract We propose to develop rectal cancer organoids (tumoroids) as individualized models and to build a large rectal cancer tumoroid repository. Research on rectal cancers is hampered by the paucity of models. Of the few existing in vivo models of rectal cancer, none place the tumors in the rectal lumen, so the models fail to mimic the correct anatomic environment and local invasion. The existing models also have not been observed to metastasize. Another problem is that we lack accurate means to predict whether individual rectal cancer patients will respond to chemotherapy or radiation, both of which are part of the current standard of care. We believe that both the paucity of models and the lack of predictive tools can be addressed by patient-derived tumoroids. Tumoroids can be grown in 3-dimensional culture ex vivo or implanted into mice, so they offer a flexible research platform. In preliminary results, we have derived tumoroid lines from multiple patients' rectal cancers and found them to resemble the corresponding patient tumors. The tumoroids, when implanted in mice endoluminally (i.e. in the rectum), formed locally invasive tumors capable of metastasis. Moreover, we found tumoroids to have clinically relevant responses to chemotherapy and radiation. Thus, drawing from these preliminary data, we hypothesize that rectal cancer tumoroids mirror the traits of their original tumors, can be used to predict patients' response to therapy, and, when implanted endoluminally into mice, can serve as an optimal model of rectal cancer. We plan to develop 100 new tumoroids, which we expect to encompass much of the diversity of human rectal adenocarcinoma. The tumoroids will be analyzed in ex vivo culture and in two mouse models: the endoluminal implantation model and a conventional flank injection model. In these settings, we will test whether the tumoroid accurately reflects its tumor of origin in terms of mutations, histology, and gene expression. We will determine whether response of the tumoroids to patient-specific chemotherapy and radiation can predict the corresponding patient's response. Of particular interest is whether individual human rectal cancers are more accurately modeled by endoluminal implantation than by flank injection. Finally, to integrate our findings into a comprehensive platform for broad use, we will develop a rectal cancer tumoroid biorepository seamlessly integrated with online pathologic, genomic, and model-specific information. The online platform will be built within our institution's cancer genomics portal, then integrated into the NCIP Hub. We have assembled a collaborative team with expertise in colorectal surgical oncology, radiation oncology, and pathology; organoids; mouse models; biostatistics; and bioinformatics. We anticipate that the proposed research will credential tumoroids as accurate models for rectal cancer research and for predicting patient responses to therapy. The large tumoroid biorepository is likely to stimulate research on new treatments for rectal cancer. The ultimate result will be new treatment options and better treatment selection for patients affected by this deadly disease.

PI Name(s) SNYDER, JOSHUA CLAIR

Title Visualizing tumor heterogeneity in an immune intact and autochthonous mouse model of breast cancer

Institution DUKE UNIVERSITY

Abstract Genetically modified mouse models of breast cancer have been used for decades as premier basic science tools for mechanistic discovery. However, the successful implementation of mouse models as surrogates of therapeutic efficacy and translational research has been challenging. One major challenge for status quo approaches is their limited ability to model the genetic heterogeneity observed in breast cancers. Metastatic and treatment resistant HER2+ breast cancers are incurable largely due to this heterogeneity, the source of which may stem from the competition and evolution of multiple oncogenic isoforms of the driver gene HER2. The objective for this proposal is to recapitulate the genetic heterogeneity of HER2 oncogenes in a genetically tractable model more closely resembling the human condition – including an intact immune system and stromal network. Published preliminary data recently described a Cancer rainbow (Crainbow) modeling system for fluorescently barcoding and expressing multiple tumor driver genes in a single immune intact mouse. The fluorescent barcode is retrieved by multispectral imaging and single-cell “omics” techniques providing a simple solution for inducing intratumor heterogeneity and visualizing its evolution. Any tumor driver gene can be incorporated into Crainbow mice. Therefore, this proposal will test the central hypothesis that modeling the oncogenic heterogeneity of HER2 in a Cancer rainbow mouse recapitulates the phenotypic heterogeneity found in treatment resistant and metastatic HER2+ breast cancers. The central hypothesis will be tested by completing four specific aims seeking to: (Aim 1) Validate a HER2 Crainbow mouse model of tumor heterogeneity, (Aim 2) Demonstrate heterogeneity within the tumor epithelium, (Aim 3) Demonstrate heterogeneity of the tumor microenvironment and its contribution to tumor biology, and (Aim 4) Demonstrate heterogeneity and differential response to therapy. HER2 Crainbow mice will provide an autochthonous mouse model of the genetic heterogeneity found in HER2+ breast cancer, all while maintaining the endogenous contributions of the tumor microenvironment to invasion and metastasis. Completing this proposal is expected to validate the HER2 Crainbow mouse as a shareable resource strain for more predictive preclinical trials and a framework for illuminating the molecular and cellular ontogeny of invasive breast cancer.

PI Name(s) SWEET-CORDERO, ERIC ALEJANDRO; HAUSSLER, DAVID H

Title Development of Advanced Preclinical Models for Pediatric Solid Tumors

Institution UNIVERSITY OF CALIFORNIA, SAN FRANCISCO

Abstract Pediatric solid tumors are a rare and highly heterogeneous collection of cancers. For many subtypes, progress in defining novel therapies has stalled over the last 10-20 years. Indeed, for most of these tumors chemotherapy continues to be the primary form of treatment and targeted therapies are not available. This lack of progress is likely due at least in part to the difficulty in developing clinical trials for individual histologic subtypes given their rarity. An alternative is to develop a robust preclinical testing program. Currently, such programs are limited by the lack of well-credentialed models that incorporate the most advanced technologies for genomic and functional characterization and do to the lack of good models for therapy-resistant and metastatic disease. Here we bring together two PIs with complimentary expertise to develop new approaches for validation and preclinical use of pediatric solid tumor animal models. Our focus is on the use of patient- derived xenografts for three most common histotypes: osteosarcoma, ewing sarcoma and rhabdomyosarcoma. PDX models are particularly well suited for studying highly heterogeneous tumors such as pediatric solid tumors. In Aim 1, we will develop novel advance PDX models that incorporate two key innovations: i) utilization of CRISPR/CAS9 technology for genetic interrogation and ii) autologous and allogeneic approaches for development of PDX models with a human immune system (hu-PDX). In Aim 2, we will develop novel computational tools to assess the similarity of PDX models to their tumor of origin and to evaluate human- mouse and mouse-mouse evolution which may impact clinical relevance. We will work closely with members of the Oncology Models Forum to develop scoring systems to assess this similarity and make these tools widely available to the modeling community. In Aim 3, we will evaluate intratumor heterogeneity during human-mouse and mouse-mouse evolution of PDX models using single cell RNAseq. We expect that the tools and models developed here will be widely applicable to other PDX models and that the specific models we develop will help facilitate preclinical research. We will make all tools and models widely accessible to the research community.

PI Name(s) WU, TZYY-CHOOU

Title Development of Novel Spontaneous HPV Cervicovaginal Carcinoma Models for Cancer Immunotherapy

Institution JOHNS HOPKINS UNIVERSITY

Abstract The identification of human papillomavirus (HPV) as a causative agent for a host of conditions, particularly cervical cancer, has led to the development of HPV-targeting therapeutics, including therapeutic HPV vaccines, for the treatment of HPV-associated malignancies. However, the potent efficacies demonstrated by the therapeutic HPV vaccine candidates in preclinical studies are often not reflected in clinical settings. This discrepancy is potentially due to the inability of existing preclinical HPV tumor models to fully replicate the biology of clinical HPV-associated cancers. We hypothesize that an ideal preclinical HPV tumor model should possess the following characteristics: 1) forms spontaneous, localized, HPV oncogenic proteins-expressing tumors; 2) displays carcinoma morphology; 3) possesses a locally immunosuppressive tumor microenvironment (TME) resembling that of clinical HPV+ tumors; 4) tumor formation should follow clinical progression starting from a precancerous to an invasive and metastatic state; 5) be applicable to different MHC class I backgrounds; and 6) the tumor-bearing mice should respond appropriately to immunotherapeutic strategies and generate anti-tumor immunity. Preliminary data: We developed a strategy for the generation of preclinical spontaneous HPV cervicovaginal carcinoma based on orthotopic injection of oncogenic plasmids encoding HPV16-E6, HPV16-E7, constitutively active Akt, luciferase reporter gene, and Sleeping Beauty Transposase (SB) into the cervicovaginal tract of mice with electroporation to enhance transfection efficiency. Subsequent expression of SB induces the integration of plasmid DNA into the genome of transfected cells, resulting in persistent oncogenes expression and spontaneous transformation of transfected cells. In a systemic immunosuppressed setting induced by short-term anti-CD3 administration, intracervicovaginal oncogenic plasmid transfection led to the spontaneous formation of HPV+ tumors with carcinoma characteristics. We propose to further optimize our model by incorporating immunosuppressive molecules that are often overexpressed in clinical cervical cancers into our spontaneous HPV cervicovaginal tumor model and eliminate the need of short-term CD3 depletion. Also, we will further utilize genetic outbred mice and HPV16 pseudovirion delivery of oncogenes for the generation of spontaneous tumors, thereby recapitulating the genetic diverse patient population and HPV16 infection-induced oncogene introduction. Furthermore, we will examine various treatment strategies, such as the combination of therapeutic HPV vaccination with inhibitors of immunosuppressive molecules, in overcoming the immunosuppressive TME for the generation of improved therapeutic antitumor responses. Impact: A novel preclinical HPV cervicovaginal cancer model that faithfully recapitulates the clinical situation would potentiate crucial immunotherapeutic and biological research for HPV-associated cancers, provide better predictions for clinical outcomes of HPV-specific immunotherapies, and permit testing of novel molecular interventions targeting immune suppressive genes.

PI Name(s) ZHU, HAO

Title Improving hepatocellular carcinoma mouse modeling by understanding the malignant potential and biology of liver cell subpopulations

Institution UT SOUTHWESTERN MEDICAL CENTER

Abstract Hepatocellular carcinoma (HCC) is the fastest growing cause of cancer-related death in the United States and one of the leading causes of death in patients with cirrhosis – the end result of any chronic liver injury. HCC is difficult to treat and outcomes have barely improved over the last 30 years, with 5-year survival under 20% and an incidence-to-mortality ratio near 1. Innovative strategies for detection, prevention, and treatment for HCC are desperately needed, the development of which will depend heavily on mouse models of liver cancer. The current models are inadequate because they do not take into account the cells of origin, which will likely have a significant impact on tumor initiation, maintenance, and therapeutic vulnerabilities. While the liver is appreciated for being a central metabolic hub and for its astounding regenerative capacity, cellular heterogeneity in the liver is mostly unexplored. Along the portal to central axis within the hepatic lobule, profound differences in gene expression, metabolism, hypoxia, and ploidy are observed. Whether or not these differences reflect differences in neoplastic potential, and whether or not they influence metabolic disease or carcinogenesis, is unclear. In recent years, there has been intense controversy about whether or not there is a liver stem/progenitor cell. To compound these debates, the injury assays used to drive HCC development often do not model common etiologies such as non- alcoholic steatohepatitis (NASH), which is emerging as the most common cause of cirrhosis in the U.S. We posit that a critical problem for HCC modeling is a lack of understanding of how different cell types contribute to cancer in the context of clinically relevant injuries. We believe that identifying the specific cellular subtypes that give rise to HCC and then being able to genetically perturb these cells is critical for us to better model HCC. Unfortunately, the field does not have mouse reagents to manipulate much of the heterogeneity in the liver. To address this, my lab has optimized CRISPR genome-editing methods to rapidly generate lineage tracing mice. Nine new CreER knock-in models that label different zone-specific and progenitor populations have been produced, effectively quadrupling the number of CreER lines available to our field. We will use these tools to trace and genetically manipulate cell types in a systematic fashion in order to identify the most important regenerative cell populations (Aim 1) and the HCC cell(s) of origin (Aim 2) in the context of clinically-relevant chemical and nutritional injuries. We will then ask if cell type specific gene manipulation of common HCC driver genes will help to uncover different transformation competencies between hepatocyte subpopulations (Aim 3). Success in this project will provide the community with a large panel of important CreER tools, allow the field to focus on important subpopulations that are more likely to transform, and reveal pathways that control tumor development in these cells.