Oncology Models Forum: 2021 Fall Seminar

DATE: Thursday, December 9, 2021
TIME: 1:30 pm – 3:00 pm EST

TIME	TITLE	SPEAKER
1:30 pm – 1:35 pm	Welcome and Introduction	Christine Nadeau, PhD NCI

Models for Studying Gynecologic Malignancies

TIME	TITLE	SPEAKER
1:35 pm – 1:55 pm	*Polymerase-mediated ultramutagenesis and carcinogenesis in mice* **PI: Diego Castrillon, MD, PhD** **UT Southwestern Medical Center**	Diego Castrillon, MD, PhD UT Southwestern Medical Center
1:55 pm – 2:15 pm	*Modeling factors associated with risk of high-grade serous carcinoma in mice* **PI: Kathleen Cho, MD** **University of Michigan**	Kathleen Cho, MD University of Michgan
2:15 pm – 2:35 pm	*Development of novel spontaneous HPV cervicovaginal carcinoma models for cancer immunotherapy* **PI: TC Wu, MD** **Johns Hopkins University**	TC Wu, MD Johns Hopkins University
2:35 pm – 3:00 pm	***Discussion***	All attendees

Mammalian Models for Translational Research: PAR- 17-245

PI Name(s): CASTRILLON, DIEGO

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.

Mammalian Models for Translational Research: PAR-17-245

PI Name(s): CHO, KATHLEEN

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

Institution: UNIVERSITY OF MICHIGAN

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.

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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.