Animal Models for Cancer Interception and Precision Prevention Virtual Workshop

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SPEAKER ABSTRACTS

PLENARY SESSION

Mechanism of Action and an Actionable Inflammatory Axis for Air Pollution Induced Non-Small Cell Lung Cancer: Towards Molecular Cancer Prevention

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Background
A mechanistic basis for non-small cell lung cancer (NSCLC) initiation in never smokers, a disease with high frequency EGFR mutations (EGFRm), is unknown. Air pollution particulate matter (PM) is known to be associated with the risk of NSCLC, however a direct cause and mechanism remain elusive.

Methods
We analysed 463,679 individuals to address the associations of increasing 2.5um PM (PM2.5) concentrations with cancer risk. We performed ultra-deep profiling of 247 normal lung tissue samples, analysed normal lung tissue from humans and mice following exposures to PM, and investigated the consequences of PM in mouse lung cancer models.

Results
Increasing PM2.5 levels are associated with increased risk of EGFRm NSCLC in England, S.Korea and Taiwan and with increased risk of mesothelioma, lung, anal, small intestine, GBM and laryngeal carcinomas in UK Biobank (HR>1.1 for each 1ug/m^3 PM2.5 increment). 18-33% of normal lung tissue samples harbour driver mutations in *EGFR* and *KRAS* in the absence of malignancy. PM promotes a macrophage response and a progenitor-like state in lung epithelium harbouring mutant EGFR. Consistent with PM promoting NSCLC in at-risk epithelium harbouring driver mutations, PM accelerates tumourigenesis in three EGFR or KRAS driven lung cancer models in a dose-dependent manner. Finally, we uncover an actionable inflammatory axis driven by IL1B in response to PM, in agreement with reductions in lung cancer incidence with anti-IL1B therapy.

Conclusions
These results shed light on the etiology of EGFRm lung cancer and suggest that oncogenic mutations may be necessary but insufficient for tumour formation. These data reveal a mechanistic basis for PM driven lung cancer in the absence of classical carcinogen-driven mutagenesis, reminiscent of models of tumour initiation and promotion proposed 70 years ago, providing an urgent mandate to limit air pollution and reveal opportunities for molecular targeted cancer prevention.

Session I

Status of the Current Cancer Prevention Research Animal Models

Carcinogen Induced and Genetically Engineered Models (GEM) for Cancer Interception

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The global cancer burden has been continuously rising and the emphasis on cancer prevention/interception research may significantly reduce cancer occurrence and minimize cancer-related deaths. Early detection of precancerous lesions and preventing/delaying their progression by interception agents may ultimately achieve the goal of cancer prevention. Progress in cancer research and drug development is highly dependent on pre-clinical model systems. The use of optimized animal models that faithfully mimic the genetic and biological evolution of human cancers, assessment of tumor progression, and interception of tumor growth have the potential to markedly improve the success of translating cancer interception in high-risk individuals. For decades, a number of rodent models of chemically induced tumors were developed for several organ-site cancers representing various histopathological and molecular similarities to human cancers that provided better insights into understanding step-wise processes of tumor progression. Importantly, carcinogen-induced lung, colon, breast, skin, and bladder rodent tumor models aided the development of several prevention/interception agents, which apply to secondary prevention, particularly for high-risk individuals. The tobacco-derived chemicals that induce rodent cancers represent high relevance to human cancer etiology, while other chemicals, though not human-relevant, may still be used for interception research. In the past two decades, genetically engineered models (GEM) development has made significant progress. GEMs representing the molecular etiology of human cancers in the appropriate cell types may be ideal for interception agent development. Several GEMs are extensively studied to understand cancer biology and for therapeutic interventions. For interception research, however, fewer GEMs, like the KRAS mutation-driven cancer models (pancreatic, lung, and colon) and MMTV-Neu model for breast cancer, and TMPRSS2-ERG transgenic models for prostate cancer, have been beneficial. In spite of the numerous advantages and playing an essential role in accelerating cancer prevention/interception efforts, there are some challenges and concerns with existing models. These highlight the need to develop better models and validate the findings in alternate models for the successful translation of prevention/interception research. This presentation will discuss the advantages and limitations of existing animal models for interception research.

Animal Models Testing Aerosolized Drug Delivery Approaches for Lung Cancer Chemoprevention

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Aerosol drug delivery in lung cancer chemoprevention offers the advantage of delivering drug directly to the site of tumor formation and also potentially reducing systemic levels of drug, thus lowering the potential for toxicity. We have reported the delivery of several chemopreventive agents including budesonide, bexarotene, epigallocatechin gallate, polyphenon E, gefitinib and Let-7 miRNA as potential strategies for lung cancer prevention in high-risk individuals. In this talk, I will focus on the characterizations of aerosolized delivery of bexarotene and Let-7 miRNA in mouse models of lung cancer. Bexarotene was selected as a candidate for aerosol delivery because of hypertriglyceridemia and hypercholesterolemia observed with oral administration. Using custom-built aerosol delivery system, we have shown that aerosolized bexarotene administered by inhalation has shown potent chemopreventive activities in three different mouse models of lung cancer (adenocarcinoma, squamous cell carcinoma and small cell lung cancer) without causing hypertriglyceridemia. We also evaluated aerosolized Let-7 miRNA as a potential candidate for lung cancer chemoprevention. We found that aerosolized let-7 miRNA exhibited a significant inhibition of a mouse model of lung cancer with no detectable side effects. Single-cell RNA sequencing of tumor-infiltrating T cells from primary tumors reveals that Let-7 post-transcriptionally suppresses PD-L1 and PD-1 expression in the tumor microenvironment, suggesting that let-7 miRNAs may promote antitumor immunity in vivo. Let-7 treatment decreases the expression of PD-1 in CD8+ T cells and reduces PD-L1 expression in lung tumor cells. These results showed the feasibility of evaluating aerosolized chemopreventive agents in mouse models of lung cancer. Aerosolized bexarotene has been successfully undergone IND enabling studies for advancing to human clinical trials.

Nonclinical Considerations for Safety Evaluation of Drugs Intended to Reduce the Risk of Cancer

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This presentation will review the current, general expectations for submission of nonclinical data to support therapeutics intended to reduce the risk of cancer provided to the Center for Drug Evaluation and Research (CDER). Expectations of the types of pharmacology and toxicology studies that support development from submission of a first-in-human investigational new drug (IND) application to submission of a marketing application will be outlined. Because some drugs evaluated for cancer prevention are drugs that have been previously assessed in clinical trials for the treatment of cancer, this presentation will also discuss differences in the expectations for the nonclinical package for drugs intended to treat cancer versus drugs intended to prevent cancer.

Toward rational selection of targeted agents to augment treatment response and prevent recurrence in triple-negative breast cancer

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Triple-negative breast cancer (TNBC) is an aggressive clinical subtype of breast cancer that is characterized by the absence of expression of the steroid hormone receptors (HR) estrogen receptor alpha (ESR1) and progesterone receptor (PGR), as well as the absence of overexpression and/or genomic amplification of oncogenic epidermal growth factor receptor 2 (ERBB2 or HER2). Unlike HR+ and ERBB2-driven tumors, which are treated with either endocrine therapy or ERBB2-targeted therapies, respectively, TNBC largely lacks biomarker-guided selection for treatment with targeted agents. Thus, treatment options for many TNBC patients remain limited to regimens using cytotoxic chemotherapies, which themselves lack predictive biomarkers for guided agent selection.

Even with the most recently used chemotherapy combinations, pCR rates still only reach 55-65%, and both local and distant disease recurrence is frequent. If systemic combination chemotherapy is to remain at the forefront of treatment for TNBC, it is critical that molecular biomarkers of differential response to individual chemotherapy and molecularly targeted agents be identified to enhance treatment response and prevent disease recurrence.

Under PDXNet funding and other grants, we have shown that assessment of candidate targeted agents in a collection of randomly selected PDX models is largely ineffective, as measurable responses to any given agent are detected in only 10-20% of the PDX used. However, if PDX models are chosen rationally based on expression levels of the target and/or evidence that the targeted pathway is active, successful agent evaluation rates improve dramatically.

Recently, using a PDX-based preclinical trial platform with 50 TNBC PDX, we and our collaborators developed a computational molecular feature selection method called CTD that allows prediction of differential response to two commonly used chemotherapy classes, taxanes and platinums. In addition, we are refining a computational method for identifying targeted agents that may augment response to chemotherapeutics and match PDX with these agents rationally. These two approaches are currently being evaluated prospectively in PDX-based preclinical trials for augmentation of treatment response and prevention of recurrence.

Optimizing mouse models for precision cancer prevention

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Precision cancer prevention takes into account the collaboration of intrinsic and extrinsic factors for influencing cancer incidence and aggressiveness in the context of the individual, as well as the recognition that such knowledge can improve early detection and more accurate discrimination of cancerous lesions. In this regard, analyses of genetically-engineered mouse models (GEMMs) should greatly augment precision prevention, since they enable longitudinal and molecular analyses of precancerous and cancerous lesions in defined genetic contexts and in environmentally-controlled conditions, which would be exceedingly difficult to accomplish solely by studying human cancer. However, the relevance of mouse models for prevention research has often been called into question.

So, why have mouse models not been fully integrated in cancer prevention? This is likely reflects actual disparities in their genetics and physiology relative to humans, as well as perceived misconceptions regarding their suitability for prevention research. In particular, because most mouse models are derived from inbred laboratory strains, they may not well suited to inform on the genetic diversity of the human population (with the notable exception of the diversity strains). Furthermore, dissimilarities in the physiology and anatomy of mice relative to humans may hinder effective analyses of the toxicity or pharmacodynamic/pharmacokinetic properties of chemopreventive agents; however, preclinical studies using mouse models can reveal mechanisms of action of such agents, lead to identification of biomarkers of response, and facilitate evaluation of combinations of agents. Furthermore, analyses of mouse models offer unique opportunities to interrogate aspects of cancer prevention that are difficult to study in humans, such as accessing pre-cancerous and/or early-stage cancers with known progressive potential.

I will present current research from my laboratory in which we have successfully used GEMMs of prostate and bladder cancer to study mechanisms of cancer initiation and progression, to identify markers to identify patients at highest risk, and to evaluate new pharmacological approaches for cancer prevention.

Statistical aspects of preclinical cancer prevention studies

Kevin W. Dodd

National Cancer Institute

The National Institutes of Health have identified a need to incorporate rigor into scientific research as a prerequisite for reproducibility. Although statistical theory has a role in the design, analysis and interpretation of results across the entire spectrum of scientific experimentation, some features of preclinical animal models deserve special attention from the statistical viewpoint. This presentation reviews the basic framework of statistical inference and explores the perils to reproducibility caused by 1) poor definition of the experimental unit and 2) multiple testing/unprotected inference that may be exacerbated by the small sample sizes generally used in animal studies.

Session II

Precision Animal Models for High-Risk Cancer Cohorts

Genetically Engineered Mouse Models of Lynch Syndrome

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Division of Cancer Prevention

Lynch Syndrome is caused by inheritance of mutations in DNA mis-match repair genes. Modeling this disease in genetically engineered mouse models is made challenging by the number of DNA mis-match repair genes, the large number of mutations that occur at each locus, complex modulation of penetrance and expressivity of individual mutations, among other factors. In the clinic, the majority of cases are due to inheritance of a mutation in MLH1 or MSH2 with autosomal dominant expression. Loss of the wild-type allele in colon crypts is associated with loss of mis-match repair capability and a cascade of mutations particularly at microsatellite regions of the genome. Carriers of MLH1 or MHS2 have a lifetime risk of colon cancer in the range of 70-80%. Carriers are also at increased risk for endometrial and other cancers. Single base insertions or deletions at coding mononucleotide repeats are particularly common and recur in different individual carriers. Neoantigens generated by these frame-shift mutations have been exploited for development of vaccines. Initial clinical trials in MSI-H colon cancer confirmed safety and immunogenicity of a trivalent frame-shift peptide vaccine in patients with MSI-H colon cancer. To evaluate preventive testing of this vaccine approach, a genetically engineered mouse model of mutant MSH2-mediated Lynch Syndrome was developed that recapitulates features of the spectrum of mutations seen in the clinic. This model demonstrated cancer prevention efficacy of a multivalent frame-shift vaccine and provided a preclinical platform for biomarker development. In order to have a model that develops tumors in an experimentally useful time-frame, it was found necessary to breed homozygous mutant mice. Thus, this model can be viewed to reflect the syndrome of constitutional mismatch repair (CMMR) seen in the clinic. This syndrome is associated with cancer development in childhood and mutations in the PMS2 gene. Modeling of specific PMS2 mutations in mice has been possible and, in combination with mutations in APC or other tumor suppressor genes, results in intestinal cancers. As with the MSH2 model mentioned above, tumorigenesis occurs over a protracted time-frame. This, together with issues in breeding sufficient numbers of mice for interventional studies, makes use of the GEMMs expensive and time-consuming. Models have yet to be developed for endometrial or other Lynch Syndrome-associated tumors. Nonetheless, available GEMMs provide a means for preclinical modeling of mechanistic phenomena that can potentially be exploited for development of preventive and therapeutic interventions.

BRCA2 mouse models for functional analysis of genetic variants and breast cancer prevention

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Breast cancer is the most common malignancy and leading cause of lethality in women. Inheritance of a deleterious mutation in breast cancer susceptibility gene, *BRCA2,* is a well-established factor associated with increased risk of hereditary breast and ovarian cancers. Individuals with mutations in *BRCA2* are also at risk for developing cancer in other organs as well. The penetrance of the disease in mutation carriers has been estimated to be 35-80%. Sequencing based genetic tests are now available to identify *BRCA2* mutation carriers. A major caveat of this approach lies in the interpretation of the risk associated with mutations that do not clearly disrupt the gene product. We have used a mouse embryonic stem (ES) cells-based assay developed in our laboratory to examine the functional significance of BRCA2 variants. To assess the physiological significance of our findings, we are also engaged in generation of novel mouse models. These models serve as powerful tools that have improved our understanding of the effect of these variants on growth and development, hematopoiesis, fertility and tumorigenesis. We have generated mice with a single amino acid substitution (Gly25Arg) in the Partner and Localizer of BRCA2 (PALB2)-binding domain and revealed the essential role of the interaction between the two proteins in the repair of DNA double strands breaks by homologous recombination as well as protection of stalled replication forks. We have generated two mouse models, each with a single amino acid alteration (Pro2510Leu and Arg3052Gln) in the BRCA2 C-terminal DNA binding domain to examine their impact on tumorigenesis. Currently, we are using CRISPR/Cas9 technology to generate mouse models to functionally characterize an important domain of BRCA2, the BRC domain containing eight BRC repeats spanning over 1100 amino acids. Several of these repeats, each consisting of 35-40 amino acids have been shown to bind to RAD51, which is essential for repair of double strand breaks by homologous recombination. In spite of such an essential function, no cancer causing missense substitution has been identified in this region, which suggests functional redundancy between these repeats. We have generated mice with a single BRC, repeat 2 or repeat 4, to examine whether the residual HR activity is sufficient for normal physiological functions including tumor suppression. Our current efforts are focused on differentiating ES cells into mammary organoids and use them to generate mammary glands in mice to determine the tumorigenic potential of BRCA2 variants.

The Interplay of Host Genetics and the Gut Microbiota on Rodent Models of Familial Colon Cancer

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Director of the Mutant Mouse Resource and Research Center at the University of Misouri

Many studies have associated certain intestinal microbial communities with the presence of colorectal cancer (CRC), highlighting particular species that either associated with a healthy colon or presence of disease. The challenge is to develop and validate *in vivo* experimental platforms that can reproducibly interrogate the mechanisms of microorganisms in relation to a defined well controlled host genome. This can reduce the translatability of the results owing to the complexity and diversity of both the human genome and microbiome. The Mutant Mouse Resource and Research Center at the University of Missouri has recognized that both the host genome and complex microbiome need to be recognized as measurable variables that should be recognized as impacting reproducibility and translatability of mouse models. As a model of this impact we investigated the role of the two complex microbiota populations that represent two of the major vendors of mice and their impact on one of the most studied mouse models of intestinal cancer – the C57BL/6-*Apc*^{Min} mouse. Through embryo rederivation we colonized two genetically isolated colonies of *Apc*^{Min} mice with two distinct complex gut microbiotas (GMs). Mice colonized with a simplified GM developed fewer adenomas, while a more complex GM resulted in higher adenoma numbers. Unexpectedly, we found that the two genetically isolated populations also had significantly different adenoma numbers reflecting changes in the host genome over a relatively small generational time that may reflect inadvertent differential selection in the two populations.

To determine if the *Apc*^{Min} mouse experiments reflected a reproducible impact of the GM on adenoma development across species the Rat Resource and Research Center at the University of Missouri rederived the *Apc*^{Pirc} model of FAP on differing complex gut microbiota. Similar to the mouse the different complex microbiomes of the rat significantly altered the early adenoma phenotype. The mouse and rat shared many taxa but there many novel taxa in the rat that seemed to be reflected in the human data sets, such Prevotella copri and *Desulfovibrio spp.*

The challenge with both models is to determine if the platform can be used in a reproducible manner or if these complex GMs were intractable to colonization of single microbes that were identified in original experiments. We chose two different significant taxa in their respective species to see if we could test the system. In the mouse we chose the sulfidogenic Bilophila species associated with higher adenoma numbers. We successfully colonized the GM that did not previously harbor Bilophila but this resulted in lower adenoma burden in the mouse. Community profiling indicated that we significnaly changed the community structure and changed the metabolic capacity of the GM. In the rat we selected one of the significant taxa for testing in a similar complex GM experiment, this time testing the impact of the biofilm forming capacity Desulfovibrio. We used a genetic isolate of a laboratory strain of *Desulfovibrio* that we could genetically alter the type-I secretion system and thus the biofilm capacity of the bacteria. We discovered that the biofilm forming strain could reduce tumor burden in the rat and again alter the community structure of the GM, especially within the mucosal layer.

The use of rodent models can reveal complex gut microbiota community impacts on fundamental biological processes, but it remains to be seen if these impacts are translatable to human disease development. The use of complex systems seems more translatable than germ free and mono-colonization experiments as the complex metabolic system is likely more important to cancer initiation and progression than any single microbe that is not itself enterotoxic. As part of the mission of the Rodent Resource Centers we are continuing to research the translatability of rodent models to human disease. We consider the complex laboratory microbiomes, along with the host genomes, as being the variables that need definition and characterization to improve reproducibility of human disease models.

Ovarian cancer models for Cancer Prevention Research

Ludmila Szabova

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Ovarian cancer is the 5th leading cause of cancer-related deaths in women in US. Women with ovarian cancer are often diagnosed at late-stage disease due to the non-specific symptoms associated with the earlier stages of the disease. The lack of early diagnosis is thus problematic, and there are no effective screening tools available for the general population. Prevention of ovarian cancer in high-risk population (BRCA1/2 carriers) often includes prophylactic bilateral salpingo-oophorectomy that affects fertility and results in negative side effects due to surgical menopause. New prevention strategies including chemoprevention are urgently needed. Genetically engineered mouse (GEM) models for ovarian cancer can serve as an excellent tool for preclinical studies testing new preventive agents. Two such GEM models will be described; one modeling development of ovarian cancer from the ovarian surface epithelium, and the second from the fallopian tube epithelium. Special considerations for each model when used in prevention studies and examples of prevention study designs and outcomes will be presented.

Precision cancer mouse model development through genome editing

Wen Xue

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The liver cancer genome is highly complex, with many point mutations, translocations, and chromosome gains and losses per tumor. Likewise, the clinical presentation of hepatocellular carcinoma (HCC), a major type of primary liver cancer, is highly heterogeneous and its diagnostic, prognostic and treatment assessment is complicated by both tumor biology and compromised liver function due to underlying chronic inflammatory liver diseases such as fibrosis and cirrhosis. To understand the effects of various cancer genes, precise animal models that incorporate both genomic changes in tumor cells and appropriate hepatic microenvironmental milieu are needed. I will describe how CRISPR-Cas9 has been used to create mouse models of liver cancer with point mutations, deletions and complex genomic rearrangements. I will highlight the progress and challenges of such approaches, and how these models can be used to understand progression of liver tumors and identify new strategies for cancer treatment. I will also discuss using CRISPR to model gene correction in liver diseases for advancing novel therapeutics. The generation of CRISPR-based liver cancer mouse models will provide a rapid avenue for functional cancer genomics and pave the way for precision cancer medicine.

Molecular insights into the mechanisms linking diet and microbial metabolites to colon cancer risk

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The aryl hydrocarbon receptor (AhR) plays a tumor-type specific pro-oncogenic or tumor suppressor-like role in cancer. In recent studies, we demonstrated that the AhR and its ligands protect against colonic inflammation and tumor formation in the colon. Transgenic and azoxymethane (AOM)/dextran sodium sulphate (DSS) induced models of colon carcinogenesis in the context of AhR knock out (KO) mice were used to investigate the colonic tissue-specific role of the AhR in colonic stem cells, organoids and tumor initiation/progression. AhR expression was associated with decreased levels of $Lgr5^{+}$ stem cells, organoid initiating capacity and inhibition of colon tumor formation in mutant $APC^{S580/+}$; $Kras^{G12D/+}$ and AOM/DSS-induced mice. In addition, AhR- and AhR ligand-mediated attenuation of FOXM1 and Wnt signaling played a role in suppressing colonic stem/progenitor cell behavior. These results were complemented by single cell transcriptomics on colonic intestinal crypts which also showed that AhR deletion promoted expression of FOXM1 regulated genes in multiple colonic cell subtypes. AhR deletion elevated single-cell entropy (a measure of differentiation potency or cell stemness) and RNA velocity length (a measure of the rate of cell differentiation) in noncycling and cycling $Lgr5^{+}$ stem cells. In general, intercellular signaling crosstalk via soluble and membrane-bound factors was perturbed in AhR null colonocytes.

Noninvasive fecal multiomic features (metabolome, 16S rRNA and host exfoliome) were assessed over time to discriminate the ability of AhR to attenuate cecal and colon tumorigenesis in mutant $APC^{S580/+}$; $Kras^{G12D/+}$ mice. Our initial findings indicate that 17 metabolites serve as effective discriminators between genotypes. Interestingly, 6 of the 17 metabolites are putative AhR ligands. These results suggest that longitudinal deep phenotyping using lumenal AhR metabolites in the gut may predict disease outcomes arising from AhR and Lgr5 related signaling.

In summary, our findings provide new evidence of the molecular function of AhR in modulating putative stem cell driver genes, cell potency lineage decisions and colonic cell-cell communication *in vivo*. Since intestinal stem cells (cells of origin for cancer) are exquisitely sensitive to extrinsic nutritional cues, our findings support the feasibility of using dietary and gut microbial-derived AhR ligands to reduce colon cancer risk.

Session III

Animal Models for Immunoprevention

Novel humanized mouse models for translational tumor immunology studies

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Owing to clinical success of immune-checkpoint blockade, immunotherapy is becoming a cornerstone of modern oncology, and immuno-oncology is at the forefront of basic cancer research. Immune checkpoint blockade unleashes naturally occurring T cells that can recognize cancer cells, by eliminating negative signals that normally hold those T cells in check to prevent autoimmune attack. Yet not all patients experience clinical benefit from these therapies and to realize the full potential of immunotherapy, combination approaches that tackle several immunosuppressive components of tumor environments are needed to activate multiple arms of the immune system. New model systems and technologies are needed to address the gaps in knowledge about basic T cell biology and the challenges faced in the clinical application of immunotherapy. Mice are often used for basic and translational research to support development and testing of immune interventions. However, further development and validation of these systems is needed for preclinical and translational studies of immunotherapy. Humanized mice, mice with a human immune system, have been successfully used for *in vivo* studies of HIV infection, cancer and immunotherapies. Yet human innate and adaptive immune responses remain suboptimal. Limited biologic cross-reactivity between murine and human cytokines is the main contributing factor to suboptimal development of human hematopoietic lineages in humanized mice. To address these research gaps, we have taken a two-pronged approach: 1. We are applying CRISPR technology to generate novel immunodeficient NSG mouse models representing a complexity of the human immune system. For example, we developed *Flt3* knockout (KO) mice to create a more permissive environment for human DC development and constructed human *IL6* knockin (KI) mice; both of which showed higher engraftment when transplanted with low number of hematopoietic progenitor cells (HPCs) and significantly improved human monocyte differentiation in vivo; 2. We are capitalizing on existing models to study human tumor immunology in vivo. For example, using NSG mice with transgenic expression of human SCF/GM-CSF/IL-3 (NSG-SGM3), we uncovered a previously unknown role of bone marrow-derived KIT+ cells in support of visceral metastasis by melanoma. Thus, humanized mice offer novel tools for basic and pre-clinical studies in human immunology.

Humanized EBV mouse models

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Epstein-Barr virus (EBV) causes a variety of different types of lymphomas in humans, including post-transplant lymphoproliferative disease (PTLD), diffuse large B cell lymphoma (DLBCL), Hodgkin lymphoma (HL) and Burkitt lymphoma (BL). EBV cannot infect mouse cells, but humanized mouse models, which support the growth of EBV-infected B cells and anti-viral T cells and NK cells, are increasingly used to examine the ability of various different components of the host immune response to inhibit EBV-induced lymphomas and to test potential immunotherapies for treatment of EBV-positive lymphomas. In addition, humanized mouse models are being used to examine the roles of different viral proteins in the development of EBV-induced lymphomas, and often reveal different results compared to traditional *in vitro* B cell transformation assays. This talk will review the use of humanized models to study host immune control of EBV-induced B cell lymphomas, to develop novel therapies, and to define mechanism(s) by which EBV induces lymphomas *in vivo*. The pros and cons of humanized mouse models for understanding pathways by which EBV causes lymphomas in humans and studying new treatments and prevention strategies will also be discussed.

LOSS OF COMPLEMENT C3a RECEPTOR (C3aR) IN A NOVEL MODEL OF SPONTANEOUS COLORECTAL TUMORIGENESIS: POTENTIAL FOR IMMUNOPREVENTION AND THERAPETIC INTERVENTIONS

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Colorectal cancer (CRC) is the third leading cause of cancer-related deaths, the third most commonly diagnosed cancer worldwide and has been showing a consistent yet puzzling increase in the young population. More than 50% patients with earlier diagnosis experience progressive disease during treatment, which makes CRC a significant public health burden.

Despite the inflammatory nature of the majority of CRC, only a minority is currently amenable to immunotherapy. Genetic susceptibility, environmental exposure, lifestyle factors, immune inflammation and microbiota are all known players in CRC development. Further, in line with the pivotal role of the intestinal barrier in preserving gastrointestinal health, accumulating evidence indicate that the impaired function of the intestinal barrier is associated with CRC development. Alterations in innate sensing mechanisms at the barrier play a crucial role in intestinal inflammation and CRC and the complement is a key player in this context as it can establish extensive networks with other innate immune and adaptive pathways and can directly affect intestinal epithelial cells. Studying CRC development and therapeutic avenues has been partially hindered by the sparsity of suitable mouse models to recapitulate the human disease.

We recently analyzed publicly available datasets and reported the first evidence that patients with CRC undergo down-regulation of the C3aR, indicating that the C3a-C3aR axis is highly relevant in human CRC. We found that C3aR down-regulation is associated with methylation of the CpG island of the c3ar1 gene promoter, indicating that epigenetic modifications may affect C3aR expression in CRC. By crossing the C3aR-/- mice with $APC^{Min/+}$ mice we found that the $APC^{Min/+}$/C3aR-/- mice developed numerous polyps in the colon and showed defective intestinal permeability. Our newly generated model more closely resembles the human anatomic location and offers a tool to study the tumorigenic process from the early dysplastic phase.

Furthermore, we found that absence of C3aR resulted in significant modifications of the gut microbiota characterized by the expansion of Bacteroidetes and Proteobacteria phyla, which were sufficient to enhance colon tumor growth. The analysis of the tumor microenvironment revealed that, similar to the patients with C3aR down-regulation, the tumors originating in the colon in the absence of C3aR signaling showed marked innate and adaptive immune infiltrates. Our preliminary data indicate that these immune cells could be exploited to render these tumors susceptible to the treatment with checkpoint blockade inhibitors.

Finally, we found that C3aR is downregulated in the early stage of patients with colon cancer. Therefore, finding the mechanisms of C3aR methylation and down-regulation in the early stage may halt the causes of a latent inflammation potentially preventing the establishment of CRC.

Modeling the p53 tumor suppressor pathway in mice

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Disruption of the p53 tumor suppressor pathway commonly occurs via missense mutations, many of which exhibit gain-of-function activities such as increased tumor aggressiveness and metastasis. We have developed novel conditional mutant *p53* alleles that switch wild type p53 to mutant in a Cre-specific manner to explore the role of the microenvironment and immune system in tumor development and progression. A somatic model of breast cancer with metastases will be discussed. These models most closely simulate the genesis of somatic cancers and will thus be invaluable in testing novel therapeutic combinations.

Session IV

Next Generation Models for Prevention Research

Preclinical models of pancreatic cystic neoplasia

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Pancreatic cancer is the 3rd most common cause of cancer related mortality, with the overwhelming majority of patients presenting with advanced, surgically unresectable disease. Earlier detection and cancer interception has the greatest potential of improving cancer related mortality attributable to this otherwise lethal disease. Pancreatic cystic neoplasms (PCNs) are well established non-invasive precursor lesions of pancreatic cancer, of which intraductal papillary mucinous neoplasms (IPMNs) are the most common subtype. Retrospective studies have demonstrated that progression to invasive neoplasia on the backdrop of non-invasive PCNs has a profound and deleterious impact on prognosis, reiterating the importance of intercepting cancer in these lesions. However, standard of care surveillance of PCNs is essentially passive imaging-based surveillance, and there are scant cancer interception approaches that might revert the path to invasive neoplasia. Building the evidence base for cancer interception in PCNs will require the availability of animal models that genocopy and phenocopy the cognate human disease. The genomic landscape of IPMNs is characterized by oncogenic "hotspot" mutations of *KRAS* and *GNAS*, and loss of function mutations of the E3 ubiquitin ligase encoding gene *RNF43*. To model IPMNs in mice, we have genetically engineered two independent models – the first expressing pancreas-specific compound heterozygous oncogenic mutations of $KRAS^{G12D}$ and $GNAS^{R201C}$ – henceforth referred to as "*Kras;Gnas*" mice; and the second expressing pancreas-specific oncogenic $KRAS^{G12D}$ mutation in conjunction with bi-allelic loss of *RNF43* (henceforth referred to as "*Kras;Rnf43*" mice). Both the "*Kras;Gnas*" and the "*Kras;Rnf43*" models demonstrate multistep progression of pancreatic cystic neoplasia to invasive adenocarcinoma, phenocopying human IPMN progression. We have generated secondary reagents from these models, including cell lines and organoids, which can be used for elucidating the mechanistic underpinnings of cancer formation on the backdrop of PCNs. Moreover, the availability of autochthonous models of PCNs that faithfully recapitulate human IPMNs provides an unprecedented opportunity to both identify appropriate molecular targets for cancer interception across species, as well as to validate pancreatic cancer interception strategies in the appropriate *in vivo* context. Selected examples of how these models are being used to elucidate and validate molecular targets for cancer interception will be presented, integrating various platforms and profiling approaches such as spatial transcriptomics, MALDI and immunopeptidome analyses.

Mouse-INtraDuctal: An *in vivo* model for studying breast cancer progression

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Background: A large fraction of human DCIS (>50%) may not need the multimodality treatment options currently offered to all patients. More importantly, while we may be overtreating many, we cannot identify those most at risk for invasion/metastasis. Revealing the cellular and molecular mechanisms by which some DCIS remain indolent while others advance to invasive and metastatic breast cancers is currently a clinical unmet need. **Methods:** To address this gap, we developed the Mouse-INtraDuctal (MIND) model, by which patient-derived (PDX) DCIS epithelial cells are injected intraductally and allowed to progress naturally in mice. Single cell RNA-sequencing (scRNA-seq) was utilized to profile the DCIS epithelial and stroma cells in progressors vs. non-progressors. To distinguish between stromal (diploid) cells and tumor (aneuploid) cells, we calculated Copy Number Aberration (CNA) profiles from RNA using CopyKAT. Cell-type specific differential gene expression analysis of DCIS epithelial cells and microenvironment cell types in progressors and non-progressors was performed. We also predicted putative ligand:receptor interactions between the tumor cells and cell types in the microenvironment by CellPhoneDB. **Results:** Among 37 PDX DCIS MIND models followed for a median of 9 months, 20 (54%) grafted into 95 glands, showed *in vivo* invasive progression (progressed) while 17 (46%), injected into 107 glands, remained non-invasive (non-progressed). ScRNA-seq was performed on 13 DCIS samples including 10 progressors and 3 non-progressors. Aneuploid cells were further analyzed to identify deferentially expressed genes that were upregulated in progressors compared to non-progressors (log2 fold=1, FDR $p<0.05$). Notable genes included *NEAT1*, *EIF4EBP1*, *SCGB2A2, TFF1 and TFF3* that were upregulated in the progressors. *NEAT1*, the core structural component of the paraspeckles, is frequently overexpressed in human cancers and its expression is correlated with worse survival in cancer patients. *NEAT1* drives tumor progression by regulating genes involved in cellular growth, migration, invasion, metastasis, EMT, stemness, radio- and chemoresistance, supporting its role as a potential biomarker and therapeutic target. Further analysis using Cancer Hallmarks identified mitotic spindle, interferon signaling, DNA repair, oxidative phosphorylation and P53 pathway among the top signatures that were upregulated in the progressors. Further analysis by CellPhoneDB identified expression of several receptor/ligand interactions including CD74/MIF involved in epithelial/stromal and stromal/stromal cross talks that may play a role in DCIS invasive progression.
Conclusions: MIND models provide a realistic tool for identification of biomarkers of high-risk DCIS and for the discovery of novel therapies to block DCIS transition to invasive and metastatic breast cancers.

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Bioengineered Models of the Female Reproductive System: Understanding uterine diseases

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Advancements in technology are changing the *in vitro* models used to investigate mechanisms of disease. In order to more accurately phenocopy human biology, researchers have been moving towards three-dimensional (3D) culture techniques and microfluidic technology. We developed the first multi-organ microfluidic platform to study the female reproductive tract. A second generation version was recently developed for biological research that was cost-effective, easy-to-use, and familiar. Using this new system, termed LATTICE, we have demonstrated physiologic function of 3D cultures of reproductive tissues including murine ovarian tissues, human fallopian tube epithelium, and human endometrial organoids, as well as human adipocyte spheroids, and murine pancreatic islets. We are also using LATTICE to model diseases including polycystic ovarian syndrome and endometrial cancer. New technologies that move towards more closely modeling physiology can generate meaningful data and can better predict biological responses to insults or treatments.

Virtual experiments with multiscale agent-based models

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Model systems—whether they are cell cultures, bioengineered tissues, animals, or theoretical models—provide simplified, controlled systems where experiments can shed new insights on otherwise intractable, complex biological problems. Increasingly sophisticated experimental model systems are giving incredibly detailed glimpses into biology, but time and cost can limit their scalability. In this talk, we introduce agent-based modeling—where individual cells are represented as software objects that move and interact in simulated tissues—as an approach to create a virtual model systems for massively parallel virtual experiments. After showing some examples from cancer biology, we show new results on creating virtual models "on the fly" by automatically translating expert hypotheses into model code. We will close with discussion of current challenges and opportunities to integrate virtual and experimental model systems.

Pancreatic cancer progression using model systems

Claudia Tonelli and David Tuveson

Abstract

The optimal therapeutic response in cancer patients is highly dependent upon the histological differentiation state of their tumors. Pancreatic ductal adenocarcinoma (PDA) is a lethal cancer that harbors several distinct subtypes with preferential sensitivities to standard therapies. Using single-cell RNA sequencing, we uncover multiple preinvasive and invasive neoplastic cell subpopulations representative of intratumoral heterogeneity and cellular plasticity in a mouse model of PDA. We identify Fgfr2 in the last stage of pre-invasive cancer cells, where a highly mucinous cell state is present, and show that it is required for progression of PanIN. Further evaluation identified the ETS family transcription factor Spdef as an important regulator of the mucinous cell state. By comparative gene expression analysis of tumor differentiation states in mice and humans, we find that the Spdef program is highly expressed by human Classical PDAs. Furthermore, PDA cells expressing elevated levels of Spdef are dependent upon it for tumor progression *in vivo*. The tumor-promoting function of Spdef is recapitulated by its targets Agr2 and *Ern2*/Ire1β that regulate protein folding and endoplasmic reticulum activity and thus prevent aberrant mucus production. Following inactivation of the Spdef program, cells escape by initiating Classical-to-Basal-like tumor trans-differentiation. Our findings offer insights into the factors controlling lineage plasticity in PDA and reveal cell state-specific vulnerabilities and cellular features of resistance.

Session V

Large Animals for Translational Research

“How dogs are helping us understand cancer”

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Comparative oncology clinical trials play an important and growing role in cancer research and drug development efforts. These trials, conducted in companion (pet) animals, allow assessment of novel anticancer agents in a veterinary clinical setting that supports serial biologic sample collections and exploration of dose, schedule, and corresponding pharmacokinetic/pharmacodynamic relationships. Further, an intact immune system and natural co-evolution of tumor and microenvironment support exploration of novel immunotherapeutic strategies in these veterinary patients. Significant improvements in our collective understanding of the molecular landscape of veterinary cancers, mainly in dogs, have occurred in the last 10 years, facilitating the translational research process and supporting the inclusion of comparative studies into the drug development paradigm. Further, recent clinical trials carried out in pet dogs demonstrate how this approach can assess efficacy in a variety of settings, including but not limited to single agent or combination response rates, inhibition of metastatic progression, and randomized comparison of multiple agents in a simultaneous head-to-head fashion. Such comparative oncology studies have been purposefully included in the developmental plan for several FDA-approved and up-and-coming anticancer drugs. Challenges for this field include keeping pace with technology and data dissemination/harmonization, improving annotation of the canine genome and immune system, and generation of canine-specific validated reagents to support integration of correlative biology within clinical trial efforts.

Large Animal Platform for Earlier Pancreatic Cancer Detection and Prevention

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University of Illinois Cancer Center

There have been modest improvements in pancreatic cancer (PC) survival over the past 4 decades despite a plethora of preclinical assessments testing the efficacy of novel and/or combination therapeutic regimens. Mouse models have provided an amazing glimpse into PC etiology and the convergence of signaling cascades likely to contribute to disease initiation and progression. Much of this data has defined potential targets and points of chemoresistance though preclinical efforts have mostly failed to be recapitulated in human clinical trials. One means to extend findings in mice has led to the development of a large animal platform to provide an in vivo model that would better match human anatomy and physiology with the size to assess imaging modalities, novel in vivo detection devices, relevant drug dosing and metronomics, and extensive solid and liquid samples for multi-investigator use. To date, three PC pig models have been engineered including the first one where adenocre virus was injected retrograde into the main pancreatic duct of a pig harboring a Cre-responsive transgene to express mutant Kras and mutant p53. These pigs generated localized PC though with accompanying sarcoma. A second model has been established that employs a similar system though with adenocre virus injection into the pancreatic duct within the collecting lobe, and these pigs also developed PC but with a powerful inflammatory component that often overwhelmed these pigs. The final PC pig model resembles KPC mice in that p48 or Pdx1-Cre induced expression of mutant Kras and p53 from endogenous mouse alleles. These mice developed a PC sequela from PanIN-like lesions to invasive and metastatic disease with limited caveats as observed in the first two PC pig models. A future PC pig model can be engineered with inducible expression of human mutant Kras and p53 alleles to create pancreatic neoplasia and cancer derived from adult cells. Also, these pigs can be established in a background that has metabolic features more common to humans on a high fat diet than standard pigs or mice. Indeed, various high fat diets can be examined in this large animal platform to inform on in vivo detection methods including endoscopy, an ex vivo tool that can establish a molecular profile for surgical margins, and a keen system for training surgeons and endoscopists.

Dogs Are Not a Model: Status of a Canine Preventative Cancer Vaccine Trial (VACCS)

Doug Thamm (Colorado State University), Jenna Barton (Colorado State University), Jennifer Willcox (UC-Davis), David Vail (University of Wisconsin), Jens Eickhoff (University of Wisconsin) Stephen Albert Johnston (Calviri Inc).

Probably the most attractive solution to the problem of cancer is to develop a vaccine to prevent it. We think this may be possible and are testing the idea in the world's largest canine interventional clinical trial. What has enabled the vaccine is the discovery that tumors make frequent and shared (across patients and tumor types) mistakes in RNA processing that create frameshift neoantigens (FSPs). There are ~1.4M possible such FSPs that are bioinformatically predicted that could be produced in dog tumors. Fortunately, relative to screening these FSPs, dogs and people with tumors generate antibodies to these FSPs. We manufacture peptide arrays displaying FSPs for ease of screening. Sera from dogs with the major types of tumors were screened on arrays displaying ~30K FSPs. From this screen 31 FSPs were chosen to constitute a vaccine to be given to dogs without known cancer or history of cancer.

With this vaccine a trial was initiated (Vaccine Against Canine Cancer Study, VACCS) in May 2019. 800 owner-enrolled dogs are participating. It is an equal arm, double-blind (triple if you count the dogs) trial of up to 5 years. Dogs were enrolled at 3 leading academic veterinary cancer centers. All dogs were initially screened for pre-existing tumors, including ultrasound. The vaccine was a DNA prime with a peptide+Hiltonol boost. Dogs are given an exam every 6 months with blood collection and a boost yearly with PBMC collections. The trial is starting the 4th of 5 years. The primary end point is the number of malignant tumors in the control versus vaccine arm. The trial is powered to detect a 30% reduction. Secondary endpoints include time to diagnosis, average age at death etc.

The trial has had two independent safety reviews. No evidence of vaccine associated adverse events were noted. ~90 malignant tumors have been recorded. The first efficacy assessment is in progress. There is little known about latency of tumors in dogs. We estimate that it is on average ~2 years, which actually makes it harder to develop a preventative vaccine in dogs relative to humans. Because of the latency most tumors detected in the first 2 years probably already were present at the time of enrollment. Therefore, we do not expect to see an efficacy effect until years 3-5.

Several things of note in the trial. 1) Ultrasound screening was not useful, 2) 10x more tumors are being diagnosed at stage 1 compared to standard practice, 3) canine immunology is not as well developed as human – we had to develop assays, 4) owners are very motivated to participate in the trial – for the sake of dogs and people, 5) ~35% of the vaccine dogs did not develop an immune response to the vaccine.

We think a better vaccine can be developed. Only ~2% of the possible FSPs were sampled to create the current vaccine. We will screen all 1.4M for the next composition. The delivery was suboptimal and can be improved and simplified. A version 2 for testing is being prepared. The reason dogs are not a model: the estimated market potential in US of a dog preventative vaccine is $5B/yr.