2023 ANNUAL MEETING

December 5 - 6

ONCOLOGY MODELS FORUM

The National Center Institute's Division of Cancer Biology held the Annual Meeting of the Oncology Models Forum on December 5 and 6, 2023. This meeting was held in Rockville, Maryland, at NCI's Shady Grove Campus.

Mammalian models and their derivatives are integral components of basic cancer research. The Oncology Models Forum supports mammalian models that overcome translational deficiencies of mammalian oncology models and define new uses mammalian models or their genetics for unexplored translational challenges. Members of the Oncology Models Forum spur the development of mammalian models that advance standard practices for translational use, test approaches to validate and credential models, or challenge current practices for how models are used translationally. The demonstration of these models as robust representation of human biology that are appropriate to test questions of clinical importance will provide reliable information for patient benefit.

The purpose of the meeting was to stimulate information sharing and collaborations between Oncology Models Forum Members.

Agenda

Day 1 – December 5, 2023

Entry: 8:30 AM

Session 1: State of Modeling
9:00 – 10:35 AM
Moderator: David Gutmann
Panelists: Josh Snyder, Mukund Seshadri, Elinor Karlsson

Time	Talk Title/Subject	Speaker
9:00 – 9:15 AM	Opening Remarks	Dan Gallahan (NCI)
9:15 – 9:20 AM	Meeting Introduction	Christine Nadeau (NCI)
9:20 – 9:50 AM	Precious GEMs: Lessons learned from mouse modeling	David Gutmann (Washington University)
9:50 – 10:00 AM	State of Modeling: Murine	Josh Snyder (Duke University)
10:00 – 10:10 AM	State of Modeling: Patient-derived	Mukund Seshadri (Roswell Park Comprehensive Cancer Center)
10:10 – 10:20 AM	State of Modeling: Canine	Elinor Karlsson (Broad Institute)
10:20 – 10:35 AM	Panel Discussion	Moderated by David Gutmann

Break: 10:35 – 10:45 AM

Session 2: Modeling Translational Questions: Technology I
10:45 AM – 12:45 PM
Moderator: Jesse Boehm

Time	Talk Title/Subject	Speaker
10:45 – 11:00 AM	Spontaneous Canine Osteosarcoma: A Translationally Relevant Patient Model for Humans	Amy LeBlanc (NCI)
11:00 – 11:15 AM	Novel somatic mutant IDH glioma model identifies high-risk patients with distinct copy number and tumour microenvironment alterations	Aditya Shroff (University College London)

11:15 – 11:30 AM	Resource Building to Enhance the Canine Model in Cancer Vaccine Development	Shaying Zhao (Georgia)
11:30 – 11:45 AM	Large DNA transgenesis using the Cas9+Bxb1 toolbox in mice	Vishnu Hosur (Jackson Laboratory)
11:45 AM – 12:00 PM	Lentiviral Delivery of Oncogenes to Model Glioma in the Swine Spinal Cord	Nick Boulis (Emory University)
12:00 – 12:15 PM	Human Cancer Models Initiative (HCMI): A community resource of next-generation cancer models and associated data	Julyann Perez-Mayoral (NCI)
12:15 – 12:45 PM	Discussion	Moderated by Jesse Boehm

Lunch: 12:45 PM – 1:30 PM

Session 3: Modeling Translational Questions: Immunity

1:30 PM – 3:00 PM

Moderator: Deeann Wallis

Time	Talk Title/Subject	Speaker
1:30 – 1:45 PM	Loss of NF1 drives hormone dependent mammary carcinogenesis in a rat model with intact immune system	Deeann Wallis (University of Alabama at Birmingham)
1:45 – 2:00 PM	Mechanisms of resistance to anti-BCMA bispecific antibodies in multiple myeloma: lessons from the Vk*MYC mouse model (virtual)	Marta Chesi (Mayo Clinic Arizona)
2:00 – 2:15 PM	Developing translationally relevant genetically engineered mouse models of lung adenocarcinoma for investigations in cancer immunology	Kelli Connolly (Yale University)
2:15 – 2:30 PM	Exploring How the Intrinsic Diversity of Cancer DNA Damage Phenotypes Intersects with Immune Context	Mary Helen Barcellos-Hoff (University of California San Francisco)
2:30 – 3:00 PM	Discussion	Moderated by Deeann Wallis

Break: 3:00 PM – 3:10 PM

Session 4: Modeling Translation Questions: Therapy

3:10 PM – 4:40 PM

Moderator: Elinor Karlsson

Time	Talk Title/Subject	Speaker
3:10 – 3:25 PM	Leveraging canine spontaneous cancer to optimize the power of blood biopsy	Heather Gardner (Tufts University)
3:25 – 3:40 PM	Expansion of Tumoroid Models for Precise Treatment of the Rectal Cancer Patient	Josh Smith (Sloan-Kettering Institute of Cancer Research)
3:40 – 3:55 PM	Understanding cancer using dogs and many mammals	Kerstin Lindblad Toh (Uppsala University)
3:55 – 4:10 PM	Credentialing next-generation human glioma models for precision therapeutics (virtual)	Ryan Miller (University of Alabama at Birmingham)
4:10 – 4:40 PM	Discussion	Moderated by Elinor Karlsson

Break: 4:40 – 4:50 PM

Session 5: Early-Stage Investigator/Trainee Flash Talks

4:50 PM – 5:20 PM

Moderator: Justin Benavidez

Time	Talk Title/Subject	Speaker
4:50 – 4:55 PM	Electroporation-based genetically engineered mouse models (EPO-GEMMs) as a versatile platform for evaluating efficacy and safety of CAR T cell therapy	Zeda Zhang (Memorial Sloan Kettering Cancer Center)
4:55 – 5:00 PM	Dynamic systems modeling in HER2 Crainbow mice reveals critical inflection points of malignancy	Josh Ginzel (Duke University)
5:00 – 5:05 PM	Establishing a Clinically Relevant Mouse Model to Study Necrosis as a Primary Variable in Glioblastoma	Jiabo Li (Northwestern University)
5:05 – 5:10 PM	Genotyping Canine MHC class II genes using RNA-seq data	Nikitha Sundaresha (University of Georgia)

5:10 – 5:15 PM	Overcoming the inhibitory FcγRIIB barrier unlocks Fc-dependent activity of human anti-CTLA-4 antibodies	Lucas Blanchard (Rockefeller University)
5:15 – 5:20 PM	Enabling investigation of gene-environment interactions and cancer risk in pet dogs and cats	Kate Megquier (Broad Institute)

Poster Session: 5:20 – 7:00 PM

Day 2 – December 6, 2023

Entry: 8:30 AM

Session 6: Fidelity and Reproducibility in Animal Modeling
9:00 – 11:00 AM
Moderator: Mukund Seshadri

Time	Talk Title/Subject	Speaker
9:00 – 9:05 AM	Day 2 Welcome	Justin Benavidez (NCI)
9:05 – 9:10 AM	Office of Research Infrastructure Programs and Rodent Resources	Oleg Mirochnitchenko (NIH)
9:10 – 9:30 AM	Rat Resource and Research Center: Rats, Reproducibility, Translatability, and Best Practices	Elizabeth Bryda (University of Missouri)
9:30 – 9:45 AM	Rapid ex vivo biosensor cultures to assess dependencies in gastroesophageal cancer	Jesse Boehm (Massachusetts Institute of Technology)
9:45 – 10:00 AM	Modeling myelodysplasia	Stephanie Halene (Yale University)
10:00 – 10:15 AM	Improving the translational value of head and neck cancer patient-in-mouse models	Randall Kimple (University of Wisconsin–Madison)
10:15 – 10:30 AM	NCI Patient Derived Models Repository	Yvonne Evrard (NCI)
10:30 – 11:00 AM	Discussion	Moderated by Mukund Sheshadri

Break: 11:00 – 11:10 AM

Session 7: Modeling Translational Questions: Technology II

11:10 AM – 12:40 PM
Moderators: Josh Snyder and Elizabeth Bryda

Time	Talk Title/Subject	Speaker
11:10 – 11:25 AM	Visualizing tumor heterogeneity in an immune intact and autochthonous mouse model of breast cancer	Josh Snyder (Duke University)
11:25 – 11:40 AM	Sharing Models and the Biorepository for the Children's Oncology Group	Patrick Reynolds (Texas Tech University Health Sciences Center)
11:40 – 11:55 AM	Highly penetrant and immunogenic mouse models of non-viral HCC that are suitable for evaluation of immune checkpoint inhibitors (virtual)	Michael Karin (University of California, San Diego)
11:55 AM – 12:10 PM	Next Generation Rat Models of ER+ Breast Cancer	Yi Li (Baylor College of Medicine)
12:10 – 12:40 PM	Discussion	Moderated by Josh Snyder

Lunch: 12:40 – 1:45 PM

Wrap-Up & Next Steps (1:45 PM – 2:00 PM)

Moderators: David Gutmann, Elinor Karlsson, Jesse Boehm, Mukund Seshadri, Josh Snyder

Time	Talk Title/Subject	Speaker
1:45 – 1:50 PM	Overview of meeting discussions	Moderators
1:50 – 2:00 PM	Closing remarks	Joanna Watson (NCI)

Speaker Abstracts

Session 1 - State of Modeling

Precious GEMs: **Lessons learned from mouse modeling**

David H. Gutmann, MD, PhD, Washington University School of Medicine

Individuals with the Neurofibromatosis type 1 (NF1) cancer predisposition syndrome are prone to the development of benign and malignant tumors. The most common brain neoplasm seen in children with NF1 is a World Health Organization grade 1 glioma (pilocytic astrocytoma), which typically arises within the optic pathway. Since these tumors are rarely biopsied or resected as part of routine clinical care, the majority of our understanding of their pathobiology has derived

from the deployment of genetically engineered mouse models. These preclinical avatars have been instructive in partly elucidating the cells, signals, and circuits that underlie *Nf1* optic glioma formation and progression, as well as tumor-induced vision loss. In addition, murine *Nf1* optic glioma models have been useful for the identification and evaluation of targeted therapies and for the discovery of risk factors that predict tumor development and progression. In this presentation, I will discuss the credentialing of murine *Nf1* optic glioma models, their use for addressing questions not possible with human tumor biospecimens, and the opportunities that these platforms afford in the realm of novel therapeutics and risk factor prediction relevant to future precision medicine strategies for children with these brain tumors.

Session 2 - Modeling Translational Questions: Technology I

Spontaneous Canine Osteosarcoma: A Translationally Relevant Patient Model for Humans
Amy LeBlanc, National Cancer Institute

Canine osteosarcoma shares many features with human osteosarcoma and has been suggested as an important preclinical model with the potential to inform pediatric oncology. However, key gaps persist in our understanding of the histologic, genomic, and transcriptomic features of canine osteosarcoma. Under the NCI's Decoding the Osteosarcoma Genome of the Dog (DOG2) project, the COP is addressing these gaps through a comprehensive assessment of outcome-linked tissue samples collected through the efforts of the Comparative Oncology Trials Consortium (COTC). Canine clinical trials offer opportunities to prospectively collect invaluable biospecimens. These clinically annotated samples can be interrogated to uncover new therapeutic targets and predictive biomarkers with translational relevance for human patients. In canine patients, full autopsies are routinely completed providing access to non-tumor and tumor tissue including matched metastases. Coupled with a ten times higher incidence of osteosarcoma in the dog, the increased availability of trial samples from canine patients underscores the wealth of knowledge that can be obtained from studying tumor-bearing pet dogs as a model for pediatric osteosarcoma.

Canine osteosarcomas also demonstrate a similar metastatic progression to human patients with similar dissemination to distant tissues such as lung and bone, development of morphologically-heterogeneous tumors displaying multiple histologic subtypes including telangiectatic and giant cell-rich, and formation of a complex TME with intra- and peri-tumoral lymphocytic aggregates, diffuse macrophage infiltration, and accumulation of tumor-derived osteoid and collagenous tumor matrix. The morphologic characterization of these samples facilitated the development of an AI predictive model based on histologic subtypes and reinforced the identification of transcriptional signatures of primary tumors highlighting a role for immune infiltrates and tumor stroma in overall survival and disease-free progression.

Novel somatic mutant IDH glioma model identifies high-risk patients with distinct copy number and tumour microenvironment alterations
Aditya Shroff[1], Yu-Jui Ho[2], Guidantonio Tagliazucchi[9], Felipe Galvez Cancino[3], Melanie Clements[4], Timour Baslan[2], Gordon Beattie[5], Sergio Quezada[3], Thomas Jacques[6], Nada Jabado[7], Simona Parrinello[4], Paolo Salomoni[8*], Maria Secrier[9*], Scott Lowe[2,10*], Henning Walczak[1,11*]

Gliomas are among the most lethal human neoplasms, in part due to their marked heterogeneity. Molecular classifications of patient tumours linked majority of adult diffuse low-

grade gliomas (LGG) to gain-of-function mutations in the isocitrate dehydrogenase (IDH^{Mut}) genes, which alter the tumour epigenome via their neomorphic enzymatic function. Due to the lack of precise autochthonous preclinical progression models, there is limited understanding of the biology of the oncogenic mutant IDH-driven function in LGG, and the heterogenous crosstalk with the tumour microenvironment (TME). Here, we employed postnatal brain electroporation to develop a slowly progressing somatic mutant IDH1 (*IDH1R132H*; $IDH1^{mut}$) murine model reminiscent of LGG patient tumour development. We show that mutant IDH1, combined with loss of the tumour suppressor protein 53 (*p53*) and α-thalassemia/mental retardation syndrome X-linked (*Atrx*) genes, is sufficient to drive formation of diffuse gliomas with resulting tumours recapitulating the histopathological and molecular hallmarks of mutant IDH-astrocytomas (IDH-A) patient tumours, especially, the copy number alteration. Multi-omic based molecular characterization and data integration led to the identification of the epigenetic silencing of the targets of the polycomb regulator EZH2 and the altered composition of the TME. Applying the novel murine signature to the genomic and transcriptomic datasets derived from patient tumours, we identified a previously unrecognized subgroup of IDH^{Mut} and IDH-A tumours with significantly enriched immune-TME accompanied by distinct somatic copy number alterations. Collectively, our results provide insights into IDH-A pathogenesis, intrinsic tumour characteristics associated with TME heterogeneity and uncovering an opportunity for IDH^{Mut}-patient stratification with prognostic value.

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Resource Building to Enhance the Canine Model in Cancer Vaccine Development

Shaying Zhao

Dogs hold unique advantages in accelerating cancer vaccine development, due to their shorter life span, large population, high cancer incidence, and >300 pure breeds, along with the more relaxed FDA regulation on investigational drugs. Importantly, compared to many other cancer models, spontaneous cancers in pet dogs more faithfully represent their human counterparts, including shared hotspot mutations. However, their effective use is hampered by a lack of essential resources, including those for major histocompatibility complex class I (MHC-I) genotyping and tumor-specific neoantigen (TSNA) prediction. To address these deficiencies, we developed Kmer-based paired-end read (KPR) *de novo* assembler and genotyper, a software package for canine MHC-I genotyping using RNA-seq data. Our KPR software outperforms other popular tools examined in typing new alleles. By applying the KPR software on >1000 dogs with public RNA-seq data, we discovered over 50 new alleles, as well as dominant alleles in >10 popular breeds. Moreover, we established experimental systems and computational pipelines to characterize canine immunopeptidomes. We are developing machine learning models for cross-

species data integration to use the massive human data for canine TSNA prediction. Our work provides key resources that will significantly enhance the use of the canine model in developing cancer vaccines and other immunotherapies.

Large DNA transgenesis using the Cas9+Bxb1 toolbox in mice.
Vishu Hosur, Jackson Laboratory

One of the primary obstacles in genomic engineering is the lack of efficient and precise methods for integrating big DNA constructs into the genome. The technique of random transgenesis, which is extensively employed, exhibits a lack of precision, and is associated with a multitude of problems. The lentiviral and adeno-associated viral techniques are hindered by DNA toxicity and a limited payload capacity of fewer than 5 kb, respectively. The efficacy of homology-directed repair (HDR) procedures utilizing CRISPR-Cas9 is limited to genomic alterations within the range of 1–5 kilobases. Furthermore, currently established approaches utilizing homology-directed repair (HDR) necessitate the use of large homology arms, which are DNA sequences that facilitate the insertion of constructs. These homology arms typically have lengths ranging from 0.5 to 5 kb. The utilization of Cas9-guided transposases represents a promising novel approach for the insertion of DNA structures with a maximum length of 10 kb. However, it is important to note that the efficacy of this method has been observed exclusively in bacterial systems. Overcoming these obstacles, a novel toolkit has been recently devised, which merges RNA-guided Cas9 and the site-specific integrase Bxb1. This toolkit enables the integration of DNA constructs, spanning from 5 to 40 kb in length, into mouse zygotes with germline transmission. This innovative toolkit will provide researchers with an asset for the creation of novel mouse of cancer and other genetic disorders.

Lentiviral Delivery of Oncogenes to Model Glioma in the Swine Spinal Cord
Nick Boulis, Emory University

A large animal model is an ideal model for surgical translational studies due to its anatomical resemblance to the human spinal cord. The Göttingen minipigs were selected for this study, and the thoracolumbar spinal cord was chosen as the injection site for the oncogenic lentiviral vectors. This location offers distinct advantages, allowing the observation of clinically appreciable and quantifiable motor deficits in the hindlimb ipsilateral to the injection site as the tumor progresses. The surgical process involved a two-level laminectomy, during which the animals received two injections – one with the oncogenic vector and another with the control, spaced apart by two vertebral levels. This specific spacing was crucial to facilitate the exclusive analysis of injection sites, provide room for tumor growth, and ascertain the laterality of motor deficits. Leveraging our extensive expertise in spinal cord drug delivery and glioma modeling, our team have established a non-xenograft minipig spinal cord glioma model. Ten experimental groups have been devised to explore glioma induction, targeting specific genes such as PDGF-B, p53, CDKN2A, HRAS, F3T3, H3K27M, BRAF600E, EGFR, EGFRVIII, and PTEN through lentiviral transgene delivery. These models will serve as a resource for future surgical translational studies of glioma in brain and spinal cord. The utility of a model with a more relevant size is demonstrated by our initial work to characterize the biodistribution of chemotherapy delivered by convection enhanced delivery (CED).

Human Cancer Models Initiative (HCMI): A community resource of next-generation cancer models and associated data
Julyann Perez-Mayoral, National Cancer Institute

The Human Cancer Models Initiative (HCMI) is an international consortium founded by National Cancer Institute (NCI), Cancer Research UK, Wellcome Sanger Institute, and the foundation Hubrecht Organoid Technology. The initiative has generated patient derived Next-generation Cancer Models (NGCMs) from diverse tumor types and subtypes including rare adult and pediatric cancers as a community resource. HCMI addresses deficiencies in traditional cell lines models by collecting patients' clinical data, as well as the genomes and transcriptomes of the parent tumor, case-matched normal tissue, and the derived next-generation cancer model.

NCI's Center for Cancer Genomics (CCG) sponsors four Cancer Model Development Centers (CMDCs) and the downstream model development pipeline. All biospecimen, clinical, and molecular characterization data are quality controlled and submitted to NCI's Genomic Data Commons (GDC) for the research community. The HCMI models and culture protocols are made available to the research community through a single third-party distributor, ATCC.

The HCMI Searchable Catalog (https://hcmi-searchable-catalog.nci.nih.gov/) is an online resource that allows users to query and identify available models using various data elements including clinical and molecular characterization data, including WGS, WXS, RNA-seq, and methylation array. To date, over 300 HCMI models are available to query on the Searchable Catalog and are available to the research community through ATCC. These models have been derived from several cancer types including glioblastoma, colorectal, pediatric, gastroesophageal, pancreatic, and more. Biospecimen, clinical, and molecular characterization data are available for over 250 models at NCI's GDC, with additional cases released as the data completes the HCMI pipeline. Data, tools, and resources generated by CCG initiatives are made publicly available via the CCG website and GDC. The CCG website also provides available data types, data usage policies and guides to access data (https://www.cancer.gov/about-nci/organization/ccg).

Session 3 - Modeling Translational Questions: Immunity

Loss of *NF1* drives hormone dependent mammary carcinogenesis in a rat model with intact immune system
Deeann Wallis, University of Alabama at Birmingham

Loss of *NF1* plays a major role as an oncogenic driver in many cancer types and can be found in up to 33% of all breast cancers (BC). Loss of *NF1* is also a prognostic indicator for increased cancer risk at an earlier age, poorer outcomes, and therapeutic resistance. In addition, certain *NF1* genotypes may increase cancer risks, while others do not. NF1 is largely perceived as a classic Ras-opathy syndrome due to inactivating mutations in neurofibromin affecting Ras-MAPK signaling. However, recently it has been shown that NF1 binds estrogen receptor (ER) and acts as a transcriptional corepressor. We have generated novel rat models deficient for *Nf1* that have a very robust ER+ BC phenotype, therefore more closely recapitulating clinical tumors compared to other preclinical models. Our models include a missense allele c.3827G>A, p.R1276Q (knockin or KI) as well as a 14 base pair deletion knockout (KO) model. Phenotypic differences between our models indicate that the variant matters. Our overall goal is to characterize the phenotype of these rat models in terms of histopathology, Ras signaling, hormone signaling, immune components, and targeted drug response and compare/contrast them with what is known regarding patients with somatic or germline inactivation of *NF1* and breast cancer. Aim 1 will evaluate tumor onset, growth, histology, and molecular characterization of Ras and estrogen. Aim

2 will characterize the Nf1 deficient tumor microenvironment (TME), identify immuno-targets, and evaluate immuno-targeting with and without Ras-targeting therapeutics. Aim 3 will evaluate the role of hormones in tumor initiation, maintenance and targeting therapeutics both with and without co-targeting Ras to show synergy. As HR+ BC accounts for ~80% of patient cases, and that appropriate mammalian models with intact immune systems are lacking, we believe that our proposed studies are highly *significant* and will substantially advance the development of new therapies to this disease.

Mechanisms of resistance to anti-BCMA bispecific antibodies in multiple myeloma: lessons from the Vk*MYC mouse model

Marta Chesi, PhD, Department of Medicine, Mayo Clinic Arizona

T cell redirected therapy CAR-Ts and bispecific antibodies) has revolutionized the treatment of multiple myeloma (MM), inducing unprecedented response rates in a heavily pretreated patient population. However, a fraction of MM patients still displays primary resistance, and even patients that achieved complete responses eventually relapse. We need orthotopic, immunocompetent mouse models to investigate the biology of T cell redirected therapy in the context of a complete tumor (immune)microenvironment, and understand mechanisms of primary resistance, relapse, and treatment related toxicities.

The immunocompetent Vk*MYC genetically engineered mouse we have developed is an ideal model to study immunotherapy in MM. Based on the sporadic AID-induced activation of MYC in a single germinal center B-cell, it demonstrates biologically and therapeutically fidelity to human MM, but also captures the genomic complexity and heterogeneity of the human disease through spontaneous acquisition and selection over time of driver gene mutations, aneuploidies and complex structural variants converging on pathways important for human MM pathogenesis.

Using the Vk*MYC model we investigated the anti-MM activity of a bispecific antibody (BsAb) against CD3 on T cells and BCMA on MM cell surface and found it correlates with tumor burden, which in turn correlates with serum levels of soluble BCMA. Gamma secretase inhibition stabilizes BCMA on cell surface and improves primary response in high tumor burden setting by removing sBCMA competition for BsAb binding. Primary resistance to BsAb could also be transiently overcome by combination treatment with the immunomodulatory drug pomalidomide, known to boost T cell IL2 production. However, responses are transient due to upregulation of regulatory T cells and T cell exhaustion. In contrast, combination of BsAb with cyclophosphamide reshapes the suppressive tumor microenvironment and is curative in a fraction of mice by promoting protective immunity.

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

Kelli Connolly, Yale University

Checkpoint blockade immunotherapies have revolutionized cancer treatment as they can significantly extend survival but, in lung cancer and others, only a fraction of treated patients see these benefits. These therapies work by blocking inhibitory proteins on a specific type of intratumoral immune cell, called stem-like CD8 T cells, which in turn boosts their ability to kill cancer cells. It is not currently known why a majority of lung cancer patients do not respond but, within Kras-driven lung adenocarcinomas (LUAD), certain genetic mutations in cancer cells such as deletion of the tumor suppressors P53 or LKB1 are associated with better or worse response rates, respectively. To address these critical gaps in our knowledge, I have developed a series of

advanced genetically-engineered mouse models of LUAD in which tumor-neoantigens are induced on cancer cells within genetically distinct, commonly occurring subtypes of disease. Using these models, I have discovered that a reservoir of therapy-responsive stem-like T cells are maintained outside of tumors, in tumor-draining lymph nodes, and that stem-like T cells within the tumor are continually replenished by migration from these reservoirs. This work has led us to ask two important questions: can this reservoir be therapeutically targeted to improve therapy efficacy, and, if all patients have reservoirs of therapy-responsive cells, why don't all patients respond? Critically, how genetic mutations like LKB1 deletion might impact stem-like T cell reservoirs in Kras-driven LUAD is not understood. Future studies will combine advanced imaging techniques with multicolor flow cytometry and single cell RNA/TCR sequencing to investigate how presentation of the same tumor-neoantigen from genetically distinct LUAD subtypes might differentially impact the cell to cell interactions critical for maintenance and migration of stem like CD8 T cells within tumor-draining lymph nodes.

Exploring How the Intrinsic Diversity of Cancer DNA Damage Phenotypes Intersects with Immune Context

Jade Moore, Ines Guix, William Chou and Mary Helen Barcellos-Hoff, Department of Radiation Oncology, UCSF

Lack of diversity in most current preclinical models limits their utility for systemic evaluation of many aspects of therapeutic response, hence, a critical unmet translational requirement is a model system in which both the tumor and the immune infiltrate are defined so that determinants of therapy can be readily studied. We generated a large bank of *Trp53* null mammary carcinomas that have been extensively characterized for markers, immune infiltrate, histology and behaviors, and benchmarked for relevance to human cancer. The intrinsic molecular diversity and immune complexity of these mammary carcinomas represent a high value system for oncology. These primary tumors were expanded as mammary tumor derived transplants (mTDT) that maintain key characteristics and diversity to provide a tractable model to investigate the interaction of cancer, microenvironment and immunity. To date we have expanded mTDT from 25 *Trp53* null cancers of which the characterization of 12 mTDT has been published (1). We credentialed mTDT immune cell context using tissue agnostic, recurrent immune motifs, called immune archetypes (IA) derived from diverse human cancers (2). We interrogated the mTDT RNAseq data (n=70) using the tumor-educated immune cell gene expression signatures (TeIS) defined by immunoprofiling. Consistent with the published classification based on lymphocyte spatial distribution, mTDT range from immune rich to immune desert as reflected by TeIS, similar to the IA spectrum observed in humans. We then correlated TeIS with a score, termed βAlt, that we developed to report DNA repair competency as evidenced by predicting response to genotoxic therapy (3). The mTDT βAlt was significantly correlated with TeIS—high βAlt indicative of poor DNA repair competency was characteristically immune-poor TeIS profiles.

The analysis of mTDT reveals a new relationship between DNA damage response and immune phenotype that prompts mechanistic studies of how these components respond to therapy. These studies are essential in advancing our understanding of intrinsic DNA damage response and extrinsic immunity, which will could lead to development of new therapeutic strategies.

Session 4 - Modeling Translation Questions: Therapy

Leveraging canine spontaneous cancer to optimize the power of blood biopsy

Heather Gardner, Tufts University

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

Josh Smith, Sloan-Kattering Institute of Cancer Research

We have developed rectal cancer organoids (tumoroids) as individualized models and built a large rectal cancer tumoroid repository (n=400). Prior to our work, research on rectal cancers was hampered by the paucity of relevant models. Of the few existing *in vivo* models of rectal cancer, none placed the tumors in the rectal lumen, so the models failed to mimic the correct anatomic environment and local invasion. The existing models also had not been observed to metastasize. Another problem is that we lacked accurate means to predict whether individual rectal cancer patients would 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. To date, we have derived tumoroid lines from hundreds of patients' rectal cancers and found them to resemble the corresponding patient tumors. The tumoroids, when implanted in mice endoluminally (i.e. in the rectum), form locally invasive tumors capable of metastasis. Moreover, we found tumoroids have clinically relevant responses to chemotherapy and radiation. Thus, drawing from our growing body of data, **we hypothesize** that rectal cancer tumoroids, which 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 have developed more than 400 tumoroids, which encompass much of the diversity of human rectal adenocarcinoma. Tumoroids are being 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 have developed a rectal cancer tumoroid biorepository seamlessly integrated with online pathologic, genomic, and model-specific information. The online platform has been built within our institution's cancer genomics portal, and will be 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 our work will credential tumoroids as accurate models for rectal cancer research and for predicting patient responses to therapy. The growing tumoroid biorepository will 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.

Understanding cancer using dogs and many mammals

Kerstin Lindblad Toh, Uppsala University

The major goals of the NIH are to understand human disease, including cancers, and to translate basic biomedical research into clinical practice, thereby impacting global human health.

We have developed the dog as a model for human cancer studying both germ-line and tumor mutations. Cancers shared across dogs and humans include mammary tumors, osteosarcoma, hemangiosarcoma and lymphoma. After originally looking at inherited risk factors we have now characterized tumor mutations both in whole exome sequencing data and whole genome sequencing data. We see that the same pathways often are shared across species both for coding and non-coding mutations.

Current knowledge of cancer genomics remains biased to in depth analysis of coding regions, while non-coding mutations are understudied. To systematically search for regulatory non-coding mutations that are functional, we assessed mutations in constraint positions in the genome under the assumption that evolutionary fixation likely reflects function. To show the importance of non-coding mutations, we used whole-genome sequencing data from the International Cancer Genome Consortium and combined it with evolutionary constraint inferred from Zoonomia (240 mammals), to identify genes enriched in non-coding constraint mutations (NCCMs).

We analyzed medulloblastoma (MB), a malignant brain tumor, finding >500 genes with high levels of NCCMs. Intriguingly, several loci with NCCMs in MB are associated with different ages of onset, such as the *HOXB* cluster in young MB patients. In adult patients, NCCMs occurred in, e.g., the *WASF-2/AHDC1/FGR* locus. One of these NCCMs, when CRISPR/Cas9 edited in an MB cell line, led to increased expression of the SRC kinase gene *FGR.* It also augmented the responsiveness of MB cells to dasatinib, a SRC kinase inhibitor. These newly identified putative candidate driver mutations may aid in patient stratification in MB and could be valuable for future selection of personalized treatment options.

Credentialing next-generation human glioma models for precision therapeutics
Ryan Miller, University of Alabama at Birmingham

Despite notable success in various solid neoplasms, precision therapeutics targeting mutated kinases have failed in gliomas, the most common and deadly primary brain tumors in both adults and children. Reasons for failure include the lack of preclinical models that faithfully recapitulate the biology of mutated kinase gene-driven gliomas, including intra-tumor heterogeneity, drugs specifically designed to target invasive brain tumor cells located behind an intact blood-brain barrier (BBB), and adaptive drug resistance. We have developed and will molecularly credential novel, mutated receptor tyrosine kinase (RTK)-driven human glioma models for use in preclinical development of tyrosine kinase inhibitor (TKI)-based therapies. The foundation of our work comes from the Furnari Lab at UCSD, who developed a novel platform for engineering glioma models using CRISPR genome editing of human induced pluripotent stem cells (iPSC) and has established intra-tumor heterogeneity in RTK genes as a symbiotic driver of tumorigenesis. The Miller Lab at UAB has extensive experience in small molecule experimental therapeutics using genetically engineered glioma models, next-generation sequencing, and mass spectrometry-based targeted proteomics. The goal of our studies is to assess the glioma kinome *en masse* at multiple omic levels and explore the extent to which dynamic kinome reprogramming contributes to TKI resistance in gliomas.

Session 5 Flash Talk Abstracts

Electroporation-based genetically engineered mouse models (EPO-GEMMs) as a versatile platform for evaluating efficacy and safety of CAR T cell therapy

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Genetically engineered mouse models (GEMMs) have been pivotal in characterizing oncogenic mutations and evaluating experimental cancer therapies within native tissue contexts. However, the time and cost associated with GEMMs present challenges in rapidly investigating multiple cancer-causing mutations and therapeutic hypotheses. To address this issue, we developed a somatic tissue engineering platform known as electroporation-based genetically engineered mouse models (EPO-GEMMs). EPO-GEMMs allow us to efficiently introduce patient-relevant oncogenic mutations into various organs, resulting in the development of prostate, stomach, pancreas and ovarian tumors that faithfully replicate clinical and pathological features of human cancer.

One goal of our research is to test the suitability of our models for various preclinical applications. In this regard, we have previously shown that the urokinase plasminogen activator receptor (uPAR) is overexpressed in senescent cells and across various cancer types including pancreas, bladder, brain and ovarian carcinomas. This finding prompted us to test whether our EPO-GEMM model would serve as a useful preclinical system for evaluating CAR T cell therapy targeting uPAR as a potential cancer treatment. Leveraging EPO-GEMM-derived ovarian tumors, we demonstrated the potent anti-tumor activities of murine uPAR CAR T cells in immunocompetent mice without inducing severe adverse effects. Subsequently, we launch an exhaustive functional screening of human uPAR single-chain fragment variants (scFVs). This screening resulted in the identification of lead scFVs with subnanomolar affinity. Remarkably, human uPAR CAR T cells are capable of eradicating both orthotopic and metastatic human ovarian cancer xenograft tumors. Our ongoing efforts include assessing the long-term safety and toxicity profiles of uPAR CAR T cells using a humanized model platform. These results constitute the proof-of-concept evidence of utilizing EPO-GEMMs to create patient-relevant tumors and evaluate the efficacy and safety of new targets for CAR T cell therapy in autochthonous an immunocompetent hosts.

Dynamic systems modeling in HER2 Crainbow mice reveals critical inflection points of malignancy

Josh Ginzel, Henry Chapman, Joelle Sills, H. Kim Lyerly, Bruce Rogers, Joshua C. Snyder

Although cancer undergoes a series of morphologic changes along the malignancy cascade, previous clinical evidence suggests that many breast cancers will stall during this process while others will progress so rapidly as to avoid early detection. Here we quantitatively lineage trace the rates of progression for more than 2000 tumors across tumorigenesis and find that lesions destined for invasion are associated with wounded epithelial cells in the tumor microenvironment. Using Cancer Rainbow (Crainbow) lineage tracing, we were able to provide first-in-kind quantification of occult HER2 isoform driven tumor growth through three phases: field growth, hyperplasia, and screen detection, in addition to more classical palpation. A dynamic

mathematical model led to the observation that each step is progressively more likely to progress. However, a major difference between the metastatic and the indolent tumors was a 1000-fold difference in the progression from a hyperplastic lesion to a screen-detectable tumor. These rapid progressing, metastatic hyperplasias were associated with a hormone sensitive, “bystander epithelial cell” (BECs). These so called BECs lack expression of the transgenic oncogene, are enriched in the metastatic tumors, and retain markers of epithelial injury. Our findings provide an experimental demonstration of the importance of developing autochthonous mouse models to study the complex interplay between host and oncogenic cells throughout the ontogeny of a cancer. Ultimately, our data indicate that the critical inflection point in the disease process occurs prior to screen detection.

Establishing a Clinically Relevant Mouse Model to Study Necrosis as a Primary Variable in Glioblastoma

Jiaobo Li, Department of Pathology, Northwestern University Feinberg School of Medicine

All glioblastoma (GBM) molecular subsets share the common trait of accelerated progression following necrosis which cannot be adequately explained by cellular proliferation arising from accumulated genetic alterations. We suggest that development of necrosis is much more than a passive phenomenon related to rapid growth but rather is a driving force behind tumor microenvironment (TME) restructuring responsible for sustaining accelerated expansion. However, mechanisms related to TME restructuring and biologic progression, including glioma stem cell (GSC) enrichment and the influx and polarization of tumor-associated macrophages (TAMs), remain poorly understood due to a lack of animal models to study necrosis as a primary variable. To reveal spatio-temporal changes following the development of hypoxia and necrosis, we developed an immunocompetent RCAS/tv-a model that can be used to study TME restructuring in a precise and reproducible manner. We generate diffuse high-grade gliomas that lack necrosis by introducing RCAS-PDGFB and RCAS-Cre in a Nestin-tv-a TP53$^{flox/flox}$, PTEN $^{flox/flox}$ background mouse. We then photoactivate Rose Bengal with specific, targeted blood vessels in the glioma to induce thrombosis, hypoxia and necrosis. We are then able to visualize TME restructure and its impact on glioma growth in real time using multiphoton microscope. TAMs increase dramatically with the onset of necrosis, with preferential localization to the hypoxic zone in the peri-necrotic niche, which supports their survival. Flow cytometry on digested pre- and post-necrotic GBMs, show increased TAM influx and GSC enrichment as necrosis emerges. Our immunofluorescence staining along with in silico analysis of Ivy GAP data suggests podoplanin (PDPN) expression by GBM cells within the peri-necrotic niche causes polarization of TAMs by activating CLEC5A on TAMs to cause an immunosuppressive phenotypic switch. Collectively, this model captures glioma growth dynamics, GSC enrichment and TAM influx, and will facilitate the development of therapies that antagonize these mechanisms to improve outcomes.

Genotyping Canine MHC class II genes using RNA-seq data

Nikitha Sundaresha. University of Georgia

At least 50% of canines older than ten years will develop cancer. The incidence of cancer in dogs is comparable to humans. Canine cancer models are analogous to humans for advanced immunotherapy. However, only 20% of cancer patients respond to immunotherapy. The genetic sequence of MHC plays a role in T cells recognizing the epitopes required for response, known as MHC-restricted antigen recognition.

MHC-II genes present antigens to CD-4 T cells for recognition and are known as classical MHC-II. In classical HLA-II, alpha, and beta subunits, encoded by individual genes, exist for the three classical HLA-II molecules, DR[A/B], DQ[A/B], and DP[A/B]. However, the DP[A/B] genes are not annotated in the canine genome. As current MHC genotyping softwares like Seq2HLA is based on comprehensive HLA-II allele data and does not suffice for the canine model, our lab has previously developed an assembler and genotyper for canine MHC-I, KPR. This study aims to modify KPR for canine DLA-II molecules.

From the IPD-MHC database, we have collected 178 DLA-DRB1, 30 DLA-DQA1, and 86 DLA-DQB1 reference alleles. DLA-DRA is monomorphic, so we did not include it in our software. Upon input of paired-end RNA-seq data, setting kmer value K value and the number of iterations, N, we output assembled and genotyped DLA-DRB1, DLA-DQA1, and DLA-DQB1 alleles within the sample with the relative expression of alleles for each gene. We estimate if the assembled contig is chimeric based on the depth of the paired reads. This is our first version of the software that still requires simulation to test parameters.

We can assemble and genotype all three genes in PRJNA489087. We chose this dataset because there are more than 200 samples that pass quality control. We identified novel alleles and breed-specific alleles using this dataset.

Overcoming the inhibitory FcγRIIB barrier unlocks Fc-dependent activity of human anti-CTLA-4 antibodies

Lucas Blanchard, Rockefeller University

While antibodies targeting CTLA-4 were developed to block inhibitory signals in T cells, studies in mouse models demonstrated that Fc-dependent mechanisms, including depletion of tumor-infiltrating regulatory T cells (Tregs), are required for their complete antitumor activity. However, the two clinically available antibodies Ipilimumab and Tremelimumab do not show substantial Fc-dependent activity in cancer patients, which may limit their therapeutic efficacy. Here, we investigate the mechanisms restraining the Fc-dependent activity of fully human anti-CTLA-4 antibodies in a novel humanized mouse model expressing both human CTLA-4 and human Fcγ receptors (FcγRs). As observed in cancer patients, Ipilimumab and Tremelimumab were limited in their capacity to deplete tumor-infiltrating Tregs in humanized CTLA-4/FcγR mice, indicative of poor Fc effector function. We found that FcγRIIB, the inhibitory Fc receptor, was highly expressed in both murine and human tumors, acting as a potential barrier to Fc-dependent responses. Antibody-based blocking of FcγRIIB unlocked the Treg-depleting capacity of Ipilimumab and unleashed its antitumor activity. Alternatively, Fc-engineering of Ipilimumab to enhance binding to activating FcγRs while limiting binding to FcγRIIB significantly increased Treg depletion and antitumor activity. We obtained similar results with a Fc-optimized antibody targeting CCR8, a chemokine receptor selectively expressed on tumor-infiltrating Tregs. Our results define FcγRIIB as a major barrier limiting the Fc-dependent activity of human anti-CTLA-4 antibodies in the tumor microenvironment and support the use of humanized mouse models to characterize the in vivo activity of therapeutic human antibodies.

Enabling investigation of gene-environment interactions and cancer risk in pet dogs and cats

Kate Mecquier, Broad Institute

Spontaneous cancers in pet dogs and cats are potentially powerful models for investigating gene-environment interactions modulating cancer risk. These companion species share environments with humans and develop cancers that are genomically and clinically similar. Moreover, their shorter lifespans and accelerated disease course facilitate rapid studies. To enable these comparative gene-environment interaction studies in companion animals, we are investigating three main areas. (1) Linking inherited variation to cancer risk: We will perform whole-genome sequencing of samples collected in our direct-to-owner studies, as well as leveraging existing germline sequencing data from 674 dogs. (2) Leveraging passive environmental sampling to measure exposure: For a pilot set of 101 pet dogs, we have deployed a silicone dog tag which absorbs volatile compounds, which can then be identified using mass spectrometry. Preliminary analysis has identified multiple compounds with differential exposure levels between cancer and healthy groups. (3) Characterizing somatic mutations in normal and tumor tissues: We have identified clonal hematopoiesis of indeterminate potential in approximately 10% of dog blood samples. In parallel, we are comparing circulating cell-free DNA between healthy dogs and cats and those with cancer. Together, our work in these areas is advancing comparative studies in companion animals as a powerful framework to accelerate discovery of genetic and environmental factors and interactions leading to somatic mutations and cancer.

Session 6 - Fidelity and Reproducibility in Animal Modeling

Office of Research Infrastructure Programs and Rodent Resources
O. Mirochnitchenko, PhD, DCM/ORIP/Division of Program Coordination, Planning, and Strategic Initiatives, Office of the Director, NIH

Animal models and related resources play an essential role in biomedical discovery by enabling scientists to better understand, diagnose, prevent, and treat human diseases. The value of these models is enhanced by information about their genomic and phenotypic characteristics, which allows researchers to predict human disease outcomes more accurately. ORIP provides critical infrastructure for biomedical researchers by supporting the development and maintenance of animal models and related resources. Eliminating variability in animal research is accomplished by supporting the creation and encouraging the use of animal repositories. The Mutant Mouse Resource and Research Centers (MMRRC) and Rat Resource and Research Center (RRRC) acquire, distribute, and cryopreserve scientifically valuable, genetically engineered mouse and rat strains and embryonic stem cell lines. In addition, the facilities develop new technologies to improve the handling and use of mutant animals, including advances in assisted reproductive techniques, cryobiology, genetic analysis, phenotyping, and infectious disease diagnostics. NIH-funded rodent repositories ensure the quality and welfare of distributed animals and supply expertise to guide reliable studies. ORIP recently renewed the Pilot Centers for Precision Disease Modeling Initiative focusing on creating pipelines for research community-nominated unique human genomic variants linked to diseases for cost effective high-throughput testing in model organisms. All three Centers along with other species are developing rodent models that more precisely mirror the genotype and phenotype of human disease processes and promote the creation of new therapeutics.

Rat Resource and Research Center: Rats, Reproducibility, Translatability and Best Practices
Elizabeth Bryda, University of Missouri

The rat has always been a popular model organism species, particularly in the fields of cardiovascular research, neuroscience, behavior, toxicology, and addiction. Recent advances in genetic engineering have enabled sophisticated genetic modifications to be introduced into the rat genome in ways that were previously limited to the mouse. This has immensely increased the utility of rats as models for a wide variety of human diseases. Because the rat is larger in size than a mouse, has anatomy and physiology comparative to that of a human, and yet is relatively cost effective to maintain versus a larger mammal such as the pig, the rat represents a superior model organism. The NIH-funded Rat Resource and Research Center (RRRC) serves as a centralized repository for maintaining/distributing rat models and providing rat-related services to the biomedical community. Currently, the RRRC has over 575 rat lines, including many relevant to cancer research. All strains/stocks are given RRIDs, registered in the Rat Genome Database (RGD) to enhance visibility and archived by cryopreservation to ensure against future loss. The RRRC distributes live animals, cryopreserved sperm/embryos, and rat embryonic stem (ES) cell lines (website: www.rrrc.us). Quality control measures for all materials include extensive genetic validation and health monitoring. The RRRC works closely with investigators to provide a wide variety of services, including but not limited to colony management, health monitoring, genetic assay development/optimization, strain rederivation and cryopreservation, isolation of rat tissues, microbiota analysis and creation/characterization of genetically engineered rats. The RRRC strives to ensure research reproducibility and provide comprehensive investigator support to optimally serve the needs of biomedical researchers using rats. Funding: NIH P40 OD01106.

Rapid ex vivo biosensor cultures to assess dependencies in gastroesophageal cancer
Jesse Boehm, Massachusetts Institute of Technology

Overcoming cancer drug resistance is a major unmet clinical need as it remains challenging to accurately predict therapeutic sensitivity from baseline tumor and immune profiling. While biopsies from refractory disease are challenging to routinely obtain for research, upwards of 40% of gastroesophageal adenocarcinoma (GEA) and 20% of all cancer patients develop peritoneal carcinomatosis and ascites, a plentiful, heterogenous and molecularly representative sample format that is easily accessible since routine drains occur. However, in standard clinical practice this material is only subjected to cytology to detect malignant cells and then discarded. Bulk profiling technologies are challenging to deploy due to the highly variable single cell heterogeneity of ascites. To address these challenges, we have developed the first generation of AscitesPredict, an ‘ex vivo tumor biosensor’ technology using single cell technologies to measure cell identities and therapeutic sensitivities during a 5 day period in which viability is preserved via media supplementation with patient-matched ascites fluid. AscitesPredict utilizes high dimensional single cell image-based morphological profiling and machine learning applied to brightfield microscopy. Thus far, we have profiled over 20 GEA ascites samples, made initial assessments of technological reproducibility, and have optimized initial workflows. Our ultimate goal is to harden the technology to facilitate deployment by a wide diversity of researchers and clinicians for two driving use cases: (1) supporting preclinical drug development and (2) eventually as a functional diagnostic.

Modeling Myelodysplasia
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Humanized mouse models are a powerful tool to study human hematopoiesis and hematologic malignancies *in vivo*. Existing models have greatly improved but continue to be limited by suboptimal engraftment of stem cells with inherent growth defects, especially in bone marrow failure (BMF) disorders such as low-grade myelodysplastic syndromes (MDS). In our studies we set out to determine whether we can faithfully model MDS engraftment and its treatment in humanized mice. Previously, we presented a highly efficient MDS/AML PDX model in cytokine-humanized MISTRG mice, that express human M-CSF, IL-3, GM-CSF and THPO in the background of the SIRPα - humanized rag $^{-/-}$ IL2Rg $^{-/-}$ mice. MISTRG mice support efficient, faithful and long-term MDS hematopoietic stem and progenitor cell (HSPC) engraftment with multi-lineage representation. The bone marrow environment is critical for efficient HSC maintenance and propagation, but engraftment of human stem cells into mice requires irradiation to condition the niche. To improve upon MDS engraftment in MISTRG mice, we introduced the stem cell factor receptor c-kit$^{W41/W41}$ mutation via CRISPR/CAS9 into MISTRG6 mice that also express human interleukin 6 to engraft human HSCs without irradiation.

We will present data comparing MISTRG6kit$^{W41/W41}$ with MISTRG6 and extensive phenotypic and clonally characterized MDS from patients and MISTRG6kit$^{W41/W41}$.

Improving the translational value of head and neck cancer patient-in-mouse models
Randy Kimple, University of Wisconsin, Madison

Patient-derived model systems play an important role in the development of novel therapeutic approaches. Key decisions regarding establishment, maintenance, and use of these models may have important implications for conclusions reached using them. Factors such as site of implantation, number of passages, patient/animal gender mismatches, and others may impact the resulting biology of the model. We are using samples from a window-of-opportunity study in patients with locally advanced head and neck cancer to compare the concordance of response between patient and model system and to confirm mechanisms of resistance identified using cell line models. Histology, imaging, and single cell approaches are being used to study the impact of implantation site and passage on the biology of these squamous epithelial cancers.

NCI Patient Derived Models Repository
Yvonne Evrard, NCI

The National Cancer Institute's Patient-Derived Models Repository (NCI PDMR; https://pdmr.cancer.gov) has developed a national repository of Patient-Derived Models (PDMs) comprised of patient-derived xenografts (PDXs), in vitro patient-derived tumor cell

cultures (PDCs) and cancer associated fibroblasts (CAFs) as well as patient-derived organoids (PDOrg). These PDMs are clinically annotated with molecular information available in an easily accessible database for the extramural community. To ensure the data and models remains pertinent to current translational efforts, extensive quality control and regular quality review are built into the model development cycle. Based on feedback from researchers, new molecular features are regularly added to the public database for researchers to use. Current model characteristics include two histologic classifiers, growth characteristics, histologic assessment, WES and RNASeq files, OncoKB variant reporting, inferred ancestry calls, human leukocyte antigen (HLA) typing, molecular classification of tumors (e.g. CMS subtyping of colorectal adenocarcinoma, PAM50 signature based subtyping of breast carcinoma), microsatellite instability (MSI) assessment, and identification of clinically relevant fusions. SOPs for the pipelines used are available on the public website to allow for independent validation and use by other laboratories.

Session 7 - Modeling Translational Questions: Technology II

Visualizing tumor heterogeneity in an immune intact and autochthonous mouse model of breast cancer
Snyder Lab and Joshua C. Snyder, Duke University

The heterogeneity of HER2 isoforms may, in part, explain the heterogeneity of responses to HER2 targeted therapy. While some patients are cured, others will ultimately progress to incurable and metastatic breast cancer. Three major isoforms of HER2 include the wild-type (WT), exon-16-splice isoform (d16), and the N-terminal truncation isoform (p95). The *objective* for our Onocology Models Forum project 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. Four aims were proposed 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. Our major findings over the last year have illustrated that p95 HER2 Crainbow tumor cells, but not WT or d16, disseminate and metastasize to the lungs. We also show that d16 tumors but not p95 are responsive to HER2 targeted monoclonal antibody therapy. Surprisingly, p95 tumors are also non-responsive to receptor tyrosine kinase inhibition (RTKi). Additional single cell analysis has revealed new epithelial states associated with the most invasive tumors. Future studies are directed to fully characterizing the spatial analysis of heterogeneous cell states and the contribution of the tumor microenvironment to tumorigenesis. Altogether, our analysis illustrates the importance of immune models of tumor heterogeneity by demonstrating that HER2 isoform her underly progression to treatment resistant breast cancer.

The Children's Oncology Group Patient-Derived Cell Line and Xenograft Repository.
C Patrick Reynolds, MD Ph, School of Medicine Texas Tech University Health Sciences Center Lubbock, TX

Many aspects of laboratory research on childhood cancers depends on the study of laboratory models. In large part, the impact of research is dependent on how robust and representative of

the patient are the models studied. Patient-derived cell lines (PDCLs) and patient-derived xenografts (PDXs) are important models for carrying out biological and preclinical therapeutic studies of childhood cancer. The COG/ALSF Childhood Cancer Repository establishes and banks PDCLs and PDXs from Children's Oncology Group (COG) biobanking protocols and distributes PDCLs and PDXs to > 500 investigators in 30 countries. To date the repository has established and banked PDCLs/PDXs from 524/78 neuroblastomas, 11/2 rhabdomyosarcomas, 20/3 Ewings sarcomas, 15/26 leukemias, 7/6 lymphomas, 41/1 retinoblastomas, 1/5 Wilms tumor, and 15 brain tumors. Repository users have published 373 peer-reviewed papers using PDCLs and PDXs from the repository. Repository models have demonstrated clinical relevance, having supported clinical testing of new agents (including in a phase III study) and development of novel cancer biomarkers. PDCLs and PDXs are validated as to patient origin by short-tandem repeat profiling, growth ability and telomere maintenance mechanisms are documented, are clinically annotated, and for PDXs the ratio of human to mouse tissue determined and the PDXs certified to be free of mouse and human pathogens. The repository attempts PDCLs in various pO2 conditions, with 50% of neuroblastoma patient samples only growing in tumor or bone marrow level hypoxia. The COG Neuroblastoma Committee ensures a large sample flow to the repository, enabling generation of pre/post therapy pairs patients (19 PDCL and 3 PDX pairs). Success with establishing PDCLs and PDXs from post-mortem blood samples is high, 69% for with 35 PDCLs and 83% for 21 PDXs, ideal models to study overcoming therapy resistance. The COG repository serves as a world-wide resource for patient-derived models of childhood cancers.

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

Michael Karin, Department of Pharmacology UCSD School of Medicine

Primary liver cancer (PLC), consisting of hepatocellular carcinoma (HCC; the major PLC in adults) and intrahepatic cholangiocarcinoma (ICC; the second most common adult PLC) is a major cause of cancer related deaths. Despite their distinct histology, HCC and ICC share similar etiologies, which used to be viral (HBV, HCV) in the past but now are replaced by metabolic associated steatohepatitis (MASH; used to be called NASH). Whereas immunotherapy with checkpoint inhibitors revolutionized HCC treatment, its effectiveness is modest (15 to 25% response rates), ICC is relatively non-responsive to currently available immunotherapies. I will describe one mouse model of immunogenic HCC and a newly generated model of autochthonous ICC, which shows immune exclusion, but remains to be characterized for its immunogenicity. Both models are based on the *MUP-uPA* transgenic mouse, which due to overproduction of urokinase plasminogen activator (uPA) in hepatocytes experiences ER stress early in life that can be reignited by MASH-inducing high fat (HFD) or high fructose (HFrD) diets. Whereas HFD or HFrD fed *MUP-uPA* mice develop classical MASH-related HCC that responds to PD-(L)1 blockade, conditional activation of transcription factor NRF2 in MUP-uPA hepatocytes results in spontaneous development of ICC in mice kept on normal chow diet.

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Next Generation Rat Models of ER+ Breast Cancer

Yi Li, Baylor College of Medicine

The rat has also been a leading model for research in behavior/psychology, physiology, pharmacology, and toxicology, and for the study of a wide range of common, genetically complex human diseases. In fact, the rat was the first and most commonly used model species until late 1990s and early 2000s when routine genetic engineering of mice advanced these smaller rodents

as the most commonly used model species in biology. However, new genetic engineering techniques, especially somatic CRIPR/Cas9 technology, have now cleared the technological hurdles for generating precision rat models. This is particular important for breast cancer. Currently, mouse models can model ER-negative breast cancer well, but they rarely develop ER+ breast tumors, which comprise 70% of all human breast cancer cases. Among the few mouse lines that do develop ER+ tumors, they generally do not respond to endocrine therapy, and they do not metastasize to the bone, the most common site of metastases in patients. I will review our latest development in creating and charactering rat models of breast cancer.