

Oncology Models Forum

2021 Annual Meeting

Agenda

DAY 2

DATE: Wednesday, March 31, 2021

TIME: 11:00 am – 5 pm EST

[WebEx Link](#)

PW: Wednesday1!

| TIME | TITLE | SPEAKER |
|---|--|---|
| 11:00 am | Welcome | Joanna Watson, PhD NCI |
| Session 4: Models for Predicting and Evaluating Therapies, continued | | |
| 11:10 am – 11:30 am | <i>Integration of high dimensional datasets in an immunocompetent mammary mouse model</i> PI: Mary Helen Barcellos-Hoff, PhD University of California, San Francisco | Mary Helen Barcellos-Hoff, PhD University of California, San Francisco |
| 11:30 am – 11:50 am | <i>Investigating cancer immunology using the NINJA model</i> PI: Nikhil Joshi, PhD Yale University School of Medicine | Nikhil Joshi, PhD Yale University School of Medicine |
| 11:50 am – 12:10 pm | <i>Polymerase-based ultramutagenesis in animal cancer models</i> PI: Diego Castrillon, Ph.D. UT Southwestern Medical Center | Diego Castrillon, Ph.D. UT Southwestern Medical Center |

| Session 4: Flash Talk | | |
|--|---|---|
| 12:10 pm – 12:15 pm | <i>Patient-Derived Cell Modeling Neuroendocrine Tumors</i> PI: Xuefeng Liu, PhD Georgetown University | Seema Agarwal, PhD Georgetown University |
| 12:15 pm – 12:35 pm | Break (20 minutes) | |
| Session 5: Credentialing in Non-Mouse Models | | |
| 12:35 pm – 12:55 pm | <i>A novel adenoviral-permissive, immunocompetent hamster model to evaluate oncolytic adenoviral therapy for glioblastoma</i> PI: Frederick Lang, MD MD Anderson Center | Frederick Lang, MD The University of Texas, MD Anderson CancerCenter |
| 12:55pm – 1:15 pm | <i>Porcine Models of Pancreatic Cancer</i> PI: Mark Carlson, MD University of Nebraska Medical Center | Mark Carlson, MD University of Nebraska Medical Center |
| 1:15 pm – 1:35 pm | <i>Human basal-like breast cancer is represented by one of the two intrinsic mammary cancer subtypes in dogs</i> PI: Shaying Zhao, PhD University of Georgia | Shaying Zhao, PhD University of Georgia |
| 1:35 pm – 1:55 pm | <i>Dog as a comparative model for cancer</i> PI: Kerstin Lindbald-Toh, PhD Broad Institute of MIT and Harvard | Kerstin Lindbald-Toh, PhD Broad Institute of MIT and Harvard |
| 1:55 pm – 2:15 pm | <i>Advancing blood biopsy through the canine comparative model</i> PI: Elinor Karlsson, PhD Broad Institute of MIT and Harvard | Elinor Karlsson, PhD Broad Institute of MIT and Harvard |
| Session 5: Flash Talks | | |
| 2:15 pm – 2:20 pm | <i>Canine Osteosarcoma as a Model of DMD Deletion</i> PI: Elinor Karlsson, PhD Broad Institute of MIT and Harvard | Heather Gardner, PhD Broad Institute of MIT and Harvard |

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|---|--|--|
| 2:20 pm – 2:25 pm | <i>Enabling comparative genomics through a machine learning classifier for human and canine cancers</i> PI: Elinor Karlsson, PhD Broad Institute of MIT and Harvard | Kate Mecquier, PhD Broad Institute of MIT and Harvard |
| 2:25 pm – 2:45 pm | Break (20 minutes) | |
| Session 6: PRECINCT – Pre-medical Cancer Immunotherapy Network Canine Trials | | |
| 2:45 pm – 3:05 pm | <i>Introduction to Comparative Oncology Program</i> | Amy LeBlanc, DVM DACVIM NCI |
| 3:05 pm – 3:25 pm | <i>Introduction to PRECINCT</i> | Nicola Mason, PhD, BVM University of Pennsylvania |
| 3:25 pm – 3:45 pm | <i>Enhancing the efficacy of immunotherapy in diffuse large B cell lymphoma (DLBCL) using rational combination approaches</i> PI: Cheryl London, DVM, PhD Tufts Cummings School of Veterinary Medicine | Cheryl London, DVM, PhD Tufts Cummings School of Veterinary Medicine |
| 3:45 pm – 4:05 pm | <i>Modulation of autophagy and apoptosis in doxorubicin treated canine cardiac slices</i> PI: Cheryl London, DVM, PhD Tufts Cummings School of Veterinary Medicine | Vicky Yang, DVM, PhD Tufts Cummings School of Veterinary Medicine |
| 4:05 pm – 4:25 pm | <i>Immunotherapy Trial in Pet Dogs with High-grade Glioma Promotes Translation to Phase I Human Glioblastoma Trial</i> PI: Michael Olin, PhD University of Minnesota | Michael Olin, PhD University of Minnesota |
| 4:25 pm – 4:45 pm | <i>Comparable Safety and Efficacy Challenges for CAR-T Therapy in Human and Canine Patients with B cell Malignancies</i> PI: Nicola Mason, PhD, BVM University of Pennsylvania | Nicola Mason, PhD, BVM University of Pennsylvania |
| 4:45 pm – 5:00 pm | Discussion: Moderated by Amy Leblanc | |
| 5:00 pm | Wrap-Up | |

Mary Helen Barcellos-Hoff, PhD
University of California, San Francisco

Abstract Title: Integration of high dimensional datasets in an immunocompetent mammary mouse model

Abstract

While major strides have been made in cancer treatment, the challenge remains to determine which patients will benefit most from a therapeutic regimen, which is the ultimate goal of personalized medicine. Almost all cancers are treated with cytotoxic chemotherapy and radiation therapy but the emerging clinical success of immunoncology (IO) drugs whose success is predicated on the biology of the immune infiltrate requires new tactics to model optimal combinations. As cytotoxic therapy is a critical component of cancer patient treatment, mammalian models that represent a range of DNA damage response deficits (DDR), and hence sensitivity to different agents, would improve translational research. Likewise, the multiple mechanisms by which cancer evades the anti-tumor immunity need to be represented in translational research. Given the range of IO approaches and the diversity of DDR, a critical unmet translational requirement is a model system in which both the tumor DDR and the immune infiltrate is defined so that combinations can be readily studied. To address this gap, we developed an immune competent model in which mammary tumor derived transplant (mTDT) derived from *Trp53* null primary cancers are expanded and maintained by serial in vivo passaging.

The pattern of tumor infiltrating lymphocytes (TIL) for each was assessed on spatial distribution of CD8 lymphocytes and designated as inflamed, excluded and desert. We visualized and quantified TGF β activity using PET-CT imaging of ⁸⁹Zr-TGF β antibody. TGF β activity was significantly increased in excluded tumors compared to inflamed or desert tumors, which was supported by quantitative pathology (Perkin Elmer) of its canonical signaling target, phosphorylated SMAD2 (pSMAD2). TGF β is a potent regulator of immune cell phenotypes and function. Hence we further characterized changes in immune system by multi-parametric cytometry time of flight (CyTOF) of spleens from tumor bearing mice. Mice bearing tumors with different patterns of TIL exhibit distinct splenic profiles. The spleens of mice bearing excluded tumors contained more classical dendritic cells. In contrast, spleens of mice bearing inflamed tumors were characterized by increased macrophages and plasmacytoid dendritic cells and those of mice bearing desert tumors spleens were characterized by increased cytotoxic CD8+ T cells. Two sub-groups were identified, those in which the frequency of pSMAD2 positive cells was high (>40%) versus those in which the frequency was low (<20%).

We recently showed that TGF β endorses effective DNA repair in tumor cells but loss or inhibition of TGF β signaling results in use of alternative DNA repair that is less effective (DOI: 10.1126/scitranslmed.abc4465). Gene signatures for TGF β competency and alternative end-joining repair are anti-correlated in human cancer and were associated with outcome for patients treated with radiotherapy or chemo-radiation. This anti-correlation is reproduced in mTDT. Consistent with the functional data, excluded tumors displayed high TGF β signatures and low expression of the alternative end-joining. To test the functional outcome, we used mTDT explants to assay 53BP1 foci from unresolved DNA damage following irradiation. mTDT with high pSMAD2 expression showed complete repair whereas mTDT with low pSMAD2 expression showed high levels of residual 53BP1 foci after irradiation.

These data from a new murine breast cancer model that represents the diversity of TIL patterns and DDR observed in human breast cancer will provide a robust platform for studies of therapeutic combinations.

Investigating cancer immunology using the NINJA model

Nikhil Joshi, PhD
Yale University School of Medicine

Investigating cancer immunology using the NINJA model

Genetically engineered mouse models of cancer recapitulate cardinal features of tumor biology, often including relevant genetic alterations for human disease and autochthonous tumor development within a native tumor microenvironment. However, tumors often lack the sources of neoantigens necessary to drive potent anti-tumor immune responses that are similar to those seen in patients with different cancer types. Using the inversion inducible joined neoantigen (NINJA) platform, we have adapted the Kras LSL-G12D; p53 fl/fl model so that developing tumors express neoantigens and demonstrate that these tumors elicit anti-tumor immune responses by tumor-specific CD8 and CD4 T cells. Moreover, in comparison to lentivirally programmed neoantigens in the KP model, KP-NINJA tumors express consistent levels of neoantigens per tumor and neoantigens can be induced at time points after the inflammation associated with tumor induction has abated. Finally, because the NINJA platform is modular, tumors in most Cre-inducible cancer models can be adapted for neoantigen expression, broadening the ability to perform studies of cancer immunology in other cancer types.

Polymerase-based ultramutagenesis in animal cancer models

Hao-Dong Li¹, Changzheng Lu¹, He Zhang², Qing Hu¹, Junqiu Zhang³, Ileana C Cuevas¹, Subhransu S Sahoo¹, Mitzi Aguilar¹, Elizabeth G Maurais¹, Shanrong Zhang⁴, Xiaojing Wang⁴, Esra A Akbay^{1, 5}, Guo-Min Li^{3, 4}, Bo Li^{5, 6, 7}, Prasad Koduru¹, Peter Ly^{1, 5, 8}, Yang-Xin Fu^{1, 5, 7}, Diego H Castrillon^{1, 5, 9}

¹Department of Pathology, ²Quantitative Biomedical Research Center, Department of Population and Data Sciences, ³Department of Radiation Oncology, ⁴Advanced Imaging Research Center, ⁵Simmons Comprehensive Cancer Center, ⁶Lyda Hill Department of Bioinformatics, ⁷Department of Immunology, ⁸Department of Cell Biology, and ⁹Department of Obstetrics & Gynecology, UT Southwestern Medical Center, Dallas, Texas, USA.

Abstract

Cancer is characterized by increased mutation rate, and recent work has led to a deeper understanding of mutator phenotypes and underlying molecular mechanisms. Differences in the mutational landscape of individual cancers underlie key aspects of clinical behavior. For example, overall base substitution rate is the best predictor of immune therapy response. Most genetically engineered mouse models of cancer (GEMMs) reiterate a few driver events but fail to recapitulate the high mutational loads that are the ultimate cause of most human cancer, making GEMMs inadequate for many aspects of tumor behavior, such as immune responses. The highest base substitution rates in cancers ($\geq 100/\text{Mb}$) result from specific heterozygous single amino acid substitutions (usually P286R) in the proofreading domain of DNA polymerase epsilon (POLE), rendering POLE highly error prone. *POLE* mutations are most common in endometrial cancer but occur with lower incidence in a wide range of cancers.

We hypothesized that GEMMs could be generated by recapitulating *Pole*^{P286R} via conditional knockin. Previously, we showed that constitutive expression of *Pole*^{P286R} throughout the body triggered malignancies of diverse lineages. Mice harboring *LSL-Pole*^{P286R} and the endometrial *BAC-Spr2f-Cre* developed endometrial cancers starting at 1 year. Endometrial cancers exhibited histologic features previously reported in human *POLE* tumors, and metastasized in all mice ($p < 0.00001$, $n = 36$ vs $n = 28$ controls). Of note, 100% penetrance was achieved through a single (monoallelic) amino acid change, with experimental animals efficiently generated in a single generation. Whole-genome and targeted cancer gene panel sequencing of primary tumors ($n = 12$) and cell lines ($n = 6$) revealed distinctive mutational signatures and the highest nucleotide substitution rates described in murine cancers, with some tumors exceeding 100 base substitutions/Mb. Since most DNA replication errors are repaired by mismatch repair (MMR), and some human tumors harbor *POLE* mutations and defective MMR (dMMR), we introduced dMMR via conditional *Msh2* knockout. This further accelerated tumor progression and mortality ($p < 0.00001$, $n = 36$ *Pole* alone vs. $n = 28$ *Pole+Msh2*), revealing clear synergism. In both models, there were robust antitumor immune responses, as evidenced by increased T-cell infiltrates, accelerated tumor growth following T-cell depletion by anti-CD4 antibody, and clinical regression following anti-CTLA4/PDL1 checkpoint therapy.

In summary, this is a robust approach to model the high mutational loads intrinsic to human cancer. Expression of *Pole*^{P286R} in a specific lineage is sufficient to result in cancers with greater ease than previous strategies such as dMMR alone, and with mutational loads in the range of human cancers. The approach may be useful for diverse models and investigations.

In Vitro and In Vivo Models for Circulating Tumor Cells to Study Metastasis

PI: Dr. Seema Agarwal, Georgetown University

Seema Agarwal, PhD
Georgetown University

Circulating Tumor Cells (CTCs) play a central role in cancer metastasis. Challenges in identifying and expanding ultra-rare CTCs has limited scientific and technological advances in combating metastatic cancers. We have developed a novel method to routinely generate short-term CTC cultures. In a study with 12 metastatic breast cancer patients we achieved a 100% success rate. Importantly, we were also successful in establishing CTC Derived Xenograft (CDX) models using cultured CTCs. This technological advance will provide well characterized individual patient-derived CTCs in sufficient numbers to allow genetic and molecular characterization. Detailed genomic and functional studies using these novel *in vitro* and *in vivo* models of metastases will help to elucidate metastasis-specific gene(s)/pathway(s), therapies that target to metastatic cancer cells and personalized treatment regimens for patients.

Mark A. Carlson
Department of Surgery, University of Nebraska
“Porcine Models of Pancreatic Cancer”
NCI Oncology Models Forum
March 31, 2021, 1:40 PM

Rodent cancer models may not accurately reflect human biology because of differences in physiology, anatomy, immune response, and genetic sequence between the two species. Remarkably, only 5-8% of anti-cancer drugs that emerged from preclinical studies and entered clinical studies have been ultimately approved for clinical use. The cause of this low approval rate is multifactorial, but likely includes the less-than-optimal predictive ability of some murine models to determine the efficacy of various therapeutics in humans. Murine models also have limited utility in the development of diagnostic or interventional technology that requires an animal subject whose size approximates a human. So at present, there remains a need for improved animal models of cancer (1) are more predictive of human response to anti-cancer therapy and (2) are of adequate size for development of specific technologies. The rationale for large animal cancer models is to have platforms for diagnostic/therapeutic device development otherwise not achievable in murine models; and (2) to have a highly-predictive preclinical models in which anti-cancer therapies could be vetted/optimized prior to a clinical trial. As an example, porcine biomedical models have been used for decades in the fields of trauma and hemostasis, xenotransplantation, dermal healing, toxicology, atherosclerosis, and cardiac regeneration, to name a few. The rationale to use the pig in this cancer modeling is that this species mimics human genomics, epigenetics, physiology, metabolism, inflammation and immunology, and size remarkably well (in particular, better than mice), with reasonable compromises towards cost and husbandry. Recently, a porcine genome map was generated, and further coverage, annotation, and confirmation is ongoing. Genetic manipulation of pigs (including knockouts, tissue-specific transgenics, inducible expression) with similar tools as used in the mouse is becoming more routine, with new gene-edited porcine models continually emerging. The pig has a proven track record in biomedical research as a mimic of human biology (in particular, superior to rodents) and is relatively close to human size, all of which makes the pig favorable for cancer modeling.

Human basal-like breast cancer is represented by one of the two intrinsic mammary tumor subtypes in dogs

Tianfang Wang, Joshua Watson, Kun-Lin Ho, Yuan Feng, Burair A. Alsaihati, Sydney Twyman, William C Bastian, Shaying Zhao

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Abstract

Mammary tumors in both humans and dogs are heterogenous and consist of molecularly distinct subtypes. However, unlike human breast cancers, canine mammary tumors have not been molecularly subtyped using genome-wide data, and the dog-human subtype homology has not been established. To address this deficiency, we subtyped >250 canine mammary tumors with published RNA-seq data using various strategies, and discovered two intrinsic subtypes. Importantly, one of the subtypes significantly matches basal-like breast cancer (BLBC), via PAM50 subtyping with canine tumors alone or in combination with human tumors, as well as molecular characterization including mutation profiles. The study justifies the use of an effective dog-human comparison approach developed by our lab to achieve cancer driver-passenger discrimination for amplified/deleted genes in BLBC.

Dog as a comparative model for cancer

Kerstin Lindblad-Toh

Science for Life Laboratory, Department of Medical Biochemistry and Microbiology, Uppsala University, Sweden and Broad Institute of MIT and Harvard, Cambridge, MA, USA

Dogs, like humans, suffer from a large number of complex diseases including cancer. As dogs have been bred into isolated populations, with a small number of founders, certain strong genetic risk factors have become common in specific breeds leading to an increase of specific cancers in different breeds. For example, many large breeds, including Leobergers, get osteosarcoma at a high rate. To harness the potential of studying cancer in dogs we have first improved the canine genome and gene annotation to make it almost as good as the human genome. We have also performed genomewide association studies to find inherited risk factors, for example for mammary tumors and osteosarcoma. In addition, we have previously performed tumor normal resequencing of multiple tumor types by whole exome sequencing and are now performing whole genome sequencing to be able to detect not only coding mutations but also non-coding constraint mutations to identify mutations leading to altered gene regulation. This information will allow us to compare canine and human cancers and to better characterize cancer subtypes, which might be useful for more tailored treatment options in both species.

ADVANCING BLOOD BIOPSY THROUGH THE CANINE COMPARATIVE MODEL

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⁴ Count Me In, Cambridge, MA, USA

Pet dogs are a powerful spontaneous model for human cancers, with an accelerated clinical course but similar interventions and treatment, presenting an opportunity for rapid preclinical trials. Blood biopsy is a non-invasive method for measuring and sequencing cell-free tumor DNA (cfDNA) present in the bloodstream. Using pet dogs, we are assessing the longitudinal use of blood biopsy to track tumor genomics during treatment, as well as the potential for early detection of relapse and identification of therapeutic vulnerabilities. We assessed the performance of blood biopsy in nine canine tumor types, measured the correlation between large-scale somatic copy number aberrations in osteosarcoma tumor samples and matched cfDNA samples, and tested whether phlebotomy site or timing affect yields. We developed a new capture panel for exome sequencing of blood biopsy samples, and have performed exome capture and sequencing of 12 blood biopsy samples in a small pilot study. Finally, we completed a longitudinal study of 10 dogs with lymphoma, collecting blood biopsies at treatment, remission, and relapse, and found a correlation between blood biopsy tumor fractions and treatment response. Our studies demonstrate the feasibility of blood biopsy in dogs, the high concordance between cfDNA and tumor samples, and suggest that blood biopsy is a promising method for monitoring response to therapy. Development of blood biopsy in dogs has the potential to accelerate clinical application of this technology in human medicine and to facilitate further high-impact comparative translational studies in the dog model.

Canine Osteosarcoma as a Model of *DMD* Deletion

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Canine osteosarcoma (OS) recapitulates the high structural complexity observed in human cancers such as OS. *DMD* deletions have recently been identified in 50% of spontaneous canine OS tumor samples, with analogous somatic *DMD* deletions recognized across several human cancers associated with aggressive biologic behavior. Therefore, we are leveraging canine OS to interrogate the role of *DMD* in tumor biology and identify points of therapeutic vulnerability. Whole genome sequencing of established canine OS cell lines detected *DMD* deletions in 3/8 (38%) of the cell lines, with a predominance of truncated dystrophin isoforms. CRISPR-Cas9 and shRNA-based technologies were subsequently used to knockout the Dp71 dystrophin isoform in a panel of canine OS cell lines. Our results suggest that dystrophin co-associates with the dystrophin-associated dystroglycan complex in canine OS, and that dystrophin deletion results in increased cell proliferation. The high incidence of naturally occurring *DMD* deletions combined with *in vitro* biologic correlatives in canine OS, support further investigation of targeting dystrophin dysregulation in canine OS as a tool to accelerate discovery in *DMD*-mutant human cancers.

Enabling comparative genomics through a machine learning classifier for human and canine cancers

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⁴Count Me In, Cambridge, MA, USA

Pet dogs are a powerful spontaneous model for human cancers. Dogs develop the same cancers that humans do, with striking similarities at the clinical, histopathological, and genomic level. Historically, comparative studies have been limited to cancers of the same histologic type. However, in some cases, cancers originating from a different cell type but carrying the same driver mutations may be useful models of cancers with the same driver. For example, the V600E mutation in *BRAF* is extremely common in canine bladder cancers, while in humans this driver mutation is more common in melanomas. In order to harness the potential of the canine model in the age of precision medicine, we must identify the closest cross-species matches at the genomic level.

To enable cross-species matching of cancers that is agnostic to histopathology, we have developed an analysis pipeline that uses a supervised learning algorithm to match cancers based on shared somatic variant features. Our approach first trains the classifier on human cancer data, then applies it to canine data to identify which human cancers the canine cancers match most closely. We have extracted over 20,000 genomic features, including mutated genes, affected pathways, and trinucleotide context, from over 20,000 human samples in 52 histologic cancer types using publicly available data from The Cancer Genome Atlas (TCGA), International Cancer Genome Consortium (ICGC), and cBioPortal. Using this data, we have trained a gradient boosting classifier (XGBoost) using the Scikit-learn package which classifies human cancers with 63% accuracy on cross-validation. Our cross-validation reveals substantial variation in accuracy by cancer type, likely reflecting variation in sample size, mutational burden, and mutational heterogeneity. Cancers with small sample sizes tend to perform poorly, including angiosarcoma and osteosarcoma, two rare cancer types of high comparative interest.

We have applied our classifier software to samples from four canine cancer types, including 47 angiosarcomas, 168 lymphomas (B and T cell), 102 osteosarcomas, and 77 melanomas. The initial results are intriguing. Notably, samples from only two canine cancer type are classified as the human cancer that would be considered the closest histological match (3% of canine melanomas and 2% of canine lymphomas). Some samples instead matched cancer types with known similarities in mutational patterns. For example, 23% of canine hemangiosarcomas were characterized as breast cancers, potentially reflecting the common occurrence of *PIK3CA* mutations in both cancer types. Other matches, for example 24% of canine osteosarcomas and 16% of canine melanomas being classified as liver cancer, are more difficult to interpret. We are currently investigating the most important features used to classify each cancer type in order to better understand what drives each classification. It is possible in some cases that lack of data due to small sample sizes may explain the low rate of matches between matching histologies. We are pursuing several approaches to help balance the amount of training data from each cancer type, including adding new data from the Count Me In initiative and other emerging datasets, sample weighting, over- and under-sampling classes, and exploring additional classification algorithms.

2021 Annual Meeting: NCI Oncology Models Forum

Amy LeBlanc, DVM DACVIM (Oncology)

Director, NCI Comparative Oncology Program

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, assessment of novel immunotherapeutic approaches, 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.

Burton JH, Mazcko C, **LeBlanc A**, et al. NCI Comparative Oncology Program Testing of Non-Camptothecin Indenoisoquinoline Topoisomerase I Inhibitors in Naturally Occurring Canine Lymphoma. *Clinical Cancer Research*; Published OnlineFirst July 30, 2018; DOI: 10.1158/1078-0432.CCR-18-1498.

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Saba C, Paoloni M, Mazcko C, ... **LeBlanc AK**. A comparative oncology study of iniparib defines its pharmacokinetic profile and biological activity in a naturally-occurring canine cancer model. *PLoS One* 2016 Feb 11;11(2):e0149194. doi: 10.1371/journal.pone.0149194.

LeBlanc AK, Mazcko C, Khanna C. Defining the value of a comparative approach to cancer drug development. *Clinical Cancer Research*. Published OnlineFirst Dec 28, 2015; doi: 10.1158/1078-0432.CCR-15-2347.

LeBlanc AK, Breen M, Choyke P, ... Khanna C. Filling in the Gaps for Man's Best Friend: Perspectives from a National Academies Institute of Medicine Workshop on Comparative Oncology. *Science Translational Medicine* 2016; 8 (325) [epub ahead of print]

Introduction to the pre-medical cancer immunotherapy network for canine trials (PRECINCT)

Nicola Mason B.Vet.Med., Ph.D.
University of Pennsylvania, School of Veterinary Medicine

Advances in our understanding of immunobiology and tumor biology together with increasing advanced genetic engineering approaches have led to unprecedented clinical responses in human patients with both solid and hematological cancers. However, further advances in clinical implementation of next generation immunotherapy strategies are hindered by the lack of clinically relevant, immune competent, spontaneous tumor models that accurately predict safety and therapeutic efficacy. Immune competent pet dogs develop spontaneous cancers with clinical and biological features similar to human malignancies and represent a parallel patient population that can be used to inform and accelerate clinical translation of next generation human immunotherapies. The primary goal of the Pre-medical Cancer Immunotherapy Network for Canine Trials (PRECINCT) is to facilitate the performance of immunotherapy clinical trials in canine cancer patients and to expedite the identification of immunological correlates of successful therapeutic responses. Since its inception in 2017, PRECINCT has established a Data Coordinating Center at the University of Pennsylvania that provides comprehensive project oversight, management and coordination for the 5 participating U01 sites and their satellite trial sites. PRECINCT has developed and maintains a Data Management System to collect, curate and share all clinical and correlative data amongst the U01 sites and with the Integrated Canine Data Commons (ICDC), the Comparative Oncology Program (COP) directors and PRECINCT Steering Committee members. The network also provides biostatistical support for study design and data analysis where needed to ensure that immunotherapy clinical trials performed within the network are strategically designed and appropriately powered to achieve meaningful clinical and laboratory data results in the most expedite way. Finally, the network provides initiative and support for the development of analytical tools that enable a deeper understanding of canine immune responses, identifying correlative immunological biomarkers and facilitating data harmonization across the network. These tools build value in the canine cancer patient as a “model” for immunotherapy. Examples of collaborative tool development within the network include the generation of a canine immune-oncology Nanostring panel comprising 800 genes that closely parallels the content and scope of the human pan-cancer immune profiling panel and the development of multiparametric, multicolor flow panels and SOPs for their use across the U01 sites. Over the past 4 years, the network has developed into a collaborative and interactive community where members share ideas, develop and evaluate innovative immunotherapeutic approaches and identify and build on natural and synergistic collaborations with the PRECINCT network and across other cancer moonshot networks including IOTN and PIDD.

Enhancing the efficacy of immunotherapy in diffuse large B cell lymphoma (DLBCL) using rational combination approaches

Gardner HL¹, McLinden G¹, Hendricks WPD², Avery AC³, Wood CA¹, London CA¹

¹Cummings School of Veterinary Medicine, Tufts University, North Grafton, MA;

²Translational Genomics Research Institute, Phoenix, AZ; ³College of Veterinary Medicine and Biomedical Sciences, Colorado State University, Ft. Collins, CO

Despite the addition of rituximab to CHOP chemotherapy in the treatment of diffuse large B cell lymphoma (DLBCL), up to 40% of human patients ultimately succumb to their disease. Additionally, those patients effectively cured have a high risk of long-term morbidities secondary to treatment. As such, novel approaches that reduce the intensity of chemotherapy while simultaneously improving outcomes through combination with small molecule inhibitors/immunotherapeutics are desirable. Unfortunately, exploration of such treatment strategies is typically relegated to patients with relapsed/refractory disease where efficacy is likely to be lower. It is now clear that DLBCL in dogs resembles the human disease with respect to clinical presentation, molecular aberrations, and genomic alterations making it a good spontaneous large animal model of disease. As such, we have been leveraging canine DLBCL to rapidly evaluate the activity of rational small molecule inhibitors targeting PI3K δ (RV1001), NAMPT/PAK4 (KPT-9274), or SUMO-Activating Enzyme (TAK-981) combined with immunotherapy (canine anti-CD20, from Elanco), with the ultimate goal of identifying the most effective combination to move forward in human patients. Using an adaptive mini-pilot trial approach in the front-line setting we are assessing the activity and toxicities of anti-CD20/targeted small molecule combinations in affected dogs and correlating clinical, biological, and genomic biomarkers to develop signatures that predict response to therapy and long-term progression-free survival. Our data demonstrate that anti-CD20 effectively depletes B cell populations in treated dogs and that treatment with RV1001 can break tolerance as evidenced by prednisone responsive transaminitis. We have identified long-term survivors in each treatment cohort, and our preliminary data suggest that a combination of low dose doxorubicin, anti-CD20, KPT-9274 and RV1001 may be associated with the long-term survival. For our first 3 cohorts, the median overall survival time for dogs was 638 days, compared to approximately 180 days for dogs that undergo similar treatment without immunotherapy. Importantly, 8/18 (44%) of the dogs are still alive at 15+ months (range 465+ to 645+ days). We have begun performing integrated genomic analysis of paired tumor/normal and matched constitutional samples from the dogs to identify candidate molecular correlates of treatment response. Matched tumor/normal whole genome sequencing confirmed previously described (TRAF3, SETD2, TP53) as well as novel candidate (MGA) driver mutations associated with canine DLBCL. We are also beginning to interrogate potential correlative biomarkers including NanoString based analysis of PBMCs using a new canine IO panel and longitudinal assessment of ctDNA in treated dogs.

Modulation of autophagy and apoptosis in doxorubicin-treated canine cardiac slices

Vicky Yang, Asma Boukhalfa, Howard Chen

Anti-cancer drug-induced cardiac injuries are becoming more prevalent in both canine and human patients with advancements in cancer treatment, either associated with known cardiotoxic drugs such as anthracycline or with newer therapies such as tyrosine kinase. Understanding cardiomyocyte response to toxic insult on a cellular level is paramount for the development of preventative treatment measures. Cardiomyocytes can respond to injury by undergoing autophagy that may aid in injury repair, or apoptosis and autosis that result in cell death. Identifying the mechanistic drivers for the switch between cardioprotective autophagy and damaging apoptosis and autosis may help researchers develop therapies to preserve cardiomyocytes during cancer treatment.

We use a novel canine cardiac slice culture system to investigate doxorubicin-induced change in autophagy, autosis, and apoptosis. This culture system allows for the study of adult canine heart tissues in response to anti-cancer drugs. Conventional cardiomyocyte culture is limited to neonatal murine cells isolated through enzymatic digestion, and adult cardiomyocytes are extremely difficult to maintain *in vitro* because of the lack of regenerative capability. Alternatively, cardiomyocytes can be generated from embryonic stem cells or induced pluripotent stem cells, but these cells maintain the fetal phenotype. The slice method preserves the structural and electrophysiological properties of the adult heart. We demonstrated that the heart slices obtained from client-owned, humanely euthanized adult canine patients maintain cell viability for up to 14 days after harvest. Cardiac slices were challenged with doxorubicin (0.5-50 μM) over a period of 30 min to 48 hours. Autophagy was assessed with autophagy-detecting nanoparticles that are activated upon entering autophagosomes and trafficking to lysosomes, as well as with expression of autophagy proteins (LC3, Beclin1), while apoptosis was assessed with TUNEL staining and histology, and autosis by rubicon expression. Cardiomyocyte injury was confirmed via cardiac troponin levels. Macroautophagy blockade was already evident at low doxorubicin concentration, leading to increased cell death. Autophagy induction with rapamycin restored autophagic flux and reduced apoptosis, while the opposite was observed with autophagy inhibitors (chloroquine). We demonstrated, using the cardiac slice culture system, that doxorubicin perturbs the autophagic flux, which results in cell death. Preservation of the normal autophagy may provide a cardioprotective strategy in cancer therapy.

Comparable safety and efficacy challenges for CAR-T therapy in human and canine patients with B cell malignancies

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Chimeric antigen receptor (CAR) T cells have shown remarkable promise in the treatment of hematological malignancies. However responses in patients with solid tumors has generally been disappointing. Furthermore, side effects including cytokine release syndrome, neurotoxicity, and on-target, off-tumor toxicity together with lack of durable response associated with the tumor microenvironment, tumor heterogeneity, target antigen escape and lack of CAR T persistence pose significant challenges to the curative potential of this approach.

Lack of clinically relevant, immune competent, spontaneous cancer models that recapitulate these challenges impedes progress in the development of next generation CART approaches designed to improve safety, efficacy and accessibility. Pet dogs develop spontaneous cancers that share similar clinical, biological, and genetic features with human cancers and represent a parallel, immunocompetent, patient population in which to evaluate CART therapies.

We have developed effective canine CAR T cell production protocols that parallel those used in human CART production and have demonstrated antigen-specific canine CAR T function *in vitro*. Systemic administration of autologous CD20-targeting CAR T cells to canine patients with B cell lymphoma reveals comparable challenges to human patients receiving CAR T therapies including cytokine release, target antigen escape, development of canine anti-murine antibodies and lack of CART persistence. Development of tools that include canine single chain fragment variable (scFv) phage display libraries to rapidly generate CAR constructs against exploratory target antigens further enables the canine cancer patient to serve as a valuable “model” in which to evaluate next generation CAR T therapies and combination therapies. Integrating pet dogs with spontaneous cancer into pre-clinical CART programs aims to improve safety and efficacy, identify correlative biomarkers of response and advance next generation approaches to increase the reach of CAR T to other tumor histologies and currently ineligible patient populations. Furthermore, this comparative approach serves to benefit both human and canine oncology patients.

No abstract submitted:

A novel adenoviral-permissive, immunocompetent hamster model to evaluate oncolytic adenoviral therapy for glioblastoma

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Immunotherapy Trial in Pet Dogs with High-grade Glioma Promotes Translation to Phase I Human Glioblastoma Trial

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