UW Carbone Cancer Center's Annual

RESEARCH RETREAT

TUESDAY, MARCH 16 • 9 AM - 4 PM
2021 VIRTUAL EVENT

ABSTRACT BOOK

Carbone Cancer Center
UNIVERSITY OF WISCONSIN
SCHOOL OF MEDICINE AND PUBLIC HEALTH

For more information:
CANCER.WISC.EDU/RESEARCH/RETREAT

Poster Presentations Breakout Sessions

Link to Poster Breakout Main Zoom Room
https://uwmadison.zoom.us/j/96215445923?pwd=NitpWnpkcWo3aTVFUGV5OGM1VU9MUT09

Once in the main Zoom room you will be able to select which session you want to attend.

Session 1: **Cancer Immunotherapy: Adoptive Cell Therapy**

Session 2: **Cancer Immunotherapy: Biologics**

Session 3: **Immune-related Adverse Events**

Session 4: **Single Cell Analyses/Cancer Stem Cells**

Session 5: **Precision Medicine**

Session 6: **Molecular Oncology**

Session 7: **Tumor Microenvironment**

Session 8: **Cancer Virology**

Session 9: **Clinical Oncology**

Session 10: **Population Health**

Poster Session 1: **Cancer Immunotherapy: Adoptive Cell Therapy**

Abstract number	Name		Title
4	Paul	Begovatz	Canine Natural Killer Cell Tracking via Fluorine Magnetic Resonance Imaging (19F-MRI) with a Clinical 1H/19F Torso Coil at 3T
25	Jeremy	Kratz	Sensitivity of HER2 Amplified Colorectal Cancer Organoids at Ex Vivo Resistance to Panitumumab and Trastuzumab
29	Nicholas	Mohrdieck	An immune co-stimulatory vaccine, with adoptive transfer of natural killer cells and immune inhibition blockade, after allogeneic bone marrow transplant, delays and reduces neuroblastoma tumor growth
55	Jiyoon	Bu	Multimodal Liquid Biopsy System for Cancer Immunotherapy Using PD-L1-Targeting Peptides
71	Sejal	Sharma	Synthetic Fusion Cytokine, GIFT4, Transduced Autologous Peritoneal Immune Cells (PICs) - a Novel Cell Therapy for Treatment of Ovarian Cancer.
72	Longzhen	Song	FIST15 enhances anti-Tumor effect of NK cells against murine osteosarcoma after Bone Marrow Transplant

Poster Session 2: **Cancer Immunotherapy: Biologics**

Abstract number	Name		Title
3	Taylor	Aiken	Short-course neoadjuvant intratumoral immunocytokine establishes immunologic memory in murine melanoma
9	Monica	Cho	CD155 blockade boosts alloreactive natural killer cell antitumor effects against osteosarcoma
10	Sarah	Emma	Mechanisms of cooperative response to bempegaldesleukin (BEMPEG) and 90Y-NM600 targeted radionuclide therapy in the treatment of a syngeneic murine model of head and neck squamous cell carcinoma
12	Arika	Feils	INV721, a bispecific SNIPER antibody targeting GD2 and B7H3, improves tumor specific immunotherapeutic antibody targeting of neuroblastoma tumors
45	Fernanda	Szewc	Alpha-tocopheryloxyacetic acid induces apoptosis of murine rhabdomyosarcoma in vitro while modulating innate and adaptive immune responses in vivo
59	Adam	Drelich	Peptide-Mediated Multivalent Nanoparticles: Promising Platforms for Cancer Immunotherapy
67	Erin	Nystuen	Estrogen receptor blockade and radiation therapy may enhance the anti-tumor response to immune checkpoint inhibitors in immunologically cold ER+ breast cancer
73	Elizabeth	Sumiec	In vivo synergy of 90Y-NM600 and Bempegaldesleukin improves anti-tumor efficacy of immune checkpoint inhibitors in syngeneic murine cancer models
75	David	Turicek	Treating post-transplant B-cell acute lymphoblastic leukemia with blinatumomab as a radiation sparing immunotherapy

Poster Session 3: **Immune-related Adverse Events**

Abstract number	Name		Title
14	Matthew	Forsberg	Human monocytes educated with exosomes from TLR4 primed mesenchymal stem cells treat acute radiation syndrome by promoting hematopoietic recovery
15	Mikayla	Foster	Psychological and Physical Function in Hematopoietic Cell Transplant Survivors with Chronic Graft-Versus-Host Disease
17	Nicholas	Hess	Identification and function of a CD4+/CD8αβ+ T cell population that is predictive of GVHD development in a xenogeneic transplant model
19	Daniel	Huff	Detection and monitoring of immune-related adverse events with 18F-FDG PET/CT in cancer patients receiving immune checkpoint inhibitors
20	Justin	Jagodinsky	Temporal analysis of type 1 interferon activation in tumor cells following external beam radiotherapy or targeted radionuclide therapy

Poster Session 4: **Single Cell Analyses/Cancer Stem Cells**

Abstract number	Name		Title
5	Joshua	Brand	Single-cell landscape of human fallopian tubes and its implications for high grade serous ovarian cancer
13	Patricia	Filippsen Favaro	Feasibility and promise of circulating tumor DNA analysis in dogs with naturally-occurring sarcoma
31	Ruben	Moreno	Cellular contributions of Lgr5 cells in MmuPV1 infected cutaneous skin
35	Sofia	Romero	Getting to the core: single-cell dynamics and subcellular trafficking of hepatitis B virus core proteins
48	Maria	Virumbrales-Munoz	3D Patient-Specific Microfluidic Models of Primary Head and Neck Cancer To Evaluate Individual Drug Responses
61	Alexa	Heaton	Intravital multiphoton microscopy of infiltrating T cell and tumor cell metabolism in a murine melanoma model
68	Autumn	Olson	Baseline culture conditions do not alter the growth or treatment response of F1. FCAKPK mouse derived cancer organoids
69	Michael	Poellmann	Circulating Tumor Cell Trends in Gastrointestinal Cancer Predict Treatment Response Using a Highly Sensitive Detection Device and Machine Learning for Enumeration

Poster Session 5: **Precision Medicine**

Abstract number	Name		Title
6	Ngai Ting	Chan	Transcriptional elongation machinery controls vulnerability of breast cancer cells to PRC2 inhibitors
22	Maggie	Keating	Topical and Systemic Administration of a Dual PI3K/mTOR Inhibitor in Mice with Established High-Grade Anal Dysplasia
24	Sam	Krabbenhoft	The oncoproteins H3 K27M and EZHIP inhibit PRC2 by conserved mechanisms in mammals and Drosophila melanogaster.
58	Rebecca	DeStefanis	BCLxL inhibition is necessary for induction of intrinsic apoptosis in PIK3CA mutant colorectal cancer
62	Nguyet-Minh	Hoang	DNMT3A contributes to ibrutinib resistance in mantle cell lymphoma
64	Chunrong	Li	Molecular Targeted Radiotherapy with 90Y-NM600 for the treatment of leukemia
66	Ashita	Nair	A Hybrid Nanoparticle System Integrating Exosomes and Poly(amidoamine) Dendrimers: Implication for an Effective Gene Delivery Platform
70	Ankita	Shahi	Effects of molecular-targeted radiotherapy with 90Y-NM600 on tumor growth and the tumor microenvironment in murine rhabdomyosarcoma
78	Meng	Xu	Tracking and Mapping Tumor-Targeted Alkylphosphocholine Metal Analogs with Mass Spectrometry Imaging

Poster Session 6: **Molecular Oncology**

Abstract number	Name		Title
7	Mo	Chen	A Nuclear p53-Phosphoinositide Signalosome Regulates Akt Activation
8	Changliang	Chen	A Novel Phosphoinositide-NRF2 Complex Regulates Oxidative Stress
18	Yang	Hu	Paclitaxel induces micronucleation and activates pro-inflammatory cGAS-STING signaling in triple-negative cancer
34	Rebecca	Reese	The role of GRHL2 in facilitating pS118-ER transcriptional activity in hormone-dependent breast cancers
46	Narendra	Thapa	Phosphatidylinositol 3-kinase Signaling is Spatially Organized at Endosomal Compartments by Microtubule-associated Protein 4
52	Tianmu	Wen	Interaction between Star-PAP and the phosphoinositide stress signaling pathway
56	Sierra	Colavito	Investigating CHK1 and AXL in claudin-low breast cancer
57	Sarah	Copeland	Mad1 Upregulation in Breast Cancer: Causes and Consequences

Poster Session 7: **Tumor Microenvironment**

Abstract number	Name	Column1	Title
16	Kristal	Gant	Characterizing Collagen Organization in Precursor Lesions of High Grade Serous Ovarian Cancer
21	Katherine	Johnson	Cancer-associated fibroblast phenotypes vary across colorectal cancers and correlate with CD8+ T-cell infiltration
27	Anna	Lippert	Impact of Cancer Associated Fibroblast Phenotypes on the Infiltration of T-lymphocytes in Early Age Onset Colorectal Cancer
28	Hannah	Micek	The Influence of Extracellular Matrix on Metastatic Multicellular Detachment in High-Grade Serous Ovarian Cancer
38	Shannon	Rush	The Rhesus Macaque: A Unique and Compelling Model for Human Endometriosis-Associated Ovarian Carcinomas
40	Shannon	Rush	Determining Parity in Immunohistochemistry (IHC) staining of Ovarian and Fallopian Tube Tissues in Humans and Non-Human Primates Rhesus Macaque and Common Marmosets
42	Shreyans	Sadangi	A Microfluidic Platform To Investigate the Effects of Skin Microenvironment on Primary Melanoma Evolution
43	Nan	Sethakorn	LumeNEXT: A microfluidic organ-on-chip model of the bone niche in solid tumor metastases to assay stroma-mediated drug resistance
63	Genevra	Kuziel	Identifying the role of fibrocytes in obesity-induced mammary gland fibrosis
76	Brenna	Walton	Obesity reduces breast epithelial TGF-B activity potentially through enhanced ECM sequestration
77	Abbey	Williams	Breast density and obesity increase mammary gland inflammation

Poster Session 8: **Cancer Virology**

Abstract number	Name	Column1	Title
36	Quincy	Rosemarie	Cellular chromatin reorganization by Epstein-Barr Virus, a human tumor virus, during its lytic phase
47	Nicholas	Van Sciver	Hippo Signaling Effectors YAP and TAZ Induce Epstein-Barr Virus (EBV) Lytic Reactivation Through TEADs in Epithelial Cells
51	Tao	Wei	Role of IQGAP1, a PI3K signaling scaffolding protein, in papillomavirus-associated head and neck tumorigenesis using an infection-based animal model
53	Andrea	Bilger	Papillomavirus infection of the oropharynx causes advanced dysplasia in immune-competent mice, and squamous cell carcinoma in HPV E5 transgenics, within 2 weeks
54	Simon	Blaine-Sauer	Studying the interplay of cervicovaginal dysbiosis and papillomavirus infection in a preclinical murine model
65	Amanda	Loke	A Novel In Vitro Culture Model System to Study Merkel Cell Polyomavirus Associated MCC Using Three-Dimensional Organotypic Raft Equivalents of Human Skin

Poster Session 9: **Clinical Oncology**

Abstract number	Name	Column1	Title
23	Aria	Kenarsary	Clinical Characteristics and Outcomes of Prostate Cancer Patients Undergoing [F- 18] fluciclovine PET/CT scan at UWMSPH – A Retrospective study
32	Sarvesh	Periyasamy	Guidance and Assessment of Histotripsy Ablations using C-Arm Imaging Systems
33	Robert	Pohlman	Validation of Microwave Ablation using Electrode Displacement Elastography
44	Jack	Shireman	Effect of Pre-Operative Stereotactic Radiosurgery on Non-Small Cell Lung Cancer Brain Metastasis: Initial Radiobiologic Analysis of DNA and RNA Genomic Profiles from Phase-II Clinical Trial NCT03398694
49	Matthew	Wagar	Postoperative venous thromboembolism in gynecologic oncology patients undergoing minimally invasive surgery: Does modality matter?
60	Michael	Fallon	Leveraging an Electronic Health Record to Support a Clinician-Driven High-Dose Methotrexate Management Platform

Poster Session 10: **Population Health**

Abstract number	Name	Column1	Title
11	Alma	Farooque	Pre-anthracycline Left Ventricular Ejection Fraction (LVEF) Assessment and Long-Term Cardiovascular (CV) Outcomes in Adolescent & Young Adult (AYA) Lymphoma Survivors
26	John	Krebsbach	Pancreatic Cancer Treatment and Outcome Disparities between Rural and Urban Patients
30	Rachel	Mojdehbakhsh	Disparities in the enrollment of racial and ethnic minorities in clinical trials of poly ADP ribose polymerase inhibitors for women's cancers
37	Shannon	Rush	Funding to Lethality Measures in 11 Years of National Cancer Institute Funding Allocation
39	Shannon	Rush	The State of K2R Grant Funding by Cancer Type from the National Cancer Institute
41	Shannon	Rush	Hormone Replacement Therapy Counseling after Prophylactic Bilateral Salpingo-Oophorectomy in High Risk Patients
50	Connor	Wang	Cancer Lethality: an important burden metric to consider for the allocation of clinical trial research funding from public, industry, and other sources

List of Abstracts

Abstract #3

Short-course neoadjuvant intratumoral immunocytokine establishes immunologic memory in murine melanoma

Taylor J. Aiken, David Komjathy, Mat Rodriguez, Arika Feils, Stephen D. Gillies, Amy K. Erbe, Alexander L. Rakhmilevich, Paul M. Sondel

Introduction: GD2 is disialoganglioside preferentially expressed in neuroblastoma and melanoma and anti-GD2 directed therapies are used clinically in neuroblastoma, with ongoing clinical trials in melanoma. We are currently developing an *in situ* vaccination approach using intratumoral (IT) delivery of an immunocytokine (IC) consisting of IL-2 linked to an anti-GD2 monoclonal antibody. While IT-IC monotherapy does not cure mice bearing established B78 melanoma tumors, it is effective when combined with local radiation therapy (RT). Here, we tested whether short course IT-IC monotherapy prior to surgical resection could result in a robust adaptive immune response preventing tumor recurrence following rechallenge after surgery.

Methods: Mice bearing 50-100mm^3 GD2-expressing melanoma (B78) tumors were treated with a 5-day course of 50μg IT-IC and complete surgical resection was performed 3 days following the final treatment. The immune infiltrate of resected tumors was assessed by flow cytometry. Rechallenge experiments consisted of either 2x10^6 B78 cells injected into the contralateral flank or 2x10^5 B16-GD2 cells injected via tail vein for pulmonary metastasis rechallenge.

Results: IT-IC treated tumors had fewer viable tumor cells, increased CD8 T-cells, and an improved CD8:Treg ratio. Rejection of B78 contralateral flank rechallenge (implanted 40 days following surgical resection of the primary tumor) was observed in 78% (7/9) of mice treated with IT-IC compared to 50% (5/10) that received surgery alone and 0% (0/5) of naïve mice. Immunologic memory was potent in neoadjuvant-treated mice early after surgery, with all mice (5/5) rejecting contralateral B78 rechallenge that occurred on the day of surgery compared to 0% (0/5) in both surgery-alone and naïve mice. Neoadjuvant IT-IC also prevented the development of B16-GD2 lung metastasis compared to naïve mice or the surgery-alone group (when the IV injected experimental metastases were given 80 days following surgery).

Conclusions: While ineffective in curing large B78 melanoma flank tumors as monotherapy, mice receiving neoadjuvant IT-IC developed robust immunologic memory preventing recurrence following surgery. The memory response was present as early as the day of surgery and was sufficient to prevent pulmonary metastasis. IT-IC should be further investigated as a neoadjuvant therapy for preventing recurrence in high-risk settings.

Abstract #4

Canine Natural Killer Cell Tracking via Fluorine Magnetic Resonance Imaging (^{19}F-MRI) with a Clinical ^{1}H/^{19}F Torso Coil at 3T

Paul Begovatz, Monica Cho, Mallery Olsen, Lawrence Lechuga, Rachel McMahon, David Vail , Christian Capitini, Sean Fain

Introduction

Immunotherapy cancer treatments are evolving which implement natural killer cells (NK cells) to target and kill cancer; and fluorine magnetic resonance imaging (^{19}F-MRI) is emerging as a non-invasive method to track immune cells *in vivo*. However, these studies have been conducted on small animal MRI systems ($B_0 \geq 7T$), and the transition to clinical 3T field strengths is needed to advance the relevancy of ^{19}F-MRI immunotherapy cell tracking. Therefore, this study set out to investigate the feasibility of NK cell tracking on a clinical 3T MRI with a ^{1}H/^{19}F torso coil, through the *in situ* reproducibility imaging of ^{19}F labeled canine NK cells in canine limbs.

Methods

Canine limbs (N=3) were obtained from a male Beagle canine (age: <2 years), which was euthanized prior to the removal of both thoracic and pelvic limbs. NK cells were obtained from 15-20ml of blood from healthy canine volunteers (N=3) and CD5lo NK fraction cells were isolated (#cells: 3x10^6, 5x10^6, 5x10^6) and then co-cultured in RPMI medium containing canine IL-21 and human IL-2 along with 8 mg/ml perfluoropolyether (PFPE) (CS-1000, Celsense Inc, PA) for 24 hours. The labeled allogeneic NK cells (100ul) were injected next to the bone of a canine limb and immediately positioned for ^{19}F/^{1}H-MRI, followed by a reproducibility measurement with canine limb and coil repositioning. ^{19}F-MRI was conducted on a 3.0T Discovery MR750w MRI (GE Healthcare, WI) with a 8-channel ^{1}H/^{19}F torso coil (MRI Tools, Germany), and a cartesian 3D fast spin echo sequence (TR: 750ms, TE: 27.4ms, ETL: 16, FOV: (250x250x30)mm, resolution: (3x2x2)mm, BW: 15.63kHz, NEX: 42, T = 42min).

Results

In each limb, cartesian 3D fast spin echo ^{19}F-MRI revealed clear signal intensities corresponding to ^{19}F labeled NK cells located at distances between 12-18mm from the coil surface. Reproducibility measurements revealed that 3x10^6 NK cells were detected with a SNR=9.6 ± 0.5 ($Mean \pm SD$), whereas 5x10^6 cells were repeatedly detected at an SNR=11.1 ± 1.4. Furthermore, overlay with proton structural images discovered that all detected ^{19}F-MRI signal intensities were found in the canine limb near the bone of PFPE labeled NK cell injection sites.

Conclusion

This study found that NK cells labeled with an FDA approved PFPE nano-emulsion were reliably detected through ^{19}F-MRI with the combination of a cartesian 3D fast spin echo imaging sequence, and a ^{1}H/^{19}F torso coil on a clinical 3T MRI within a 42-minute scan time.

Abstract #5

Single-cell landscape of human fallopian tubes and its implications for high grade serous ovarian cancer

Joshua Brand[1,2], Kate Lawrenson[3], Huy Q. Dinh[1,4]

[1]McArdle Laboratory for Cancer Research, UW Madison, WI
[2]Department of Pathology and Laboratory Medicine, UW Madison, WI
[3]Cedars Sinai Medical Center, Los Angeles, CA
[4]Department of Biostatistics and Biomedical Informatics, UW Madison, WI

High grade serous ovarian cancer (HGSOC) is the most common and most lethal form of ovarian cancer, receiving little outcome improvements over the years. HGSOC is often diagnosed at a late stage, so there is an urgent need for earlier detection leading to improved survival outcomes. Emerging molecular data of tumors and prevalence of precursor lesions suggest HGSOC development begins in the fallopian tube (FT). Characterizing FT cellular heterogeneity is vital to understanding HGSOC progression that could lead to identification of early biomarkers and therapeutic targets.

Our lab utilized single cell transcriptome technology (scRNA-Seq) to profile 53,000 cells from 12 non-malignant FT specimens. We established a computational pipeline combining existing analysis methods with a comprehensive justification based on statistical and biological criteria to identify and annotate cell-types and novel subsets. These cell-types will be characterized by biological pathway enrichment and cell-cell interaction analysis. Their gene signatures will be used to analyze hundreds of published HGSOC bulk RNA-Seq samples to identify distinct subsets associated with clinical features.

Our lab and others previously characterized the heterogeneity of FT epithelial cells and potential gene regulation pathways related to HGSOC. To the best of our knowledge, we present the first report of single-cell transcriptome of non-epithelial FT cells. Our analyses identified abundant fibroblasts, T/NK cells, and to a lesser extent, myeloid and B cells. We defined 5 fibroblast subsets with consistent representation across patients and unique gene signatures relevant to the complement system, metallothioneins, and collagen genes. T cells, including both activated and suppressive phenotypes, were highly enriched, representing nearly half of the total cells. We will present potential cell-cell communication via ligand receptor interactions and how they might influence the origin and progression of HGSOC within the newly characterized subsets.

In summary, we presented a comprehensive single-cell transcriptome map of the FT cellular ecosystem and identified key cell-types and functional pathways that influence HGSOC progression. On-going work will integrate this FT data with our in-house HGSOC tumor scRNA-Seq and large-scaled published bulk RNA-Seq together with experimental validation to gain mechanistic insights into cellular dysregulation and tumorigenesis.

Abstract #6

Transcriptional elongation machinery controls vulnerability of breast cancer cells to PRC2 inhibitors

Ngai Ting Chan[1], Junfeng Huang[2], Gui Ma[1], Hao Zeng[1,3], Yidan Wang[1], Lingjun Li[2,5] and Wei Xu[1,4]

[1] McArdle Laboratory for Cancer Research, University of Wisconsin-Madison, Madison, WI 53706, USA

[2] School of Pharmacy, University of Wisconsin-Madison, Madison, WI 53705, USA

[3]Present address: H3 Biomedicine, Cambridge, MA 02139, USA

[4] UW Carbone Cancer Center, School of Medicine and Public Health, University of Wisconsin-Madison, Madison, WI 53705, USA

[5] Department of Chemistry, University of Wisconsin-Madison, Madison, WI 53706, USA

Running title: CTR9 demarcates H3K27me3 repressive domains

Keywords: PAFc, Polycomb repressor complex 2, transcription, H3K27me3, Ctr9

*Corresponding Author:

Dr. Wei Xu

McArdle Laboratory for Cancer Research, Wisconsin Institute for Medical Research, 1111 Highland Ave, Madison, WI 53705

Phone: 608-265-5540

Fax: 608-262-2824

Email: wxu@oncology.wisc.edu

ABSTRACT

CTR9 is the scaffold subunit in PAFc (Polymerase associated factor complex), a multifunctional complex employed in multiple steps of RNA Pol II-mediated transcription. CTR9/PAFc is well known as an evolutionarily conserved elongation factor regulating many actively transcribing genes by directly interacting with corresponding histone modifications enzymes. However, its function in counteracting repressive histone markers has not been appreciated. Using inducible and stable CTR9 knockdown breast cancer cell lines, we discovered that the amount of H3K27me3 is strictly controlled by CTR9. Quantitative profiling on histone modifications revealed a striking increase of H3K27me3 level upon loss of CTR9. Moreover, loss of CTR9 leads to genome-wide expansion of H3K27me3 as well as increased recruitment of PRC2 on chromatin. Mechanistically, CTR9 depletion triggers a PRC2 subtype switching from PRC2.2 to PRC2.1 which exhibits higher PRC2 methyltransferase activity and thus promoting H3K27me3 spreading. Regulation of H3K27me3 and PRC2 signaling by CTR9 is dynamic since all these changes could be reversed by CTR9 re-expression. Furthermore, some genes displaying H3K27me3 increase upon loss of CTR9 contain putative CTR9 binding, indicating that transcription defects caused by CTR9 loss may catalyze H3K27me3 spreading. As a consequence, CTR9 depletion generates vulnerability that renders breast cancer cells hypersensitive to PRC2 inhibitors. Our findings that CTR9 demarcates PRC2-mediated H3K27me3 levels and genomic distribution provide a unique mechanism of transition from transcriptionally active to repressive chromatin states and shed light on the biological functions of CTR9 in development and cancer.

Abstract #7

A Nuclear p53-Phosphoinositide Signalosome Regulates Akt Activation

Mo Chen[1], Suyong Choi[1], Tianmu Wen[1], Changliang Chen[1,2], Narendra Thapa[1], Vincent L. Cryns[2], and Richard A. Anderson[1,*]

[1]University of Wisconsin-Madison, School of Medicine and Public Health, 1300 University Avenue, Madison, WI 53706, USA

[2]Department of Medicine, University of Wisconsin Carbone Cancer Center, University of Wisconsin-Madison, School of Medicine and Public Health, 1111 Highland Avenue, Madison, WI 53705, USA

*Correspondence to: Richard A. Anderson, 3750 Medical Sciences Center, 1300 University Avenue, Madison, WI 53706. Phone: 608-262-3753; Fax: 608-262-1257; E-mail: raanders@wisc.edu

Background

The tumor suppressor p53 is activated by a wide range of cellular stressors, protecting the genome from genotoxic stress, oxidative stress, hypoxia, and oncogene activation. Most cellular stressors induce accumulation of wild-type p53 in the nucleus for the transcriptional regulation of gene networks that lead to cell cycle arrest, DNA damage repair, senescence, apoptosis, and/or metabolic reprogramming. *TP53* is the most commonly mutated gene in cancer and results in a gain-of-function protein that promotes tumor progression. We have recently discovered that p53 is a novel phosphoinositide (PI)-binding protein. The PI kinase PIPKIα binds to mutant p53 and stress-induced wild-type p53 and transfers its product PI4,5P2 to the C-terminus of p53, which stabilizes p53 by recruiting small heat shock proteins. However, the functional role of the nuclear p53-PI complex and whether other PIs modify it is unknown.

Methods

We have utilized immunofluorescent staining (IF), immunoprecipitation (IP), Western Blot (WB), proximity ligation assay (PLA), and microscale thermophoresis (MST) to test the interactions of p53, PIs, PI-modifying enzymes, and PI-downstream targets under stressed conditions. Different nuclear markers in combination with confocal or super-resolution microscopy were used to locate the functional site and molecules involved in the PI-p53 association.

Results

Here we show that genotoxic stress induces the interaction of Akt and the Akt-activating kinases PDK1 and mTORC2 with p53, leading to the nuclear activation of Akt. The PI3-kinase IPMK binds to mutant p53-PI4,5P2 to produce p53-PI3,4,5P3, a reaction that is reversed by the 3- phosphatase PTEN. In the membrane-free nucleoplasm, p53-PI3,4,5P3 binds Akt, PDK1 and mTORC2 to sustain Akt activation and subsequently recruit and phosphorylate downstream targets such as FOXOs. The expression levels of mutant p53 level are tightly correlated with activated Akt and FOXOs in the nucleus. Moreover, silencing IPMK inhibits the assembly of p53- PI3,4,5P3 and sensitizes cancer cells to genotoxic stress.

Conclusion

We have discovered a novel nuclear p53-PI signalosome that regulates Akt activation in response to genotoxic stress that is entirely distinct from the canonical membrane-based PI 3-kinase/Akt pathway. The *de novo* synthesis of PI3,4,5P3 linked to p53 via the nuclear PI-p53 signalosome activates the stress-induced nuclear Akt pathway and protects tumor cells from genotoxic stress.

Additionally, the p53-PI signalosome directly connects p53 and Akt, two of the most commonly altered cancer pathways, for the first time. As such, the discovery of this alternative pathway for nuclear Akt activation has profound therapeutic implications for cancer.

Abstract #8

A Novel Phosphoinositide-NRF2 Complex Regulates Oxidative Stress

Changliang Chen[1,2], Mo Chen[2], Narendra Thapa[2], Suyong Choi[2], Tianmu Wen[2], Richard A. Anderson[2] and Vincent L. Cryns[1, #]

[1]Department of Medicine, University of Wisconsin Carbone Cancer Center, University of Wisconsin-Madison, School of Medicine and Public Health, 1111 Highland Avenue, Madison, WI 53705, USA.

[2]University of Wisconsin-Madison, School of Medicine and Public Health, 1300 University Avenue, Madison, WI 53706, USA

Correspondence to: Vincent L. Cryns, 4144 MFCB, 1111 Highland Avenue, Madison, WI 53705. Phone: 608-262-4786; Fax: 608-263-9983; E-mail: vcryns@wisc.edu

ABSTRACT

Oxidative stress is a condition in which the balance between production and disposal of reactive oxygen or nitrogen species is altered. Cancer cells have elevated levels of reactive oxygen species due to oncogenic alterations that fuel their rapid growth. The transcriptional activator nuclear factor erythroid 2-related factor 2 (NRF2) is a master regulator of the antioxidant response that is aberrantly activated in many tumors, enabling their survival. Although mutations in KEAP1 stabilize NRF2 in a subset of cancers, additional regulators of NRF2 are likely to contribute to its aberrant activation in cancer. Here, we show that the stability of stress-induced NRF2 is regulated by the type I phosphatidylinositol phosphate kinase PIPKIγ and its product phosphatidylinositol 4,5-bisphosphate (PtdIns(4,5)P2). Nuclear PIPKIγ binds to NRF2 in response to oxidative stress, resulting in the production and association of PtdIns(4,5)P2 with NRF2. PtdIns(4,5)P2 binding promotes the interaction between NRF2 and the small heat shock proteins HSP27 and αB-crystallin. Silencing PIPKI-γ or sHSPs destabilizes NRF2,

pointing to a critical role of this complex in regulating NRF2 stability and function. Taken together, we have discovered an unexpected role for phosphinositide kinases and their lipid second messengers in regulating NRF2 and the antioxidant response in cancer, thereby implicating these molecules as novel drug targets.

Abstract #9

CD155 blockade boosts alloreactive natural killer cell antitumor effects against osteosarcoma

Monica Cho, Madison Phillips, Longzhen Song, Amy Erbe-Gurel, Christian Capitini

Background
Pediatric patients with relapsed and refractory osteosarcoma have poor prognoses with few treatment options. Allogeneic bone marrow transplant (BMT) has not yet shown a graft-versus-tumor (GVT) effect for osteosarcoma. Natural killer (NK) cells demonstrate antitumor activity against osteosarcoma, but adoptively transferred NK cells have limited cytotoxicity and persistence in vivo. To enhance an NK-specific GVT effect, we propose blocking the CD155 checkpoint molecule, which is overexpressed on osteosarcoma and can engage both activating and inhibitory NK cell receptors. The impact of CD155 blockade on GVT and graft-versus-host-disease (GVHD) is unknown.

Methods
NK cells from C57BL/6 (B6) mice were expanded with recombinant IL-15/IL-15R and analyzed by flow cytometry. Cytotoxicity assays were performed with IL-15 expanded B6 NK cells and K7M2 murine osteosarcoma with blockade of CD155 and CD155 ligands. To test NK cell infusion and CD155 blockade treatment after allogeneic BMT, BALB/c mice were lethally irradiated, transplanted with allogeneic B6 bone marrow, and challenged with luciferase-expressing K7M2 on day 0. At day 7, mice received IL-15 expanded B6 NK cells intravenously with either anti-IgG control or anti-CD155 antibody intraperitoneally and IL-2 subcutaneously on days 7 and 11. Mice were monitored for tumor growth by bioluminescence, and GVHD toxicity using weight loss and clinical scores.

Results
Compared to unexpanded murine NK cells, IL-15 expanded NK cells (n = 6) show increased expression of NKG2D (65.33 ± 10.77% NKG2D$^+$, p = 0.0077; 1030 ± 177.0 MFI, p = 0.0101) and an increased ratio of CD155 activating (CD226) to inhibitory (TIGIT) ligand expression (11.71 ± 4.121, p = 0.0362). In cytotoxicity assays with IL-15 expanded allogeneic NK cells (n = 3 replicates), CD155 blockade enhances K7M2 osteosarcoma lysis (60.62 ± 3.19%, p = 0.0189) compared to IgG control (29.01 ± 7.66%). CD226 blockade decreased tumor killing (10.62 ± 8.51%, p = 0.0053) compared to CD155 blockade. Allogeneic murine NK cell infusion and anti-CD155 antibody treatment after allogeneic BMT decreased tumor area under the curve by 44.3% compared to IgG control, without exacerbating GVHD.

Conclusions
These findings demonstrate that CD155 blockade enhances an allogeneic NK cell-specific GVT effect against osteosarcoma without exacerbating GVHD. CD155 blockade may improve allogeneic BMT and NK cell adoptive immunotherapy as a combination treatment for osteosarcoma, and perhaps other pediatric sarcomas.

Abstract #10

Mechanisms of cooperative response to bempegaldesleukin (BEMPEG) and 90Y-NM600 targeted radionuclide therapy in the treatment of a syngeneic murine model of head and neck squamous cell carcinoma

Sarah Emma, Amber M. Bates, Reinier Hernandez, Joseph J. Grudzinski, Ian R. Marsh, Justin C. Jagodinsky, Bryan P. Bednarz, Alexander A. Pieper, Elizabeth G. Sumiec, Erin J. Nystuen, Gustavo A. Sosa, Ravi B. Patel, Jamey Weichert, Zachary Morris

The purpose of this study was to evaluate mechanisms of cooperative therapeutic effects of bempegaldesleukin (BEMPEG; NKTR-214) and 90Y-NM600 in head and neck squamous cell carcinoma (HNSCC). BEMPEG is a first in class, CD122-preferential interleukin-2 pathway agonist tailored to stimulate antitumor immunity through promoting the activation and proliferation of CD8+ T and NK cells. Targeted radionuclide therapy (TRT) delivered at low doses to sites of metastatic cancer can enhance immune susceptibility in immunologically "cold" tumors such as the MOC2 syngeneic mouse HNSCC model. We hypothesized that combining BEMPEG and 90Y-NM600 would cooperate to increase immunosusceptibility and immune cell infiltration to create a more favorable tumor immune microenvironment.

MOC2 tumors were engrafted in the flank of C57BL/6 female mice. Mice were randomized into one of eight treatment groups for a survival study utilizing varying combinations of BEMPEG, 90Y-NM600, and anti-CTLA4. In vivo dosimetry was performed prior to treatment day 1 using the Monte Carlo based RAPID platform. Serial 86Y-NM600 PET/CT imaging indicated that the dose delivered to the tumor site was ~12 Gy. Cohorts of mice in a parallel study were treated with PBS (control), BEMPEG, 90Y-NM600, or the combination of BEMPEG and 90Y-NM600. Flow cytometry, qPCR analysis, and multiplex cytokine analyses were used to evaluate tumors collected at day 14.

In this immunologically "cold" murine HNSCC model, a complete tumor response was observed in 62.5% of mice treated with the triple combination therapy of BEMPEG, 90Y-NM600, and anti-CTLA4. In comparison to all single therapy groups, mice treated with BEMPEG and 90Y-NM600 had increased CD8+ T cell tumor infiltrate and 90Y-NM600 induced increased expression of the IL2βγ receptor, CD122, on the surface of CD8 T cells. Tumors from mice treated with BEMPEG and 90Y-NM600 had increased expression of genes associated with tumor cell immune susceptibility, a type 1 interferon response, tumor immune cell recruitment, activation of cytotoxic T lymphocytes, and production of immune stimulatory cytokines. These results suggest a synergistic interaction between BEMPEG and 90Y-NM600 that improves the immune microenvironment in this difficult to treat murine model of HNSCC.

Abstract #11

Pre-anthracycline Left Ventricular Ejection Fraction (LVEF) Assessment and Long-Term Cardiovascular (CV) Outcomes in Adolescent & Young Adult (AYA) Lymphoma Survivors

AUTHORS: Alma Farooque, BA; Cibele B. Carroll, MD MPH; Amye Teevarwerk, MD; Priyanka Pophali, MD

BACKGROUND: Anthracycline chemotherapy, commonly used in lymphoma treatment, has an established long-term risk of cardiac toxicity. There are no specific guidelines for CV screening and follow-up of AYA patients treated with anthracyclines. Pediatric guidelines focus on long-term imaging surveillance, while for adults, LVEF assessment prior to anthracyclines is recommended. Multiple studies have demonstrated LVEF assessment rarely impacts treatment decisions, especially in the absence of CV symptoms/risk factors, adds to unnecessary costs and delays treatment initiation. Our study aimed to determine the pre-treatment LVEF assessment practices in AYA lymphoma patients treated with anthracyclines and its association with long-term cardiotoxicity.

METHODS: Survivors diagnosed with lymphoma >5 years ago and treated with anthracyclines at age 15-39 years were identified in a retrospective single institution registry. To ensure adequate follow-up, at least 2 follow-up visits during 2015-19 were required. Data abstracted on eligible subjects included documentation of pre-treatment LVEF evaluation, clinical rationale and treatment regimen. CV risk factors and events were collected pre-treatment and during follow-up. Descriptive statistics were used to summarize data.

RESULTS: 64/115 (56%) of AYA lymphoma patients underwent pre-treatment LVEF assessment. Rationale for/against LVEF assessment was rarely documented: low CV risk was recorded as rationale for no LVEF assessment in 2 subjects. Among AYAs who underwent pre-treatment LVEF assessment, no significant abnormalities were detected and no changes in subsequent treatment plans were found. During median follow-up of 6.7 (inter-quartile range 5.4-9.5) years, 6/115 (5%) experienced CV events. Only 2 (1.7%) survivors experienced potential anthracycline-related CV events including cardiomyopathy, congestive heart failure, and atrial fibrillation. Both had other CV risk factors- family history, smoking, obesity, hyperlipidemia. Four (3.5%) survivors experienced CV events with clear alternative etiology e.g. sepsis/symptom burden. There was no correlation between having pre-treatment LVEF assessment and occurrence of CV events. 13/115 (11.3%) developed new CV risk factors.

CONCLUSIONS: Pre-treatment LVEF assessment is done inconsistently in AYA lymphoma patients but does not appear to impact treatment regimen or predict late cardiotoxicity. CV events in long-term AYA lymphoma survivors are rare but evaluation of CV risk factors, early detection and management may be more important than focusing on LVEF assessment.

References:

1. Watts RG, George M, Johnson WH. Pretreatment and routine echocardiogram monitoring during chemotherapy for anthracycline-induced cardiotoxicity rarely identifies significant cardiac dysfunction or alters treatment decisions. *Cancer*. 2012;118(7):1919-1924. doi:10.1002/cncr.26481
2. Jeyakumar A, Dipenta J, Snow S, et al. Routine cardiac evaluation in patients with early-stage breast cancer before adjuvant chemotherapy. *Clin Breast Cancer*. 2012;12(1):4-9. doi:10.1016/j.clbc.2011.07.006
3. O'Brien P, Matheson K, Jeyakumar A, Anderson K, Younis T. The clinical utility of baseline cardiac assessments prior to adjuvant anthracycline chemotherapy in breast cancer: a systematic review and meta-analysis. *Breast Cancer Res Treat*. 2019;174(2):357-363. doi:10.1007/s10549-018-05114-7
4. Truong SR, Barry WT, Moslehi JJ, Baker EL, Mayer EL, Partridge AH. Evaluating the Utility of Baseline Cardiac Function Screening in Early-Stage Breast Cancer Treatment. *Oncologist*. 2016;21(6):666-670. doi:10.1634/theoncologist.2015-0449

Abstract #12

INV721, a bispecific SNIPER antibody targeting GD2 and B7H3, improves tumor specific immunotherapeutic antibody targeting of neuroblastoma tumors

Feils AS, Erbe AK, Rosenkrans Z, Gerhardt D, Hammer B, Felder M, Bercher M, Engle JW, Barnhart TE, Hank JA, Glaser B, Green R, Hernandez R, Sondel PM

Introduction

Immunotherapy with anti-disialoganglioside mAb (anti-GD2), dinutuximab, is part of the standard treatment for children with high-risk neuroblastoma, the most common extracranial solid tumor in children. Yet, dinutuximab treatment causes severe neuropathic pain in patients, as GD2 is not only expressed on tumor cells but peripheral nerves as well. A separate tumor antigen, B7H3, is commonly overexpressed on neuroblastoma, with minimal expression on most normal cells and no expression on nerves. To mitigate toxicity resulting from off-tumor binding to nervous tissue, we developed a bispecific SNIPER antibody, INV721, to simultaneously target these two tumor antigens.

Methods

INV721 was engineered with the individual Fab arms targeting GD2 and B7H3 to have low-moderate affinity, such that the antibody only binds with high affinity when both arms concurrently bind to their respective antigen. INV721 was titrated to assess binding efficacy to GD2/B7H3-expressing tumors via flow cytometry. The ability of INV721 to induce antibody-dependent cellular cytotoxicity (ADCC) and target GD2/B7H3-expressing tumors was evaluated with Incucyte spheroid-killing assays. *In vivo* biodistribution of SNIPER binding was measured by positron emission tomography (PET) imaging with ^{89}Zr-labeled INV721 compared to ^{89}Zr-labeled dinutuximab.

Results

Antibody titrations showed binding of INV721 to GD2+/B7H3+ neuroblastoma cells, even at the lowest amount, 1.25ng/million cells. Minimal binding was observed to cells that do not express both antigens. Incucyte assays confirmed that INV721 is capable of inducing ADCC in GD2+/B7H3+ cells. A positive correlation was observed between the titration binding curve dissociation constant (Kd) for INV721 and the respective ADCC activity. PET imaging revealed an increased uptake of INV721 in GD2+/B7H3+ tumors compared to GD2-/B7H3- and GD2+/B7H3- or GD2-/B7H3+ tumors, further confirming the binding specificity to double positive targets *in vivo*.

Discussion

Our *in vitro* and *in vivo* data confirm that INV721 is effective at simultaneously targeting the GD2 and B7H3 tumor antigens; resulting in greater binding specificity and tumor killing via ADCC. Continued efforts to assess whether INV721 is associated with reduced neuropathic pain are ongoing. Our goal is to enhance the tumor-specificity with this bispecific SNIPER antibody and reduce the toxic/painful side-effects, ultimately resulting in improved tolerance and clinical benefit.

Abstract #13

Title: **Feasibility and promise of circulating tumor DNA analysis in dogs with naturally-occurring sarcoma**

Postdoctoral Trainee: Patricia Filippsen Favaro
Authors: Patricia Filippsen Favaro, Samuel D. Stewart, Bradon R. McDonald, Jacob Cawley, Tania Contente-Cuomo, Shukmei Wong, William P.D. Hendricks, Jeffrey M. Trent, Chand Khanna, Muhammed Murtaza

Keywords: cell-free DNA, biomarker, comparative oncology

Background: Comparative studies of naturally-occurring canine cancers have provided new insight into many areas of cancer research. The inclusion of pet dogs in the development and validation of circulating tumor DNA (ctDNA) diagnostics may be uniquely informative for human translation for many reasons, including: high incidence of certain spontaneous cancers, repeated access to blood and tumor from the same individuals during the course of disease progression, and molecular heterogeneity of naturally-occurring cancers in dogs.

Methods: We present a feasibility study of ctDNA analysis performed in 9 healthy dogs and 39 dogs with either benign or malignant splenic tumors (hemangiosarcoma) using shallow whole genome sequencing (sWGS) of cell-free DNA. To enable detection and quantification of ctDNA using sWGS, we adapted two informatic approaches and compared their performance for the canine genome.

Results: At presentation, mean cfDNA tumor fraction in dogs with malignant splenic tumors was 11.2%, significantly higher than dogs with benign lesions (3.2%; p 0.001), achieving an AUC of 0.84. ctDNA fraction was 14.3% and 9.0% in dogs with metastatic and localized disease, respectively, although this difference was not statistically significant (p 0.227). In analysis of paired samples, ctDNA fraction decreased from 11.0% to 7.9% after resection of malignant tumors (p 0.047).

Conclusion: Our results demonstrate that ctDNA analysis is feasible in dogs with hemangiosarcoma using a cost-effective approach such as sWGS. Future studies are underway to validate these findings, and further evaluate the role of ctDNA to assess burden of disease and treatment response during drug development.

Abstract #14

Human monocytes educated with exosomes from TLR4 primed mesenchymal stem cells treat acute radiation syndrome by promoting hematopoietic recovery

Matthew Forsberg, John Kink, Peiman Hematti, Christian Capitini

Total body irradiation is often used as a conditioning regimen for bone marrow transplants but can cause life threatening damage to host tissues especially the bone marrow. Developing a cellular therapy that can protect the bone marrow from acute radiation syndrome and stimulate hematopoiesis is a priority for patients exposed to therapeutic or even accidental radiation injury. In this study, exosomes derived from MSCs stimulated with the TLR4 agonist lipopolysaccharide (LPS) were used to alternatively activate human monocytes, termed LPS EEMos, as a potential novel radioprotective cellular therapy. LPS EEMos expressed higher levels of PD-L1 ($p<0.0001$), and lower levels of CD16 ($p<0.01$), CD86 ($p<0.01$), and CD206 ($p<0.0001$) by flow cytometry compared to monocytes educated with exosomes from unstimulated MSCs (EEMos). Using qPCR, increased gene expression in LPS EEMos of IL-10 ($p<0.05$), IDO ($p<0.001$), FGF2 ($p<0.05$), IL-15 ($p<0.05$), and IL-6 ($p<0.0001$) were detected compared to EEMos. Using a xenogeneic radiation injury model, infusion of human LPS EEMos in to NSG mice 4 hours after lethal radiation led to reduced clinical scores and an increased survival at 40 days post-infusion, as compared to infusions of PBS, EEMos, and monocytes alone, all of which led to worse clinical scores and 0% survival with uniform death by 20 days ($p<0.05$). Complete blood cell counts in LPS EEMo recipients showed leukocyte, erythrocyte and platelet counts equivalent to non-irradiated mice, demonstrating complete restoration of hematopoiesis. In vitro co-culture experiments showed that LPS EEMos were also able to improve the survival of Irradiated human CD34+ haemopoietic stem cells. Due to toxicity concerns, the TLR4 binding, lipid a analogue CRX was used instead of LPS to stimulate MSCs prior to exosome isolation in additional mouse experiments. Results from these experiments showed that the CRX-EEMos provided mice with the same radioprotective effect as LPS-EEMos. Infusion of LPS or CRX EEMos may be a useful strategy to protect the bone marrow from acute radiation syndrome by expression of anti-inflammatory molecules and cytokines that promote hematopoiesis/engraftment.

Abstract #15

Psychological and Physical Function in Hematopoietic Cell Transplant Survivors with Chronic Graft-Versus-Host Disease

Mikayla A. Foster, Mark B. Juckett, Peiman Hematti, & Erin S. Costanzo

Background: Chronic graft-versus-host disease (cGVHD) is a frequent complication of allogeneic hematopoietic cell transplantation (HCT). While clinical manifestations are well characterized, little is known about the impact on psychological and physical well-being.

Methods: A longitudinal dataset tracking hematologic cancer patients pre- and post-HCT was used to compare psychological and physical well-being for allogeneic HCT patients who did and did not develop cGVHD. Participants (N=251) completed measures of depression, anxiety, and well-being (IDAS), fatigue intensity and interference (FSI), pain intensity and interference (BPI), and sleep disturbance and duration (PSQI) at 6 months and 1 and 3 years post-HCT. T-tests were used to compare scores for participants who had developed cGVHD to those that had not at each assessment point. ANOVA was employed to determine whether scores varied by cGVHD severity.

Results: During the study period, 109 participants (43.4%) were diagnosed with cGVHD (NIH consensus criteria) at a median of 191 days post-HCT (range = 80-707). There were few differences on psychological measures. The exception was that cGVHD patients reported more anxiety at 1-year post-HCT (t=-2.28, p=.02). There were more notable differences in physical symptoms. Those with cGVHD reported greater fatigue intensity at 6 months and 3 years (t=-2.25 and -2.03, ps<05), greater pain intensity and interference at 1 and 3 years (t=-2.02 and -2.41, ps<.05), and shorter and more disturbed sleep at 6 months and 3 years post-HCT, respectively (t=-2.12 and -2.35, ps<.05). Follow-up analyses focused on the subset with active cGVHD, as defined by those on immunosuppressants, showed the same pattern of findings. Only one association was seen between cGVHD severity and symptom scores: those with moderate-to-severe cGVHD reported poorer overall sleep as compared to those with mild cGVHD at 6 months post-HCT (F(1,50)=6.10, p=.02).

Conclusion: Among allogeneic HCT survivors, those who develop cGVHD are at risk for greater impairment in physical function relative to HCT survivors without cGVHD, regardless of cGVHD severity. At the same time, those with cGVHD demonstrate relatively resilient psychological function. Findings further suggest that fatigue, pain, and sleep may be important intervention targets to improve function and quality of life for those with cGVHD.

Abstract #16

Characterizing Collagen Organization in Precursor Lesions of High Grade Serous Ovarian Cancer

Kristal L. Gant[1], Alexander N. Jambor[2], Eric C. Rentchler[2], Manish S. Patankar[1,4], and Paul J. Campagnola[2,3]

kgant@wisc.edu, ajambor@wisc.edu, rentchler@wisc.edu, patankar@wisc.edu, and pcampagnola@wisc.edu

[1]Department of Obstetrics and Gynecology, [2]Department of Biomedical Engineering, [3]Department of Medical Physics, [4]Carbone Cancer Center, University of Wisconsin-Madison, Madison, WI.

Background: Ovarian cancer is the most lethal of the gynecological cancers and is the fifth leading cause of cancer deaths in women. This is due to a lack of early detection tools. There is a need for a thorough understanding of extracellular matrix alterations in relation to metastasis. Previous data has shown a significant difference in collagen fibril orientation in the ovarian tumor microenvironment as compared to normal ovarian and peritoneal tissues. However, the collagen arrangement in precursor lesions such as p53 signatures and serous tubal intraepithelial carcinomas (STICs) has not previously been explored. Perhaps there are changes that occur in collagen early on in high grade serous ovarian cancer that may shed light on disease progression.

Hypothesis: There is a rearrangement of collagen fibrils within the ovarian tumor microenvironment early in ovarian cancer development that allows for tumor advancement.

Methods: Normal, and Serous Tubal Intraepithelial Carcinomas (STIC), and High Grade Serous Ovarian Cancer (HGSOC) were analyzed by Second Harmonic Generation (SHG) Microscopy for differences in and reorganization of collagen fibrils. Image analyses of SHG images were completed by ImageJ, MatLab, and Origin programs. Statistical and discriminatory analyses were completed in Statistical Analysis System (SAS).

Results: Imaging studies showed an incremental change in collagen fibrils as normal tissues transition to HGSOC. There was a significant difference between normal and HGSOC tissues while a subtle difference between normal and STIC tissues. Normal, p53 signatures, and STIC tissues overlapped but statistical differences between them suggested there may be a trend towards collagen remodeling and micro-environmental changes, even in the earliest stages of ovarian pathology that are not detectable with current diagnostics. Image analyses and statistical tests provided classification accuracies for normal, p53 signatures, and STICs as 62%, 100%, and 78%, respectively. Our ongoing studies will focus on analyzing the collagen organizational patterns in both precursor and cancer lesions, and improving classification accuracies for all groups.

Conclusion: Collectively, these efforts will allow us to visualize extracellular matrix alterations that may potentially develop into collagen signatures prior to HGSOC development and aid in understanding overall tumor biology and HGSOC etiology.

Abstract #17

Identification and function of a $CD4^+/CD8\alpha\beta^+$ T cell population that is predictive of GVHD development in a xenogeneic transplant model

Nicholas Hess, Peiman Hematti, Jenny Gumperz

Background: Graft-vs-host disease (GVHD) is mediated by donor reactive T cells that have a hierarchical classification based on CD4 and CD8 expression. While CD4 and CD8 lineages are thought to have fixed expression, $CD4^+/CD8\alpha\beta^+$ double positive (DP) T cells have recently been reported in cases of human cancers and autoimmune diseases though the lack of a suitable model system has hindered their research.

Methods: In this study, we transplanted primary human graft tissue into non-conditioned immunodeficient mice and observed the development of a human DP T cell population that was not present in the starting grafts. Furthermore, we are collecting leftover blood samples from allogeneic HSCT patients to confirm the development and association of DP T cells with clinical GVHD.

Results: DP T cells developed irrespective of graft tissue (peripheral blood or bone marrow), accessory cells (i.e. isolated T cells) and immunodeficient mouse strain (NSG and NBSGW). An increase in the percentage of DP T cells in the blood of these mice is correlated and predictive of GVHD development. We also observed that DP T cells are functionally active with elevated IFNγ and TNFα secretion compared to CD4 and CD8 single positive T cells. DP T cell also express cytotoxic machinery components including NKG2D and perforin/granzyme. Interestingly, transplantation of isolated $CD4^+$ cells did not result in the development of DP T cells while a robust population developed after transplantation of isolated $CD8^+$ T cells. We have also identified DP T cell development in clinical HSCT patients who have developed GVHD though additional patients are still required before statistical analysis of DP as a biomarker of GVHD can be performed.

Conclusion: In conclusion, we have defined a novel human T cell population that is correlated with xenogeneic and clinical GVHD. Ongoing studies will further test our hypothesis that DP T cells are the primary effector population responsible for GVHD pathology. Not only with understanding the development of DP T cells add to our understanding of human T cell biology but DP T cells may also serve as a predictive biomarker of GVHD allowing for preemptive treatment before clinical GVHD pathology arises.

Abstract #18

Paclitaxel induces micronucleation and activates pro-inflammatory cGAS-STING signaling in triple-negative cancer

Yang Hu , Baraa K. Manasrah , Stephanie M. McGregor , Robert F. Lera , Roshan X. Norman , John B. Tucker , Christina M. Scribano , Rachel E. Yan , Kari B. Wisinski , Amye J. Tevaarwerk , Ruth M. O'Regan , Lee G. Wilke , Beth A. Weaver , Ning Jin , Mark E. Burkard

Taxanes remain one of the most effective medical treatments for breast cancer. Recent trials have coupled taxanes with immune checkpoint inhibitors (ICIs) in triple-negative breast cancer (TNBC) patients with promising results. However, the mechanism linking taxanes to immune activation is unclear. To determine if paclitaxel could elicit an antitumoral immune response, we sampled tumor tissues from patients with TNBC receiving weekly paclitaxel (80 mg/m2) and found increased stromal tumor-infiltrating lymphocytes (sTILs) and micronucleation over baseline in 3 of 4 samples. Mechanistically, paclitaxel operates by inducing chromosome missegregation on multipolar spindles during mitosis. Consequently, post-mitotic cells are multinucleated and contain micronuclei, which can activate cyclic GMP-AMP synthase (cGAS) and induce a type I interferon response reliant on the stimulator of interferon genes (STING) pathway. Other microtubule-targeting agents (MTAs), eribulin and vinorelbine, recapitulate this cGAS/STING response and increased the expression of immune checkpoint molecule, programmed death-ligand 1 (PD-L1), in some types of TNBC cells. To test the possibility that MTAs sensitize tumors that express cGAS to ICIs, we identified ten TNBC patients treated with PD-L1 or PD-1, seven of whom also received MTAs. Elevated baseline cGAS expression significantly correlated with treatment response in patients receiving MTAs in combination with ICIs. Our study identifies a mechanism by which MTAs can potentiate an immune response in TNBC. Further, baseline cGAS expression may predict treatment response in therapies combining MTAs and ICIs.

Abstract #19

Detection and monitoring of immune-related adverse events with ^{18}F-FDG PET/CT in cancer patients receiving immune checkpoint inhibitors

Daniel Huff, Hamid Emamekhoo, Scott Perlman, Robert Jeraj

Background

Immune checkpoint inhibitors (ICI) improve patient outcomes. However, many patients experience serious, potentially fatal, immune-related adverse events (irAE) as side effects. irAE cause organ inflammation and can require treatment cessation as management. Additionally, growing evidence suggests irAE development and ICI response may be related. Molecular imaging with ^{18}F-FDG PET/CT, which is used to assess response in ICI patients, can also play a role in monitoring irAE. In this work,we focus on detecting irColitis in the bowel, but also present preliminary findings for irPneumonitis and irThyroiditis.

Methods

We retrospectively analyzed ^{18}F-FDG PET/CT scans of 40 patients receiving ICI for melanoma, lymphoma, or lung cancer at UWCCC. A convolutional neural network was trained to segment the bowel, lungs, and thyroid from low dose CT and used to quantify organ ^{18}F-FDG uptake. SUV metrics (SUV_{max}, SUV_{mean}, SUV_{total}, percentiles of SUV histogram) were compared to clinical diagnoses of irAE. Differences in SUV metrics between irAE and non-irAE groups were assessed with Wilcoxon sign-rank tests and Receiver Operating Characteristic (ROC) analysis.

Results

irColitis was observed in 7/40 (18%) of reviewed patients. Median time to clinical irColitis diagnosis was 140 days after treatment start (range:13-302). The most predictive biomarker of irColitis was the 95th percentile of the bowel SUV histogram ($SUV_{95\%}$, AUROC=0.86). Patients who later received a clinical diagnosis of immune-related colitis had a significantly higher increase in $SUV_{95\%}$ from baseline to first on-treatment PET than patients who did not experience colitis (p=0.023). The optimal cutoff for detecting irColitis was $\Delta SUV_{95\%}$>+40% (Sensitivity=75%, Specificity=88%). For irColitis patients, elevated bowel PET uptake was seen a median of 115 days prior to clinical diagnosis (range: 30-206). However, in 2/7 colitis patients, the diagnosis of colitis occurred prior to the first on-treatment PET. In an expanded cohort (N=70), similar performance for detecting irThyroiditis (AUROC=0.96) and irPneumonitis (AUROC=0.99) was observed.

Conclusion

Increased ^{18}F-FDG uptake in normal organs is a useful early indicator of immune-related adverse events in patients receiving immune checkpoint inhibitors. While standard-of-care ^{18}F-FDG PET/CT provides sufficient information for irAE prediction, it could be further improved by acquiring follow-up PET more quickly after treatment start.

Abstract #20

Temporal analysis of type 1 interferon activation in tumor cells following external beam radiotherapy or targeted radionuclide therapy

Justin C. Jagodinsky, Amber M. Bates, Reinier Hernandez, Joseph J. Grudzinski, Ian R. Marsh, Ishan Chakravarty, Ian S. Arthur, Luke M. Zangl, Ryan J. Brown, Erin J. Nystuen, Sarah E. Emma, Caroline Kerr, Won Jong Jin, Peter M. Carlson, Jonathan W Engle, Eduardo Aluicio-Sarduy, Todd E. Barnhart, Trang Le, KyungMann Kim, Bryan P. Bednarz, Jamey P. Weichert, Ravi B. Patel, and Zachary S. Morris

Background: Radiation (RT) activates a type 1 interferon (IFN1) response which is critical for synergy with immune checkpoint blockade (ie anti-CTLA4). Clinical interest in utilizing systemically administered targeted radionuclide therapy agents (TRT) is growing as these agents can be used to target multiple sites of disease including micro-metastases. It is unclear how IFN1 activation induced by continuous delivery of RT during exponential decay of a TRT source will compare to that induced following instantaneous external beam RT (EBRT). Here we report the time course of IFN1 response following RT *in vitro* and *in vivo.*

Methods: We utilized murine models of melanoma (B16, B16 STING knockout, B78), and head and neck squamous cell carcinoma (MOC2). For *in vitro* studies, EBRT was prescribed to 2.5 Gy, 12 Gy, or 3 fractions of 8 Gy. For *in vivo* studies, RT (TRT or EBRT; 2.5 Gy or 12 Gy cumulative absorbed dose) was delivered when mean tumor size was 100-150 mm^3. For TRT, we used ^{90}Y conjugated to NM600, an alkylphosphocholine analog that exhibits selective uptake and retention in tumor cells. Cells or tumors were harvested at 1d, 7d, and 14d post RT and RNA was isolated. Gene expression of *Ifn-β* and IFN response elements was quantified by qPCR.

Results: We observed significant IFN1 activation in all cell lines, with peak activation in B78, B16, and MOC2 cell lines occurring 7, 7, and 1 days, respectively, following RT for all doses. This effect was STING-dependent. *In vivo* delivery of EBRT and TRT to B78 and MOC2 tumors resulted in a comparable time course and magnitude of IFN1 activation. In the MOC2 model, the combination of ^{90}Y-NM600 and anti-CTLA-4 therapy reduced tumor growth and prolonged survival compared to single agent therapy.

Conclusions: We report the time course of the STING-dependent IFN1 response following RT in multiple murine tumor models. We show the potential of TRT to stimulate IFN1 activation that is comparable to that observed with EBRT of equivalent cumulative dose. Further evaluation of the timing and magnitude of IFN1 response following EBRT and TRT may be critical to the optimal integration with immunotherapies.

Abstract #21

Cancer-associated fibroblast phenotypes vary across colorectal cancers and correlate with CD8+ T-cell infiltration

Katherine Johnson, Philip B Emmerich, Cheri A Pasch, Linda Clipson, Kristina A Matkowskyj, Dustin A Deming

BACKGROUND: Cancer-associated fibroblasts (CAFs) exhibit divergent phenotypes in their expression profiles and function, altering tumor progression and treatment response. Across cancer types, two CAF subsets have been consistently identified: myofibroblastic and non-myofibroblastic. Here we examine podoplanin (PDPN), α-smooth muscle actin (αSMA), and collagen as potential CAF subtype markers in colorectal cancers (CRCs). These markers were compared with versican (VCAN), an immunoregulatory proteoglycan associated with an immune excluded tumor microenvironment.

METHODS: A tissue microarray containing tissue from 122 CRC patients was stained using Masson's Trichrome (collagen) or immunohistochemistry for αSMA, PDPN, VCAN and versikine (Vkine, a proteolytic fragment of VCAN). Quantification of the stains (0-3+) was performed by at least two observers in consultation with a surgical pathologist. CD8 staining was quantified as the number of tumor infiltrating CD8+ lymphocytes (TILs) per high power field (HPF). Tissue cores were sequenced for 107 patients using the Qiagen Comprehensive Cancer Panel. Mutations were identified using Strelka and cross-referenced to ClinVar.

RESULTS: Stromal staining varied across CRC sections, but was independent of disease stage. Cancers with high collagen or αSMA had reduced TILs (collagen 0-1+: 8.0 TILs/HPF, 2-3+: 3.0, p=0.02; αSMA 0-1+: 7.4, 2-3+: 3.6, p=0.04), with a similar trend for PDPN (0-1+: 6.5, 2-3+: 3.5, p=0.1). VCAN proteolysis predominant (VPP; VCAN 0-1+ and Vkine 2-3+) samples, previously reported to associate with higher CD8+ TILs, had lower collagen vs VCAN proteolytic weak (VPW; mean collagen score 1.6 vs 2.2, p=0.002) and lower αSMA (VPP: 1.7 vs VPW: 2.1, p=0.02). Within VPW tumors, CD8+ TILs were higher in low PDPN (4.1 vs 1.5 TILs/HPF, p = 0.02) and low αSMA tumors (4.8 vs 1.6, p = 0.009), with a similar trend in low collagen tumors (3.7 vs 1.9, p = 0.1). Collagen was more abundant in *TP53*-mutant cancers (p=0.03, Chi-Square Test).

CONCLUSIONS: The stromal microenvironment of CRC is highly variable across patients with diverse CAF densities and phenotypes. The makeup of this microenvironment has potential clinical impacts, as it correlates with immune cell infiltration. Further investigation into CAF biology could reveal therapeutic targets to alter immune surveillance of cancers.

Abstract #22

Topical and Systemic Administration of a Dual PI3K/mTOR Inhibitor in Mice with Established High-Grade Anal Dysplasia

M Keating, M Ziolkowski, T Moyer, L Gunder, M Finlay, P Bertrang, EH Carchman

Introduction

Anal dysplasia and anal cancer are ever-growing health problems. Current treatments for precancerous anal lesions are poorly tolerated by patients and have high recurrence rates. We have previously shown the importance of autophagy, via mTOR/PI3K pathway inhibition, in preventing the progression of anal dysplasia to cancer. This study investigates the efficacy of both topical and systemic administration of a dual mTOR/PI3K inhibitor in preventing anal cancer in mice with established high-grade anal dysplasia.

Methods

K14E6/E7 mice, containing HPV16 E6 and E7 oncogenes in their epithelium, began treatment at 25 weeks of age. Based on timecourse data,these mice exhibit high-grade anal dysplasia around 25 weeks. Mice were treated weekly with the topical carcinogen, 7,12 dimethylbenz[a]anthracene (DMBA), to promote anal carcinogenesis. Samotolisib was administered topically(1% w/v) at the anus or given systemically(4.5 mg/kg) via oral gavage daily for 20 weeks. Mice were randomly assigned to groups: control, DMBA only, topical or systemic Samotolisib and topical or systemic Samotolisib with DMBA. Mice were monitored daily for overt tumors and sacrificed at 45 weeks of age. Anuses were harvested, processed and stained (immunohistochemistry) for pS6 and pAKT. Tumor incidence and protein expression were assessed using two-sided Fisher's t-test and one-way ANOVA with Tukey post-hoc, respectively.

Results

No control mice developed overt tumors, while 21/30 (70.0%) DMBA only mice developed tumors. Only one of 30 mice (3.33%) treated topically with Samotolisib developed an overt tumor and zero of the systemic Samotolisib. Mice given topical Samotolisib with DMBA and Systemic Samotolisib with DMBA developed overt tumors at a rate of 27/29 (93.1%) and 4/11 (36.5%) respectively. Differences in tumor incidence were significant between topical Samotolisib with DMBA and DMBA only (p-value=0.042) treatments, but not between systemic Samotolisib with DMBA and DMBA only (p-value=0.743). There was a significant difference between topical Samotolisib with DMBA and systemic Samotolisib with DMBA treatments (p-value= 0.001). pS6 protein expression was significantly different between topical Samotolisib with DMBA and control (p-value=0.036) and DMBA only (p-value= 0.038). There was no significant difference in the level of pAKT protein expression.

Conclusions

For the prevention of anal cancer, neither systemic or topical Samotolisib were effective in preventing cancer developmen, and in fact topical Samotolisib appears to promote carcinogensis in mice with high-grade dysplasia.

Abstract #23

Clinical Characteristics and Outcomes of Prostate Cancer Patients Undergoing [F-18] fluciclovine PET/CT scan at UWMSPH – A Retrospective study

Aria Kenarsary[1], Hamid Emamekhoo MD[2], John Floberg MD, PhD[1]

[1]Department of Human Oncology – Division of Radiation Oncology, UWSMPH
[2]Department of Medicine–Division of Hematology, Medical Oncology and Palliative Care, UWSMPH

Background: Rising prostate-specific antigen (PSA) after definitive therapy of localized prostate cancer (PC) such as radical prostatectomy (RP) or radiation therapy (RT) indicates disease (dx) recurrence. Determining local, regional, and/or distant sites of PC recurrence with conventional imaging such as CT or bone scan remains challenging. PSA rise without radiographic evidence of dx recurrence is called biochemical recurrence (BCR) [1,2,3]. Newer imaging modalities with higher sensitivity, such as [F-18]fluciclovine (Axumin) positron emission tomography (PET) scan, can detect a lower dx volume.

Objectives: In this project, we aimed to evaluate the Axumin scan findings, treatment approach, and outcomes in PC patients (pts) at the University of Wisconsin.

Methods: 84 PC pts who had Auxmin scans, with at least 20 months follow-up, were included in this retrospective study. Clinical/pathological dx characteristics, treatment, and outcomes data were collected via a review of the electronic medical records.

Results: Of the 84 pts, 94% were Caucasian, 4.8% African American, and 1.2% Hispanic. Median age at diagnosis was 64 (46–84 yo). The median interval between the initial PC diagnosis and Axumin scan was 34 months (1-345-months). Complete data was available for review on 79 scans. The most common indication for obtaining an Axumin scan included: BCR following RP (35, 44.2%), BCR following initial RT (18, 22.8%), BCR following RP & salvage RT (7, 8.9%), BCR following RP & adjuvant RT (3, 3.8%), initial diagnosis (9, 11.4%), and other indications (7, 8.9%). Auxmin findings included: 19 negative (Neg) scans (24.0%), 49 positive (Pos) (62.0%), 10 with equivocal findings (12.7%), and 1 unknown (1.3%). 17 pts with Pos/equivocal findings underwent biopsy (bx) which resulted in 12 Pos bx confirming PC (70.6%), 3 Neg bx (17.6%), 1 non-diagnostic bx (5.9%), and 1 with unknown results (5.9%). Of the pts who received additional therapy following Axumin scan, 42 pts received RT, 2 pts had surgical resections of the Pos Auxmin lesions, and 3 pts who had Axumin scan at initial diagnosis received systemic treatment for metastatic dx.

Conclusion: This study was a descriptive analysis of PC patients who received Auxmin PET scans at our institution. This will serve as a foundation for future studies investigating the prognostic utility of Axumin scan in BCR of PC.

Abstract #24

The oncoproteins H3 K27M and EZHIP inhibit PRC2 by conserved mechanisms in mammals and Drosophila melanogaster.

Sam Krabbenhoft, Truman J. Do, Siddhant U. Jain, Peter W. Lewis, and Melissa M. Harrison

Background: Among the deadliest and most common pediatric brain tumors are diffuse intrinsic pontine glioma (DIPG) and posterior fossa ependymoma type A (PFA). Most DIPG tumors harbor a lysine-to-methionine mutation at residue 27 on histone H3 (H3 K27M). Nearly all PFA tumors feature elevated expression of the protein EZHIP. While these tumors arise from different cell types and have distinct molecular drivers, both originate during early development and feature a reduction of histone H3 trimethylation at lysine 27 (H3K27me3), a mark deposited by Polycomb repressive complex 2 (PRC2). Crucially, however, DIPG and PFA tumors retain H3K27me3 at sites of PRC2 recruitment. While H3 K27M and EZHIP are both competitive inhibitors of PRC2, it remains unclear why they are preferentially enriched in DIPG and PFA, respectively. To better understand how these oncoproteins lead to discrete tumor types, it is imperative to study them in the context of organismal development. PRC2, whose component subunits were discovered in *Drosophila*, is highly conserved among metazoans, making flies an outstanding model to address these fundamental questions.

Methods: Building on data generated in mammalian cell lines, we utilized *Drosophila* cell culture to test the conservation of PRC2 inhibition by H3 K27M and EZHIP. We examined the genome-wide distribution of H3K27me3 using ChIP-seq. Using transgenic expression of H3 K27M or EZHIP, we probed PRC2 inhibition and developmental dysregulation in larval and adult tissues. We have also begun to screen for molecular pathways that mediate oncoprotein phenotypes with RNAi knockdown.

Results: Similar to what has been observed in mammals, in *Drosophila* cells EZHIP expression caused a global H3K27me3 reduction with limited methylation retained at sites of PRC2 recruitment. Furthermore, expression of H3 K27M or EZHIP inhibited PRC2 *in vivo*, causing mutant phenotypes in the eye and wing. Using RNAi, we have identified preliminary molecular pathways whose knockdown modifies oncoprotein phenotypes.

Conclusion: *Drosophila* are an excellent model to address unresolved questions about DIPG and PFA tumorigenesis given the remarkable conservation of PRC2 and wealth of tools. Our data demonstrate that these proteins function by mechanisms conserved between flies and humans, allowing us to systematically identify modifiers of oncoprotein phenotypes.

Abstract #25

Sensitivity of HER2 Amplified Colorectal Cancer Organoids at Ex Vivo Resistance to Panitumumab and Trastuzumab

Jeremy D. Kratz, Lucas Zarling, Aishwarya Sunil, Suhjah Rehman, Katherine A. Johnson, Sarbjeet K. Makkar, Cheri A. Pasch, Nicole Lassen, Kayla K. Lemmon, Linda Clipson, Sam J. Lubner, Melissa C. Skala, Dustin A. Deming

Background: HER2 amplification is an emerging biomarker in colorectal cancer (CRC) representing ~4% of metastatic cases. The sequencing therapies between EGFRi versus HER2 inhibition in low copy number HER2 amplified CRC remains uncertain. Patient-derived cancer organoids (PDCOs) allow an *ex vivo* method to assess treatment sensitivity. We examined treatment sensitivity of a HER2 amplified PDCO at baseline and following resistance to panitumumab and trastuzumab.

Methods: Following IRB-approval, fresh CRC tissue was cultured to maturation. Molecular profiling was performed per institutional standard by StrataNGS. Subcultures were treated with stepwise (20%) increase to physiologic Cmax of panitumumab (230ug/mL) and trastuzumab (180ug/mL). Threshold for escalation was median relative growth of +20% at 96h. Sensitivity was assessed on primary culture (RC1), panitumumab resistance (RC1-P) and trastuzumab resistance (RC1-T) using 96h of physiologic CMax panitumumab, trastuzumab, and combination trastuzumab/pertuzumab. Response was assessed at 96h in comparison to control using effect size (GΔ).

Results: Molecular profiling revealed HER2 copy number of 14 and pathologic alteration in TP53 (p.E51*) with no concurrent alterations in RAS, RAF, or PIK3CA. Time to resistance was similar between panitumumab (55 days) and trastuzumab (51 days). RC1 had baseline growth (+116%) which was reduced with single agent panitumumab (+17%, GΔ=1.40) with intermediate sensitivity to trastuzumab (+48%, GΔ=0.95) and trastuzumab/pertuzumab (46%, GΔ=0.99). Normalized NADH/FAD ratio revealed significant metabolic response to panitumumab (-20%, GΔ=0.66) and trastuzumab/pertuzumab (-35%, GΔ=1.16) with insignificant effect of single agent trastuzumab (-14%, GΔ=0.46). Following resistance to panitumumab, RC1-P had persistent growth with trastuzumab (+68%) which improved in combination trastuzumab/pertuzumab (+34%, GΔ=1.16). Following resistance to trastuzumab, RC1-T was insensitive to EGFRi with panitumumab including persistent growth (+58%, GΔ=0.70) and unchanged metabolism (+2%, GΔ=-0.10)

Conclusions: Therapeutic dose escalation in a single PDCO of HER2 amplified CRC suggests improved sensitivity to EGFRi and dual HER2 targeting with trastuzumab/pertuzumab. Resistance to EGFRi resulted in persistent sensitivity to dual HER2 inhibition using trastuzumab/pertuzumab, however resistance to single agent trastuzumab. Resistance to trastuzumab resulted in future insensitivity to EGFRi. Molecular profiling at resistance revealed no pathologic alterations in EGFR or ERBB2 signaling, with ongoing analysis of transcriptional changes by RNAseq.

Abstract #26

Pancreatic Cancer Treatment and Outcome Disparities between Rural and Urban Patients

John Krebsbach, Amy Taylor MD, Andrea Schiefelbein MSPH, Jienian Zhang MA, John Hampton MS, Amy Trentham-Dietz PhD, Melissa Skala PhD, John Eason PhD, & Noelle LoConte MD

Background: Pancreatic ductal adenocarcinoma (PDAC) has one of the lowest survival-rates of all cancers (5-year survival of 8.5%). Wisconsin is frequently in the top quartile of states for pancreatic cancer incidence and mortality. National studies demonstrate that treatment and outcome disparities exist between rural and urban PDAC patients. We aimed to measure disparities in treatment course and overall survival between PDAC patients living in rural and urban counties to identify potential interventions to minimize these disparities.

Methods: Data was obtained from the UW-Health Cancer Registry. Patients were aged 18 and above and diagnosed with adenocarcinoma, NOS or infiltrating duct carcinoma, NOS between 2004 to 2016. Sequential models of adjusted Cox proportional hazard regression were performed to describe the association between overall survival (OS) and variables of interest including rurality, race/ethnicity, treatment course, and payer. Treatment course was defined as no treatment, chemoradiation, or surgery with/without chemoradiation.

Results: 1,569 patients were included. 38.6% of patients were diagnosed with metastatic disease at presentation. OS was 11.6 months (0.01-147.5). Non-Hispanic black (NHB) patients experienced an increased risk of death (Hazard Ratio (HR)=1.88; 95% Confidence Interval (1.23-2.88)) as did patients categorized as other race/ethnicity (HR=1.32; (1.10-1.60)) compared to Non-Hispanic white (NHW) patients when treatment was excluded from modeling. After adding treatment course and insurance status, NHB patients had similar risk of death (HR=1.41; (0.92-2.17)) compared to NHW Patients. Medicaid patients (HR=1.41; (1.01-1.95)), unknown/uninsured patients (HR=2.62; (1.71-4.02)), and Insurance, NOS patients (HR=1.28; (0.98-1.67)) experienced increased risk of death compared to managed care patients. Rural patients experienced a similar risk of death when compared to non-rural patients (HR=1.00; (0.99-1.01)).

Conclusions: Disparities in overall survival exist across race/ethnicity and insurance status for PDAC patients at UW-Health. Disparities were mitigated when patients had similar treatment course and payer. Ensuring equitable treatment by providing guideline-based care, increasing insurance access, and improving financial support programs for patients of color and underinsured/uninsured patients may reduce these disparities. We found no disparity for rural patients, suggesting improving transportation to a comprehensive cancer center may reduce rural cancer treatment disparities. Our investigation of these disparities continues across colorectal and liver cancers.

Abstract #27

Impact of Cancer Associated Fibroblast Phenotypes on the Infiltration of T-lymphocytes in Early Age Onset Colorectal Cancer

Anna Lippert, Katherine A. Johnson, Philip B. Emmerich, Cheri A. Pasch, Linda Clipson, Kristina A. Matkowskyi, Wei Zhang, Dustin A. Deming

Background:
Incidence of early age onset colorectal cancer (EAO CRC) has increased by 2% each year since the 1990s, with the rate expected to double by 2030. Despite this, little research has been done to understand the immune and stromal environments of EAO CRC. There are two distinct phenotypes of cancer associated fibroblasts (CAFs): myofibroblastic (myCAFs) and non-myofibroblastic (non-myCAFs). Here, we evaluate both CAF environments and immune infiltrating cells in context of EAO CRC.

Methods:
153 CRC patient samples (60 EAO CRC) were obtained with matching adjacent normal tissue. Slides were stained via immunohistochemistry (IHC) for the CAF subtype markers αSMA, FAP, PDPN, and MMP2, by Masson's Trichrome for collagen, and quantified on an intensity scale from 0-3. MyCAF and non-myCAF scores were calculated by averaging the scores of αSMA and collagen, or FAP, PDPN, and MMP2, respectively. These scores were split into low (average score <2) and high (average score ≥2) groups. CD4 and CD8 IHC stains were quantified as the number of tumor infiltrating lymphocytes (TILs) per high power field in the epithelial compartment.

Results:
Patients with low myCAF and non-myCAF scores display the highest average number of $CD4^+$ (10.6) and $CD8^+$ (10.3) TILs across both age groups. Cancers with both myCAF and non-myCAF high scores had reduced average $CD4^+$ and $CD8^+$ TILs when compared to both scores being low (p value: 0.018 for $CD8^+$ TILs, <0.001 for $CD4^+$ TILs). Cancers that show myCAF high and non-myCAF low scores show the overall lowest average $CD8^+$ TILs. There are significantly higher $CD4^+$ TILs in the 50+ age group in all CAF phenotypes except non-myCAF high (p-values 0.01 myCAF low, 0.05 myCAF high, 0.007 non-myCAF low).

Conclusions:
There are differences in the stromal and immune microenvironments between both age groups of CRC. Increased myCAF and non-myCAF scores are associated with T cell exclusion, but the extent of this varies across age groups. This difference in T-cell exclusion implicates the importance for further research into the stromal and immune microenvironments of EAO CRC.

Abstract #28

The Influence of Extracellular Matrix on Metastatic Multicellular Detachment in High-Grade Serous Ovarian Cancer

Hannah Micek, Carine Renner, Yicheng Ma, Salina Loer, Alix Rosenblatt, Bianca Barredo

Background:

High grade serous ovarian cancer (HGSOC) is the most frequent and fatal type of ovarian cancer with only a 47% five-year survival rate. While single cells and multicellular aggregates are both found in HGSOC patient ascites, it is thought that aggregates have a greater metastatic potential as they have increased resistance to anoikis and chemotherapy. There is increasing evidence that aggregates are formed through a multicellular detachment process off primary and/or metastatic sites, but the variables that influence this process are unknown. The extracellular matrix (ECM) in the HGOSC tumor microenvironment (TME) undergoes dramatic changes with tumor progression, including an increase in fibrillar collagens and bulk stiffness. These changes in the ECM are associated with a worse prognosis and a highly metastatic phenotype. We aim to understand the
relationship between the ECM and multicellular detachment of aggregates in HGSOC.

Methods:

An *in vitro* model was developed to isolate aggregates produced by multicellular detachment from cells seeded on a substrate. Proliferation of cells was assessed through EdU labeling. Immunohistochemistry was done to determine presence of ECM in aggregates and nascent protein labelling was done using an azide-containing methionine analog. A microfluidic device was employed to flow aggregates over mesothelial cells to measure adhesion of aggregates with incorporated ECM to a mesothelial layer.

Results:

Multicellular detachment of aggregates from a monolayer increased with substrate stiffness and collagen concentration, though proliferation remained constant across these conditions. Diverse ECM proteins were present in aggregates isolated from both the *in vitro* model and ascites samples. Using nascent protein labelling, it was found that this ECM was produced by the cells in the aggregate post-detachment. ECM incorporated into aggregates promoted attachment of aggregates to a mesothelial layer.

Conclusions:

The ECM of a mature metastatic HGSOC TME promotes multicellular detachment of aggregates. ECM produced by aggregates post-detachment may promote re- attachment to mesothelial cells to facilitate further metastatic spread.

Abstract #29

An immune co-stimulatory vaccine, with adoptive transfer of natural killer cells and immune inhibition blockade, after allogeneic bone marrow transplant, delays and reduces neuroblastoma tumor growth

Nicholas R. Mohrdieck[1], Paul D. Bates[1], Sean P. Rinella[1], Chloe A. King[1], Christian M. Capitini[1,2]

University of Wisconsin-Madison School of Medicine and Public Health[1], Carbone Cancer Center[2]

High risk neuroblastoma remains a challenge to cure despite multi-modality treatment, with only 50% survival. "Tumor specific" therapies are needed to improve survival. Common neuroblastoma treatment strategies involve the use of autologous bone marrow transplant (BMT). A more effective BMT strategy is using an allogeneic BMT, which is MHC-mismatched, but graft-versus-host disease (GVHD) is often a worry. Natural killer (NK) cells are lymphoid cells within our innate immune system that have cytotoxic effects against tumor cells and can also stimulate a T-cell mediated response against tumors. The immune co-stimulatory vaccine, AgN2a 4P, is an irradiated aggressive variant of the murine neuroblastoma cell line Neuro-2a, that is engineered to express four co-stimulatory markers: CD54, CD80, CD86, and CD137L[1]. AgN2a 4P can induce an anti-tumor effect against neuroblastoma *in vitro* and *in vivo*, through stimulation of T-cells and NK cells via the co-stimulatory markers. *In vitro*, T-cells and NK cells stimulated by AgN2a 4P have greater cytotoxic effects against neuroblastoma, than T-cells and NK cells alone[2]. *In vivo*, giving the AgN2a 4P vaccine after allogeneic BMT, with adoptive transfer of donor-derived NK cells, induces a stronger anti-tumor response than giving the AgN2a 4P vaccine alone[2]. This combination therapy did not cause GVHD but was not curative. One limitation in activating both NK and T-cells is that they can become exhausted, which abrogates their function. Inhibition of immune checkpoints that regulate exhaustion may keep NK and T-cells activated. Our objective was to determine if the addition of anti-PD1 blockade to our combination therapy would reverse any exhaustion induced on NK and T-cells during vaccination, and thus enhance anti-tumor effects *in vivo*. Our results prove that addition of immune inhibition blockade, via anti-PD1, to our combination therapy significantly delayed and reduced neuroblastoma tumor growth, without GVHD incidence. The AgN2a 4P vaccine, with adoptive transfer of NK cells and immune inhibition blockade, after allogeneic bone marrow transplant, is a novel approach to reducing neuroblastoma tumor growth, as previous studies have not been successful at curing pediatric malignancies with anti-PD1 or the use of allogeneic bone marrow transplant.

1. Johnson BD, Gershan JA, Natalia N, Zujewski H, Weber JJ, Yan X, Orentas RJ. Neuroblastoma cells transiently transfected to simultaneously express the co-stimulatory molecules CD54, CD80, CD86, and CD137L generate antitumor immunity in mice. J Immunother. 2005 Sep-Oct;28(5):449-60.

2. Mohrdieck NR, Rinella SP, Tippins KE, Bates PD, Capitini CM. Combining and engineered costimulatory vaccine with NK cells induces an anti-tumor effect against murine neuroblastoma *in vitro* and after bone marrow transplant *in vivo* [abstract]. *Journal for ImmunoTherapy of Cancer* (2019) Vol **7**, 282: P487.

Abstract #30

Title: **Disparities in the enrollment of racial and ethnic minorities in clinical trials of poly ADP ribose polymerase inhibitors for women's cancers**

Authors: [1]Rachel P. Mojdehbakhsh, MD, MS; [1]Matthew K. Wagar, MD; [1]Amy Godecker, PhD; [2]Laurel W. Rice, MD; [2]Lisa Barroilhet, MD, MS

[1]Department of Obstetrics and Gynecology, University of Wisconsin School of Medicine and Public Health, Madison, WI, United States.

[2]Division of Gynecologic Oncology, University of Wisconsin School of Medicine and Public Health, Madison, WI, United States.

Objectives: Disparities persist in the enrollment of diverse racial and ethnic groups in clinical trials for ovarian and breast cancers. We sought to analyze the enrollment of participants by race/ethnicity in phase II and III clinical trials involving poly ADP ribose polymerase (PARP) inhibitors for ovarian and breast cancers and compare these to the racial/ethnic prevalence of ovarian and breast cancer in the United States.

Methods: This study was designed as a retrospective review of clinical trials registered with Clinicaltrials.gov. Studies included evaluated PARP inhibitors for the treatment of ovarian, fallopian tube, primary peritoneal, and breast cancers. Enrollment rates for clinical trials were stratified by race/ethnicity and cancer type. Enrollment fractions (EF) were calculated using prevalence data from the Surveillance, Epidemiology, and End Results (SEER) Database. Odds ratios (OR) and 95% Confidence Intervals (CI) were calculated to compare minority enrollment rates to Non-Hispanic (NH) White enrollment rates.

Results: Forty-eight trials were identified, 17 of which met inclusion criteria (12 ovarian, 2 breast and 3 breast and ovarian). For ovarian cancer trials, enrollment fractions were 1.5% for NH-White, 0.3% for NH-Black, 0.5% for Hispanic, and 2.4% for Asian/Pacific Islander. For breast cancer trials, the EF for NH-White participants was 0.005%, for NH-Black patients was 0.008%, for Hispanic patients was 0.003%, and for Asian/Pacific Islander patients was 0.009%. Patients identified as NH-Black and Hispanic were significantly underrepresented compared to those identified as NH-White in ovarian cancer trials (OR 0.23, 95% CI [0.18-0.29] and OR 0.3, 95% CI [0.25-0.38] respectively, *p*-value of <0.0001). In breast cancer trials, both NH-Black and Asian/Pacific Islander groups were significantly overrepresented compared to NH-White (OR 1.54, 95% CI [1.02, 2.33] p= 0.042, and OR 1.81, 95% CI [1.01, 3.26] p=0.047 respectively).

Conclusions: NH-Black and Hispanic participants are significantly underrepresented in clinical trials evaluating PARP inhibitors for ovarian cancer compared to NH-White cohorts. Phase II and III trials assessing PARP inhibitors for ovarian and breast cancer do not accurately represent the populations diagnosed with these malignancies. Enrollment strategies are needed to increase diversity in PARP inhibitor clinical trials for women's cancers.

Abstract #31

Cellular contributions of Lgr5 cells in MmuPV1 infected cutaneous skin

Ruben Moreno, Paul Lambert

Background Study of the papillomavirus' life cycle has been carried out in a variety of vertebrates, including rabbits and cows. A papillomavirus that naturally infects and causes disease in *mus musculus* was recently discovered. This allows the use of genetically engineered laboratory mice to pursue new approaches to uncover the mysteries of papillomavirus associated diseases. Specifically, since mouse papillomavirus can cause squamous cell carcinoma (SCC), it is possible to address questions related to the origins of papillomavirus-associated disease within the mouse hair follicle. The hair follicle is composed of many progenitor cell populations, though much attention has been paid to the Lgr5 progenitor cell population. Recently, a study in transgenic mice expressing human papillomavirus oncogenes were found to have tumors derived from Lgr5 progenitor cell populations via lineage tracing. This led us to believe perhaps Lgr5 progenitor cells may make a cellular contribution to lesions arising in mouse cutaneous tissue infected with mouse papillomavirus.

Methods 6–8-week-old Lgr5CreER^{T2}/Rosa26LSLtdTomatoRed mice were treated topically with 4-OH Tamoxifen to induce labeling of Lgr5 progenitor and future progeny populations. 72 hours later, mice were scarified and infected on their ears with mouse papillomavirus. 4 months later, tissue was harvested, formalin-fixed, paraffin embedded and sectioned to perform H&E and immunofluorescent analysis. Lesions were then analyzed by performing immunofluorescence and determining percent of tdTomato+ cells divided by number of K14+ cells within a lesion (mean+/-SE). Diagnosis of lesions as SCCs are awaiting final approval from a pathologist.

Results Immunofluorescent analysis of these animals' dysplasias were low for tdTomato+ cells (6.9% +/-1.1), indicating they were composed primarily of non-Lgr5 progenitor cells. SCCs, however, were substantially positive for tdTomato+ cells (44%+/-5.7), indicating squamous cell carcinomas arise from Lgr5 progenitor cells. Comparison of the cellular composition of tdTomato+ cells in dysplasias vs SCCs reveals the difference to be statistically significant ($p<.05$ via two-sided Wilcoxon Rank Sum).

Conclusion Mouse papillomavirus associated squamous cell carcinomas arise from Lgr5 progenitor cells.

Abstract #32

Guidance and Assessment of Histotripsy Ablations using C-Arm Imaging Systems

Sarvesh Periyasamy, Martin Wagner, John Swietlik, Emily Knott, Annie Zlevor, Fred Lee, Tim Ziemlewicz, Paul Laeseke

Objective

Histotripsy is a newly developed non-invasive, non-thermal focused ultrasound ablation technique. Histotripsy is currently guided by conventional ultrasound, which is often limited by a poor acoustic window (e.g., blockage of ultrasound beam or large body habitus). The purpose of this study is to determine the feasibility of using C-arm x-ray imaging systems to guide and assess histotripsy treatments.

Methods

Four 50 kg domestic swine underwent histotripsy ablations of the liver (n=5), spleen (n=1), kidney (n=1), and muscle (n=1). Histotripsy ablations were also performed in an ex-vivo phantom model (n=3). Pre-procedure non-contrast CBCTs of the liver were acquired at inspiratory and expiratory phases. To evaluate the feasibility of C-arm for histotripsy ablation target location prediction, CBCT and fluoroscopic images were acquired of the therapy transducer positioned for a targeted ablation and a 3D to 2D pose estimation of the transducer was used to estimate the ablation location. To evaluate the feasibility of a motion-model based approach for predicting histotripsy ablation zone enlargement due to respiratory motion, a 3D deformable registration technique was used to predict respiratory motion during treatment and subsequent deformation of the ablation volume. Post-procedure non-contrast and contrast-enhanced CBCTs and conventional CTs were acquired to assess conspicuity and size of the ablation zone.

Results

The accuracy of the estimated ablation zone center using CBCT and fluoroscopy was 0.72±0.21 mm in an ex-vivo phantom model and 3.59±1.17 mm in an in-vivo porcine model. Relative to actual ablation zones, histotripsy ablation zone predictions using motion-models had lower mean percent errors of 4.7% (cranial-caudal), 8.6% (anterior-posterior), and 4.1% (medial-lateral) when compared to predictions without motion modeling (16%, 12.5%, 8.4%). Finally, post-histotripsy CBCT images had contrast-to-noise ratios (non-contrast 1.5±0.4, contrast-enhanced 2.4±0.7) comparable or better to those found using conventional CT (0.6±0.2, 3.5 ±0.8). Ablation zone dimension measurements between CBCT and conventional CT were similar with a mean percent difference of 6.31±1.04% and an intra-class correlation coefficient of 0.89.

Conclusions

C-arm based imaging techniques accurately predicted histotripsy ablation zone locations, estimated the effects of respiratory motion, and depicted the final treatment zone. Integrating C-arm imaging into the histotripsy workflow may ultimately improve the efficacy and safety of the procedure.

Abstract #33

Validation of Microwave Ablation using Electrode Displacement Elastography

Robert Pohlman, James L. Hinshaw, Timothy J. Ziemlewicz, Meghan G. Lubner, Shane A. Wells, Fred T. Lee Jr., Marci L. Alexander, Kelly L. Wergin, Tomy Varghese

Background

Liver cancer is a leading cause of cancer related deaths, however primary treatments such as surgical resection and liver transplants may not be viable options for many patients. Minimally invasive image-guided microwave ablation (MWA) provides a locally effective treatment option for these patients with an impact comparable to surgery for both cancer specific and overall survival. MWA efficacy is correlated with accurate image guidance, however conventional modalities such as B-mode ultrasound and computed tomography (CT) have limitations. Alternatively, ultrasound elastography has been utilized to demarcate post-ablation zones yet has limitations for pre-ablation visualization due to variability in strain contrast between cancer types.

Methods

This study attempts to characterize both pre-ablation tumors and post-ablation zones using electrode displacement elastography (EDE) for 13 patients with hepatocellular carcinoma or liver metastasis. Typically, MWA ablation margins of 0.5 – 1.0 cm are desired, which are strongly correlated with treatment efficacy.

Results

Results demonstrate an average estimated ablation margin inner quartile range of 0.54 – 1.21 cm with a median value of 0.84 cm. These treatment margins lie within or above the targeted ablative margin indicating the potential for using EDE for differentiating index tumors and ablated zones for use during clinical ablations. We also obtained a high correlation between corresponding segmented cross-sectional areas from contrast-enhanced computed tomography (CECT), the current clinical gold standard, when compared to EDE strain images with R^2 values of 0.97 and 0.98 for pre- and post-ablation regions.

Conclusion

For the 13 patients in this study, electrode, or antenna displacement elastography was successful in delineating pre-ablation index tumors and post-ablation ablation zones for differentiating tumors and respective ablation zones and validating ablative margins. Further work using 3D ultrasound and elastographic data sets are necessary along with accurate registration of the pre-ablation tumor with the ablation zone to verify that the margins obtained are consistent around the entire circumference of the malignant tumor as well as registration with corresponding CECT images to verify size and location of pre-ablation tumors and post-ablation zones obtained using EDE.

Abstract #34

The role of GRHL2 in facilitating pS118-ER transcriptional activity in hormone-dependent breast cancers

Authors: Rebecca Reese, Kyle Helzer, Elaine Alarid

Background: GRHL1, GRHL2, and GRHL3 are nuclear transcription factors (TFs) that regulate epithelial differentiation. In breast cancer, GRHL2 expression is higher in tumors than in normal mammary tissue, and high GRHL2 expression is associated with poor prognosis and tamoxifen resistance. High GRHL2 expression is also specifically associated with estrogen receptor positive (ER+) breast tumors over ER- breast tumors. Cistromic analysis by our group of ER phosphorylated at serine 118 (pS118-ER), a form of transcriptionally active ER, identified an enrichment of the GRHL motif near pS118-ER binding sites. Despite these findings, the mechanistic link between GRHL2 and ER is not well-defined.

Methods: Protein and mRNA expression of the GRHL TFs was assessed in several ER+ and ER- breast cancer cell lines. RNA-seq was performed in the ER+ cell lines MCF7 and T47Ds treated with estrogen (E2) and/or siGRHL2 to identify E2/GRHL2 co-regulated genes. The pS118-ER ChIP-seq from our lab was integrated with a published GRHL2 ChIP-seq to identify pS118-ER/GRHL2 co-occupancy sites. ChIP-qPCR was used to assess the effect of the loss of GRHL2 on pS118-ER recruitment in response to E2 near co- regulated genes.

Results: GRHL2 is the most highly expressed GRHL TF with specifically high expression in ER+ cell lines. *GRHL2* expression was also detected in patient-derived metastatic breast cancer cells. Principle component analysis of RNA-seq data revealed MCF7 gene expression is more strongly influenced by E2 than by GRHL2, whereas in T47Ds gene expression is affected more equally by the two factors. Despite this, both cell lines demonstrate a similar percentage of genes co-regulated by both E2 and GRHL2. ChIP-qPCR revealed three patterns of E2-inducible pS118-ER recruitment to DNA upon the loss of GRHL2: normal, reduced, and failed recruitment.

Conclusions: GRHL2 is more highly expressed in ER+ cells than ER- cells, adding further evidence to a specific role for GRHL2 in ER+ breast cancer. ChIP-qPCR data reveals GRHL2 modulates the transcriptional activity of ER, as pS118-ER recruitment either failed or was reduced near E2/GRHL2 co- regulated genes when GRHL2 is removed. These studies demonstrate that GRHL2 can influence the gene signature of ER+ breast cancer cells by modulating E2-dependent ER DNA-binding.

Abstract #35

Getting to the core: single-cell dynamics and subcellular trafficking of hepatitis B virus core proteins

Sofia Romero, Nuruddin Unchwaniwala, Daniel D. Loeb, Nathan M. Sherer

Hepatitis B virus (HBV) is a reverse-transcribing DNA virus that infects the liver and is a leading cause of hepatocellular carcinoma. Although a vaccine offers protection against infection, it is not therapeutic and the incidence of HBV infections in the U.S. has increased over the last decade . Thus, determining novel HBV-host interactions that support infection is needed to develop curative anti-HBV strategies. During replication, HBV pregenomic (pg)RNA is packaged by Core protein (Cp) into nucleocapsids where pgRNA is reverse-transcribed to generate relaxed-circular (rc)DNA. Here, we performed a comprehensive analysis of Cp subcellular trafficking over time in Huh7 hepatocellular carcinoma cells using immunofluorescence, live-cell imaging, and biochemical assays for three replication conditions: (I) WT HBV genomes, (II) genomes encoding an assembly incompetent $Cp^{(Y132A)}$, and (III) genomes encoding functional Cp but lack the pgRNA packaging signal (Eps-). Although HBV cores are thought to form in the cytoplasm, we unexpectedly observed high levels of high-order Cp assemblages accumulating preferentially in the nucleus at early time points (24h) followed by a marked shift to the cytoplasm at 48h. This transition was not observed for either $Cp^{(Y132A)}$ or WT Cp encoded by Eps- pgRNA. Live cell imaging revealed Cp nucleus-to-cytoplasm re- localization occurs through two mechanisms; (I) predominantly by nuclear escape during mitosis followed by cytoplasmic retention or (II) punctuated release of nuclear Cp in discrete “burst” events occurring at the nuclear membrane. Cytoplasmic retention of Eps- pgRNA- derived Cp assemblages was rescued by expressing Eps+ pgRNA, suggesting that pgRNA and Cp trafficking are interdependent. These results reveal that nucleocytoplasmic transitions of Cp/capsids are prevalent, typically occur in conjunction with nuclear membrane breakdown; and are regulated by viral RNA.

Abstract #36

Cellular chromatin reorganization by Epstein-Barr Virus, a human tumor virus, during its lytic phase

Quincy Rosemarie & Bill Sugden

Background:
Epstein-Barr Virus (EBV) is known to contribute to several lymphomas and carcinomas, and its lytic phase has been found to be important for tumor progression and maintenance. A key event in EBV's lytic phase is viral DNA amplification which occurs in replication factories within the nucleus. As these factories form, the cellular DNA moves to the periphery of the nucleus, constituting a reorganization of cellular chromatin (ROC). ROC occurs in other families of viruses and is likely important for viral life cycles. We aim to characterize EBV's ROC and elucidate its mechanism of formation.

Method:
ROC is assayed visually by fluorescence microscopy of cells carrying fluorescently tagged histones or stained with DAPI. We investigated the role of the co-occurring viral amplification factories for ROC formation. EBV-positive cells were induced to enter the lytic phase, treated with ganciclovir to inhibit lytic DNA amplification, and assayed for ROC by fluorescence microscopy. We then investigated the role of EBV's DNA synthesis machinery in ROC using a library of 293 cells carrying EBV with single gene knockouts of its lytic replication genes. Cells were induced to enter the lytic phase with or without trans-complementation of the missing replication gene, and assayed for ROC.

Results:
Ganciclovir treatment of lytic cells successfully inhibits the formation of amplification factories but not ROC, indicating that continuous DNA synthesis is not required for ROC. However, *oriLyt*, EBV's lytic origin of replication, is required for ROC and supports ROC in *trans*. Given that oriLyt supports ROC in *trans* and EBV's late lytic genes require *oriLyt* in *cis*, it is apparent that late genes are dispensable for ROC. We then tested two of EBV's seven lytic replication genes and found that they are required for ROC.

Conclusion:
EBV's lytic reorganization of chromatin can occur independently of amplification factories, but requires the viral lytic origin of replication. Several early genes involved in DNA replication are also required, but late genes are dispensable. We are further investigating the mechanism of EBV's ROC, a likely important phenomenon in the life cycle of this human tumor virus.

Keywords: Epstein-Barr Virus, chromatin reorganization, lytic phase.

Abstract #37

Funding to Lethality Measures in 11 Years of National Cancer Institute Funding Allocation

Shannon K Rush, MD; Shitanshu Uppal, MBBS; Connor Wang, MD; Trang Q Le, PhD; Roxana A Alexandridis, MS PhD; Laurel W Rice, MD; Ryan J Spencer, MD MS

Objectives:

We sought to quantify funding to lethality (F:L) ratios, track changes in those ratios over time, and compare F:L to other measures of allocation, including funding to incidence (F:I), funding to mortality (F:M) and funding to years of life lost (F:YLL).

Methods:

We collected NCI funding allocation from the NCI research portfolio database, and incidence (I) mortality (M) rates and years of life lost (YLL) from the Surveillance, Epidemiology and End Results (SEER) database for 19 cancers from 2007-2017. We calculated yearly F:L as total NCI funding divided by (M/I x (YLLx100)). We ranked each median funding ratio by cancer and slope of change, compared to the three gynecologic cancers reported (cervix, ovary and uterus). We also compared ratios by Pearson correlation tests and performed linear regressions of ratio slope changes, using uterus, cervix and ovary as referents. All studies were completed in R, version 4.0.2.

Results:

Highest F:L ratios were breast, prostate and melanoma despite these being 12^{th}, 17^{th} and 16^{th} in terms of lethality. Ovary, uterus and cervix ranked 12th, 15th and 13^{th} in F:L ratios, but were 5^{th}, 13^{th} and 6^{th} by lethality. All three gynecologic cancers experienced negative slope of change of F:L over the 11 years. Ovary, Cervix and Uterus also experienced negative slope of change for F:I, F:M, and F:YLL barring ovary with positive slope of change for F:M. For F:L, the slopes of change were significantly less for ovary, cervix and uterus compared to breast and melanoma, though significantly less negative than prostate. F:L ratio was strongly positively correlated to F/YLL with Pearson correlation coefficient of 0.86 ($p<0.001$), weakly positively correlated to F:M (Pearson coefficient 0.14, $p=0.04$), and weakly negatively correlated to F:I (Pearson coefficient -0.14, $p=0.042$). Sensitivity analyses looking at last 5 years of reported funding largely confirm trends.

Conclusions:

F:L ratios correlate with other funding allocation measures, and highlight inadequate NCI funding to gynecologic cancers. Trends of the last 5 years are consistent with trends of the last 11 years.

Abstract #38

The Rhesus Macaque: A Unique and Compelling Model for Human Endometriosis-Associated Ovarian Carcinomas

Shannon K Rush, MD; Stephanie McGregor, MD, PhD; Paul Weisman, MD; Heather Simmons, DVM; Hanna Jens; David H. Abbott, PhD; Jon E. Levine, PhD; Joseph W. Kemnitz, PhD; Manish Patankar, PhD

Objective: Clear cell ovarian carcinoma and endometrioid ovarian carcinoma (EnOC) are considered endometriosis-associated ovarian cancers (EAOCs). Given no higher order animal model currently exists for these cancers, we propose a novel animal model, the rhesus macaque (*Macaca mulatta*), for better understanding malignant transformation from endometriosis to EAOCs.

Methods: The Wisconsin National Primate Research Center (WNPRC) maintains a multigenerational colony of Indian-derived rhesus macaques with well-documented incidence of spontaneous endometriosis. WNPRC researchers developed a model in which estrogen receptor-α was knocked down in the hypothalamus (hypoER-α) of adult rhesus females. Standard hemotoxylin and eosin (H&E) stained slides of reproductive tissues (uterus, cervix, fallopian tube, ovary) from formalin fixed and paraffin embedded tissues were reviewed by human and veterinary pathologists. Select slides underwent immunohistochemical staining for PAX-8, Wilm's Tumor 1 (WT-1), estrogen receptor alpha (ER- α) and progesterone receptor (PR).

Results: Twelve animals were evaluated, 6 hypoER-α and 6 control. Age at necropsy and characteristics associated with rhesus endometriosis were comparable between groups. Endometriosis was present in 4/6 hypoER-α females but in only 1/6 controls. HypoER-α animals had endometriosis in the uterus (2 cases), cervix (1 case), fallopian tube (2), ovary (2) and colon (1). The control animal had endometriosis in the uterus. ARID1A was retained in all tissues tested, including cases with endometriosis. WT-1 was positive in both epithelium and stroma for all endometriosis lesions. ER- α was negative in HypoER-α but not control endometriosis; PR was patchy positive in normal endometrium. PAX-8 stained rhesus tissue as would be expected in human analogs. One EnOC was identified in a hypoER-α animal that also had endometriosis.

Conclusions: We have successfully created a nonhuman primate model that increases endometriosis penetrance. We also demonstrated parity between rhesus and human female endometriosis and EAOC by gross and histologic examination. Immunohistochemistry demonstrates WT-1 consistently stains endometrial glands and stroma in endometriosis. There was one animal with an EAOC in association with endometriosis. The rhesus macaque poses a higher order animal model for ongoing research in EAOC.

Abstract #39

The State of K2R Grant Funding by Cancer Type from the National Cancer Institute

Shannon K Rush, MD; Shitanshu Uppal, MBBS; Connor Wang, MD; Trang Q Le, MS; Roxana A Alexandridis, PhD; Laurel W Rice, MD; Ryan J Spencer, MD MS

Objectives:

Given that K grant funding secured in early career correlates with success in achieving R funding, we aim to describe the state of K and R funding by cancer type through time, hypothesizing that those cancer sites receiving more K grant allocation also receive greater R grant allocation.

Methods:

We queried the National Cancer Institute (NCI) Research portfolio from 2007-2017 for K and R grant sums by year and 19 cancers. Yearly K means, medians and sums were summarized for each cancer and trended over time, as were yearly K number of studies. R sums were similarly trended using linear regression with year as the independent variable. The relationship between K and R was assessed using multivariable linear regression with K totals by year interaction, adjusting for cancer and applying a Box-Cox transformation of R totals to gain linearity. Statistical analyses were performed in R, v.4.0.2.

Results:

Median yearly number of funded Ks range from 0-85 and total dollars from $0-10.66 million. Breast, leukemia and lung (85, 47, 45 respectively) have the highest median number of yearly Ks; and breast, lung, and brain ($10.66, 5.95, and 5.85 million) have the highest total yearly K amounts. Cervical, ovarian and uterine cancer ranked 11, 9, and 14 in median number of yearly funded Ks over the 19 cancers, and 14, 13, and 17 in median yearly K amounts. By total yearly R medians, the highest funded cancers were breast, lung, and colorectal, while cervix, ovary and uterus ranked 11, 10, and 16. Cervix, ovarian, and uterine cancer ranked 14, 7, and 15 in K growth across all cancers, and 17, 7, and 12 in R growth across cancers. K funding is significantly associated with growth in R funding ($p=0.021$) adjusting for all cancers.

Conclusions:

K and R funding demonstrate stagnant growth in gynecologic cancer. Gynecologic cancers are being funded by Ks at lower rates and amounts than other cancers, and this correlates with lower rates and amounts at the R grant level. Early career investigators foster innovation, thus supporting advocacy and mentorship for K funding in gynecologic cancer.

Abstract #40

Determining Parity in Immunohistochemistry (IHC) staining of Ovarian and Fallopian Tube Tissues in Humans and Non-Human Primates Rhesus Macaque and Common Marmosets

Shannon K. Rush, MD; Stephanie McGregor, MD, PhD, Paul S. Weisman, MD, Heather A. Simmons, DVM, DACVP, Hanna K. Jens, Jon E. Levine, PhD; David H. Abbott, PhD, Joseph W. Kemnitz, PhD, Manish S. Patankar, PhD

Objective: Non-human primates (NHP) such as rhesus macaque (Macaca mulatta, MM) and common marmosets (Callithrix jacchus, CJ) have been utilized in various areas of biomedical research for decades, but apparently not for the study of ovarian cancer. We aim to establish parity of MM and CJ ovarian and fallopian tube tissues to human ovarian and fallopian tube tissues by use of immunohistochemical (IHC) staining.

Methods: We performed IHC on MM, CJ and human ovarian and fallopian tube tissues with eight markers typically used for evaluation of ovarian cancer and its precursor lesions: MUC16, mesothelin, PAX8, ARID1A, EpCAM, estrogen receptor (ER), progesterone receptor (PR), and WT-1. We then reviewed all tissues with both human and NHP pathologists to establish parity in staining among the different species.

Results: The ovaries and fallopian tubes stained in similar pattern to humans for MUC16, PAX8, ARID1A, and mesothelin. EpCAM stained neither the MM nor the CJ ovarian and fallopian tube tissues, contrary to staining patterns seen in human tissues. PR staining was not definitively positive for any of the MM or CJ tissues, contrary to human ovaries and to a lesser extent human fallopian tubes. ER staining was positive as expected for all three species.

Conclusion: IHC staining of ovary and fallopian tubes establishes parity between the MM, CJ and human species. This lends support to expanding ovarian cancer preclinical research into NHP models using both rhesus and marmoset.

Abstract #41

Hormone Replacement Therapy Counseling after Prophylactic Bilateral Salpingo-Oophorectomy in High Risk Patients

Shannon K. Rush, MD; Connor Wang, MD; Sarah R. Beilke, MS4; Amy L. Godecker, PhD; Makeba L. Williams, MD; Amy Trentham-Dietz, PhD; Lisa M. Barroilhet, MS MD

Objective: Women at high risk of breast and ovarian cancer are counseled to undergo prophylactic bilateral salpingo-oophorectomy (BSO) and to consider prophylactic mastectomy (MAS) to reduce cancer risk. Hormone replacement therapy (HRT) mitigates the negative effects of surgical menopause. We sought to understand what factors influence how women at high risk of breast and ovarian cancer are counseled about HRT at the time of prophylactic BSO.

Methods: This is a retrospective chart review of women seen at the Hereditary Breast and Ovarian Cancer Clinic who underwent prophylactic BSO. Those undergoing BSO for ovarian cancer were excluded. We collected information related to age at BSO and MAS, type of surgery, reason for surgery and pathologic findings. We reviewed whether HRT counseling was performed the three months before and after surgery and whether HRT was recommended. All statistical analysis was performed via STATA IC v15. Chi square compared categorical and t-test compared continuous variables. P-values were significant at <0.05.

Results:
One hundred seventy-one women were included. Median age at BSO was 46 years (range 13-74); median age at MAS was 44.5 years (range 28-74). There were 83 *BRCA1*, 57 *BRCA2*, and 3 *BRCA1* and *BRCA2* mutation carriers; remaining women had other mutations or strong family histories. Only 65 women (38%) undergoing BSO had HRT counseling documented. Of those who received counseling, 28 were recommended for and 30 against HRT, with 7 receiving other recommendations. HRT recommendations were not associated with whether a MAS or hysterectomy had been performed. Younger age at BSO was significantly associated with receiving HRT counseling and with receiving positive recommendation.

Conclusions: A majority of patients undergoing BSO do not receive counseling surrounding HRT use. Prior surgeries that would impact HRT type and use do not seem correlated with receipt of counseling for or against HRT, but younger age was significantly associated with receipt of counseling and with HRT being recommended.

Abstract #42

A Microfluidic Platform To Investigate the Effects of Skin Microenvironment on Primary Melanoma Evolution

Jose M Ayuso[1*] , Shreyans Sadangi[2*] , Marcos Lares[2] , Shujah Rehman[3,4,5], Mouhita Humayun[1] , Kathryn M Denecke[1] , Melissa C Skala[3,4,5], David J Beebe[1,4,5], Vijayasaradhi Setaluri[2,6].

1. Department of Pathology & Laboratory Medicine, University of Wisconsin, Madison, WI, USA.

2. Department of Dermatology, University of Wisconsin-Madison, Madison, WI, USA.

3. Morgridge Institute for Research, 330 N Orchard Street, Madison, WI, USA.

4. Department of Biomedical Engineering, University of Wisconsin, Madison, WI, USA.

5. The University of Wisconsin Carbone Cancer Center, University of Wisconsin, Madison, WI, USA.

*These authors contributed equally to the work

Background: The transition of primary melanoma, localized to the skin, to metastatic melanoma is a complex process. Whereas the focus of research on melanoma progression has been centered on tumor cells themselves, the role of the skin microenvironment on the evolution of primary melanoma has not been adequately studied. Similarly, the influence of communication between the primary tumor and skin stroma on melanoma treatments is also not well understood. Therefore, further investigation is needed to understand the relationship between the tumor and the microenvironment to understand the development and progression of cutaneous melanoma.

Methods: In this study, we utilized a microfluidic device to investigate the effect of epidermal keratinocytes and dermal fibroblasts on the cellular dynamics of primary melanoma cells. The microfluidic platform utilizes an air wall based cell patterning, thereby obviating the need for a hydrogel barrier, porous membranes, or external equipment to create a boundary between different cellular compartments. This allows us to co-culture primary melanoma cells in the presence of dermal fibroblasts and epidermal keratinocytes. Employing this model, we investigated the cross-talk between melanoma cells and stromal cells.

Results: Presence of fibroblasts and keratinocytes in the proximity of primary melanoma altered the morphology and growth pattern of the tumor cells. Analysis of secretome showed

that the pattern of secreted chemokines was also altered, with changes in expressions of multiple factors implicated in tumor progression. Optical metabolic imaging demonstrated that the three cell types when cultured together exhibited a different metabolic signature than when cultured in isolation. In addition, the presence of stromal cells facilitated a metabolic shift in melanoma cells, underscoring the contribution of the skin microenvironment on primary melanoma evolution.

Conclusion: Taken together, our results suggest that the progression of primary melanoma is modulated by the interaction of tumor cells and the skin microenvironment. These data suggest that altering skin microenvironment can influence melanomagenesis.

Abstract #43

LumeNEXT: A microfluidic organ-on-chip model of the bone niche in solid tumor metastases to assay stroma-mediated drug resistance

Authors: Nan Sethakorn, Sheena Kerr, Ravi Yada, Jose Ayuso-Dominguez, Adeline Ding, Erika Heninger, Jacques Galipeau, Peiman Hematti, David Beebe, Joshua M. Lang

Presenting author: Nan Sethakorn
Lab: Lang/Beebe

Category: Basic science

3 keywords:
- Microfluidic bone niche
- Bone metastases
- Drug resistance

Abstract (<350 words):

Background: Bone metastases frequently occur in multiple solid tumors and are a major cause of morbidity and mortality. The complex stroma contributes to drug resistance and immunosuppression, and improved modeling of the human bone niche will advance approaches to treat this incurable disease. We present a microfluidic model called LumeNEXT incorporating primary human bone stromal cells including osteoblasts, osteoclasts, adipocytes, mesenchymal stem cells, fibroblasts, and functional microvasculature. Within the chip, we can introduce cancer spheroids and immune cells to recapitulate the metastatic bone microenvironment. The chip design enables live cell imaging to monitor viability and responses to chemical perturbations.

Methods: Primary bone cells were obtained via bone marrow aspirate and cultured from healthy donors or patients with localized prostate cancer, under IRB protocol. Differentiated cells were then encapsulated in a 3D extracellular matrix within the LumeNEXT device, with subsequent formation of luminal structures lined with human iPSC-derived endothelial cells. Imaging of live or fixed cells performed by infusion of fluorescent agent using epifluorescence and confocal microscopy.

Results: Multiple types of primary bone stromal cells can be co-cultured with endothelial cells, macrophages, and cancer spheroids in the LumeNEXT chip, and maintain viability. Endothelial cells form functional microvessels that can be used to seed additional cell types or infuse drug therapies. Multiple orthogonal readouts are available. Cell viability and protein biomarkers can be assessed via live and fixed cell fluorescence microscopy, conditioned media can be collected for analysis of secreted paracrine factors, and 3D cultures can be harvested for transcriptomic analyses.

Conclusions: The LumeNEXT platform enables multi-endpoint analyses to interrogate novel drug monotherapies or combination therapies using primary human *ex vivo* cultures. Our novel approach can be tailored to any microenvironment and tumor type to facilitate drug discovery and identify stroma-mediated mechanisms of resistance. Future directions will involve modification of the stromal compositions tailored to patient-specific signatures and incorporation of immune compartments to enable *ex vivo* assessment of novel immunotherapy drugs and combinations.

Abstract #44

Effect of Pre-Operative Stereotactic Radiosurgery on Non-Small Cell Lung Cancer Brain Metastasis: Initial Radiobiologic Analysis of DNA and RNA Genomic Profiles from Phase-II Clinical Trial NCT03398694

Jack M. Shireman, Mario Henriquez, Wei X. Huff, Gina Monaco, Namita Agrawal, Gordon Watson, and Mahua Dey

Background: With improved systemic therapy that has limited impact on the intracranial compartment, the incidence of brain metastasis (BM) from solid cancers is rising and negatively impacting patient's overall survival (OS). Treatment varies based on presentation, however, for patients with <4 symptomatic BMs current clinical practice involves surgical resection followed by stereotactic radiosurgery (SRS) to the resection cavity. Post-operative SRS is associated with increased risk of leptomeningeal disease (LMD) in the follow-up period. We hypothesize that pre-operative SRS will decrease the incidence of LMD and increase patient's OS by delivering a lethal dose of radiation to tumor cells before they are disturbed by surgical resection. In a Phase II clinical trial (NCT03398694) we are treating patients with 1-4 symptomatic BMs with pre-operative SRS while collecting DNA and RNA sequencing data from core and peripheral edges of the resected tumor to examine the genomic effects of SRS on tumor.

Methods: We identified non-small cell lung cancer (NSCLC) BMs, the most prevalent histology type, for initial analysis. The resected tumor specimens were divided into two groups: 'center' and 'periphery' with respect to the center of SRS treatment with periphery within 50% isodose line. Previously resected untreated NSCLC BMs were used as control. DNA and RNA were isolated from all the samples for sequencing.

Results/Conclusions: Genomic analysis indicated that 4 out of 5 (80%) and 3 out of 5 (60%) patients showed increase in DNA indels and SNP's in central vs peripheral tumor locations. RNA-seq demonstrated significant differences in transcriptomic profiles between control and treated samples (DE Genes 192 and 62 respectively), however, the number of differentially expressed genes between treated center and peripheral tumor samples was minimal. Gene ontological analysis indicated overexpression of WNT and BMP signaling pathways typically involved in neuronal development hinting that adaptation to the brain microenvironment was occurring after metastasis establishment. ($p < .001$, $p < .01$). From our initial analysis we conclude that SRS produces significant genomic changes at DNA and RNA levels with some differences between peripheral and central locations. Corelating our biological findings with clinical outcome data from the trial will be instrumental in establishing a more effective treatment strategy for BM patients.

Abstract #45

Alpha-tocopheryloxyacetic acid induces apoptosis of murine rhabdomyosarcoma in vitro while modulating innate and adaptive immune responses in vivo

Fernanda Szewc [1], M.Sc., Longzhen Song [1], Ph.D., Sean P. Rinella [1], M.P.H., C.C.R.P., Christopher Dubay [2], Ph.D., Emmanuel T. Akporiaye [3], Ph.D., William L. Redmond [2], Ph.D., Christian M. Capitini [1,4], M.D.

[1]Department of Pediatrics, University of Wisconsin School of Medicine and Public Health, Madison, WI, USA; [2]Earle A. Chiles Research Institute, Portland, OR, USA; [3]Veana Therapeutics, Portland, OR, USA; [4]Carbone Cancer Center, Madison, WI, USA

Background

Relapsed pediatric sarcomas have a poor prognosis with no available curative options. Alpha-Tocopheryloxyacetic acid (a-TEA) is a redox-silent analog of alpha-tocopherol that induces apoptotic and immunogenic cell death of tumor cells at doses that are not harmful to healthy normal cells. In a first-in-human clinical trial, a-TEA was well tolerated in adults with advanced solid tumors (NCT02192346), but has not yet been studied in pediatric sarcoma. We used a murine model of rhabdomyosarcoma (M3-9-M RMS) to assess the in vitro and in vivo anti-tumor effects of a-TEA.

Methods

In vitro studies were performed on the M3-9-M RMS cell line to measure a-TEA-mediated apoptosis using flow cytometry (Annexin V+/7AAD+ cells) and live cell imaging (Annexin V+ cells). In vivo studies involved orthotopic implantation of luciferase + M3-9-M tumor cells into syngeneic C57BL/6 recipients. Once tumors were palpable, mice were randomized to a control diet or a-TEA supplemented chow for 21 days and evaluated for bioluminescence, tumor growth and overall survival. Gene expression of tumor infiltrating and splenic T cells were analyzed by bulk RNA-Seq and flow cytometry respectively.

Results

M3-9-M RMS treatment with 2.5-100 uM a-TEA induced apoptosis in a dose-dependent manner within 24 hours ($p < 0.05$) as measured by flow cytometry and live cell imaging. In-vivo studies with the M3-9-M RMS mouse model showed that recipients of a-TEA chow had 30-40 % reduced tumor growth ($p<0.01$) and bioluminescence ($p<0.05$), leading to prolonged survival (> 4 weeks) compared to recipients of matched control chow ($p<0.05$). Spleen cells isolated from a-TEA-fed tumor-bearing mice demonstrated increased levels of IFN??+ cells, CD4+ T-cells, Ki-67 proliferation, and decrease in splenic CD11b+ arginase-1+ ($p<0.01$) and PD-L1+ cells ($p<0.05$) compared to their counterparts on the control diet. Gene set enrichment

analyses of excised RMS tumors after a-TEA treatment revealed increased gene expression of CD24, EP300, CXCR4, and c-Jun as compared to tumors from mice fed control chow.

Conclusion

These data indicate that a-TEA mediates apoptosis of RMS in vitro and suppresses in vivo tumor growth, leading to prolonged survival likely via enhanced activation of adaptive immunity through CD4+ T cells and suppression of innate immunity through regulation of myeloid cell subsets. Furthermore, a-TEA may have direct effects on tumor cell proliferation through EP300 and c-Jun as well as indirect effects on tumor growth by regulation of immune cell recruitment through CD24 and CXCR4 gene expression. Administration of a-TEA as a potential salvage treatment for RMS is warranted.

Acknowledgments

The study was supported by NIH TL1 TR002375 (FS), St. Baldrick's Stand up to Cancer (SU2C) Pediatric Dream Team Translational Research Grant SU2C-AACR-DT-27-17, NIH/NCI R01 CA215461, American Cancer Society Research Scholar Grant RSG- 18-104-01-LIB, and the Midwest Athletes Against Childhood Cancer (MACC) Fund (CMC). SU2C is a division of the Entertainment Industry Foundation. Research grants are administered by the American Association for Cancer Research, the scientific partner of SU2C. The contents of this article do not necessarily reflect the views or policies of the Department of Health and Human Services, nor does mention of trade names, commercial products, or organizations imply endorsement by the US government.

Ethics Approval

The University of Wisconsin-Madison Animal Care and Use Committee approved all protocols (M005915).

Abstract #46

Phosphatidylinositol 3-kinase Signaling is Spatially Organized at Endosomal Compartments by Microtubule-associated Protein 4

Narendra Thapa, Mo Chen, Hudson T. Horn, Suyong Choi, Tianmu Wen, Richard A. Anderson

ABSTRACT

The current dogma in agonist-stimulated PI3K/Akt signaling indicates that PI 3-kinase is activated at the plasma membrane where receptors are activated and $PI4,5P_2$ is concentrated. Challenging this dogma, we show that $PI3,4,5P_3$ generation and activated Akt are largely confined to endomembrane upon receptor tyrosine kinase activation. This is regulated by microtubule-associated protein 4 (MAP4), an interacting partner of PI3Kα that controls localization of

membrane vesicle-associated PI3Kα to microtubules. The microtubule-binding domain (MTBD) of MAP4 binds directly to the C2 domain of the p1α10catalytic subunit. MAP4 controls the interaction of PI3Kα with activated receptors at endosomal compartments along microtubules. Loss of MAP4 culminates in the loss of PI3Kα targeting and loss of PI3K/Akt signaling downstream of multiple agonists. The MAP4-PI3Kα assembly defines a mechanism for spatial control of agonist-stimulated PI3K/Akt signaling at internal membrane compartments linked to the microtubule network.

Abstract #47

Hippo Signaling Effectors YAP and TAZ Induce Epstein-Barr Virus (EBV) Lytic Reactivation Through TEADs in Epithelial Cells

Nicholas Van Sciver, Makoto Ohashi, Nicholas P. Pauly , Jillian A. Bristol, Scott E. Nelson, Eric C. Johannsen, and Shannon C. Kenney

Abstract

The Epstein-Barr virus (EBV) human herpesvirus is associated with B-cell and epithelial-cell malignancies, and both the latent and lytic forms of viral infection contribute to the development of EBV-associated tumors. Here we show that the Hippo signaling effectors, YAP and TAZ, promote lytic EBV reactivation in epithelial cells. The transcriptional coactivators YAP/TAZ

(which are inhibited by Hippo signaling) interact with DNA-binding proteins, particularly TEADs, to induce transcription. We demonstrate that depletion of either YAP or TAZ inhibits the ability of phorbol ester (TPA) treatment, cellular differentiation or the EBV BRLF1 immediate-early (IE) protein to induce lytic EBV reactivation in oral keratinocytes, and show that over-expression of constitutively active forms of YAP and TAZ reactivate lytic EBV infection in conjunction with TEAD family members. Mechanistically, we find that YAP and TAZ directly interact with, and activate, the EBV BZLF1 immediate-early promoter**.** Furthermore, we demonstrate that YAP, TAZ, and TEAD family members are expressed at much higher levels in epithelial cell lines in comparison to B-cell lines, and find that EBV infection of oral keratinocytes increases the level of activated (dephosphorylated) YAP and TAZ. Finally, we have discovered that lysophosphatidic acid (LPA), a known YAP/TAZ activator that plays an important role in inflammation, induces EBV lytic reactivation in epithelial cells through a YAP/ TAZ dependent mechanism. Together these results establish that YAP/TAZ are powerful inducers of the lytic form of EBV infection and suggest that the ability of EBV to enter latency in B cells at least partially reflects the extremely low levels of YAP/TAZ and TEADs in this cell type.

Abstract #48

3D Patient-Specific Microfluidic Models of Primary Head and Neck Cancer To Evaluate Individual Drug Responses

María Virumbrales-Muñoz, Karina M. Lugo Cintrón, José M. Ayuso, Mouhita Humayun, Max M. Gong, Sheena Kerr, Suzanne M. Ponik, Paul M. Harari, David J. Beebe.

Background

Head and neck squamous carcinoma (HNSCC) is a highly heterogeneous group of tumors. HNSCC patients often present with lymph node metastasis at the time of diagnosis. Lymph node metastasis is also a key prognosis factor for HNSCC. It is known that high intra-tumor lymphatic density and tumor lymphangiogenesis correlate with lymph node metastasis. Anti-angiogenic therapies could be a promising strategy to improve HNSCC, which currently has few effective therapeutic options. The high inter-patient heterogeneity of these tumors makes it challenging to predict treatment success for individual patients. Therefore, a precision medicine approach may be needed to improve treatment decision rationale.

Methods

We combined a microfluidic 3D tubular lymphatic vessel model with patient-specific tumor-derived HNSCC fibroblasts from three different patients. Using these models, we studied the lymphangiogenic response resulting from fibroblast-lymphatic vessel crosstalk. Using these models, we evaluated lymphatic vessel permeability, angiogenic sprouting and studied the RNA profile of lymphatic vessels in the presence of the HNSCC tumor- derived fibroblasts. Further, we leveraged this model to identify patient-specific druggable targets (e.g., IGF) and test their efficacy *in vitro*.

Results

We demonstrated that lymphatic vessels were conditioned in a patient-specific manner by the tumor-derived fibroblasts. Further, this conditioning was different from that of normal tonsillar fibroblasts. We found that the presence of tumor-derived fibroblasts induced vessel sprouting, altered vessel permeability, and increased expression of pro- lymphangiogenic genes as compared to lymphatic vessel monocultures. We also identified distinct gene expression and functional responses in the fibroblast-conditioned lymphatic vessels consistent with the patient tumor stage and lymph node status. Finally, we found that the effectiveness of an anti-IGF antibody in the models was highly patient- specific. Further, in one of the patients, the treatment resulted in a compensatory pro- angiogenic mechanism, consistent with resistance to treatment.

Conclusion

Our patient-specific microfluidic 3D models of HNSCC provided insight into the pro-angiogenic signaling mechanisms present in the HNSCC microenvironment. Moreover, our models could play a key role in developing new treatments to improve HNSCC prognosis and integrated into a precision medicine approach to treating HNSCC.

Abstract #49

Postoperative venous thromboembolism in gynecologic oncology patients undergoing minimally invasive surgery: Does modality matter?

Matthew Wagar, Janelle Sobecki MD MA, Thevaa Chandereng PhD, Ellen Hartenbach MD, Sumer K Wallace MD

Background: Minimally invasive surgery (MIS) is increasingly utilized for gynecologic cancers. While rates of venous thromboembolism (VTE) after MIS are low, various guidelines recommend extended chemoprophylaxis for gynecologic oncology patients undergoing MIS. Our objective was to determine incidence of 90-day postoperative VTE in patients undergoing MIS for gynecologic malignancies and to determine differences in the incidence of VTE by MIS modality.

Methods: We collected demographic and perioperative variables for all patients undergoing MIS (robot-assisted, multi-port laparoscopy, single-port laparoscopy) for gynecologic cancers between January 2014 and December 2018 at our institution. Patients <18 years, with benign pathology, or on preoperative anticoagulation were excluded. Chi-square, Fisher's exact test, and one-way ANOVA were performed to determine risk factors related to VTE occurrence.

Results: We identified 818 patients who underwent MIS with median age 61. Most had Stage I disease (81.4%) and uterine cancer (81.6%). Five VTE events occurred within 90 days following surgery (0.6%). Incidence of 90-day VTE did not differ between MIS modalities (p=0.6). Patients with longer OR times (p=0.004) were more likely to experience VTE. Age, smoking status, BMI, type of cancer and stage were not significant risk factors for VTE.

Conclusion: The incidence of postoperative VTE in patients with gynecologic cancers undergoing MIS is low and does not differ by MIS modality. Given the very low incidence of postoperative VTE, extended chemoprophylaxis is unlikely to benefit patients with gynecologic malignancies undergoing MIS procedures.

Abstract #50

Cancer Lethality: an important burden metric to consider for the allocation of clinical trial research funding from public, industry, and other sources

Connor Wang, MD[a], Shannon Rush, MD[a], Shitanshu Uppal, MD[b], Laurel Rice, MD[a], Ryan Spencer, MD MS[a]

[a]Department of Obstetrics and Gynecology, Division of Gynecologic Oncology, University of Wisconsin School of Medicine & Public Health, Madison, WI

[b]Department of Obstetrics and Gynecology, Division of Gynecologic Oncology, University of Michigan, Ann Arbor, MI

Background: To identify trends and discrepancies between the number of clinical trials funded by public, industry, and other (individuals, universities, and community-based) organizations and measures of individual- and population-level burdens.

Methods: Clinicaltrials.gov was queried for US trials initiated from 2007-2017 in 18 cancers. For each cancer, incidence and mortality were obtained from the CDC, and years of life lost (YLL) from SEER database. Trials were categorized by funders and analyzed by incidence (I), mortality (M), YLL, and lethality (L, years of life lost per new diagnosis). Standardized ratios were generated as studies funded by public (#P/I,#P/M,#P/YLL,#P/L), industry (#I/I,#I/M,#I/YLL,#I/L), and other sources (#O/I,#O/M,#O/YLL,#O/L). Mean annual ratios for GYN cancers were compared to others using Wilcoxon ranksum tests. Rates of change (ROC) for each funder were analyzed by linear regression.

Results: While breast and prostate cancers rank 11th and 17th in Lethality, the mean ratios for breast and prostate are the two highest across all 3 funders. Ovary ranks 4th in Lethality with 11.14 years of life lost per new diagnosis, yet it ranks 12th in mean #P/L (2.086 - lower than 10 others at $p<0.05$), 10th in #I/L (2.886-lower than 9 others at $p<0.05$), and 14th in #O/L (1.557-lower than 13 others at $p<0.05$). Cervix is 7th most lethal, but its ratios rank 16th by all funders (#P/L=1.373, #I/L=0.886, #O/L=1.108). Uterus is 12th most lethal with ratios ranking 8th (#P/L=5.755), 11th (#I/L=3.387), and 12th (#O/L=4.246). Over 11 years, the #P/L ratios for 4 cancers (uterus, testicular, brain, esophagus) had a significant negative ROC ($p<0.05$), 13 were stagnant. For #I/L, 9 (including ovarian) had positive ROCs. None had negative ROCs. In #O/L, 7 had positive ROCs and 11 were stagnant.

Conclusions: GYN cancers have significantly fewer initiated trials when compared by Lethality across funding categories. Lethality is an important measure of individual burden and should be considered for funding allocation. Within all cancers, there is a significant trend towards rising industry- and other-funded trials and a trend towards decreasing and stagnating publicly-funded studies. This data can be used to investigate reasons for the differential allocation of resources in clinical trials.

Abstract #51

Role of IQGAP1, a PI3K signaling scaffolding protein, in papillomavirus-associated head and neck tumorigenesis using an infection-based animal model

Tao Wei[1], Suyong Choi[2], Darya Buehler[3], Denis Lee[1], Ella Ward-Shaw[1], Richard A. Anderson[2], Paul F. Lambert[1]

[1]McArdle Laboratory for Cancer Research, University of Wisconsin School of Medicine and Public Health, Madison, Wisconsin.

[2]University of Wisconsin-Madison, School of Medicine and Public Health, Madison, Wisconsin.

[3]Department of Pathology and Laboratory Medicine, University of Wisconsin School of Medicine and Public Health, Madison, Wisconsin.

Background: 25% of head and neck squamous cell carcinoma (HNSCC) is associated with human papillomavirus (HPV) infection. Mutations in PI3K signaling are highly implicated in HPV-associated HNSCCs. IQ motif-containing GTPase activating protein 1 (IQGAP1), a PI3K pathway scaffolding protein, is overexpressed and contributes to HNSCC. IQGAP1 is necessary for HPV-induced PI3K signaling both *in vitro* and *in vivo*. Blocking IQGAP1-mediated PI3K signaling reduces HPV-positive HNSCC cell survival and migration. Therefore, we hypothesized that IQGAP1 could promote papillomavirus (PV)-associated HNSCCs.

Methods: We have recently developed an infection-based model for studying PV-associated HNSCC using a mouse papillomavirus (MmuPV1). By incorporating cofactors such as the carcinogen 4-nitroquinoline (4NQO) and UVB irradiation, this model captures events from infection to the development of invasive carcinoma in mice. Both wild-type (*Iqgap1*$^{+/+}$) and *Iqgap1-deficient* (*Iqgap1*$^{-/-}$) mice were either mock-infected with PBS or infected with MmuPV1, then treated with UVB and 4NQO in their drinking water. Oral swab samples from infected mice were collected and examined by qPCR for viral presence. Mice were harvested and 6-months post-infection for histological analysis.

Results: We first discovered MmuPV1 infection highly induced PI3K signaling in keratinocytes, possibly in an IQGAP1-dependent manner. Utilizing the MmuPV1-infection model, we tested the role of IQGAP1 in MmuPV1-associated HNSCC. At 3-week post-infection, qPCR amplifying the MmuPV1 E2 gene from oral swab samples showed signs of infection in both MmuPV1-infected *Iqgap1*$^{+/+}$ and *Iqgap1*$^{-/-}$ groups at similar levels. At 6-month post-infection, MmuPV1-infected *Iqgap1*$^{+/+}$ mice developed higher tumor phenotypes than both mock-infected groups. Interestingly, MmuPV1-infected *Iqgap1*$^{-/-}$ mice developed lower tumor incidence and lower tumor multiplicity than the MmuPV1-infected *Iqgap1*$^{+/+}$. The MmuPV1-infected *Iqgap1*$^{-/-}$ mice also developed less severe neoplastic disease than their *Iqgap1*$^{+/+}$ counterparts. We detected MmuPV1 signals in both infected groups through *in situ* hybridization, indicating the presence of the virus at the endpoint. The arising tumors also showed features of HPV-infection and HPV-associated cancer.

Conclusion: Our study reported for the first time that IQGAP1 plays a role in PV-associated HNSCC.

Abstract #*52*

Interaction between Star-PAP and the phosphoinositide stress signaling pathway

Tianmu Wen, Mo Chen, Richard A. Anderson

Background

Speckle targeted PIPKIα regulated-poly(A) polymerase (Star-PAP) is a noncanonical poly(A) polymerase localized to nuclear speckles. It controls the expression of about 40% of human genes and also regulates microRNA and small nuclear RNA. Previous studies demonstrate Star-PAP being regulated by PIPKIα-mediated phosphatidylinositol-4,5-bisphosphate ($PI(4,5)P_2$) binding upon oxidative and genotoxic stress signals, which is required for the cleavage and polyadenylation of the mRNAs for vital proteins such as HO-1, NQO1, and BIK. Several other nuclear proteins, including p53 and SF-1, were also found to bind with and regulated by phosphoinositides (PIs) and their kinases.

Methods

We utilize immunofluorescence (IF) staining and proximity ligation assay (PLA) to study the *in situ* interactions between Star-PAP and different PIs and kinases, as well as in vitro binding assay and microscale thermophoresis (MST) to test their direct bindings and binding affinities.

Results

Here, we show that Star-PAP also binds with PI4P and $PI(3,4,5)P_3$, their respective kinases phosphatidylinositol 4-kinase 2-alpha (PI4K2A)， inositol polyphosphate multikinase (IPMK), the phosphatase and tension homolog (PTEN), as well as small heat shock proteins HSP27 and αBcrystallin. These interactions are stimulated *in vivo* upon oxidative and genotoxic stress treatment and direct binding *in vitro*.

Conclusion

It can be implied there is a sequential modification that starts from PI4P, which is phosphorylated to $PI(4,5)P_2$, and further phosphorylated to $PI(3,4,5)P_3$. Our data indicate Star-PAP is modified by a PI stress signaling pathway. This opens up functional studies for the various PIs-binding status of Star-PAP as well as a potentially universal PI signaling pathway for nuclear proteins.

Abstract #53

Papillomavirus infection of the oropharynx causes advanced dysplasia in immune-competent mice, and squamous cell carcinoma in HPV E5 transgenics, within 2 weeks

Andrea Bilger, Ella T. Ward-Shaw, Rong Hu, and Paul F. Lambert

Human head and neck cancers of the oropharynx (the middle section of the throat) are the most common HPV-related cancers in the US. To study the development of these cancers in a mouse model, we have used the mouse papillomavirus MmuPV1 to generate oropharyngeal lesions in both immune-deficient and immune-competent strains of mice.

Using the Greer "Pick" skin allergy testing device as a viral delivery tool, and "Mickey's Space Helmet" to provide oral access during isoflurane anesthesia, we have shown previously that infection of immune-deficient animals with mouse papillomavirus in both the soft palate and the base of the tongue in the oropharynx results in moderately to severely dysplastic lesions within 21 weeks. Mouse papilloma virus lacks the E5 oncogene found in the human HPV 16 and 18 papillomaviruses most commonly associated with oropharyngeal squamous cell carcinoma. Our laboratory previously generated E5-transgenic mice, which slowly develop spontaneous skin tumors that do not progress to cancer over the course of a 15-month study. We have previously shown that the E5 transgene accelerates the development of cutaneous and cervical tumors, and increases the proportion of squamous cell carcinomas, in mice infected with MmuPV1. Infecting both immune-competent non-transgenic and E5-transgenic mice of the FVB strain, we now show that non-transgenic FVB mice, infected with mouse papillomavirus at the base of the tongue near the circumvallate papilla, develop advanced dysplasia within only two weeks of infection. Strikingly, we also show that 30% (3/10) of E5-transgenic mice infected at the base of the tongue develop oropharyngeal cancer within 2 weeks of infection. Occurring in the absence of carcinogenic cofactors such as 4-NQO or UV irradiation, this is the most rapid development of cancer we have observed among our animal models of papillomavirus-induced disease.

Abstract #54

Studying the interplay of cervicovaginal dysbiosis and papillomavirus infection in a preclinical murine model

Authors: Simon Blaine-Sauer, Megan Spurgeon, Elizabeth Townsend, Lindsay Kalan, and Paul F. Lambert

Keywords: microbiome, papillomavirus, cervical cancer

Abstract:

Growing evidence supports a strong correlation between dysregulation of the cervicovaginal microbiome and HPV infection, persistence, and the development of neoplastic disease and cervical cancer. Thus far, this association has been primarily studied at the clinical and population level by correlating the composition of the microbiome with HPV and disease status in women. However, human patient studies are limited in their ability to allow controlled research on the directional, temporal, and mechanistic aspects of this relationship.

Our laboratory has developed an infection-based model of cervicovaginal disease using a murine papillomavirus, MmuPV1. We combined this model with an established protocol for inducing cervicovaginal dysbiosis using oral antibiotics administered in the drinking water. Female mice were pre-treated with antibiotic or control water for two weeks, then infected in the reproductive tract with MmuPV1. Cervicovaginal lavages were collected approximately every two weeks for 6 months in order to longitudinally assess viral burden and microbiome composition. Tissues were collected at the 6-month study endpoint for histopathological disease scoring and biomarker analysis.

While we hypothesized that dysbiosis would augment MmuPV1 infection, we observed that antibiotic-treated mice consistently had decreased viral loads. Ongoing experiments will assess the effect of dysbiosis on neoplastic disease, as well as how the microbiome composition changes in response to antibiotic-mediated dysbiosis and MmuPV1 infection. We are also investigating the effects of inducing dysbiosis in mice already harboring persistent MmuPV1 infections. Our novel murine model provides an adaptable and tractable pre-clinical platform to further elucidate the interplay between papillomaviruses and the microbiome.

Abstract #55

Multimodal Liquid Biopsy System for Cancer Immunotherapy Using PD-L1-Targeting Peptides

Woo-jin Jeong; Luke J. Kubiatowicz; Ashita Nair; Adam J. Drelich; Michael J. Poellmann; Randall J. Kimble; and Seungpyo Hong

Circulating tumor cells (CTCs) and exosomes have demonstrated their potential use as a biomarker for predicting treatment responses to immunotherapy. However, the currently available antibody-based liquid biopsy assays lack the sensitivity and specificity required to obtain clear indications. Our study devised and integrated systematic peptide engineering strategies to enhance the binding avidity and specificity of a β-hairpin peptide (pL1) isolated from programmed death 1 (PD-1), which was utilized as a capture agent for targeting PD-L1 on CTCs and exosomes. Specifically, we investigated the effect of: i) poly(ethylene-glycol) linkers as spacers; ii) the secondary peptide structure upon immobilization onto substrates in different conformations; and iii) removing biologically redundant amino acid residues from the peptide. The optimized pL1 configuration captured PD-L1-expressing tumor cells and tumor-derived exosomes at significantly improved efficiencies *in vitro* compared to their whole antibody counterparts (aPD-L1), which was translated into significantly improved performance in a clinical pilot study with great potential to be clinically impactful. The results presented herein facilitates the use of CTC/exosomes as biomarkers for personalized and response-adaptive immunotherapy cancer treatment.

Abstract #56

Investigating CHK1 and AXL in claudin-low breast cancer

Sierra Colavito

Background: The claudin-low breast cancer subtype is enriched for characteristics of tumor-initiating cells, and across a differentiation spectrum are most similar to mammary epithelial stem cells. This subtype is molecularly similar to cells that have undergone an epithelial to mesenchymal transition (EMT) and overlap with the recently characterized mesenchymal and mesenchymal-stem-like (MSL) sub-classifications of triple-negative breast cancer. These cancers are often triple-negative and have a poor prognosis, and few effective targeted treatment options exist for patients with these cancers. In order to determine possible drivers of proliferation in MSL breast cancer, we conducted an inhibitor screen in human mammary epithelial cells induced to undergo an EMT through constitutive knockdown of E- cadherin. The screen revealed that a Checkpoint Kinase 1 (CHK1) inhibitor is more active against the HMLE-shEcad cells compared to controls. CHK1 is a serine-threonine kinase that acts to control two checkpoints during the cell cycle, both at the intra-S phase and G2/M transition.

Methods: MSL breast cancer cells were treated with CHK1 inhibitors, and DNA damage assessed by immunoblotting for pH2A.X and p53BP1, both indicators of DNA double-strand breaks. Apoptosis, sphere forming ability, and colony formation were also analyzed, as well as a DNA fiber analysis conducted.

Results: Treatment of MSL breast cancer cells with CHK1 inhibitors results in DNA damage, cell-cycle defects characterized by a prolonged S phase, increased apoptosis, and decreased colony forming and sphere-forming abilities. When combined with a pro-apoptotic agent (BCL2 inhibitor) the results are super-additive.

Conclusions/Future Directions: These data indicate that CHK1 may be an effective therapeutic target in patients with MSL breast cancer. However, resistance to targeted therapies remains a leading problem in the clinic. Therefore, we are currently investigating possible resistance mechanisms by MSL breast cancer cells to CHK1 inhibition. We have identified elevated activation of the AXL receptor tyrosine kinase in MSL breast cancer cells with acquired resistance to CHK1 inhibitors.

Abstract #57

Mad1 Upregulation in Breast Cancer: Causes & Consequences

Sarah Allen, Jun Wan, Avtar Roopra, Beth Weaver

Mitotic arrest deficient-1 (Mad1), an essential component of the mitotic checkpoint, is upregulated in 20% of breast cancer patients. Upregulation of Mad1 causes chromosome missegregation during mitosis, a hallmark of cancer, as well as destabilization of the p53 tumor suppressor. Upregulation of Mad1 is sufficient to promote orthotopic tumor growth in immunocompromised animals. Patients with high levels of Mad1 mRNA expression have a poorer prognosis than patients with intermediate or low levels of Mad1. Unlike many core kinetochore proteins, Mad1 is not transcriptionally regulated by the transcription factor FoxM1. Thus, the mechanism for upregulating Mad1 in cancer, as well as the functional consequences, remain unknown. Bioinformatics analysis identified Histone Deacetylase 1 (HDAC1) as a likely cofactor involved in Mad1 transcriptional regulation. Consistent with this, HDAC inhibition with Trichostatin A (TSA) or Valproic Acid (VPA) increases Mad1 mRNA and protein levels 6- to 10-fold in multiple breast cancer cell lines. HDAC inhibition also induces Mad1 nuclear puncta, a localization pattern seen in primary breast cancer and breast cancer cell lines following Mad1 upregulation. Genetic approaches are currently being used to validate that HDAC1 negatively regulates Mad1 expression. To determine the consequences of Mad1 upregulation, we have generated a tetracycline (tet)-inducible Mad1 mouse model by inserting a tet responsive promoter and HA tag before the first coding exon of the Mad1 gene. Doubly heterozygous mice containing one allele of tet-inducible Mad1 and one allele of a ubiquitously expressed tet-transactivator (rtTA-M2) show inducible expression of HA-Mad1 following one week or one month of exposure to the tet analog doxycycline. Ongoing experiments will define the impact of Mad1 upregulation on mitotic fidelity and p53 levels and determine whether upregulation of Mad1 is sufficient to induce tumorigenesis in an immunocompetent setting.

Abstract #58

BCLxL inhibition is necessary for induction of intrinsic apoptosis in PIK3CA mutant colorectal cancer

Rebecca DeStefanis, Alyssa DeZeeuw, Gioia Sha, Susan N. Payne, Christopher P. Babiarz, Devon Miller,
Demetra P. Korkos, Cheri A. Pasch, Linda Clipson, Kristina A. Matkowskyj, and Dustin A. Deming

Colorectal cancer (CRC) is a leading cause of cancer related death with *PIK3CA* mutations occurring in ~18% of all cases. Mutations in this gene lead to constitutive activation of the phosphoinositide-3 kinase (PI3K) oncogene. Previously we have shown that MTORC1/2 inhibition is sufficient to induce a therapeutic response both *in vitro* and *in vivo* with minimal induction of apoptosis. BCL-xL is a well-known negative regulator of apoptosis in solid tumors. We therefore investigated whether inhibition of the BCL-2 family, and more specifically BCL-xL, would enhance therapeutic response and induction of apoptosis.

Murine-derived cancer organoids (MDCOs) were generated from invasive colon adenocarcinomas of *Apc* and *Pik3ca* transgenic mice (F1 (FVBxB6) $Apc^{fl/+}$ $Pik3ca^{H1047R}$). MDCOs were allowed to mature for 24 hours, baseline brightfield images were taken and therapeutic agents added at concentrations outlined below. Median relative change in organoid diameter after 48 hours of treatment was determined. *In vivo* response was measured in F1 (FVBxB6) $Apc^{fl/+}$ $Pik3ca^{P110*}$ mice as change in endoscopic tumor lumen occlusion over 14 days. Immunoblotting (IB) and immunofluorescence (IF) were utilized to evaluate for induction of apoptosis.

Navitoclax (ABT-263, BCL-2/BCL-xL/BCL-w inhibitor, 250nM) was evaluated alone and in combination olorectal cancer (CRC) is a leading cause of cancer related with a panel of MTORC1/2 inhibitors (BEZ-235 (BEZ), TAK-228 (TAK), copanlisib (Cop), 200nM). Navitoclax did not induce a treatment response as a single agent. Enhanced response was seen with the combination compared to the MTORC1/2 inhibitors alone (Bez 56% vs combo -100%, $p<0.001$; TAK -27% vs combo -100%, $p<0.001$; Cop -16% vs -100%; $p<0.001$). Results were confirmed *in vivo* with TAK-228 (1mg/kg/day) or BEZ-235 (30mg/kg/day), alone and in combination with navitoclax (80mg/kg/day) with the greatest reduction in lumen occlusion of colon tumors in the combination therapy (TAK/ABT: control +11%, ABT-263 +0.5%, TAK -25%, and combo -33%, $p=0.5$ TAK vs combo. BEZ/ABT: control +15%, navitoclax +1%, BEZ -15%, and combo -42%, $p<0.003$ BEZ vs combo). IB of cleaved PARP, a main cleavage target of cleaved caspase 3 (CC3) once apoptosis is induced, and IF of CC3 confirmed induction of apoptosis was highest in the combination therapy in both *in vitro* and *in vivo* studies. This induction was found as early as 6 hours post treatment in the MDCOs. To confirm inhibition of BCL-xL was the primary anti-apoptotic protein necessary for this induction of apoptosis, MDCOs were treated with copanlisib (200nM) alone or in combination with WEHI-539 (BCL-xL inhibitor, 250nM) or ABT-199 (BCL-2 inhibitor, 250nM). An enhanced sensitivity was observed when MTORC1/2 inhibition was combined with the inhibition of BCL-xL compared to BCL-2. These studies indicate that BCL-xL signaling reduces MTORC1/2 inhibitor response and targeting BCL-xL in combination with MTORC1/2 enhances both the treatment response and the induction of apoptosis in PIK3CA mutant CRC.

Abstract #59

Peptide-Mediated Multivalent Nanoparticles: Promising Platforms for Cancer Immunotherapy

Adam Drelich, Woo-jin Jeong, Mari Iida, Kourtney Kostecki, Jiyoon Bu, Ashita Nair, Seungpyo Hong, Deric Wheeler

Background:
Immunotherapy has grown to become a cornerstone of modern oncology and one of the "five pillars" of cancer treatment. The checkpoint blockade of immunoinhibitory receptors and their ligands, like the PD-1/PD-L1 pathway, has played a significant role in this success. However, current therapies are based on full-size monoclonal antibodies. While efficacious, antibodies are hindered by key disadvantages, including incompatibility with site-specific conjugation, low thermodynamic stability, and high manufacturing cost and complexity, further complicating efforts to develop new therapies to overcome limited patient response rates. Peptides, small chains of amino acids, are one promising alternative. Despite their advantages, such as target affinity and selectivity, isolated peptides typically lack innate folding structures which often leads to hampered binding. To overcome these issues, we have studied peptide-conjugated hyperbranched polymeric nanoparticles as a next step in cancer immunotherapy.

Results:
Utilizing G7 PAMAM dendrimers conjugated to PD-L1 binding peptides, derived from human and mouse PD-1 sequences, we measured a multivalent binding affinity for PD-L1 via surface plasma resonance (SPR) five orders of magnitude stronger than free peptides and equivalent to antibodies. Our peptide-dendrimer conjugates also demonstrated enhanced efficacy in promoting co-cultured Jurkat T-cell activation when treated to 786O cancer cells. Using circular dichroism (CD), FTIR, and 3D modelling, this was hypothesized to be the result of a volume exclusion effect on the dendrimer surface which promoted native β-hairpin peptide structure, enhancing their overall binding potential. Our conjugates were then treated to immunocompetent BALB/c mice injected with 4T1 breast cancer with improved efficacy, distribution and half-life, and safety than antibody treatment. We then applied this same strategy to novel polymeric dendron micelles, developing a preliminary nanoparticle platform for further enhanced cancer immunotherapy.

Conclusion:
Peptides are a promising alternative to antibodies for checkpoint inhibition, provided that their binding affinity can be enhanced. We have demonstrated that this is possible through conjugation of peptides to hyperbranched polymeric nanoparticles, leading to stabilization of native protein structure, enhanced multivalent binding, and ultimately efficacy equivalent to or surpassing antibodies. Combined with other polymeric platforms, this strategy has the potential to open a new focus in cancer immunotherapy.

Abstract #60

Leveraging an Electronic Health Record to Support a Clinician-Driven High-Dose Methotrexate Management Platform

Michael Fallon, Mikala Hillis, Mary Mably, Jessica Branson, Chris Nemergut, Christopher Fletcher

Abstract:

Background/Rationale:

- High-dose methotrexate is any dose of methotrexate greater than or equal to 500 mg/m^2 which is considered universally lethal unless appropriately rescued. Toxicities, including myelosuppression, gastrointestinal toxicity, neurotoxicity, and nephrotoxicity occur in direct proportion to duration of exposure. UW Health developed and implemented a high-dose methotrexate supportive care platform which includes a clinical practice guideline, decision support tools, comprehensive order sets, operational policies, and continual staff education. All components of this platform were built into the electronic health record (EHR) and include high-dose methotrexate specific chemotherapy orders, labs, hydration, nursing instructions, monitoring parameters, intravenous fluid and leucovorin dosing adjustment algorithms, and discharge criteria.

Methods:

- Retrospective chart review of adult patients who received high-dose methotrexate before and after the implementation of our institutional supportive care platform was performed. Patients treated between January 2014 and December 2015 were included in the pre-implementation group, while those treated between July 2016 and June 2020 were included in the post-implementation group, allowing for a 6-month washout period. The incidence of AKI during an encounter with high-dose methotrexate administration was evaluated for a significant difference between groups using a chi-square test. Secondary outcomes were evaluated for a significant difference between groups using a two-sided t-test and a Fisher's exact test, respectively.

Results:

- The incidence of AKI during an encounter with high-dose methotrexate administration (n=322) prior to this platform was 16.3% compared to 11.4% post-implementation (p=0.16). The mean length of stay was 6 days prior to this platform and 5 days post-implementation (p= 0.079). The incidence of encounters with methotrexate level greater than 5 umol/L at 42 to 54 hours following the start of methotrexate was 2.4% before and 1.4% after implementation of our EHR-supported platform (p=0.42).

Conclusions/Discussion:

- Our study demonstrates that use of the EHR can facilitate implementation of timely supportive care measures to decrease length of stay, while numerically reducing the incidence of AKI and improving the rate of methotrexate clearance. Though not statistically significant, these differences could suggest a trend toward improved patient morbidity.

Abstract #61

Intravital multiphoton microscopy of infiltrating T cell and tumor cell metabolism in a murine melanoma model

Alexa R. Heaton[1,2], Tiffany M. Heaster[3], Anna Hoefges[2], Alexander L. Rakhmilevich[2], Amy K. Erbe[2], Peter R. Rehani[1], Angelica F. Lopez[3], Paul M. Sondel[2], Melissa C. Skala[1,3]

[1] Morgridge Institute for Research, 330 N. Orchard Street, Madison, Wisconsin, United States, 53715
[2] University of Wisconsin, Department of Human Oncology, 1111 Highland Ave., Madison, Wisconsin, United States, 53705
[3] University of Wisconsin, Department of Biomedical Engineering, 1415 Engineering Drive, Madison, Wisconsin, United States, 53706

Background: Intravital multiphoton microscopy (IMM) provides single-cell imaging within intact living systems. IMM of the autofluorescent metabolic co-enzymes NAD(P)H and FAD, or optical metabolic imaging (OMI), provides *in vivo* label-free imaging of metabolic changes. The metabolism of tumor cells and immune cells is closely associated with cancer progression[1–3], so we aim to study metabolic trends throughout administration of an established, effective, triple-combination immunotherapy within murine melanoma tumors.[4] This therapy includes 12 Gy external beam radiation, intratumoral administration of a hu14.18-IL2 immunocytokine (anti-GD2 mAb fused to IL2), and intraperitoneal administration of anti-CTLA-4 leading to *in situ* vaccination and cure of GD2+ murine tumors.[4] Previous work has shown that a T cell response is critical to the efficacy of this therapy[4,5], so we created mCherry-labeled T cell mouse models to study T cell response. Here, IMM was used to image concurrent tumor and T cell metabolic trends, T cell infiltration, and tumor microenvironment composition.

Methods: We created mCherry-labeled T cell reporter mouse models through CRISPR/Cas9 knock-in and Cre-*lox* genetic modifications. We then implanted syngeneic B78 (GD2+) melanoma cells intradermally into the flanks of these mice to induce measurable tumors. Mice were anesthetized, skin flap surgery performed, and tumors imaged at varying time points using IMM. Murine tissues were also harvested and analyzed via flow cytometry and immunofluorescence to confirm mCherry expression in mouse models, infiltrating immune cell populations, and IMM findings.

Results: Here we demonstrate the feasibility of our IMM platform to perform single-cell resolution imaging *in vivo*. We establish that our reporter mouse models enable clear identification and tracking of mCherry+ populations. In addition, we show that label-free OMI provides metabolic trends and structural information *in vivo*. Overall, we demonstrate concurrent imaging of intravital tumor cell and T cell populations within the tumor microenvironment.

Conclusions: Our preliminary results suggest that the combination of IMM and our mCherry mouse models with OMI allows for concurrent imaging of T cell infiltration and metabolic trends.

With continued work, this imaging platform has the potential to provide dynamic, metabolic information on tumor cell and immune cell populations to inform further immunotherapy development.

References

(1) Renner, K.; Singer, K.; Koehl, G. E.; Geissler, E. K.; Peter, K.; Siska, P. J.; Kreutz, M. Metabolic Hallmarks of Tumor and Immune Cells in the Tumor Microenvironment. *Front. Immunol.* **2017**, *8* (MAR), 1–11. https://doi.org/10.3389/fimmu.2017.00248.

(2) Mockler, M. B.; Conroy, M. J.; Lysaght, J. Targeting T Cell Immunometabolism for Cancer Immunotherapy; Understanding the Impact of the Tumor Microenvironment. *Front. Oncol.* **2014**, *4* (May), 1–11. https://doi.org/10.3389/fonc.2014.00107.

(3) Ghesquière, B.; Wong, B. W.; Kuchnio, A.; Carmeliet, P. Metabolism of Stromal and Immune Cells in Health and Disease. *Nature* **2014**, *511* (7508), 167–176. https://doi.org/10.1038/nature13312.

(4) Morris, Z. S.; Guy, E. I.; Francis, D. M.; Gressett, M. M.; Werner, L. R.; Carmichael, L. L.; Yang, R. K.; Armstrong, E. A.; Huang, S.; Navid, F.; Gillies, S. D.; Korman, A.; Hank, J. A.; Rakhmilevich, A. L.; Harari, P. M.; Sondel, P. M. In Situ Tumor Vaccination by Combining Local Radiation and Tumor-Specific Antibody or Immunocytokine Treatments. *Cancer Res.* **2016**, *76* (13), 3929–3941. https://doi.org/10.1158/0008-5472.CAN-15-2644.

(5) Morris, Z. S.; Guy, E. I.; Werner, L. R.; Carlson, P. M.; Heinze, C. M.; Kler, J. S.; Busche, S. M.; Jaquish, A. A.; Sriramaneni, R. N.; Carmichael, L. L.; Loibner, H.; Gillies, S. D.; Korman, A. J.; Erbe, A. K.; Hank, J. A.; Rakhmilevich, A. L.; Harari, P. M.; Sondel, P. M. Tumor-Specific Inhibition of In Situ Vaccination by Distant Untreated Tumor Sites . *Cancer Immunol. Res.* **2018**, *6* (7), 825–834. https://doi.org/10.1158/2326-6066.cir-17-0353.

Abstract #62

DNMT3A contributes to ibrutinib resistance in mantle cell lymphoma

Nguyet-Minh Hoang, Yunxia Liu, Lixin Rui

Mantle cell lymphoma (MCL), a B-cell tumor comprising approximately 6% of non-Hodgkin lymphoma, remains incurable with the current standard immunochemotherapy. Ibrutinib, a selective inhibitor for Bruton tyrosine kinase (BTK) in the BCR signaling pathway, has elicited responses in 68% of refractory or relapsed MCL patients and promises to be a valuable therapeutic option. Primary or acquired resistance to Ibrutinib, however, impacts the long-term survival of these patients; understanding ibrutinib-resistance and identification of mechanisms to overcome resistance are unmet clinical needs. Our study focuses on *de novo* DNA methyltransferase 3A (DNMT3A) whose expression and activity are higher in ibrutinib- resistant MCL patients. It is also known that ibrutinib resistant cells show elevated expression of MYC target genes and increased oxidative phosphorylation compared to ibrutinib sensitive cells. We hypothesize that DNMT3A is involved in ibrutinib resistance in MCL. We found that DNMT3A physically interacts with MYC in MCL cells. CRISP/ Cas9-mediated DNMT3A knockout downregulates both MYC targets and genes that are involved in mitochondrial oxidative phosphorylation. Mechanistically, our DNMT3A ChIP-sequencing demonstrated a significant motif enrichment for MEF2B, a transcription factor that physically interacts with MYC to enhance transcriptional activities of MYC. Functionally, overexpression of DNMT3A increases mitochondrial activities, including increased membrane potential and mitochondrial reactive oxygen species as well as oxygen consumption rate. DNMT3A knockout sensitizes ibrutinib resistant cells to ibrutinib treatment. Conversely, overexpression of DNMT3A induces ibrutinib resistance in ibrutinib sensitive cells. In conclusion, our data provide evidence that DNMT3A overexpression contributes to ibrutinib resistance through the MEF2B-MYC axis, which regulates mitochondria function and oxidative phosphorylation in MCL. The study suggests that targeting DNMT3A is a potential therapeutic strategy to overcome ibrutinib resistance in MCL.

Abstract #63

Identifying the role of fibrocytes in obesity-induced mammary gland fibrosis

Genevra Kuziel

Obesity significantly increases breast cancer risk. In addition, obese breast cancer patients have an overall worsened prognosis. The complex relationship between obesity and breast tumor growth and aggressiveness is still being examined. Within breast adipose tissue, obesity causes a state of chronic, macrophage-driven inflammation. Chronic inflammation is associated with increased extracellular matrix (ECM) deposition, however, the underlying mechanisms are not completely understood. In mouse models, increased collagen within the mammary microenvironment is a risk factor for tumor formation, as well as more aggressive tumors. To understand how obesity affects collagen deposition in the mammary gland, we used a diet-induced obesity mouse model. We demonstrated that there was significantly greater collagen deposition around the mammary ducts of high-fat diet (HFD) fed mice compared to control diet (CD) fed mice. To investigate how myeloid lineage cells contribute to this fibrosis, CD11b+ cells were sorted from mammary glands of CD- and HFD-fed mice using magnetic beads. When cultured, a population of these sorted CD11b+ cells formed adherent colonies, and colony formation was significantly increased in the CD11b+ cells isolated from HFD-fed mice. When examined further, these colonies expressed myofibroblast markers smooth muscle actin (SMA) and collagen I. These colony-forming CD11b+ cells are consistent with fibrocytes, which have been studied in both chronic inflammatory and fibrotic diseases, but have not been studied in obesity. Fibrocytes are a multipotent, bone-marrow-derived population of cells that originate as myeloid progenitor cells and have characteristics of both macrophages and myofibroblasts. Consistent with an increase in fibrocytes in the mammary gland, myeloid progenitor cells (CD45+CD11b+CD34+) were significantly increased in the bone marrow of HFD-fed mice, indicating an upregulation in the progenitor cell population for both monocytes/macrophages and fibrocytes. Together, our results suggest that chronic inflammation within the obese mammary gland leads to increased fibrocyte numbers, as well as increased collagen deposition around mammary ducts. The effects of obesity on breast tissue fibrosis, as well as the cells responsible for this fibrosis, must be elucidated to better address the risk of breast tumor development in obese women.

Abstract #64

Molecular Targeted Radiotherapy with 90Y-NM600 for the treatment of leukemia

Chunrong Li, Reinier Hernandez, Joseph J Grudzinski, Carolina A Ferreira, Jamey P Weichert, Bryan P Bednarz, Mario Otto

Targeted radionuclide therapy (TRT) is an attractive approach that employs radiolabeled molecules to specifically deliver radiation to primary or metastatic tumors. NM600 is a novel tumor-selective alkyl phosphocholine analog that has been shown unique ability to selectively target a variety of cancer types. Here we evaluated the anti-leukemic effect of targeted radionuclide therapy with ^{90}Y-NM600 in various murine xenograft models of human leukemia.

Methods: CLR1501 (fluorescent NM404-BODIPY) was used to characterize uptake of NM600 in human leukemia cell lines *in vitro*. Luciferase-expressing human leukemia cells (MOLT4, KG1, K562, 2×10^6 cells/mouse) were injected intravenously into immune-deficient NRG mice to establish humanized leukemia xenograft models. In a minimal disease model, ^{90}Y-NM600 was injected intravenously into mice one day after injection with leukemia cells. In a model representing established disease, animals were injected with ^{90}Y-NM600 two weeks after leukemia cell inoculation and disease development. Leukemia burden over time for each model was monitored using bioluminescence imaging, peripheral blood count measurements and flow cytometry.

Results: Leukemia cell lines exhibited 2-3 fold increased CLR1501 uptake and retention compared to normal lymphocytes. In the minimal disease models, single dose treatment with 220 μCi ^{90}Y-NM600 reduced tumor burden, delayed disease development and progression, and led to statistically significant prolonged median survival. In established disease, two separate doses of 200μCi ^{90}Y-NM600 (on day 14 + day 35) prolonged median survival in a Molt4 leukemia model. Single injection of ^{90}Y-NM600 up to 400μCi had minimal and manageable hematological toxicities in NRG mice.

Conclusion: Taken together, our studies to date confirm that treatment of human leukemia in murine xenograft models with ^{90}Y-NM600 is feasible, safe and leads to prolonged survival. In ongoing experiments, combination approaches combining ^{90}Y-NM600 with other anti-cancer agents are being explored to obtain maximum anti-leukemic synergy.

Abstract #65

A Novel In Vitro Culture Model System to Study Merkel Cell Polyomavirus Associated MCC Using Three-Dimensional Organotypic Raft Equivalents of Human Skin

Amanda Loke, B. Jack Longley, Paul F. Lambert, Megan E. Spurgeon

Background

Merkel cell polyomavirus (MCPyV) is a human polyomavirus causally linked to the development of Merkel cell carcinoma (MCC), an aggressive malignancy that largely arises within the dermis of the skin. Despite the significant advances in developing tools to study MCPyV+ MCC, the field of MCPyV+ MCC research still lacks an in vitro model system to study the dynamics of MCC biogenesis, cell–cell interactions, and molecular mechanisms in a physiologically relevant, three-dimensional (3D) tissue context. Recent studies suggest that MCPyV infects the human skin, most likely epidermal keratinocytes or dermal fibroblasts followed by integration of viral genome into the host genome. In this study, we recapitulate the histopathology of human MCC tumors in vitro using an organotypic (raft) culture system that is traditionally used to recapitulate the dermal and epidermal equivalents of skin in three dimensions (3D).

Methods

Briefly, dermal equivalents with either fibroblasts or a mixture fibroblats and MCPyV+ MCC cells were suspended in collagen were generated and plated onto transwells. In some setups, to generate an intermediate layer, MCPyV+ MCC cells were suspended in collagen (supplemented with 10% 10× F-12 media, 10% FBS, 100 µg/mL penicillin/streptomycin, and 10 N sodium hydroxide) and added onto the dermal equivalent 24 h before the addition of keratinocytes. To form the epithelial layer, either epithelial cells or MCPyV+ MCC cells in collagen were added. Four days post generation of the epithelial layer, rafts were lifted and cultured with cornification. The rafts were then harvested and the presence and organization of MCC cells within these dermal lesions were characterized through biomarker analyses.

Results & Conclusions

In the optimal culture condition, MCPyV+ MCC cells were embedded in collagen between the epidermal equivalent comprising human keratinocytes and a dermal equivalent containing fibroblasts, resulting in MCC-like lesions arising within the dermal equivalent. Interestingly, co-culture of MCPyV+ MCC together with keratinocytes specifically within the epidermal equivalent of the raft did not reproduce human MCC morphology, nor were any keratinocytes necessary for MCC-like lesions to develop in the dermal equivalent. This 3D tissue culture system provides a novel in vitro platform for studying the role of MCPyV T antigens in MCC oncogenesis, identifying additional factors involved in this process, and for screening potential MCPyV+ MCC therapeutic strategies.

Abstract #66

A Hybrid Nanoparticle System Integrating Exosomes and Poly(amidoamine) Dendrimers: Implication for an Effective Gene Delivery Platform.

Ashita Nair[1], Jason Bugno[1], In-San Kim[2], Ick Chan Kwon[2], and Seungpyo Hong[1]
[1]Pharmaceutical Sciences Division, School of Pharmacy, University of Wisconsin, Madison, WI 53705
[2]Biomedical Research Center, Korea Institute of Science and Technology, Seoul, Republic of Korea 02792

Introduction: Nanoscale drug delivery systems for cancer treatment have demonstrated the potential to target therapeutics while reducing their systemic toxicity. However, several biological and physical barriers, such as immunogenicity of the delivery system and undesirable biodistributions, have hindered their fast translation. To address these issues, we have developed an exosome-dendrimer hybrid nanoparticle (NP) platform to combine the advantageous biological properties of exosomes (e.g. non-immunogenicity and favorable biodistribution) and dendrimers (e.g. well-defined hyperbranched structure and efficient tumor penetration) into a single NP system.

Materials and Methods: Exosome-dendrimer hybrid NPs, consisting of exosomes derived either from MCF7 cells or mesenchymal stem cells (MSC) and functionalized poly(amidoamine) (PAMAM) dendrimers, were prepared using sonication and characterized in terms of loading capacity, size, cytotoxicity, and cellular interactions.

Results and Discussion: Our results indicate that the loading of dendrimers into exosomes is dependent on dendrimer size and charge. Hybrid NPs inherited the size (~150 nm), surface charge (-10 mV), and surface protein markers (CD81, CD63) of exosomes. Importantly, hybrid NPs enhanced cellular internalization of amine-terminated PAMAM dendrimers ($p < 0.05$), while substantially reducing cytotoxicity (113.3% vs. 35.6% of cell viability at 500 nM, $p < 0.05$), as compared to positively charged free dendrimers. Additionally, the cell targeting properties of the exosome, specifically the homing property of mesenchymal stem cell derived exosome to sites of inflammation, was also shown to impart the hybrid NPs with tumor cell specificity. These advantageous properties of hybrid NPs were leveraged for use as a gene delivery vehicle, resulting in enhanced oligonucleotide delivery (over 2-fold) to cancer cells, compared to dendrimers alone.

Conclusions: The exosomes and dendrimers were hybridized successfully with the hybrid NPs inheriting the membrane properties of the exosomes. Hybridization altered the normal cellular interaction and cytotoxicity of the cationic dendrimers with the exosome aiding in the cell uptake process. The hybrid NPs also increased the cell uptake of oligonucleotides with the potential to be a very useful gene delivery tool. Our results demonstrate that the beneficial characteristics of individual exosomes and dendrimers can be integrated to generate a multifaceted NP platform, proposing a novel NP design strategy.

Abstract #67

Estrogen receptor blockade and radiation therapy may enhance the anti-tumor response to immune checkpoint inhibitors in immunologically cold ER+ breast cancer

Erin Nystuenm Amber M. Bates, Kathleen A. O'Leary, Sarah E. Emma, Elizabeth G. Sumiec, Linda A. Schuler, Zachary S. Morris

Metastatic, hormone therapy-resistant breast cancers expressing estrogen receptor (ER+) account for most breast cancer deaths. A lack of tumor-infiltrating lymphocytes (TILs) and low tumor mutation burden render most ER+ breast cancers immunologically "cold" and unresponsive to immune checkpoint inhibitors (ICI). Radiation therapy (RT) can augment tumor immune susceptibility, but RT may also increase immunosuppressive cell recruitment to the tumor. Recent studies demonstrate that ER inhibition may antagonize the trafficking and activation of myeloid derived suppressive cells (MDSCs) in tumors. We hypothesized that combining RT and a selective ER degrader (SERD), fulvestrant, would cooperate to relieve immunosuppression, increase tumor immune susceptibility, and facilitate response to ICI in a syngeneic murine model of ER+ breast cancer. Using a cell line (TC11) developed in the Schuler laboratory, cells were transplanted to caudal mammary fat pads of female FVB/N mice. When tumors reached ~200mm^3, mice were treated with combinations of vehicle, fulvestrant (250mg/kg sc, weekly), 8Gy RT on 3 consecutive days (days -1, 0, 1), and/or anti-PDL1 (200µg, IP, at days 3, 6, and 9). Tumors were collected at day 26, and cytokines in tumor lysates were evaluated using a multiplex immunoassay. In a parallel experiment, tumors were collected at day 10 for qPCR and flow cytometry analysis. Fulvestrant with RT slowed tumor growth compared to either treatment alone. RT elicited a type-1 interferon response in TC11 tumors as measured by upregulated expression of *Mx1, Pdl1, Oas2, Oas3, Trex1,* and *IfnB* mRNAs peaking at 3-5 days post RT and persisting to day 10. Cytokine analysis from tumor lysate revealed significantly increased IP-10 in tumors from mice treated with both RT and fulvestrant compared to controls, and similar trends were seen for IL6, VEGF, and MCSF. With the addition of RT and fulvestrant to anti-PDL1, IL2, IL3, IL6, IL7, IL17, and IP-10 were significantly increased compared to anti-PDL1 alone. Combined RT and fulvestrant may reduce immunosuppression compared to RT alone. These results demonstrate a cooperative interaction between RT and fulvestrant in favorably modulating the TME in an immunologically cold, hormone-therapy resistant, murine ER+ breast cancer model. Further experiments are needed to explore these immune effects.

Abstract #68

Baseline culture conditions do not alter the growth or treatment response of F1. FCAKPK mouse derived cancer organoids

Autumn Olson, Rebecca A. DeStefanis, Alyssa DeZeeuw, Gioia Sha, Jeremy

Kratz, Dustin A. Deming

Colorectal cancer is a leading cause of cancer related deaths and there has been an increased focus on developing therapies targeted to the mutational profile of the individual patient. Mouse derived cancer organoids (MDCOs) are used as a model for drug response testing of specific mutational profiles. Through retrospective analysis, we aim to validate the culture conditions of the MDCO model.

Colorectal cancer MDCOs were derived from several *Apc* and *Pik3ca* mutant mice (Fc^{1} $Apc^{fl/+}$ $Pik3ca^{H1047R/+}$). We analyzed 40 studies from our lab that evaluated the response of F1. FCAKPK MDCOs to various PI3K pathway inhibitors. Altogether, we analyzed 3,152 individual MDCOs. In each study, baseline images of the MDCOs were taken prior to treatment, and following a 48-hour incubation period, the same MDCOs were imaged again. Our analyses focused on determining if baseline culture conditions such as passage number, plating density, location within the Matrigel droplet, and from which mouse the MDCOs were derived from altered the growth or treatment response. The retrospective analysis was completed on the individual organoid level by analyzing the control organoids and treated organoids separately to evaluate growth and treatment response, respectively. The relative change in diameter over 48 hours was compared to each culture condition.

There were no significant differences in growth due to the baseline culture conditions for the control MDCOs. The R^2 values comparing the relative change in diameter over 48 hours to the culture conditions of passage number, plating density, location within the Matrigel droplet, and from which mouse the MDCOs were derived from were 0.0103, 0.0243, 0.0001, and 0.0295, respectively. Likewise, there were no significant differences in the response to PI3K pathway inhibition of the MDCOs due to standard culture conditions. The R^2 value comparing the percent relative change in diameter to the same culture conditions as above were 0.1174, 0.0979, 0.0221, and 0.1267, respectively.

MDCOs are a useful tool in modeling drug response of specific mutational profiles which in effect aids in developing targeted approaches to cancer treatment. We validated that most baseline culture conditions do not alter the growth or treatment response of F1. FCAKPK MDCOs.

Abstract #69

Circulating Tumor Cell Trends in Gastrointestinal Cancer Predict Treatment Response Using a Highly Sensitive Detection Device and Machine Learning for Enumeration

Michael J. Poellmann[1,2], Jiyoon Bu[1], Stanley Liu[2], Andrew Z. Wang[2,3], Joseph M. Caster[4], and Seungpyo Hong[1,2,5,6]

1. Pharmaceutical Sciences Division, University of Wisconsin – Madison
2. Capio Biosciences, Madison, WI
3. Department of Radiation Oncology, University of North Carolina at Chapel Hill
4. Department of Radiation Oncology, University of Iowa
5. Department of Biomedical Engineering, University of Wisconsin – Madison
6. Yonsei Frontier Lab and Department of Pharmacy, Yonsei University, Seoul, KOREA

Purpose: A highly sensitive system for purifying circulating tumor cells (CTCs) was used to monitor gastrointestinal patients during treatment. We hypothesized that trends in CTC numbers would correlate with clinical outcomes.

Experimental Design: Patients with rectal or pancreatic adenocarcinoma undergoing radiation therapy, with or without chemotherapy, were eligible for enrollment. Peripheral blood samples were collected at multiple points during and following treatment. CTCs were purified using a combination of biomimetic cell rolling on recombinant e-selectin and dendrimer-mediated multivalent immunocapture targeting epithelial cell adhesion molecule (EpCAM), epidermal growth factor receptor (EGFR), and human epidermal growth factor receptor 2 (HER2). A machine learning algorithm was developed, trained, and validated to efficiently count the high numbers of CTCs present on the capture surfaces following immunocytochemical staining.

Results: Samples drawn from 27 subjects either prior to receiving therapy or early in chemotherapy had a median 50 CTC ml^{-1} whole blood (range 0.6-541.6). Select samples were also stained for CDX2 to confirm that CTCs were derived from the gastrointestinal system. Additionally, trends in CTC counts before and after radiation treatment correlated with clinical response. Two of three patients with progressive disease had >10x increase in CTCs over the course of radiation treatment, while 3 of 4 patients with complete response and 5 of 7 patients with partial response to treatment had decreasing CTC trends.

Conclusions: The results suggest that highly sensitive CTC capture through biomimetic cell rolling and multivalent immunorecognition mechanisms may be a useful measure of the success of radiation treatment for gastrointestinal cancer.

Abstract #70

Effects of molecular-targeted radiotherapy with ^{90}Y-NM600 on tumor growth and the tumor microenvironment in murine rhabdomyosarcoma

Ankita Shahi[1], Hassler Rengifo[2], Reinier Hernandez[3], Joseph Grudzinski[4], Christopher Massey[4], Qianqian Zhao[5], Jamey P. Weichert[4], Bryan Bednarz[3], Gopal Iyer[2], Mario Otto[1]

[1]Department of Pediatrics, Carbone Cancer Center, [2]Department of Human Oncology, [3]Department of Medical Physics, [4]Department of Radiology, [5]Department of Biostatistics and Medical Informatics, and School of Medicine and Public Health, University Wisconsin-Madison, Madison, WI, USA

Background: Rhabdomyosarcoma (RMS) is the most common pediatric soft tissue sarcoma. Patients with metastatic disease or relapse are confronted with a poor prognosis. We investigated the anti-tumor effects of a novel tumor-targeted radiopharmaceutical, the alkyl phosphocholine (APC) analog ^{90}Y-NM600, in a syngeneic murine RMS model. In addition, we tested the immunomodulatory effects of the CXCR4 antagonist AMD3100 for potential synergy with targeted radiotherapy (TRT).

Methods: Using a fluorescent APC analog of NM600 as a surrogate (CLR1501), we verified the compound's *in vitro* uptake in RMS cell lines by flow cytometry. Using the positron-emitting analog ^{86}Y- NM600, we determined in vivo tumor uptake and performed dosimetry with PET/CT. To evaluate anti-tumor efficacy, we established orthotopic tumor grafts with the murine M3-9-M RMS line in the thigh musculature of AJ.B6/J hybrid mice. Cohorts of tumor-bearing mice were treated with ^{90}Y-NM600 (250µCi), AMD3100 (3mg/kg, intraperitoneal injection daily for 21 days), or a combination of both. Control mice received vehicle only. Treatment-related immunological effects on the tumor microenvironment were evaluated by IHC, qPCR, and next-generation sequencing (NGS).

Results: RMS cell lines sequestered and retained significantly higher amounts of CLR1501 than normal cells in vitro. PET/CT demonstrated tumor-selective uptake and retention of ^{86}Y- NM600 in flank tumors at 44 hours post-injection. *Ex vivo* biodistribution experiments showed higher tumor selective uptake in tumors compared to normal organs. A single dose of ^{90}Y-NM600 led to a significant decrease in tumor growth compared to vehicle or treatment with AMD3100 alone. Both ^{90}Y-NM600 and combination treatment increased the number of tumor-infiltrating lymphocyte subsets (CD8, CD4) and decreased MDSCs and Tregs. Gene expression study demonstrated upregulation of IFNɣ, p53, and downregulation of immunosuppressive markers such as IL-10, PD-L2, TGFβ2, IL-6 in samples treated with ^{90}Y-NM600 alone and in combination with AMD3100 compared to the control group in a time-dependent fashion. Mechanistically, NGS data revealed that APC analogue activated lipid-related metabolic pathways followed by several immune-modulatory pathways over time suggesting that early activation and entry of lipid analogue results in perturbations of immune pathways through radiation effects.

Conclusion: Our preliminary results demonstrate that TRT with ^{90}Y-NM600 leads to reduced tumor growth and improved survival in a syngeneic, orthotopic model of RMS. Our data suggest a time- dependent modulation of the tumor immune microenvironment that might contribute to TRT's anti- tumor effects.

ABSTRACT #71

Synthetic Fusion Cytokine GIFT4 - engineered Autologous Peritoneal Immune Cells a Novel Cell Therapy for Treatment of Ovarian Cancer

Presenter Name: Sejal Tarun Sharma, Dr. Pradyut Paul, Dr. Arvinder Kapur, Dr. Lisa Barroilhet, Dr. Jacques Galipeau, Dr. Manish Patankar

Background: Ovarian Cancer is the 7th most common cause of cancer globally with ~22,000 new cases and ~17,000 deaths observed each year in United States alone. In most patients, it is diagnosed at an advanced stage (Figo stage III/IV), limiting the benefits of available therapeutic modalities. Synthetic fusion cytokines (fusokines) have potential as a novel cytokine-based immunotherapeutic. Such fusokines are chimeras of two functionally unrelated cytokines. One such fusokine, Gift4, consists of GM-CSF and a common γ-chain receptor interleukin IL-4 and has shown promising tumoricidal effects that foreshadows its potential use for eradication of ovarian cancer. In our current approach, we hypothesize that autologous peritoneal immune cells (PIC) genetically modified to express the fusokine, GIFT4, will result in the hyperactivation of immune cells that can efficiently serve as a novel cell therapy against HGSOC.

Aim: To develop efficient methods of lentiviral transduction of patient-derived immune cells to stably express Gift4 fusokine followed by fundamental phenotyping and functional characterization of Gift4-weaponized PICs.

Methods: Splenocytes collected from naïve mice were transduced with LV-mGIFT4 and probed for GFP expression. Gift4-engineered splenocytes were intravenously administered into 3 mice at 10 x 10^6, 20x10^6, or 50x10^6 cells each and kept under observation for 2 weeks to determine the maximum tolerated dose (MTD). The efficiency of transduction was confirmed by Flow cytometry using EGFP reporter.

Results: Splenocytes were used for transduction method development. The splenocytes showed successful transduction with GFP expression using flow cytometry. Administration of these transduced splenocytes determined maximum tolerable dose to be 50 million cells without any adverse effects on recipient health.

Conclusion: Successful splenocyte transduction validated the protocol for transducing PICs. These data also suggest that mGift4 is non-toxic at significantly higher dose. Our goal is to use mGift4-engineered PICs as an immune cell therapeutic along with other available therapies for the treatment of ovarian cancer.

Abstract #72

FIST15 enhances anti-Tumor effect of NK cells against murine osteosarcoma after Bone Marrow Transplant

Longzhen Song, Anne Wong, Paul D. Bates, Kirsti L. Walker and Jacques Galipeau, Christian M. Capitini

NK cell-based immunotherapies have been tested in several clinical trials of osteosarcoma (OS) combined with stimulating cytokines and gained some success. However, the immunosuppressive molecular in tumor microenvironments (TME) could attenuate the anti-tumor effect of NK cells, and even turn NK cells to pro-tumor side by immunosuppression molecular. TGF-β is a crucial component of the negative immune regulatory network of TME. Fused cytokine FIST15, which is designed to function as a stimulating cytokine and be a TGF-β trap in the same time, has not been tested for OS. We hypothesized that FIST15 would enhance anti-tumor effect of NK cells against OS. First, Balb/c mice were lethally irradiated and transplanted with Balb/c T cell depleted bone marrow cells (BMC). We then established an OS pulmonary metastases model by intravenously injection of luciferase+ K7M2 OS cells. When the total flux of bioluminescence was around 1×10^6 p/s, some recipients also received adoptive transfer of Balb/c NK cells with or without FIST15 (~3 μg/dose). Syngeneic BMT recipients of NK cells and FIST15, which had longer survival time, showed significantly less pulmonary metastases on gross pathology and reduced bioluminescence as compared to mice who did not receive FIST15 ($p < 0.05$). On Day 5 after treatment, larger number of NK cells appeared in lungs and bronchoalveolar lavage fluid (BALF) than both control group and IL-15/IL-15R treated group, suggesting FIST15 could improve NK cell migration in to tumor site. FIST15 showed higher efficacy than IL-15/IL-15R only when lung metastasis reached later stage. We then started to treat mice when the total flux of bioluminescence was around 1×10^8 p/s, isolated NK cells from lungs on Day 7 after treatment, and compared gene expression profile of NK cells in lungs of control group, group treated with NK cells and FIST15, and group treated with NK cells and IL-15/IL-15R by bulk RNA sequencing. Through GO analysis of different expressed genes (DEGs) between FIST15 group and IL-15/IL-15R group, the top ten downregulated pathways were angiogenesis and angiogenesis related pathways. And the top ten upregulated pathways were cell cycle related pathways and NK cell effector function related pathways. KEGG analysis of DEGs resulted in similar conclusion. In conclusion, FIST15 is an effective fused cytokine against OS when combined with adoptive NK cell infusion after syngeneic BMT, probably through reducing the proangiogenic effect of NK cells in TME of OS, meanwhile increasing NK cell proliferation and killing ability.

Abstract #73

In vivo synergy of ^{90}Y-NM600 and Bempegaldesleukin improves anti-tumor efficacy of immune checkpoint inhibitors in syngeneic murine cancer models

Elizabeth G. Sumiec[1], Amber M. Bates[1], Reinier Hernandez[2], Joseph J. Grudzinski[2], Ian R. Marsh[2], Sarah E. Emma[1], Erin J. Nystuen[1], Justin Jagodinsky[1], Alexander A. Pieper[1], Gustavo A. Sosa[1], Bryan Bednarz[2], Ravi B. Patel[4], Jamey Weichert[2,3], Zachary Morris[1,3]

[1]Department of Human Oncology, University of Wisconsin School of Medicine and Public Health, University of Wisconsin-Madison, Madison, WI

[2]Department of Radiology, University of Wisconsin School of Medicine and Public Health, Madison, WI
[3]University of Wisconsin Carbone Cancer Center, University of Wisconsin-Madison, Madison, WI
[4]University of Pittsburgh Hillman Cancer Center, University of Pittsburgh, Pittsburgh, PA

We have observed in preclinical studies that the delivery of low dose targeted radionuclide therapy (TRT) therapy to sites of primary and metastatic cancer can improve the anti-tumor immune response to immune checkpoint inhibition (ICI) with anti-CTLA4 and anti-PDL1. NM600, an alkylphosphocholine that can be radiolabeled with ^{90}Y, is selectively taken up and retained in most cancer cells following intravenous injection. Bempegaldesleukin (BEMPEG) is a first in class, CD122-preferential IL2 pathway agonist that can stimulate an immune response. Herein, we report the results of experiments combining low dose TRT with BEMPEG to increase immune activation, enhance the response to ICIs, and improve translational potential.

C57Bl/6 female mice were subcutaneously engrafted in the flank with the murine head and neck squamous cell carcinoma (HNSCC) cell line, MOC2. In vivo dosimetry performed using the Monte Carlo based RAPID platform following serial ^{86}Y-NM600 PET/CT imaging demonstrated that 100 µCi of ^{90}Y-NM600 would deliver ~8 Gy to the MOC2 tumor. Mice bearing MOC2 tumors (mean volume ~100 mm^3) received combinations of ^{90}Y-NM600 (100 µCi, day 1 IV), BEMPEG (16 µg, days 6, 15, and 24 IV), and anti-CTLA4 (200 µg, days 4, 7, and 10 IP) using a 2x2x2 study design. Tumor growth and survival were monitored. Blood was analyzed weekly on the Abaxis VetScan HM5 to evaluate toxicity. In a parallel 2x2x2 survival study, an orthotopic model generated by engrafting MOC2 tumors in the cheek was treated with combinations of BEMPEG, ^{90}Y-NM600, and anti-PDL1 (200 µg, days 4, 7, and 10, IP). Mice were euthanized when >25% weight loss was observed. Similar experiments using ^{90}Y-NM600 and BEMPEG were performed in additional syngeneic mouse cancer models including SCC7 (HNSCC), 4T1 (breast), LLC (lung), and Panc02 (pancreatic).

In the immunologically “cold” MOC2 HNSCC model, 62.5% of mice treated with ^{90}Y-NM600 (TRT), BEMPEG, and anti-CTLA4; and 44.4% of mice treated with ^{90}Y-NM600, BEMPEG, and anti-PDL1 experienced complete tumor response and no observable primary or metastatic disease at day 60. In mice treated with ^{90}Y-NM600 and BEMPEG, tumor regression followed by escape was seen without ICI. No mice receiving single or dual treatment combinations exhibited a complete tumor response. Comprehensive whole blood analysis did not show any major hematologic toxicities. This treatment was explored in mice bearing SCC7, 4T1, Panc02, and LLC tumors, and similar trends were seen.

Combination of ^{90}Y-NM600, BEMPEG, and ICI displays robust anti-tumor activity that prevents metastatic disease progression and prolongs survival in spontaneously metastatic, immunologically “cold” tumor models. Clinical studies are warranted to test the safety and efficacy of this promising combined modality treatment regimen.

Abstract #74
Not applicable

Abstract #75

Treating post-transplant B-cell acute lymphoblastic leukemia with blinatumomab as a radiation sparing immunotherapy

David Turicek, Christian Capitini, Nicholas J. Hess, Sean Rinella

Background

B-cell acute lymphoblastic leukemia (B-ALL) is an aggressive cancer of the bone marrow featuring proliferation of immature B-cells. While current treatments have attained survival rates of 85%, those that relapse or have refractory disease are difficult to cure. Novel approaches tend to evade radiation because of its toxicity. Previous research has shown γδ T cells do not cause GVHD yet remain as potent cytotoxic agents. Blinatumomab, a bi-specific T-cell engager (BiTE), induces cytolytic synapses between cytotoxic T cells and cancer cells by linking CD19 with CD3. Although past research proved efficacy of blinatumomab in patients that relapse after a T cell replete alloHSCT, the efficacy of blinatumomab in patients following an alpha/beta (αβ) T-cell-depleted alloHSCT is yet to be established. This project will assess whether blinatumomab will activate $CD3^+$ human gamma/delta (γδ) T cells against $CD19^+$ B-ALL *in vitro* and *in vivo*.

Methods

We will isolate human γδ T cells from peripheral blood using ficoll density centrifugation and a negative selection magnetic cell separation kit. After expansion with MNC and CD3/CD28 for 21 days, we will determine the purity of γδ T cells by flow cytometry ($CD3^+$ and $CD4^-/CD8^-$). γδ T cells will be co-cultured with three $CD19^+$ B-ALL lines and a $CD19^-$ T-ALL line as a negative control with varying concentrations of blinatumomab. γδ T cell-mediated killing will be monitored by IncuCyte and flow cytometry (CD69 and CD107a). We will retro-orbitally transplant B-ALL cell lines expressing luciferase into NSG mice and tracked in peripheral blood samples and IVIS. After establishing disease progression, we will combine γδ T cells with blinatumomab to determine the γδ T cell-mediated clearance of B-ALL *in vivo.*

Results

We believe blinatumomab will increase the ability of γδ T cells to mediate B-ALL lysis. Furthermore, we expect the *in vivo* experiments to reveal the cell trafficking patterns of ALL and demonstrate blinatumomab can increase the GVL effect of γδ T cells by prolonging survival of mice.

Conclusion

Promising results would translate to clinical settings by offering a novel approach to avoid the otherwise lethal toxicity associated with irradiation in the treatment of B-ALL post-alloHSCT.

Abstract #76

Obesity reduces breast epithelial TGF-B activity potentially through enhanced ECM sequestration

Brenna N. Walton, Tamara Chamberlin, Lisa M. Arendt

Obesity is a rising epidemic, with rates tripling since 1970. Roughly 39% of adults are overweight and 13% are obese. Obesity is a risk factor for multiple diseases and disorders, including breast cancer. Because breast cancer is the second leading cause of death due to cancer in women, increased risk of breast cancer due to obesity is a major concern. Transforming growth factor beta (TGF-B) is a protein involved in modulating mammary epithelial cell proliferation and can be regulated by decorin protein. TGF-B has been shown to promote breast cancer, however its activity in the context of obesity is not well understood. Here, obese women with a BMI>30 kg/m^2 were observed to have decreased TGF-B activity in mammary epithelial cells compared to lean women (BMI<25 kg/m^2), with increased decorin in the extracellular matrix (ECM) surrounding breast lobules. Decorin and TGF-B were increased in isolated ECM of reduction mammoplasty tissue as compared to whole tissue extracts. When adipose-derived stromal cells were treated with TGF-B, we observed an increase in smooth muscle actin (SMA), a myofibroblast marker. Culturing these cells on the isolated ECM with vehicle enhanced SMA expression, while treatment with a TGF-B inhibitor reduced SMA expression. These results suggest that increased decorin within the ECM sequesters TGF-B, leading to reduced TGF-B activity within epithelial cells. Decreased TGF-B activity in epithelial cells may lead to increased epithelial cell proliferation. Determining the roles of TGF-B in the obese microenvironment will aid in understanding breast cancer risk in obese women.

Abstract #77

Breast density and obesity increase mammary gland inflammation

Abbey E. Williams[1], Erica Hoffmann[2], Julia Warren[3], Suzanne Ponik[2,3], and Lisa M. Arendt[1,2,4]

[1]Comparative Biomedical Sciences Graduate Program, University of Wisconsin- Madison

[2]Cellular and Molecular Pharmacology Graduate Program, University of Wisconsin-Madison

[3]Department of Cell and Regenerative Biology, University of Wisconsin-Madison

[4]Department of Comparative Biosciences, University of Wisconsin-Madison

Recent epidemiological studies suggest that the risk factors of breast density and obesity together increase breast cancer risk in premenopausal women, and these women have a worsened prognosis following breast cancer diagnosis. Although the underlying causes of increased risk associated with dense breasts and obesity have individually been explored, little is known about how these underlying risk factors interact together to enhance breast cancer risk. To model breast density, we used heterozygous Col1a1^{tmjae} mice, that have a mutation that limits collagen degradation, leading to increased mammary collagen deposition. Heterozygous (het) mice or wild type (wt) litter mates were fed either a control diet (CD) or a high fat diet (HFD) to induce obesity. We quantified F4/80-positive macrophages within mammary glands using immunohistochemistry. We observed significantly increased numbers of macrophages in mammary glands of CD/het, HFD/wt and HFD/het mice compared to CD/wt mice. We observed significantly enhanced picrosirius red stained collagen surrounding mammary ducts from CD/het, HFD/wt, and HFD/het mice compared to those from CD/wt mice. Notably, HFD/het mice had significantly more collagen surrounding ducts than in mammary glands from CD/het mice. These results may suggest that obesity enhances collagen deposition and macrophage-driven inflammation within dense breast tissue in the mammary glands of non-tumor-bearing mice.

Abstract #78

Tracking and Mapping Tumor-Targeted Alkylphosphocholine Metal Analogs with Mass Spectrometry Imaging

Meng Xu, Caroline Kerr, Zachary S. Morris, Lingjun Li

Introduction

Targeted radionuclide therapy (TRT) employs a radio-labelled vector to selectively deliver radiation doses to tumor cells without harming normal tissues. NM600 is a TRT vector. It is an alkylphosphocholine (APC) analog featuring a DOTA chelator labeled with different radioactive isotopes. To characterize the correlation among NM600 chelates, radiobiological markers, the type I IFN response, and tumor infiltrating immune cells, mass spectrometry imaging (MSI) is employed to map the distribution of NM600 and its ^{90}Y, ^{177}Lu and ^{225}Ac chelates, and to quantify these species *in situ*.

Method

MOC2 tumors were engrafted by subcutaneous flank injection of 1 x 10^6 tumor cells in female and male C57BL/6 mice. When the tumors reached an average volume of 1245 mm^3, 250, 25, 2.5, or 0 µM (control) unlabeled NM600 was administered intravenously (*n*=2]) and harvested 72 hours after. The cryo-sectioned tumors were coated with $3.2*10^{-3}$ mg/mm^2 alpha-Cyano-4-hydroxycinnamic acid (CHCA) matrix and analyzed with a MALDI TOF/TOF mass spectrometer with a step size of 100 µm. The standards of unlabeled NM600 were mixed with the same matrix and analyzed with MALDI MS to evaluate their ionization efficiency.

Preliminary Results

The unreacted NM600 standards were dissolved in methanol. They were detected from 100 ng/mL to 10 µg/mL at m/z 913.6 under either polarity using MALDI MS. However, when its concentration went above 10 µg/mL, a signal saturation was observed. The tumor sample treated with 250 µM NM600 and control were sectioned, and 20 µg/mL NM600 was spotted to one section from each sample before matrix coating. Although the spotted regions were clearly imaged, the distribution of NM600 was not resolved in the treated tissue sample. A receiver operating characteristic (ROC) curve was plotted between the spotted region in the treated sample and in control at m/z 913.6, and the resulting area under curve (AUC) value was 0.52, showing no significant change in the signal intensity. Therefore, the concentration of NM600 was below 20 µg/mL in the sample treated with 250 µM NM600.

Conclusion

We present a new approach to plot the distribution of the metal chelate of an APC analog with on-tissue MS imaging.