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Targeting Homologous Repair to Overcome Genotoxic Therapy Resistance in Pancreatic Cancer

Adam Wolfe¹, Oscar Zuniga¹, Kirk West¹ and Justin Leung¹¹University of Arkansas for Medical Sciences

OBJECTIVES/GOALS: Pancreatic ductal adenocarcinoma (PDAC) is a relatively radioresistant disease, and inhibition of DNA homologous recombination (HR) repair in combination with radiation therapy (RT) is a potentially attractive strategy to overcome radioresistance. We have found that the expression of the HR protein RAD18 is upregulated in PDAC cells. **METHODS/STUDY POPULATION:** Standard clonogenic assays, ³H2aX foci staining, HR-GFP reporter assay, and western blot analysis of DNA damage response proteins were performed in MIA-PaCa2 (MP2) and PANC-1 cells following knockdown of RAD18 in cells via short hairpin RNA (shRNA). Drug targeting of RAD18 was achieved through the use of a USP-7 inhibitor, P5091. Cells with or without stable knockdown of RAD18 were implanted orthotopically in the pancreas of athymic nude mice and treated with sham radiation or radiation to a dose of 20 Gy in 5 daily fractions once tumors reached 100-150 mm³. **RESULTS/ANTICIPATED RESULTS:** Stable knockdown of RAD18 in MP2 and PANC-1 resulted in decreased radiation clonogenic survival in vitro (dose enhancement factor (DEF)=1.52 and 1.51, respectively), decreased DNA repair after radiation as measured by the increased number of ³H2aX nuclear foci assay at 6, 12, and 24 hours (all p<0.05), decreased HR activation following DNA damage via an HR-GFP reporter assay (p=0.039), and increased tumor growth delay following radiation in vivo (p<0.001). P5091 treatment of both MP2 and PANC-1 resulted in efficient knockdown of RAD18, which was confirmed through western blotting, qRT-PCR, and luciferase reporter assays. P5091 increased radiosensitization, ³H2aX nuclear foci remained elevated at 12 and 24 hours (p<0.05), and HR repair was also reduced (p=0.014). **DISCUSSION/SIGNIFICANCE:** Herein, we show the HR repair protein RAD18, and that modulation of RAD18 expression correlates with in vitro and in vivo radiosensitization through altered HR-mediated DNA repair. USP7 inhibition successfully reduced RAD18 expression and resulted in enhanced radiosensitization.

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The Role of Mechanosensitive Ion Channels in Primary Open Angle Glaucoma

Wendy Liu¹, Ying Zhu¹, Tatjana Jakobs² and Janey Wiggs²¹Stanford University and ²Massachusetts Eye and Ear

OBJECTIVES/GOALS: Intraocular pressure is the most significant risk factor for glaucoma. Mechanosensitive proteins may have a critical role in transducing mechanical stimuli that ultimately lead to death of retinal ganglion cells. Our goal is to use genetic and functional approaches to discover mechanosensitive ion channels that mediate the progression of glaucoma. **METHODS/STUDY POPULATION:** Association data, obtained using a logistic regression model that included age, gender and population substructure as co-variables, for 2,576 SNPs located in the PIEZO1 and PIEZO2 genomic regions were extracted from the NEIGHBORHOOD genome-wide association study results for primary open angle

glaucoma (POAG) (3,853 cases and 33,480 controls) and the subset of cases with intraocular pressure (IOP) measurements > 21mmHg (high-tension, HTG) (1868 cases and 33,480 controls). Rare coding PIEZO1 and PIEZO2 variants were evaluated using logistic regression and SNP data from the Human Exome array in 2606 POAG cases and 2606 controls and the subset of 1868 HTG cases and 2606 controls. Immunohistochemistry was used to characterize the expression of Piezo1 and Piezo2 in mouse eye sections. **RESULTS/ANTICIPATED RESULTS:** Exome data analysis identified two protein-altering variants associated with lower glaucoma risk (P<0.05): a PIEZO1 missense allele (Arg1527His; OR= 0.18, P= 0.001) and a variant disrupting a splice donor site (c.1107+1G>C; OR=0.38, P= 0.02), that prematurely truncates the protein. Investigation of the NEIGHBORHOOD GWAS dataset identified nominal association with common PIEZO2 variants (minor allele frequency > 0.3) in POAG overall (top SNP rs264179, P= 0.008) and in the HTG subgroup (top SNP rs264160, P= 0.001). The associated PIEZO2 SNPs are significantly associated with gene expression in lymphocytes (P< 1x10⁻⁸) with the risk allele correlated with decreased gene expression. Piezo1 and Piezo2 are expressed in many ocular tissues in the mouse, including cornea, ciliary body and retina. **DISCUSSION/SIGNIFICANCE:** We identify rare, protein-altering PIEZO1 variants associated with lower glaucoma risk and show that Piezo1 and Piezo2 are broadly expressed in the eye. Common variants influencing PIEZO2 expression also show nominal association with POAG risk. Inhibition of Piezo1 or augmentation of Piezo2 could be novel therapeutic strategies for glaucoma.

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Omega-3 and omega-6 fatty acids attenuate platelet reactivity in postmenopausal women

Adriana Yamaguchi¹, Livia Stanger², Amanda Prieur², Rachel Tav³, Cody Freedman⁴, Theodore R. Holman⁴ and Michael Holinostat^{2,5}¹University of Michigan Medical School, ²Department of Pharmacology, University of Michigan Medical School, Ann Arbor, MI, ³Cranbrook Academy, West Bloomfield, MI, ⁴Department of Chemistry and Biochemistry, University of California Santa Cruz, Santa Cruz, CA and ⁵Department of Internal Medicine, Division of Cardiovascular Medicine, University of Michigan Medical School, Ann Arbor, MI

OBJECTIVES/GOALS: This study aimed to investigate the mechanistic effects of fish oil (ω -3 fatty acids) or evening primrose oil (ω -6 fatty acids) supplementation on platelet reactivity in postmenopausal women. **METHODS/STUDY POPULATION:** Postmenopausal women were recruited from the Ann Arbor community and the University of Michigan Medicine Center. All subjects were recruited under study protocols approved by the University of Michigan IRB between November 2015 and March 2017. We conducted a randomized, double-blind, two-period crossover trial, consisting of a 60-day supplementation period followed by a 14-day washout period in between and at the end of the study. Subjects were treated daily in random order with 2g of fish oil supplement and 2g of evening primrose oil. Blood was drawn at baseline, post-supplementation, and after washout. The effects of fatty acid supplementation on platelet aggregation, dense granule secretion and activation of basal integrin α IIb β 3 were assessed following supplementation and washout period. **RESULTS/ANTICIPATED RESULTS:** The study started with 90

postmenopausal women. A total of 78 subjects completed the study, with 12 subjects dropping out due to non-compliance and medical reasons. Supplementation with fish oil attenuated the thrombin receptor PAR4-induced platelet aggregation, whereas primrose oil supplementation attenuated aggregation mediated by PAR4 or collagen. Supplementation with ω -3 or ω -6 fatty acids decreased platelet dense granule secretion and attenuated basal levels of integrin α IIb β 3 activation. Post-washout following supplementation with primrose oil, the thrombin receptor PAR1-induced platelet aggregation was similarly attenuated. For either treatment, the observed effects post supplementation on dense granule secretion and basal integrin activation were sustained after the washout. **DISCUSSION/SIGNIFICANCE:** Postmenopausal women are at increased risk for a cardiovascular event due to platelet hyperactivity. This study indicates that supplementation with ω -3 and ω -6 fatty acids may offer significant protection for postmenopausal women against cardiovascular diseases and occlusive thrombotic events by reducing platelet reactivity.

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Identification of *Trichomonas vaginalis* 5-nitroimidazole resistance targets to inform future drug development

Keonte Graves¹, Jyoti Sharma³, Colin Reily^{2,3}, Hemant Tiwari⁴, Vinodh Srinivasasainagendra⁴, W. Evan Secor⁵, Jan Novak³ and Christina A. Muzny²
¹CCTS, ²Department of Medicine, The University of Alabama at Birmingham, ³Department of Microbiology, The University of Alabama at Birmingham, ⁴Department of Biostatistics, The University of Alabama at Birmingham and ⁵Division of Parasitic Diseases and Malaria, Center for Global Health, Centers for Disease Control and Prevention

OBJECTIVES/GOALS: 5-nitroimidazoles are the only FDA-approved medications for *T. vaginalis* treatment. Resistance has been observed in 5-10% of cases, but may be rising. We aimed to delineate mechanisms of resistance in isolates of *T. vaginalis* using transcriptome profiling of resistant and sensitive *T. vaginalis* isolates. **METHODS/STUDY POPULATION:** *T. vaginalis* isolates (4 metronidazole (MTZ)-resistant were grown in triplicate in Diamond's Trypticase-Yeast-Maltose medium. MTZ susceptibility testing confirmed MTZ MLCs of *T. vaginalis* isolates. Total RNA extraction was done using Trizol reagent (Invitrogen; Carlsbad, CA); according to the manufacturer's instructions. RNA sequencing (RNAseq) and bioinformatics analyses were performed to identify significantly differentially expressed genes (DEGs) in MTZ-resistant vs. sensitive isolates. Subsequent qPCR was performed to confirm and extend RNAseq data and gene targets related to 5-nitroimidazole resistance. **RESULTS/ANTICIPATED RESULTS:** RNAseq identified key DEGs in MTZ-resistant vs. sensitive isolates. DEGs from MTZ-resistant isolates included those involved in metabolic pathways relevant to 5-nitroimidazole resistance such as energy production (glycolytic enzymes) and oxygen-scavenging (thioredoxin). Other DEGs included those encoding transcription factors (MYB DNA-binding protein), ribosomal proteins (30S, 40S, 50S, 60S), protein kinases (CAMK, ser/thr, CMGC), Ankyrin repeat proteins, surface proteins (Surface antigen BspA-like) and various uncharacterized hypothetical proteins. RT-qPCR experiments confirmed reduced expression of genes encoding ferredoxin (drug activation) and flavin reductase 1 (oxygen scavenging) in MTZ-resistant *T. vaginalis* isolates as compared to MTZ-sensitive isolates. **DISCUSSION/SIGNIFICANCE:** In this study, we identified several DEGs in resistant *T. vaginalis* isolates. Further studies with large number of isolates representing a

broad range of MTZ-susceptibility patterns are needed to identify genes that may represent new targets for future drug development.

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A CTS Team Approach to Modeling Migration and Suppression of CCR2+/CX3CR1+ Myeloid Cells in Glioblastoma

Hannah Anderson¹, Gregory P. Takacs², Christian Kreiger², Defang Luo², Libin Rong³, Jeffrey K. Harrison² and Tracy Stepien³
¹University of Florida, ²Department of Pharmacology and Therapeutics, University of Florida and ³Department of Mathematics, University of Florida

OBJECTIVES/GOALS: Evaluate the migration and immune suppressive functions of CCR2+/CX3CR1+ myeloid-derived suppressor cells (MDSCs). Integrate experimental data and biologically relevant mathematical models of infiltrating MDSCs in the context of glioblastoma (GBM). **METHODS/STUDY POPULATION:** CCR2+/CX3CR1+ cells were enriched from bone marrow obtained from CCR2(+RFP)/CX3CR1(+GFP) glioma-bearing mice to evaluate their immune-suppressive phenotype and ability to migrate to CCL2 and CCL7. Fluorescent imaging and quantification were performed on a range of tumor sizes to acquire vasculature, tumor, T cell, and MDSC densities. A system of ordinary differential equations was constructed to represent the temporal dynamics of glioma cells, T cells, and MDSCs within the tumor microenvironment. The Approximate Bayesian Computation method was used to determine probability distributions of important parameters, such as the suppression rate of T cells by MDSCs. **RESULTS/ANTICIPATED RESULTS:** CCR2+/CX3CR1+ M-MDSCs isolated from the bone marrow of tumor-bearing mice suppress CD8+ T cell proliferation and IFN γ production. CCR2+/CX3CR1+ cells migrate to recombinant and KR158B glioma sourced CCL2 and CCL7. Parameter values determined by the Approximate Bayesian Computation method agreed with parameter values from experimental data. This result further validated the structure and results of the mathematical model when performing computer simulations; thus, we can predict CCR2+/CX3CR1+ M-MDSC infiltration over time. **DISCUSSION/SIGNIFICANCE:** The immune-suppressive microenvironment in GBM contributes to poor outcomes despite standard of care. This study integrates biological and mathematical models to better understand infiltrating immune-suppressive cells, namely CCR2+/CX3CR1+ M-MDSCs. Future directions include modeling immunotherapies.

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Antibody function, antigenic target and glycans determine the transfer of herpes simplex virus (HSV) antibodies (Abs) from mothers to newborns and transfer is altered by SARS-CoV-2*

Aakash Mahant Mahant¹, Fatima A. Estrada Trejo¹, Jennifer T. Aguilan¹, Simone Sidoli¹ and Betsy C. Herold¹
¹MSC

OBJECTIVES/GOALS: Murine and clinical data suggest that antibody-dependent cellular cytotoxicity (ADCC) is associated with greater protection against disseminated neonatal HSV disease. To quantify the relative transfer of Abs with different functions and targets, we conducted a prospective study of mother-infant term and