>0.25 and P-value <.05 to be included in the network graph, resulting in 323 connections and 3 identified clusters. Max weight loss and baseline BMI were in a cluster enriched by unsaturated fatty acid biosynthesis (P<.0001) and arachidonic acid (P=.01) metabolic pathways but not linked to inflammation cytokines. The five other cachexia symptoms were in a cluster with 4 cytokines (C-reactive protein, interleukin 6, IL10, IL1, Tumor necrosis factor receptor 2) and enriched by aminoacyl tRNA (P<.01) and valine biosynthesis (P=.02). We observed no meaningful differences when we stratified the analysis by human papillomavirus. DISCUSSION/SIGNIFICANCE: Cachexia symptoms in head and neck cancer may be linked to specific metabolic dysregulation—weight loss and BMI were linked to fatty acids; fatigue, anemia and others were linked to amino acids and inflammation. This information may allow for the recognition of a cachexic-metabolic subtype or provide novel targets for metabolic intervention.

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Immunotherapy Sensitization via Tumor Acidosis Mitigation by Esomeprazole Monitored with MRI

Grace Abigail Murley[1], Shivanand Pudakalakatti[2], William Padron[2], Muxin Wang[2], Ryan Armijo[2], Jorge Delacerda[2], Abishai Dominic[2], Renee Chin[2,3,3], William Schuler[2], Kunal Rai[2,3], Marty Pagel[2,3] and Pratip Bhattacharya[2,3]
[1]UTHealth GSBS/MD Anderson Cancer Center; [3]the University of Texas Health Science Center at Houston, and [2]MD Anderson Cancer Center

OBJECTIVES/GOALS: Acidity and the lactate-to-pyruvate ratio correlate with immunotherapy resistance. AcidoCEST MRI and hyperpolarized magnetic resonance spectroscopy (HP-MRS) measure extracellular pH and lactate-to-pyruvate ratio. We will establish a baseline for these biomarkers then observe changes after combination esomeprazole and immunotherapy. METHODS/STUDY POPULATION: We used multiple melanoma models created via serial in vivo passage under immunotherapeutic pressure (FVAX, CTLA-4, PD-1, PD-L1). We used four of these corresponding to 25%, 50%, 75% and 100% resistance (TMT, F2, F3, and F4, respectively). HP-MRS was performed two weeks post implantation in male BL6 mice with AcidoCEST MRI 2-3 days later. Tumors were implanted in additional mice and grown for 1 week. We used esomeprazole as a possible immunotherapy sensitizer. Esomeprazole (or PBS) alone and in combination with immune checkpoint blockade (ICB; αCTLA-4, αPD-1) was then conducted every 3 days for 3 doses. ICB was administered 3h after esomeprazole. AcidoCEST MRI was performed the day after the final dose of combination therapy and 3h after esomeprazole (or PBS) alone. HP-MRS was performed 2-3 days after acidoCEST MRI. RESULTS/ANTICIPATED RESULTS: There was a statistical increase in the lactate-to-pyruvate ratio of the F4 group compared with TMT, F2, and F3 groups ($p < 0.05$). The TMT, F2, and F3 groups did not differ significantly. The extracellular pH (pHe) of the TMT group was statistically lower than the F2 and F4 groups ($p < 0.05$). The pHe did not differ significantly between the TMT and F3 groups nor the F2, F3, and F4 groups. The lactate-to-pyruvate ratio and pHe after combination treatment with esomeprazole and ICB did not differ compared to PBS+ICB control. Treatment with esomeprazole alone generated higher lactate-to-pyruvate ratio compared with PBS alone. Tumor volume curves and survival curves of mice bearing F4 tumors treated with esomeprazole combination with ICB showed no difference compared with PBS+ICB, PBS alone, and esomeprazole alone. DISCUSSION/SIGNIFICANCE: We differentiated between the 100% and 25% resistant models with both pHe and lactate-to-pyruvate ratio, although the pHe was counterintuitive. Esomeprazole was ineffective, but other potential sensitizers exist. A non-invasive clinical imaging tool and sensitizer would permit more personalized treatment plans so treatment is more effective.

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Extracellular-in-frame deletions and kinase domain duplications are novel, gain-of-function mutations in fibroblast growth factor receptor genes in cancer

Leah Stein[1], Julie W. Reeser[1], Michele R. Wing[1], Chunjie Li[1], Karthikeyan Murugesan[2], Dean Pavlick[2], Emily L. Hoskins[1], Raven Vella[1], Georgia Rigsbee[1] and Sameek Roychowdhury[1]
[1]The Ohio State University and [2]Foundation Medicine

OBJECTIVES/GOALS: There are gain-of-function genomic alterations in FGFR genes that guide personalized treatment in some patients with cholangiocarcinoma (10%) and bladder cancer (30%) who can benefit from targeted therapies. We sought to evaluate other genomic alterations in cancer involving FGFRs and assess whether they are gain-of-function. METHODS/STUDY POPULATION: We collaborated with Foundation Medicine Inc (FMI), for the assessment of 300,000 sequenced tumors and a retrospective analysis of recent publications, to identify novel candidate FGFR alterations. We propose to transiently transfect HEK293T cells with an empty vector (EV), FGFR1-4 wild-type (WT), and these variants and use a luminescent-proximity based high-throughput assay, AlphaLISA, and Western blot to assess FGFR and phosphorylated downstream signaling proteins, FRS2, AKT and ERK, and their sensitivity to FGFR inhibitors: pemigatinib, erdafitinib, futibatinib, RLY-4008, and TYRA-200. RESULTS/ANTICIPATED RESULTS: Through our collaboration we identified >100 novel candidate FGFR1-4 variants of unknown significance (VUS) including extracellular-in-frame deletions (EIDs), kinase domain duplications (KDDs), insertions/deletions (INDELs), short number variants (SNVs), and truncations. Immunoblot analysis confirmed the presence of desired EV, FGFR WT, and VUS' in HEK293T cells. We anticipate the FGFR EIDs and KDDs to display an increased presence of in the respective pFGFR, pFRS2, pERK, and pAKT as compared to the EV and FGFR WT by both immunoblot and AlpahLISA analysis. Additionally, we anticipate the VUS' to be sensitive to FGFR inhibitors: pemigatinib, erdafitinib, futibatinib, RLY-4008, and TYRA-200 using the AlphaLISA assay. DISCUSSION/SIGNIFICANCE: These findings suggest that the novel FGFR VUS' are capable of constitutive activation of FGFR kinase activity, and they preliminary demonstrate that these newly identified FGFR alterations are therapeutically targetable. Thus, providing rationale for further clinical evaluation to identify new cohorts of FGFR inhibitor responders.

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Muscle Protein Synthesis and Whole-Body Protein Balance Following Ingestion of Beef or a Soy Protein Based Meat Alternative

David D. Church[1], Katie R. Hirsch[3], Shiloah A. Kviatkovsky[2], Arny A. Ferrando[2], Gohar Azhar[2] and Robert R Wolfe[2]
[1]University of Arkansas for Medical Sciences; [2]Department of Geriatrics, Donald W. Reynolds Institute on Aging, Center for Translational Research in Aging & Longevity, University of Arkansas for Medical Sciences, Little Rock, AR [72205], USA and [3]University of

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South Carolina, Department of Exercise Science, Arnold School of Public Health,Carolina, Columbia, USA

OBJECTIVES/GOALS: We endeavor to investigated the hypothesis that muscle protein synthesis (MPS) is stimulated more after consumption of a 4-ounce beef patty as compared to 4- and 8-ounces of a soy protein based meat alternative (SPBMA) and if a greater stimulation is related to differences in the responses of plasma essential amino acid (EAA) concentrations. METHODS/STUDY POPULATION: Participants were aged 18 to 40 years of age with a BMI between 20 and 32 kg/m2. Written informed consent was obtained from all participants, and approved by UAMS IRB. Participants were assigned to one of three intervention groups via a single-blinded permuted block randomization, stratified for sex: 4 oz beef patty; 4 oz SPBMA; 2 x 4 oz (8oz) SPBMA. The impossible burgerTM was selected as it is primarily soy protein, a high-quality plant protein, and specifically designed to mimic a beef burger. Stable isotope were infused to assess protein metabolism. Appropriate muscle and blood samples were obtained. Enrichment and plasma EAA concentrations were measured with mass spectrometry. ANOVA's on the change from basal to postprandial were used to identify group difference, significance was accepted at $p < 0.05$. RESULTS/ANTICIPATED RESULTS: The MPS increase from basal to postprandial indicated a significant main effect of group ($p = 0.026$), with the beef group (0.020 ± 0.016%/hour) being significantly greater than the 4oz SPBMA (0.003 ± 0.010%/hour; $p = 0.021$) but not the 8oz PBMA group (0.013 ± 0.016%/hour; $p = 0.454$). Similar results were observed for whole-body protein synthesis, where the beef group ($p = 0.042$) and 8oz SPBMA ($p = 0.033$) were significantly greater than the 4oz SPBMA ($p = 0.021$). Whole-body protein balance was significantly greater in the 8oz SPBMA as compared to 4oz of beef and SPBMA. Lastly, we observed a significantly relationship ($p = 0.046$; $r = 0.411$) between the maximal plasma EAA concentration and change in MPS, indicating the greater rate of MPS following 4oz of beef is mediated by an higher increase in plasma EAA concentrations. DISCUSSION/SIGNIFICANCE: In conclusion, 4oz of beef stimulates muscle protein FSR more than 4oz of a SPBMA. A common SPBMA can stimulate increase in protein metabolism, however, greater amounts are required as compared to beef protein. Further, the change in the muscle protein FSR response was significantly correlated with the maximal EAA concentration.

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Transcriptomic analysis of Influenza A infected lung organoids reveals Warburg-like phenotype*

Emily Hartsell[1], Justin T. Roberts[2], Adeyeye I. Haastrup[2] and Raymond J. Langley[2]

[1]The University of South Alabama and [2]The University of South Alabama Department of Pharmacology

OBJECTIVES/GOALS: The CDC estimates that Influenza infections account for an average of 420,000 hospitalizations and 34,700 deaths in the U.S. each year. This project explores the underlying mechanisms of the infectious process of Influenza A in human lung organoids by examining the differential transcriptomic expression compared to uninfected controls. METHODS/STUDY POPULATION: Lung organoids were cultured from differentiated human bronchial epithelial cells from lung transplant donors on an air-liquid interface until they were confirmed to contain both mucous producing and ciliated cells. Lung organoids are ideal models in translational science due to their structural and functional characteristics which closely mimic those of in vivo human epithelial tissue. Half the organoids were exposed to Influenza A pH1N1 for 72h; the other half served as uninfected controls. RNA was isolated from both groups and sequenced using the Oxford Nanopore MinION which generates full length reads. Reads were aligned to the human reference genome (GRCh38.p14) using Minimap2. RESULTS/ANTICIPATED RESULTS: The MinION sequenced an average of 3.24m reads per sample and a total of 13,128 genes were relevantly expressed (defined as greater than 1 read per million in at least half the samples). ANOVA with a 5% false discovery rate (Benjamini and Hochberg correction) revealed 5,417 differentially expressed genes between infected and control groups. Within this subset, we identified downregulation of mucociliary clearance, mitochondrial and ÃŸ-oxidation, peroxisome, and glutathione replenishment genes. We further identified upregulation in inflammatory markers, lactate dehydrogenase enzymes, and several s100 proteins. The downregulation of mitochondrial and β-oxidation markers and the upregulation of lactate dehydrogenase enzymes revealed a Warburg-like phenotype which has not previously been reported. DISCUSSION/SIGNIFICANCE: This study reveals a novel Warburg-like phenotype in Influenza A infection alongside downregulated mucociliary clearance and upregulated inflammatory processes. These findings improve our understanding of Influenza A infection and point to potential therapeutic targets to advance precision medicine approaches to treatment.

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Treatment experience and symptom burden in multiple myeloma: interim results of a longitudinal electronic patient-reported outcomes study

Mihir Patel[1], Anneli Nina, Brenda Branchaud, David Campbell, Kris Herring and Thomas LeBlanc

[1]Duke University School of Medicine and [2]Duke Cancer Institute

OBJECTIVES/GOALS: Patients with multiple myeloma (MM) experience significant disease- and treatment-related symptom burden, especially with higher lines of therapy (LOT). We used a remote symptom monitoring app to characterize overall symptom profile, symptom bother, and quality of life (QOL) among patients with MM across LOT and longitudinally. METHODS/STUDY POPULATION: We used Carevive PROmpt, a symptom monitoring app for cancer patients. From 11/10/22 to 9/27/23, we enrolled 84 adult patients with MM of any stage and anywhere in the treatment continuum from Duke Health MM clinics. Participants received weekly symptom surveys while on active treatment. Per prior studies, we defined heavily pretreated patients as those on current LOT ≥4. Our sample had a mean (SD) age of 63.7 (10.8) years and was 56.0% male; 73.8% had a prior bone marrow transplant, 40.5% were on LOT ≥4 (53.6% on LOT <4, 6.0% missing), 58.3% were on triplet therapy or higher. For 14 symptoms, we described the prevalence of moderate to very severe (MOD-VS) symptoms based on LOT overall and over time. We also described responses to "How bothersome are treatment side effects?" and "Overall QOL over the past week" based on LOT. RESULTS/ANTICIPATED RESULTS: Surveys continued for a mean (SD) of 14.9 (9.6) weeks (range: 44). The top 5 MOD-VS symptoms ever experienced were fatigue

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