>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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