findings may provide translational opportunities for novel fibroid treatments.

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Defective uromodulin polymerization and peptide excretion in a natural canine model of kidney stones

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OBJECTIVES/GOALS: Using a natural canine model of kidney stone disease, we previously identified a pathogenic variant in the uromodulin gene (UMOD) that imparts a dramatic risk for calcium oxalate (CaOx) stones. This study was designed to characterize the effects of the pathogenic variant on uromodulin processing, specifically polymerization and peptide excretion. METHODS/STUDY POPULATION: Uromodulin polymerization status and peptides were measured in random urine samples from CaOx stone-forming dogs with the pathogenic UMOD variant and breed-, sex-, and age-matched healthy control dogs. Polymerization status was determined using an ultracentrifugation protocol and Western blotting in 6 CaOx cases and 3 controls; relative abundance of the polymerizing and nonpolymerizing forms was evaluated. Uromodulin peptide abundances were measured by LC-MS/MS with 4 dogs per group; results were summed to determine total uromodulin peptide excretion for each dog, and individual peptide abundances were calculated as a percentage of the total. Polymerization status and peptides were compared between groups. RESULTS/ANTICIPATED RESULTS: Dogs with the pathogenic UMOD variant had abnormalities in both uromodulin polymerization and peptide processing. The polymerization data showed that the polymerizing form of uromodulin was abundant in all healthy controls but absent or severely reduced in most dogs with the variant. In contrast, nonpolymerizing uromodulin was detected in all dogs with no observed difference between those with and without the variant. The peptidomics data showed that stone-forming dogs with the pathogenic UMOD variant lacked a peptide cleavage site, resulting in the loss of two common peptides that terminate at that site and the presence of longer peptides that span the site. DISCUSSION/SIGNIFICANCE: These findings implicate uromodulin polymerization and peptide processing defects in kidney stone risk. Future studies will define the mechanisms through which these defects affect stone formation, ultimately informing development of novel preventative therapies.

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Ligament Engagement and In-Situ Force During Multiplanar Loading of the Medial Knee Ligaments

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OBJECTIVES/GOALS: Load sharing across the arc of knee flexion of the medial knee ligaments (MKLs) is not well understood. The goal of this research is to characterize ligament engagement and in-situ force within the deep and superficial medial collateral ligament (dMCL, sMCL) and the posterior oblique ligament (POL) in response to externally applied multiplanar loads. METHODS/STUDY POPULATION: Ten human cadaveric knees, 5 male and 5 female, age 32±7 (25-42) [mean±SD (range min-max)] years, were mounted to a force sensor and a 6-degree-of-freedom robotic arm. Knee kinematics, before and after serial dissection of the sMCL, dMCL, and POL, were recorded from 0-30 degrees during applied isolated external rotation, valgus angulation, and anterior tibial moments, and the force (Newtons, N) borne by each structure was measured via the principle of superposition. Loads in the dMCL, sMCL, and POL will be compared across each knee and at each flexion angle with paired t-tests and repeated-measures analysis of variance with Tukey post hoc testing. Ten knees will provide >99% power to detect differences of 5N ± 3% at p=0.05, which is considered the threshold for clinically meaningful force differences. RESULTS/ANTICIPATED RESULTS: Our anticipated results include characterization of the means and standard deviations of the in-situ forces within the dMCL, sMCL, and POL in response to externally applied valgus angulation, tibial external rotation, and anterior-directed tibial loading at 0, 15, and 30 degrees of knee flexion. Our statistical analysis will determine if there are clinically meaningful differences (5N ± 3%) in the loads within each ligament at different knee flexion angles and will also provide data regarding differential relative ligament engagement for each applied force scenario, which is an indication of the percentage of contribution that each structure contributes to knee stability during application of forces and torques to the knee. DISCUSSION/SIGNIFICANCE: Data on ligament engagement and in-situ forces will help clinicians better diagnose potentially injured ligaments when they observe pathological knee laxity in an injured patient. Our results will also inform future computer modeling studies on injury mechanisms, individual anatomical variability, and surgical planning.

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Investigating the metabolic-inflammatory mechanisms of cachexia symptoms in head and neck cancer patient plasma via multiomics integration of the metabolome, lipidome, and inflammation cytokines

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OBJECTIVES/GOALS: Cachexia is the involuntary and irreversible loss of muscle and fat and is a major cause of morbidity and mortality in head and neck cancer (HNC). It remains a poorly understood disease diagnosed by weight loss and a confluence of symptoms. We explored the metabolic and inflammatory mechanisms of cachexia symptoms via an multiomics network algorithm. METHODS/STUDY POPULATION: Prior to chemoradiotherapy, HNC subjects completed questionnaires and donated blood for untargeted (metabolites) and targeted (lipids and cytokines) assays. Metabolites and lipids were measured by liquid chromatography mass spectrometry. Cytokines were measured by multiplex assays. We plotted a multiomics network graph by estimating partial least squares correlations amongst metabolites, lipids, cytokines, and common cachexia symptoms—max percent weight loss over 1 year, baseline BMI, fatigue, performance, albumin, hemoglobin, and white blood cell count. To interpret the network, an algorithm identified highly correlated clusters of metabolites-lipids-cytokines-symptoms representing possible biological relatedness, which were functionally annotated via metabolic enrichment analysis. RESULTS/ANTICIPATED RESULTS: In 123 subjects (59 years of age, 72% male, 84% white, avg weight loss of 13%), we analyzed 186 metabolites, 54 lipids, 7 cytokines and 7 cachexia symptoms. We required a correlation

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>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

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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

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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

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