Tarloxotinib (TRLX), is a prodrug that releases an irreversible EGFR/HER2 tyrosine kinase inhibitor (TRLX-TKI) under pathophysiologically hypoxic conditions. Non-small cell lung cancer (NSCLC) has been characterized as a hypoxic disease and approximately 15% of lung adenocarcinomas harbor EGFR mutations. While most EGFR mutations predict for response to several FDA-approved tyrosine kinase inhibitors, in-frame insertions in exon 20 of EGFR are activating mutations in the tyrosine kinase domain that have significantly decreased sensitivity to EGFR inhibitors and currently have no approved targeted therapies. We derived and characterized three human lung adenocarcinoma cell lines with different EGFR exon20 insertions in order to accelerate development of targeted therapies for this mutation class. Using these novel cell lines, we evaluated tarloxotinib as a therapeutic agent for tumors harboring this type of mutations. We demonstrate that our three patient derived cell lines: CUTO14 (p.A767_V769dupASV), CUTO17 (p.N771_H773dupNPH) and, CUTO18 (p.S768_770dupSVD) are dependent on EGFR for cell proliferation using shRNA mediated knockdown. Our results show that EGFR exon 20 insertion cell lines are resistant to gefitinib; however, treatment with afatinib or TRLX-TKI reduces cell proliferation and signaling in a similar manner. The IC50 values for the three cell lines were 203nM, 89nM and 709 nM for afatinib and 208nM, 33nM and 345nM for TRLX-TKI, respectively. The prodrug form of tarloxotinib has minimal effect on cell proliferation in these models, consistent with the necessity for hypoxia-induced activation (to TRLX-TKI). Importantly, we evaluated the effect of tarloxotinib in vivo using murine xenograft models of CUTO14 and CUTO17. After four weeks of treatment, afatinib did not alter tumor growth compared to untreated tumors, whereas treatment with tarloxotinib induced significant tumor regression. The in vivo data suggest that the activated TKI of tarloxotinib is accumulating to biologically active concentrations in tumors following cleavage of tarloxotinib under hypoxic conditions. We conclude that our EGFR exon 20 insertions cell lines represent novel models for the investigation of therapeutic strategies for this mutation class. These cell lines have the ability to develop tumors in vivo and show reduced sensitivity to current EGFR TKIs mimicking the lack of response in patients with these mutations. Finally, we demonstrate that tarloxotinib can overcome intrinsic EGFR exon 20 mutation resistance to standard EGFR TKIs.
Vascular dysfunction heralds the onset of diabetic cardiovascular disease. We have reported that vascular mitochondrial dysfunction contributes to impaired vasoreactivity in diabetes. The botanical compound (-)-epicatechin (EPICAT) is a known vasodilator. We hypothesized that EPICAT restores vasodilation by bolstering mitochondrial function in the context of high glucose via support of endothelial nitric oxide synthase (eNOS) activity and mitigation of reactive oxygen species (ROS). To test this hypothesis, we examined the impact of EPICAT on vasoreactivity ex situ and endothelial cell function in vitro. EPICAT significantly increased endothelium dependent acetylcholine-stimulated vasodilation (64.7±4%, p=0.002). HUVEC cells were incubated with 7 mM (normal glucose, NG) or 30 mM glucose (HG) with or without 0.1 µM EPICAT for 30 min, 2, 4-6 hrs, and 24 hrs. Mitochondrial superoxide was detected via electron spin resonance spectroscopy, and respiration and hydrogen peroxide were measured using Oroboros. Mitochondrial superoxide production was elevated in HG cells (2 hrs=1.63 ± 0.17 µM/mg (HG) vs. 1.18 ± 0.07 µM/mg (NG), p=0.017 and 4-6 hrs=1.46 ± 0.16 µM/mg (HG) vs. 1.18 ± 0.07 µM/mg (NG), p=0.09). Mitochondrial respiration was greater in cells exposed to HG for 2 hrs in state 3 (23.8%) and uncoupled (14.5%), and hydrogen peroxide was higher in HG treated cells, while both metrics were unchanged after 6 hours, and respiration was decreased after 24 hours. Cells pretreated with EPICAT had elevated rates of respiration (state 3=73.4% and uncoupled=55.2%) without a commensurate increase in superoxide, and EPICAT dampened HG superoxide generation after 2 hours by 17.0%. Cells pretreated with EPICAT and exposed to HG had significantly elevated eNOS protein expression (p=0.001). Our data suggest that EPICAT potentiates vasodilation by acutely activating eNOS, leading to an increase in mitochondrial respiration while mitigating excess ROS production.
Background: To help address the opioid epidemic in the United States we sought to determine if analgesic prescribing patterns of physicians in the United States differed from those in other countries and, if such differences existed, whether they were reflected by differences in patients' perception of pain control.

Methods: We performed a cross-sectional study on medical inpatient units in four hospitals in the United States and seven in other countries. Outcomes included the proportion of patients for whom opioid and non-opioid analgesics were prescribed and patients' assessment of their pain levels and their satisfaction with, and beliefs about, pain control.

Results: We surveyed 981 patients, 503 in the United States and 478 in other countries. 697 (71%) patients surveyed did not report taking a prescription opioid prior to coming to the hospital (opioid naïve) and 278 (28%) patients surveyed did report taking a prescription opioid prior to coming to the hospital (opioid tolerant). Patients in the United States in both the opioid naïve and opioid tolerant groups reported greater levels of pain than those in other countries and were more likely to have opioids prescribed before (38% in the U.S. vs 18% in all other countries) and in the opioid tolerant patients, less likely to have non-opioids prescribed (76% versus 90%). After adjusting for variables associated with opioid use, a larger proportion of patients, both opioid naïve and opioid tolerant, in the U.S. than in other countries were prescribed opioids during their hospitalization and at the time of discharge (63% vs 43% or 94% vs 77% and 34% vs 15% or 83% vs 62%, respectively, p <0.05). No difference was observed with respect to patients' satisfaction with pain control but beliefs and expectations about pain control differed.

Conclusions: Physicians in the United States prescribe opioids more frequently, and non-opioids less frequently than physicians in other countries but no differences in patients' satisfaction with pain control was observed despite patients having differing beliefs about and perceptions of pain. Efforts to reduce the opioid epidemic should include addressing physicians' inpatient analgesic prescribing practices as well as patients' expectations regarding pain control.
The HLA-DRB1 locus represents the greatest genetic risk factor for susceptibility for rheumatoid arthritis (RA), a chronic autoimmune disease that leads to extensive joint destruction. We have refined this genetic association to a single amino acid polymorphism at position 71 that can regulate susceptibility or resistance, depending on the presence of a basic or acidic residue. A basic residue (K/R) promotes preferential binding of citrullinated arthritogenic peptides. Peptide binding studies demonstrated that mutating DRβ1*04:01 at position 71 from lysine to glutamic acid (found in RA-resistant alleles e.g. DRB1*01:03 and *04:02) completely abrogates the preferential binding of citrullinated vimentin66-78 and citrullinated α-enolase11-25, as compared to their native forms, and eliminates binding of collagen258-272. As such, this single amino acid substitution renders the arthritogenic peptide-binding profile of DRβ1*04:01 nearly identical to that of the resistant allele, DRβ1*04:02. The strong correlation between peptide binding and genetic susceptibility to RA suggests that eliminating the binding preference for citrullinated peptides by HLA gene editing will attenuate the disease.

Although a DRB1-mismatched hematopoietic stem cell transplant could be used to treat RA, the risk of graft-versus-host disease is too risky to warrant such a therapy. However, an autologous hematopoietic stem cell transplant in which a single nucleotide of DRB1*04:01 has been edited at position 71 (K71 to E71) could potentially treat the disease by abrogating the ability of antigen-presenting cells to bind arthritogenic peptides. We therefore hypothesize that an autologous hematopoietic stem cell transplant, in which the patient’s DRB1*04:01 is edited using the CRISPR/Cas9 system to DRB1*04:01K71E, could render the host resistant to RA with very little risk of graft-versus-host disease.

The CRISPR/Cas9 system consists of a single-guide RNA, which contains a scaffold sequence necessary for Cas9-binding and a user-defined “targeting” guide sequence, and the CRISPR-associated endonuclease, Cas9. Cas9 introduces double-stranded breaks at the target site which the cell repairs by non-homologous end joining, which often creates insertion or deletion mutations (indels), or by homologous directed repair (HDR), which introduces a specific desired sequence change. We have identified two guide sequences (208/fwd and 185/rev) that target DRB1*04:01 and their specificity was tested by lentiviral transduction of T2 cells lines that express different human HLA molecules. Since indels are often formed by Cas9 editing, we expected a decrease of HLA-DR expression in cell lines where Cas9 was active. HLA-DR expression in the DRB1*04:01 cell line decreased from 95% to 20% or 12% after Lentivirus transduction with the 208/fwd or 185/rev guides, respectively. Control cell lines expressing T2-DRB1*04:02 and T2-DRB1*08:01 showed no loss of HLA-DR expression demonstrating specificity of the guide RNAs. Experiments using an ssDNA template to promote HDR and introduce the K71E gene edit are in process.

Finally, since HLA-DRB1*04:01K71E has not been seen in nature, we have produced a DRB1*04:01K71E transgenic mouse which demonstrates this modified allele is compatible with life. This tool will be vitally important to demonstrate that stem cells from DRB1*04:01K71E mice transplanted into HLA-DRB1*04:01 mice do not cause graft-vs-host disease and can render the mice resistant to RA.
LAMP-2B regulates human cardiomyocyte function by mediating autophagosome-lysosome fusion.

Autophagy plays a crucial role in cell homeostasis and function. Two types of autophagy have been well studied. Macroautophagy (referred to as autophagy hereafter) is mediated by double-membrane autophagosomes that enclose cytosolic cargoes, followed by fusion with late endosomes/lysosomes for degradation. Chaperone-mediated autophagy (CMA) is a process of chaperon-dependent selection of cytosolic proteins that are translocated into the lysosome for degradation. LAMP-2A, the A isoform of lysosome-associated membrane protein 2 (LAMP-2), functions as an essential receptor for CMA by interacting with chaperon proteins. Alternative splicing of pre-LAMP-2 mRNA produces three isoforms: LAMP-2A, LAMP-2B, and LAMP-2C. These LAMP-2 isoforms share an identical N-terminal lysosomal luminal domain, but have distinct transmembrane and cytosolic domains at the C-terminus.

In non-cardiomyocytes, syntaxin 17 (STX17) is required for autophagosome-lysosome fusion by interacting with synaptosome-associated protein 29 (SNAP29) and vesicle-associated membrane protein 8 (VAMP8). Whether cardiomyocytes (CMs) utilize the STX17-dependent mechanism to mediate autophagic fusion remains elusive. Using CMs derived from induced human pluripotent stem cells (hiPSC-CMs) and genome editing-based approaches, we identified previously undefined biological and molecular roles for LAMP-2B in the control of fusion between autophagosomes and endosomes/lysosomes. Remarkably, LAMP-2B functions independently of STX17. Instead, LAMP-2B interacts with autophagy related 14 (ATG14) and VAMP8 through its C-terminal coiled coil domain (CCD) to promote autophagic fusion. Knockout of LAMP-2B in hiPSC-CMs decreased colocalization of ATG14 with VAMP8, and autophagosome-lysosome fusion. Forced expression of LAMP-2B in LAMP-2B deficient hiPSC-CMs restored autophagic signaling. In addition, LAMP-2B-deficient hiPSC-CMs recapitulated the metabolic and contractile defects observed in hiPSC-CMs derived from patients with Danon disease. Danon disease represents a severe form of hypertrophic/dilated cardiomyopathy (HCM/DCM). Most of patients dies due to heart failure and/or arrhythmias. The mean ages in years of the first symptom, diagnosis of cardiac myopathy, and death are 12, 13, and 19 in men and 28, 30, and 35 in women, respectively, highlighting the rapid progression of cardiac deterioration caused by the disease. Danon disease has been associated with mutations in X-linked LAMP-2 gene. However, mechanisms by which LAMP-2 deficiency leads to cardiomyopathy and heart failure are poorly understood. While it has been postulated that LAMP-2B deficiency causes Danon cardiomyopathy because mutations in the LAMP-2B were found in a few patients, there are no prior experimental evidences. Given the similarity of phenotype between Danon and LAMP-2B knockout hiPSC-CMs, we conclude that LAMP-2B deficiency is sufficient and necessary to cause Danon cardiomyopathy.

Therefore, our findings not only reveal a STX17-independent autophagic fusion mechanism mediated by LAMP-2B in human CMs, but also provide a molecular mechanism underlying cardiomyopathy in Danon patients and a foundation for targeting defective LAMP-2B-mediated autophagy to treat this patient population.
Optimizing multiple myeloma patient-derived xenografts to test anti-CD46 antibody drug conjugate for curative potential.

BACKGROUND: Multiple myeloma (MM) is incurable, with relapse inevitable in almost all patients. We found anti-CD46 antibody-drug conjugate (CD46-ADC) potently and selectively inhibits MM cell line growth in vitro and in vivo, and eliminates patient MM cells from ex vivo culture (Sherbenou et al, JCI 2016). A problem in MM is that no current therapy is capable of eliminating minimal residual disease (MRD), and thus there currently is no cure. In theory, an ADC could be curative if the target is expressed on MRD. CD46 is an intriguing novel candidate for this, since it is a functional antigen that is genetically upregulated in MM. Notably, studies using cell lines do not reflect complexities of human MM, like MRD. Thus, we implemented a patient-derived xenograft (PDX) model of MM to facilitate testing of CD46-ADC elimination of MRD in vivo.

METHODS: Due the dependence of MM cells on their microenvironment, the mouse model utilizes subcutaneously implanted human fetal bone grafts, as described (Keizai et al, Blood 1992). The efficiency of engraftment of myeloma samples using this approach has been reported to be 33% (Kim et al, Leukemia 2012). We used this model to test whether relapsed, late-stage disease would engraft better than newly diagnosed samples. Thus, we injected 3 newly diagnosed versus 3 late-stage patient samples (n = 3-4 mice/per sample) into human bone grafts in NSG mice. Mice with long term sustained engraftment were treated with CD46-ADC 5 mg/kg IV for 4 doses.

RESULTS: Of mice harboring bone grafts and injected with patient samples, we observed engraftment in 8/19 mice (42%) via ELISA monitoring of mouse serum for restricted human free light chains matching the injected samples. At study termination, flow cytometry of the bone grafts confirmed light chain restricted myeloma cells in mice with detectable serum free light chain by ELISA. Surprisingly, the selected newly diagnosed samples engrafted more efficiently than the relapsed/refractory samples (70% vs 11%). Notably, a patient sample harboring a c-myc translocation engrafted in 3/3 mice. CD46-ADC treatment reduced engraftment (n = 2), but engraftment decreased in all mice by study termination at 30 weeks.

CONCLUSION: Our data suggest that treatment naïve patient samples engraft more efficiently than heavily pretreated samples in MM PDX that harbor human bone grafts. The further optimization of MM PDX will allow testing of CD46-ADC and other preclinical drugs for curative potential.
**Hyunmin Kim**

**Name of Presenter**
Hyunmin Kim

**Email address**
Hyun.Kim@ucdenver.edu

**Daytime phone or cell #**
720 472 0036

**Division**
Medical Oncology

**Status**
Junior Faculty

**Research Category**
Basic Science

**Title of Abstract: (no more than 200 characters with spaces)**
SALSA: Systematic Alternative Splicing Analysis Pipeline for Detecting Cryptic 3' Splice Site Usage in SF3B1 Mutant Cancer RNA-seq

**Please copy and paste your abstract here: (no more than 500 words)**

Alternative splicing (AS) of RNA is an essential function in cells to facilitate proper processing of pre-mRNAs into protein-coding genes, and alterations in AS have been associated with multiple diseases and tumorigenesis. Recent cancer genomics studies identified recurrent spliceosome mutations in multiple cancers, specifically hot-spot mutations of SF3B1 (i.e. R625, K666 and K700) were found at high frequency in myelodysplastic syndrome, chronic lymphocytic leukemia, breast cancer, pancreatic cancer, uveal and mucosal melanomas. SF3B1 mutant induces alternative 3' splicing through utilization of a cryptic 3'ss and branch point sequence, generates AS that could be: (i) translated into new aberrant proteins; and (ii) RNA degradation through nonsense-mediated mRNA decay (NMD). However, it remains unclear whether the different hot-spot mutations in SF3B1 induce distinct AS patterns in a particular gene set or cancer type. To study the AS induces by SF3B1 mutation, we developed SALSA, a novel AS analysis tool that can detect altered 3' splice sites in RNA-seq. We applied SALSA for the analysis of SF3B1-mutant RNA-seq data in TCGA cancer patients. We identified a set of genes that are enriched in cryptic 3' splice sites, and validated these AS in our in-house SF3B1-mutant RNA-seq data. We also predicted SF3B1 splicing rules regulating the AS-NMD in these samples, and examined the functional enrichment of these AS-NMD transcripts. We concluded that SF3B1-mutant cancers are using AS as a mechanism to promote tumor growth and survival. Some of these altered proteins could be exploited as cancer therapy in SF3B1-mutant cancer patients.
CPT1A and AR blockade result in differential regulation of genetic and metabolic pathways in castration resistant prostate cancer cells.

Abstract: Although localized prostate cancer (PCa) is highly curable, men who develop metastatic castration-resistant PCa (mCRPC) have limited survival and identification of novel therapies for mCRPC is critical. Our group has shown that lipid oxidation via carnitine palmitoyltransferase (CPT1A) supports growth and resistance to antiandrogen therapy in PCa models. However, the molecular connection between CPT1A activity and the androgen receptor (AR) is unknown. The goal of this study was to investigate the genetic and metabolic mechanisms behind the CPT1A-AR interaction. We used PCa cell models resistant to the anti-androgen therapy (LNCaP-MDVres, LNCaPC42, 22Rv1, PC3). The fat oxidation inhibitors (FAOis) etomoxir and ranolazine, as well as the antiandrogen enzalutamide (MDV3100) were used to treat the cells for 48 hours, followed by gene expression (GSEA), metabolomics and lipidomics analyses.

Mechanistically, we found that ranolazine and enzalutamide treatments decreased CPT1A expression (0.5-fold p< 0.01, n=3) and increased the AR-full length to ARv7 variant ratio (ARFL/ARv7). ARv7 variant was strongly downregulated in response to treatments (0.3-fold, p<0.001). These results were paralleled by genetic models of CPT1A deficiency. Microarray pathway analysis of treated LNCaP-MDVres cells revealed that cell cycle and DNA replication were common pathways downregulated in FAOis and enzalutamide treatments (KEGG pathway by GSEA, FDR q-value < 0.01; n=3 biological replicates/treatment), while only MAPK signaling pathways were significantly upregulated with both etomoxir and ranolazine treatments (FDR q-value < 0.05), underscoring common pathways of the FAOis. Particularly, the DUSP1 phosphatase was upregulated with the FAOis, and this was confirmed with RTPCR and western blotting assays. On the other hand, enzalutamide treatments significantly upregulated allograft rejection and ascorbate and aldarate metabolism pathways (FDR q-value <0.002). Metabolomics analysis revealed a significant decrease in acyl-carnitines content with etomoxir and ranolazine treatments (p< 0.01, n=3). In contrast, enzalutamide treatment resulted in upregulation of acyl-carnitines (p<0.05), suggesting possible stimulation of fat oxidation. Significant changes were also observed with pyrimidine nucleotide metabolism and TCA cycle intermediates, which parallel the microarray pathway analysis results. Enzalutamide treatment in the AR negative PC3 cells was used as control for the interpretation of the metabolic findings. Since AR isoforms have been associated with different lipid programs, we hypothesize that FAOi blockade induces a compensatory increase in the ARFL/ARv7 ratio that may restore sensitivity to enzalutamide.

Sources of research support: NIH, ACS-RSG
**Title of Abstract:** The Role of Neonatal Fc Receptor in the Expression of Major Histocompatibility Complex Type II in Glomerular Endothelial Cells

Glomerulonephritis (GN) requires intrinsic renal cells that express Major Histocompatibility complex type II (MHCII) to propagate the disease. Glomerular endothelial cells (GEnC) can express MHCII but it is unclear if they can process and present antigen. Neonatal Fc receptor (FcRn) is a trafficking protein that is required for antigen processing and presentation that is known to be expressed in endothelial cells. It has been demonstrated that when FcRn is globally knocked out, GN is prevented. The exact mechanism by which knocking out FcRn offers protection from glomerulonephritis is incompletely understood. We believe that GEnC participate in immune kidney disease by expressing MHCII under inflammatory conditions and that they require FcRn for antigen presentation on MHCII. Determination of the renal cells involved in antigen-antibody presentation is important in understanding the pathophysiology of immune-mediated GNs and in developing targeted therapies for these diseases.

We first isolated and purified GEnC from wild type (WT) & FcRn knockout (KO) mice by isolating glomeruli and growing the glomerular cells. We then used von Willebrand factor (vWF) coated dynabeads to isolate the endothelial cells. The cells were characterized using IF for vWF and a podocyte specific marker (WT1). Once both cell lines were characterized & purified, we used a temperature sensitive SV40 virus to conditionally immortalize both the WT and KO GEnC. The cells were grown under permissive conditions at 33oC and differentiated at 37oC. Quantitative PCR (qPCR) was used to demonstrate appropriate expression of endothelial cell markers as well as verify the expression of FcRn in WT and absence of FcRn in KO GEnC. To determine whether inflammatory stimuli could alter expression of MHCII, we treated GEnCs with IFNγ and TNFα, then used flow cytometry to evaluate for the expression of CD146 (an endothelial cell marker) and MHCII. We also used qPCR to evaluate mRNA expression of MHCII, a MHCII chaperone protein (CD74), and transcript regulator protein (CIITA) to evaluate for any difference in level of expression of MHCII or its key transcriptional components.

We demonstrated that IFNγ and TNFα significantly upregulated expression of MHCII in both WT and KO GEnCs by flow cytometry. However, the percentage of KO GEnC that express MHCII was significantly less than that in the WT. We also discovered the mRNA expression of MHCII, CD74, and CIITA were all decreased in KO compared to WT GEnC.

These findings suggest that the absence of FcRn alters the transcription of MHCII. This finding may provide new insight and offer potential new targets for therapies involving immune mediated kidney diseases.
Leveraging the Electronic Health Record to Seamlessly Capture Quality

I. Background: Prospective quality measurement is critical to the delivery of high quality palliative care and value-based payment. The foundational work of Measuring What Matters and The Joint Commission (JC) Advanced Certification in Palliative Care has led the way in establishing a set of national quality palliative care metrics. However, there remains a limited understanding of how to implement palliative care quality measures into routine real-world clinical settings and merge seamlessly into clinician workload.

II. Aim Statement: To integrate QDACT into the EHR, streamlined into the clinician workflow to improve efficiency of data collection of national quality measures and clinical charting.

III. Methods: Five guiding principles were identified for the integration of national quality measures into the EHR (Epic) that can be adapted to other EHR systems. The five guiding principles for the EHR-QDACT integration were: 1) data would be entered by the clinician at each patient encounter; 2) the final product would be user friendly; 3) different disciplines could access QDACT for improved efficiency and integration into the clinical workflow; 4) data would auto-populate into clinical notes for improved efficiency; and 5) Epic reports could be built to fulfill The JC mandatory performance measures reporting requirements.

IV. Results: EHR-QDACT integration led to improvements and ease of documenting the 5 JC Palliative Care mandatory measures from December 2016 to June 2017: (1) PAL-01 Pain Screening and PAL-03 Dyspnea Screening stayed at 100% documentation pre- and post-integration; (2) PAL-02 Pain Assessment documentation increased from 57% to 100%; (3) PAL-04 Treatment Preferences and Goals of Care documentation increased from 56% to 98%; and (4) PAL-05 Treatment Preferences on the Discharge Document increased from 41% to 93%.

V. Conclusions and Implications: QDACT was successfully integrated into the EHR, streamlined into clinician workflow to improve efficiency of data collection and clinical charting, and fulfilled the documentation and tracking of The JC Advanced Certification in Palliative Care mandatory measures.
RATIONALE: Chronic spontaneous urticaria (CSU) involves urticarial lesions present for ≥6 weeks without an apparent trigger. CSU patients are basopenic, and symptom severity correlates with the degree of basopenia. Omalizumab, anti-IgE mAb, is approved for CSU treatment, and basophil numbers increase as symptoms improve with treatment. Basophil FcεR1 expression decreases with omalizumab, but this doesn’t explain its efficacy, as this does not correlate with clinical improvement. We hypothesized that there are differences in the basophil proteome of CSU subjects who respond to omalizumab, compared to non-responders, and that understanding the proteome of basophils in CSU could shed light on the pathophysiology of CSU.

METHODS: This was an open label, proof of concept study that examined the basophil proteome of subjects with CSU and its relationship to omalizumab response. We enrolled subjects with CSU, symptomatic despite standard doses of 2nd generation H1 antihistamines, who received open label omalizumab (300mg SQ q4 weeks, 1-4 doses). Subjects were considered responders to omalizumab if they had a significant reduction in UAS7 score after treatment.

To assess the basophil proteome, peripheral blood was collected prior to omalizumab treatment and basophils were purified via Percoll gradients and cell sorting. After sorting, cells were frozen at -80°C. Samples were processed at the same time, proteins were identified by mass spectrometry (MS), and over-represented biological ontologies were determined.

RESULTS: Of the 7 subjects with CSU enrolled, four subjects responded to omalizumab, with reduction of median UAS7 from 30 to 13 (p<0.05) and three subjects did not respond, with median UAS7 increased from 29 to 34. Three controls without CSU were also analyzed.

We identified 322 consistently expressed proteins in basophils from subjects with CSU. There were 30 proteins found exclusively in responders, 196 proteins found exclusively in non-responders and 96 shared proteins. There was consistency in proteins expressed within each group, particularly the responders.

The 30 proteins found only in responders were primarily intermediate filament and cytoskeletal or adhesion proteins. 11 (3%) of total proteins found in basophils of subjects with CSU were not found in controls, and were found consistently only in responders (p<0.001).

Pathway analysis revealed that proteins of intermediate filament pathways were strongly represented in responders compared to non-responders (p=1.6x10^-35). Of 196 proteins found in non-responders but not responders, 24 were associated with metabolic processes, 12 were involved in oxidative processes and 11 were related to the ubiquitin-proteasome system. Pathway analysis revealed that proteins with catabolic processes, response to oxidative stress and associated with unfolded protein binding were over-represented in non-responders (p=2.8x10^-33, 3.3x10^-13 and 6 X 10^-13, respectively) compared to responders.

CONCLUSIONS: Our findings demonstrate that basophils from omalizumab responders are stressed, but appear to be mounting a protective response by overexpressing intermediate filament proteins. Similarly, basophils from nonresponders are stressed, but cellular responses are different, with overexpression of proteins associated with catabolic processes, oxidative stress and unfolded protein pathways. Thus, differential responses to cellular stress may reflect differences in the pathophysiology of the disease in patients who ultimately do or don’t respond to omalizumab.
Title of Abstract: Tracing the Innate Genetic Evolution and Spatial Heterogeneity in Treatment Naïve Lung Cancer Lesions

Extensive intratumor heterogeneity (ITH) have been observed in individual patient tumors by large-scale sequencing analyses. ITH can contribute to drug resistance and cancer metastasis. Distinct microenvironments provide selection advantages to sub-population of cells for cancer metastasis. We hypothesized that ITH can contribute to metastasis by disseminating different sub-populations of cancer cells to distant sites. We collected tumor specimens and non-cancer tissues from treatment naïve autopsied patients to study the innate genetic evolution and spatial heterogeneity. Our cohort consists of four (two adenocarcinoma and two squamous cell carcinoma) NSCLC patients and one SCLC patient. Each patient had 5–9 primary and metastatic lesions. Comprehensive data analyses were performed on the RNA-seq that include gene expression and pathway analyses, fusion detection and somatic variants detection. Global unsupervised clustering of expression data reveals the NSCLC patients clustered together from the SCLC, and the two adenocarcinoma and squamous cell carcinoma patients formed two clusters. Within each patient, metastatic lesions clustered according to the distant metastatic sites. Pathway analysis and somatic mutation analysis in individual patients revealed that, in general, the primary lesion is distinct from metastatic lesions in NSCLCs. For the SCLC patient, distant metastases and lymph node metastases clustered according to different parts of the primary tumors. We also identified KIF5B-RET fusion as a founder mutation in all tumor specimens obtained from a never-smoking adenocarcinoma patient. This study provides evidence that ITH contributes to distant metastasis sites based on the similarity and the heterogeneity between primary and metastatic lesions in lung cancer patients.
<table>
<thead>
<tr>
<th>Name of Presenter</th>
<th>Juan N Lessing</th>
</tr>
</thead>
<tbody>
<tr>
<td>Email address</td>
<td><a href="mailto:juan.lessing@ucdenver.edu">juan.lessing@ucdenver.edu</a></td>
</tr>
<tr>
<td>Daytime phone or cell #</td>
<td>6099334633</td>
</tr>
<tr>
<td>Division</td>
<td>Hospital Medicine</td>
</tr>
<tr>
<td>Status</td>
<td>Junior Faculty</td>
</tr>
<tr>
<td>Research Category</td>
<td>Outcomes Research</td>
</tr>
<tr>
<td>Title of Abstract: (no more than 200 characters with spaces)</td>
<td>The LOOP Project: A Multicenter, Interdisciplinary Initiative to Improve Diagnostic Reasoning and Patient Safety through Consistent, Rapid Feedback</td>
</tr>
</tbody>
</table>
| Please copy and paste your abstract here: (no more than 500 words) | BACKGROUND: Development of expertise in diagnostic reasoning is one of the most important goals of medical education, and feedback is fundamental for improving performance. There are many barriers to effective feedback for trainees about clinical decisions, including multiple transitions of care and fragmented health care systems. Trainees lack opportunity to consistently determine what happens to patients they admit to hospital or to follow a patient throughout entire hospital course. We must create intentional mechanisms to standardize feedback about clinical decisions across transitions of care for medical trainees. The LOOP Project is a multicenter, interdisciplinary initiative to standardize diagnostic feedback and promote a culture of continuous improvement in diagnostic reasoning.  

OBJECTIVE: Formalize peer-to-peer feedback to close diagnostic loops to improve clinical reasoning.  

INNOVATION DESCRIPTION: This project included 6 medicine and 1 pediatric programs. Resident participation was encouraged but voluntary. Pre and post surveys were administered to determine trainees’ self-efficacy in diagnostic reasoning and giving/receiving feedback, and satisfaction with current level of feedback. Teams who assumed care of patients after night admissions were encouraged to send feedback forms to admitting teams, comparing initial and subsequent diagnostic impressions. Feedback forms were gathered, coded, and categorized for content to capture changes in diagnosis over initial days of care for hospitalized patients.  

RESULTS: Frequency of giving feedback increased and residents were more confident in their ability to identify strategies to decrease diagnostic errors in their own practice (3.23 vs 3.52 on a 5 point scale, p<0.05). Residents were increasingly satisfied with the quality, frequency and value of diagnostic feedback (p<0.05). 478 feedback forms were completed; 21% of forms indicated diagnostic refinement, 11% evolution of disease, and 12% had a major diagnostic change. 44% of patients had at least some diagnostic change indicated in the form.  

DISCUSSION: A standardized effort to give rapid, peer feedback about diagnostic reasoning is feasible and effective at improving trainee’s self-efficacy in diagnostic error identification as well as satisfaction with feedback. Given the degree of change in diagnosis from night to day, diagnostic feedback is a moral imperative. Work-flow congruent methods to encourage feedback are important for success/sustainability. |
### Background:
Clinical images are photographs that serve as succinct and memorable lessons. Importantly, patient right to privacy dictates appropriate consent be obtained prior to publication. Significant disagreement exists surrounding what makes a photograph identifiable, and thus, when consent is required. Variability in consent requirements makes preparation, submission and successful publication more difficult for authors.

### Objective:
To characterize the consent process and requirements among top internal medicine journals that publish clinical images.

### Methods:
We identified the top 10 internal medicine journals, by impact factor, that publish exam-based images. We analyzed Instructions to Authors, journal consent form (if available), as well as all supplemental materials sent by journal editor(s) as response to email query asking about consent rules and policies for clinical image submissions. Study results were shared with each journal's editor to ensure that results accurately reflect the respective journal's policies.

### Results:
Publicly available information was available from all journals and query response was received from 7/10 (70%), of journals. All require written patient consent for clinical image based manuscript submissions. However, only half (50%) explicitly state that patient consent is required even if the patient is not identifiable, and several indicated that consent is only required if the patient is identifiable. Half (50%) request or require use of their own consent form. Half (50%) require consent form with patient signature be submitted to be archived by the journal, while the other half request that control be maintained by the author in order to maximize patient anonymity. Only 4/10 (40%) explicitly state that the discounted practice of black-out bars to cover eyes is not an acceptable form of anonymization. 2/10 (20%) require that the patient be given right to review the submitted material before proceeding with consent.

### Discussion:
Consent requirements of top journals that publish clinical images shows widespread, and non-rational, variability. This makes it more difficult to obtain appropriate patient consent and represents a barrier to authors. Inconsistent consent requirements represent an avoidable burden to patients who may be contacted multiple times to complete journal specific consent forms. A universal consent process and form is needed.
**Title of Abstract:**
Treatment of a murine model of human lupus with a monoclonal antibody that blocks binding of C3d to its receptors decreases anti-DNA autoantibodies and proteinuria: implications for the CR2:C3d interaction as a therapeutic target in lupus and other autoimmune diseases

**Abstract:**

The primary B cell receptors for antigen (Ag)-bound complement C3 fragments are complement receptor 1 (CR1/CD35) and complement receptor 2 (CR2/CD21). In mice, CR1 and CR2 are derived through alternative splicing from a common gene (Cr2). CR1 is the primary receptor for the C3b and C4b fragments of C3 and C4, while CR2 binds the TED domain within C3d and iC3b. The absence of CR1/CR2 in Cr2−/− mice impairs immunological responses to foreign and some self Ags due to the lack of CR2/CD19 costimulatory signals on B cells and impaired Ag retention on follicular dendritic cells. Given this role, one might expect an autoimmune enhancing role for CR1/CR2 in systemic lupus erythematosus (SLE) through amplification of responses to C3b/C3d-bound self-Ags. However, studies in murine models of SLE performed on a Cr2−/− background have demonstrated enhanced lupus-related autoimmunity. One potentially confounding factor with use of Cr2−/− mice is that CR1 is a receptor for C4b, which is itself necessary to maintain tolerance to lupus autoantigens in humans and mice. Previously, only rat anti-mouse monoclonal antibodies (mAbs) to CR2/CR1 have been available, which we found are neutralized due to their immunogenicity in mice. To further study the impact of long term therapeutic interruption of CR2:C3d interactions, we have developed novel non-immunogenic mouse anti-mouse mAbs targeted to CR2:C3d-specific ligand-receptor interactions. The first is a non-B cell depleting mAb that recognizes CR2 and blocks its interactions with C3d without directly affecting CR1 interactions with C4b or C3b. The second mAb recognizes the C3d fragment and blocks its interaction with CR2 without directly affecting C3b or C4b interactions with CR1. Using the MRL/lpr model of SLE, we have found that treatment with anti-CR2 mAb did not improve disease outcome biomarkers. Conversely, a single injection of anti-C3d mAb durably reduced anti-dsDNA antibody production (mean 383 R.U. in mAb 3d8b injected mice and 949 R.U. in control PBS injected mice, p<0.05) and proteinuria (mean 13115 mg albumin/dL/creatinine (g/dL) compared to 101759 in PBS injected mice, p<0.05). The reduction correlated with reduced BUN levels (46±10 compared to 72±38, p<0.05). We conclude that blocking C3d/TED domain receptor interactions through ligand-directed interruption of binding represents a potential new therapeutic approach in patients with SLE and potentially other autoantigen-driven disorders.
**Title of Abstract:** THE "CLINICIAN VOICE": UTILIZING A NOVEL DIGITAL PLATFORM FOR HOSPITALIST ENGAGEMENT AROUND THE EXPERIENCE OF CLINICAL WORK

**Background**
The rate of burnout among health care providers is unacceptably high and recent scholarship has advocated for organization-facing interventions to tackle this problem. To that end, our large academic hospital medicine group has developed a multi-modal strategy to identify high-yield interventions for provider well-being. Part of this initiative involved piloting a digital survey tool with the goal of monitoring hospitalists’ experience of clinical work and prioritizing improvement opportunities.

**Purpose**
We utilized a novel (Waggl) software platform to develop a "Clinician Voice" pulse survey in order to garner hospitalist engagement around their clinical work experience and prioritize improvement opportunities.

**Description**
The Waggl platform offers unique features compared to traditional engagement surveying methodologies. For one, survey length on the platform is limited to 5 quantitative questions and 1 qualitative question. In addition, Waggl includes an interactive component whereby participants prioritize their own qualitative answer as compared to answers entered by their colleagues in an A-B voting format (Figure 1.). Over time, this creates a "crowdsourced" priority list related to qualitative responses. Prior to our pilot, we consulted key hospital stakeholders, including the Chief Medical Officer, and committed to an overall engagement process to follow up the survey with an inclusive dialog of the results. Over 7 days, all physicians and advanced practice hospitalists in our group were invited to participate in a two question survey using their browser or mobile device. The first question asked participants to rate the likelihood that they would recommend their clinical workplace to a friend on a quantitative 0-10 scale. The second question asked “What would make it more likely for you to give a 10 rating on the first question?” 24 hospitalists (34%) participated in the survey and cast 147 priority votes. An in-person dialog was held at a subsequent hospitalist faculty meeting to review the survey process and better understand the factors contributing to the priorities that emerged from the survey. Outputs from the survey and dialog were shared with hospital executives to inform decision making and used by hospitalist leadership for strategic planning. As a result of the pilot, the process was adopted across several other clinical specialties.

**Conclusions**
Interactive digital survey instruments can be leveraged to assess frontline work experience, create productive dialogue between frontline clinicians and leaders, and identify improvement opportunities.
Molecular Mechanisms Modulating the Oxidative State of Leukemia Stem Cells

Mohammad Minhajuddin; Shanshan Pei; Brett Stevens; Binian Adane; Haobin Ye Nabilah Khan; Amanda Winters; Maura Gasparetto; and Craig T Jordan.

Hematology Division, Department of Medicine, University of Colorado Denver-Anschutz Medical Center, Aurora, CO, USA

Please copy and paste your abstract here: (no more than 500 words)

Reactive oxygen species (ROS) are continuously generated through various biochemical pathways, moderate levels promote cell proliferation and survival, whereas severe increase in ROS leads to cell death and contributes to disease development. In normal stem cells, intracellular ROS is tightly controlled and is maintained at low levels, for long-term self-renewal and survival. Like normal stem cells, the redox state in Leukemia Stem Cell (LSC) is very important for its survival. We have published that majority of functionally defined leukemia stem cells (LSC) are characterized by low levels of reactive oxygen species (ROS-Low). In the present study, we sought to determine the molecular mechanisms responsible for maintaining the redox state of leukemia stem cells (LSC).

We employed primary human acute myeloid leukemia (AML) samples and redox-sensitive Cell Rox dye, that provide broader measure of intracellular redox levels. AML specimens were sorted into subsets of low and high endogenous ROS, defined as 20% dimmest (ROS-Low) and brightest (ROS-High) fluorescence distribution.

Our preliminary data indicate that the energy sensor molecule AMPK, is selectively activated in ROS-Low cells, as shown by phosphorylation of Thr172 on AMPKα1 by western blotting. This data led us to hypothesize that AMPK may be involved in regulating the metabolic state of ROS-low cells. To test this hypothesis, we created a lentiviral vector that co-expresses GFP and shAMPKα1. This vector was introduced into primary AML specimens and GFP-positive cells were isolated for analysis. The strategy achieves 80-90% reduction in expression AMPKα1. We first analyzed the functional consequence of AMPKα1 knockdown by transplanting lentivirally-transduced cells into immune deficient NSG mice. Successful engraftment in the bone marrow of primary AML cells is considered as gold standard for measuring LSC activity and is commonly employed as a functional readout for malignant stem cells. We observe a dramatic reduction in the engraftment of shAMPKα1 transduced primary AML cells compared to vector control, indicating that AMPK is required for in vivo maintenance of the LSC population. Furthermore, knocking down AMPKα1 in CD34+ cells derived from normal human umbilical cord blood did not effect the engraftment in NSG mice, implicating that AMPK does not affect normal HSC function.

To better understand the mechanism driving loss of LSC potential, we examined two key metabolic properties associated with AMPK, regulation of fatty acid oxidation (FAO) and regulation of NADPH production. There is a nearly 50% reduction in FAO activity upon knockdown of AMPK in primary AML cells. Moreover, we also measured relative levels of NADPH and NADP+ in control vs. AMPK knockdown Molm13 cells. We observed reduced levels of NADPH and increased levels of NADP+, indicating that overall reducing equivalents are suppressed upon loss of AMPK. Finally, overall ROS levels are increased upon AMPK knockdown, consistent with the change in metabolic status and increased oxidative state expected from the loss of NADPH. Overall, these studies emphasize the central role of AMPK in modulating the unique redox regulatory mechanisms of LSC, and might be used as an effective strategy to eliminate these cells and develop better therapeutic regimens.
**Name of Presenter**  
Stefan Law, Manuel Diaz, Ethan Cumbler, Read Pierce, Patrick Kneeland

**Email address**  
Manuel.Diaz@ucdenver.edu

**Daytime phone or cell #**  
847-912-1287

**Division**  
Hospital Medicine

**Status**  
Junior Faculty

**Research Category**  
Clinical

**Title of Abstract: (no more than 200 characters with spaces)**  
SOMETHING AWESOME: ELEVATING STORIES OF AWE AMONG COLLEAGUES IN THE EVERYDAY WORK OF ACADEMIC HOSPITAL MEDICINE

**Background**  
The healthcare workforce suffers from high levels of burnout, disengagement, and perceived isolation due to constant and unpredictable stresses. While multifactorial, the extent to which clinicians’ experience wellness and maintain resilience in a complex work environment is in part driven by connection to purpose and consciousness of elements of everyday activities that give joy. Indeed, studies in positive psychology practices have demonstrated the impact such practices can have on overall wellness. At the same time, applied positive psychology interventions remain rare among clinicians, and many clinical groups are uncertain how to undertake this work.

**Purpose**  
Improving workplace culture, resilience, and shared sense of purpose by implementing a protected narrative session to highlight uniquely positive moments ("Something Awesome") during monthly hospitalist group meetings.

**Description**  
After introducing the concept of “Something Awesome” by relating a exemplar story at a monthly meeting, we sent all group members a call via email for submissions of brief stories highlighting moments of awe and joy. Submissions are reviewed monthly by a core group of our faculty who possess experience in storytelling and narrative medicine. Those faculty choose 1-2 stories to share at the next month’s meeting. We save unselected stories for potential sharing at a later date. Selected storytellers meet with the core faculty to practice the story, and are given feedback to help clarify the narrative or increase its impact. Stories are recorded and transcribed for performance of qualitative and quantitative analysis of themes and content. Since initiation of “Something Awesome” in February of 2017, 12 stories have been shared by physicians, advanced practice providers, and administrative staff. These cover a variety of themes (Figure 1). The average length of each story was 7 minutes. In 7-point Likert scale assessments of “Something Awesome,” group members report that it is a valuable addition to the monthly meeting (6.1/7 mean), that perception of opportunities to share positive experiences with the group have increased (4.4/7 pre, 5.8 post), and that a culture of sharing negative experiences has decreased (4.6/7 pre, 4.1 post).

**Conclusions**  
Incorporating scheduled and protected opportunities for hospitalist group members to share moments of joy and awe is a simple, low-cost intervention that promotes attention to positive experiences with potential to impact culture of the work environment.