12th Annual Lung Day Day

Abstract Sessions

And Abstracts
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# Abstract Session 1
**Room 1 (Abstracts 1-5)**

**Time:** 11:45 a.m. – 12:45 p.m.

**Moderators:** Justin Oldham and Yuanjun (Steven) Shen

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**Time:** 11:45 a.m. – 12:45 p.m.

**Moderators:** Nicholas Kenyon and Javier Garcia

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<th>Abby Ray, Bennett Penn</th>
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| 24. | 1:05-1:15 | Impact of Ergothioneine Pretreatment on Naphthalene Toxicity in the Mouse Lung | Veneese J Brown, L Ding, L Yin, PC Edwards, X Ding, LS Van Winkle |
| 25. | 1:15-1:25 | A Novel Function of the MARCKS/AXL Axis in Tobacco Smoking-Mediated Macrophage Polarization and Pulmonary Fibrosis | David C Yang, Jun Zhang, Ji-Min Li, Ching-Hsien Chen |
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| 30. | 1:15-1:25 | Feraheme Iron Oxide Magnetic Nanoparticles Inhibit Neutrophil Inflammatory Response and Recruitment | Gustavo Garcia, Scott I Simon |
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| 34. | 1:05-1:15 | Clinical Registry Infrastructure and Early Data from UCD Comprehensive COPD Clinic | Daniel Tompkins, Krystal Craddock, Tina Tham, Maya Juarez, Jimmy Nguyen, Marcy Dolan, Michael Dirks, Karla Ramirez, Michael Schivo, Brooks Kuhn |
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A Novel TSC2 dependent YAP/mTOR pathway in Pulmonary Vascular Remodeling and Pulmonary Hypertension.

Yuanjun Shen¹, Tatiana Kudryashova¹, Dmitry Goncharov¹, Theodore Avolio², Baojun Chang², Andressa Pena², Ana Mora², Jeffery Baust², Andres Chavez Barragan², Amab Ray², Evelyn Okorie², Sara Grunblatt², Matt Kart², Analise Rhode², Elena Goncharova¹
¹Division of Pulmonary, Critical Care and Sleep Medicine, Department of Internal Medicine, School of Medicine, University of California, Davis, California; ²Heart, Lung, Blood and Vascular Medicine Institute, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania.

Rationale: Pulmonary arterial hypertension (PAH) is a deadly disease with no cure. It is characterized by increased proliferation and survival of resident cells in small pulmonary arteries (PA) and pulmonary vascular remodeling. Tuberous sclerosis complex 2 (TSC2) is a key upstream negative regulator of mTOR complex 1 and cell growth; however, its role in PAH is not known. In this study, we aimed to evaluate the role and mechanisms of regulation and function of TSC2 in PAH. Methods and results: Immunohistochemical and immunoblot analyses showed that TSC2 is deficient in PA vascular smooth muscle cells (PAVSMC) in small PA from human PAH lungs and rodents with Sugen5416/Hypoxia (SuHx)-induced PH. Hemodynamic and morphological analyses showed that, compared to same-age and sex controls, 9-weeks-old male mice with smooth muscle-specific Tsc2 down-regulation (SM22-Cre-Tsc2²⁺/⁻) develop spontaneous remodeling of small PAs and PH as evidenced by significantly higher PA medial thickness, systolic right ventricular pressure (sRVP) and RV hypertrophy. Four out of six 9-weeks-old female SM22-Cre-Tsc2²⁺/⁻ mice also developed PH. TSC2 deficiency was reproduced by maintaining human non-diseased PAVSMC on substrates with pathological stiffness and was required for stiffness-induced proliferation and activation of YAP/TAZ-mTOR axis. TSC2 loss was further supported via YAP feed-forward loop. Depletion of TSC2 by siRNA reproduced PAH features in human non-diseased PAVSMC, permitted YAP/TAZ-mTOR activation, and increased PAVSMC growth and proliferation via modulating extracellular matrix (ECM) composition. Re-expression of TSC2 in human PA PAVSMC reversed such effects. The ECM, produced by human shRNA-TSC2-infected non-diseased PAVSMC, up-regulated YAP-mTOR and induced growth of non-diseased PAVSMC, which was reversed by ATN-161, integrin α5β1 (fibronectin receptor) inhibitor. "Diseased" ECM also induced growth of PA adventitial fibroblasts, but not endothelial cells. Pharmacological restoration of TSC2 by Sir1 activator SRT2104 down-regulated YAP/TAZ, mTOR and ECM production, inhibited proliferation and induced apoptosis in human PA PAVSMC. Anti-proliferative and pro-apoptotic effects of SRT2104 in human PA PAVSMC were blocked by siRNA-induced TSC2 depletion. Orally administered SRT2104 (100mg/kg; 1 week in mice or 5 weeks in rats) restored Tsc2 in pulmonary VSMC, resolved pulmonary vascular remodeling and occlusion of small PAs, and reversed sRVP, pulmonary arterial pressure, RV hypertrophy and RV contractility in two rodent models of SuHx-induced PH. Conclusion: Our data demonstrate that TSC2 acts as a critical integrator of ECM composition and stiffness with pro-proliferative signaling in PAH PAVSMC and suggest that restoration of functional TSC2 is a potentially attractive therapeutic option to treat PAH.
Abstract #1 - Room 1 (11:45 a.m. -11:55 p.m.)

Pre-recorded Presentation [CLICK HERE](#)
Glucose drives lipogenesis and proliferation of human pulmonary arterial vascular smooth muscle cells in pulmonary arterial hypertension

Lifeng Jiang1, Dmitry Goncharov1, Yuanjun Shen1, Baojun Chang2, Andressa Pena2, Tatiana Kudryashova1, Elena Goncharova1

1Division of Pulmonary, Critical Care and Sleep Medicine, Department of Internal Medicine, School of Medicine, University of California, Davis, California, 2Pittsburgh Heart, Lung, and Blood Vascular Medicine Institute, Division of Pulmonary, Allergy and Critical Care Medicine, Department of Medicine, University of Pittsburgh, Pittsburgh, Pennsylvania.

Rationale: Pulmonary arterial hypertension (PAH) is characterized by a progressive increase in pulmonary arterial vascular smooth muscle cells (PAVSMC) proliferation, leading to pulmonary vascular remodeling, increased PA pressure and premature death of heart failure. Lipogenesis plays important role in proliferative diseases, including cancer. The status and role of lipogenesis in hyper-proliferative PAVSMC in PAH, however, is not clear, and the synthetic source remains to be elucidated. In this study, we aimed to determine the role of lipogenesis in PAH PAVSMC proliferation and evaluate the mechanism(s) of its regulation as it relates to PAH.

Methods and results: Immunoblot analysis of early-passage distal human PAVSMC from non-diseased (control) and PAH subjects (n=5 subjects/group) showed that ATP-citrate lyase (ACL), acetyl-CoA carboxylase (ACC), and fatty acid synthase (FASN), key enzymes driving biosynthesis of fatty acids, were significantly upregulated in PAVSMC from human PAH lungs compared to non-diseased controls. Human PAH PAVSMC had higher unstimulated growth in culture compared to non-diseased cells, which was preserved in lipid-free conditions. In contrast, treatment with an allosteric inhibitor of ACC TOFA (5-tetradecyloxy-2-furoic acid) significantly decreased proliferation of human PAH PAVSMC, suggesting that PAVSMC in PAH have increased lipogenesis that is required for unstimulated proliferation. To determine the mechanism(s) of increased lipogenesis in human PAH PAVSMC, we employed immunocytochemical analysis of neutral lipids using a fluorescent probe (BODIPY). Briefly, PAH PAVSMC were cultured in lipid-free media and BODIPY staining was used to visualize neutral lipid accumulation. We found that both accumulation of intracellular lipids and proliferation of PAH PAVSMC in lipid-free conditions were suppressed by the treatment with 2-Deoxy-D-glucose (2-DG), a non-metabolizable analog of glucose. Further supporting importance of glucose in PAH PAVSMC lipogenesis, co-treatment with glucose metabolite pyruvate significantly attenuated 2DG-dependent inhibition of lipid synthesis and proliferation of human PAH PAVSMC.

Conclusion: Taken together, our data demonstrate that PAH PAVSMC do not require exogenous lipids to sustain increased growth and have an ability generate endogenous lipids in a glucose-dependent manner. We also show that inhibition of lipogenesis reduces PAH PAVSMC proliferation. These data indicate a novel metabolic link between glucose, lipogenesis and proliferation of PAH PAVSMC and provide potential target pathway for therapeutic intervention.
Targeting Arginine Metabolism for Mitigating Idiopathic Pulmonary Fibrosis Progression

Linhui Li\textsuperscript{1,2}, Ji-Min Li\textsuperscript{1}, David C. Yang\textsuperscript{1}, Lisa Franz\textsuperscript{1}, Nicholas J. Kenyon\textsuperscript{1}, Ching-Hsien Chen\textsuperscript{1,2}

\textsuperscript{1}Department of Internal Medicine, Division of Pulmonary and Critical Care Medicine and Center for Comparative Respiratory Biology and Medicine, University of California Davis, Davis, California, USA, \textsuperscript{2}Division of Nephrology, Department of Internal Medicine, University of California Davis, Davis, California, USA

Rationale: Idiopathic pulmonary fibrosis (IPF) is a chronic, progressive, and irreversible interstitial lung disease. Disruption of metabolic pathways has been recognized as a driver for many respiratory diseases, and metabolic reprogramming is also noted in lung fibrosis. However, the profiling of altered levels of metabolic enzymes and metabolites in IPF remain to be established. Herein, we aimed at characterizing and targeting metabolic pathways of lung fibrosis progression.

Methods: The primary lung fibroblasts isolated from healthy individuals (n = 6) and IPF patients (n=10) were subject to untargeted metabolomics analysis using gas chromatography–time-of-flight mass spectrometry for further integrated analysis of metabolomics. Saline-treated and bleomycin-induced lung fibrosis mice were fed a control (arginine-containing) diet or an arginine-free diet. Mice lungs were collected and processed for histology analysis (H&E and Masson’s Trichrome staining) and collagen content assays at day 28.

Results: We applied the metabolic set enrichment analysis (MSEA) and identified the top fifty metabolic pathways significantly altered between normal and IPF lung fibroblasts. Among these pathways, we noticed upregulation of fructose and mannose metabolism, propanoate metabolism, citrate cycle metabolism, pyrimidine metabolism, purine metabolism, arginine biosynthesis, and glutamate metabolism in IPF lung fibroblasts. Data from pathway enrichment analysis of this metabolomic study not only revealed a number of metabolites involved in amino acid-metabolic pathways but also confirmed a significant associate of IPF with amino acid metabolism, particularly arginine biosynthesis. We next carried out a bleomycin-induced pulmonary fibrosis mouse model with arginine-free diet to test if targeting arginine reprogramming regulates fibrotic progression. The histological analysis of bleomycin-exposed lung sections demonstrated extensive structural changes in mice with arginine-containing diet, whereas administration of arginine-free diet significantly reversed fibrotic lesions caused by bleomycin. Notably, bleomycin-induced upregulation of hydroxyproline levels and collagen content were significantly suppressed in bleomycin-exposed mice receiving arginine-free diet.

Conclusions: Our data suggest the critical role of metabolic regulation in IPF lung fibroblasts with an indication of targeting arginine metabolism as a promising therapeutic strategy for pulmonary fibrosis.

This abstract is funded by: DoD W81XWH1910831 (Log#KC180170), NIH T32 HL007013-42 and UCOP grants Tobacco-Related Disease Research Program (T29IR0704 and T31DT1849).
Investigating PCSK6 as a biomarker in patients with Idiopathic Pulmonary Fibrosis

Vivian Vo¹, Angela Linderholm¹, Imre Noth², Shwu Fan Ma², Justin Oldham¹
¹University of California, Davis- Davis, CA/US, ²University of Virginia- Charlottesville, VA/US

**Rationale:** Idiopathic Pulmonary Fibrosis (IPF) is a progressive fibrotic interstitial lung disease with poor survival that represents an ongoing challenge for doctors due to limited treatment options. A considerable number of resources are directed at research concerning disease progression, and survival because of its complexity and the number of risk factors involved. Notably, the presence of gene variants has been linked to the progression of IPF. This study seeks to quantify and delineate a correlation between protein expression of PCSK6 and its implication for survival in patients with IPF. By performing a genome wide association study, we may establish preliminary data associated with IPF survival and identify PCSK6 as a potential prognostic biomarker and therapeutic target.

**Methods:** Human blood samples collected from patients from two independent cohorts, UC Davis and University of Chicago, were processed to isolate plasma. A PCSK6 ELISA detection assay was used to measure protein concentration. The results were then analyzed with patient outcome data.

**Results and Conclusion:** According to these results, PCSK6 protein concentration in peripheral blood predicted IPF progression, making it clinically relevant. Further research will be directed towards understanding the role PCSK6 plays in IPF progression and its potential as a predictive biomarker.
Rationale Patients with connective tissue disease-associated interstitial lung disease (CTD-ILD), chronic hypersensitivity pneumonitis (CHP), and unclassifiable ILD (uILD) experience variable clinical course, though many develop progressive fibrosing ILD (PF-ILD) characterized by irreversible lung function, decline, and death. PF-ILD is currently classified by antecedent worsening of pulmonary function, symptoms, and/or radiographic disease burden, but cannot be diagnosed prospectively. We hypothesized that plasma protein biomarkers would predict 12 month progression-free survival consistent with PFILD.

Methods This prospective cohort study included patients with CTD-ILD, CHP and uILD recruited at 3 US centers divided into discovery (n=385) and validation (n=204) cohorts. Blood was collected from subjects at study entry, and all subjects received serial pulmonary function testing. Near-term progression was defined as a relative decline of ≥10% forced vital capacity (FVC) predicted, death, or lung transplant within 12 (+/- 3 months) of blood draw. Plasma proteins were measured using a semiquantitative proteomic panel comprised of 368 proteins of inflammation (Olink, Uppsala, Norway). Protein biomarkers were individually tested for association with near-term progression using simple logistic regression. Those with significant association corrected for multiple testing (FDR <0.05) in the discovery cohort were advanced for testing in the validation cohort. Multiple logistic regression was used to adjust for clinical characteristics.

Results Seventeen protein biomarkers maintained association with near-term progression after serial analysis in the discovery and validation cohorts. αvβ6 integrin (OR 3.13, CI 2.31-4.23), PLAUR (OR 3.28 CI 2.24-4.80) and Keratin 19 (OR 2.0, CI 1.67-2.38) showed the strongest associations. Biomarker association with near-term progression remain significant after patient stratification by ILD subtype, and after adjustment for age, sex, ILD subtype, FVC% predicted, and DLCO% predicted. In patients with definite or probable usual interstitial pneumonia (UIP) pattern on high resolution computed tomography (HRCT), αvβ6 integrin predicted near-term progression with OR 9.73 (4.47-21.16) compared to other HRCT morphologies that had OR of approximately 2.

Conclusion We have identified 17 circulating protein biomarkers that predict near-term progression-free survival in a multicenter cohort of patients with CTD-ILD, CHP, and uILD, and are robust to clinical characteristics and underlying ILD diagnosis. αvβ6 integrin showed the greatest ability to predict near-term progression in patients with UIP pattern on HRCT.
Abstract #5 - Room 1 (12:25 p.m. - 12:35 p.m.)
Pre-recorded Presentation

Table 1. Association between validated inflammatory biomarkers and PF-ILD phenotype

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<tr>
<th>Protein</th>
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<th>Validation Cohort (n=204)</th>
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<tr>
<td></td>
<td>OR</td>
<td>CI</td>
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<tr>
<td>AGER</td>
<td>0.65</td>
<td>0.50-0.85</td>
</tr>
<tr>
<td>ANGPTL4</td>
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<td>1.25-2.45</td>
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<td>CXCL17</td>
<td>1.68</td>
<td>1.28-2.24</td>
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<td>DPP10</td>
<td>1.85</td>
<td>1.34-2.59</td>
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<td>FCAR</td>
<td>1.71</td>
<td>1.27-2.33</td>
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<tr>
<td>HGF</td>
<td>2.29</td>
<td>1.53-3.49</td>
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<td>IL17c</td>
<td>1.39</td>
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<td>ITGB6</td>
<td>3.17</td>
<td>2.20-4.70</td>
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<td>KRT19</td>
<td>2.1</td>
<td>1.69-2.66</td>
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<td>FASLG</td>
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<td>MMP10</td>
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<td>PLUR</td>
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<td>1.34-2.84</td>
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<td>PRSS8</td>
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<td>SCGB3A2</td>
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<td>SPON1</td>
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<td>TGFA</td>
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<td>1.62-3.77</td>
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<tr>
<td>TNF11B</td>
<td>1.85</td>
<td>1.24-2.82</td>
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Abbreviations: AGER = Advanced Glycation End Products Receptor; ANGPTL4 = Angiopoietin-like 4; DPP10 = Dipeptidyl Peptidase 10; FCAR = Fc fragment of IgA Receptor; HGF = Hepatocyte growth factor; ITGB6 = αβ6 integrin; KRT19 = Keratin 19; LRRN1 = Leucine-rich repeat neuronal protein 1; MMP10 = Matrix metalloprotease 10; PLUR = Urokinase plasminogen activator surface receptor; PRSS8 = Prostasin; SCGB3A2 = Secretoglobin 3A2; SPON1 = Spondin 1; TGF-a = Transforming growth factor a; TNF11B = Osteoprotegerin

Figure 1: Volcano plot of protein biomarkers statistical significance versus odds ratio for PF-ILD phenotype.
Role of Surfactant Protein D (SP-D) in COVID-19

Samarth Sandeep\textsuperscript{1}, Jun Yang\textsuperscript{2}, Melissa Teuber\textsuperscript{2}, Angela Linderholm\textsuperscript{2}, Sona Aramyan\textsuperscript{1}, Cindy B. McReynolds\textsuperscript{2} Resmi Karalasseril Ravindran\textsuperscript{2} Imran H Khan\textsuperscript{2} Nam Tran\textsuperscript{2} Angela Haczku\textsuperscript{2}

\textsuperscript{1}Iff Technologies \textsuperscript{2}University of California, Davis, School of Medicine

\textbf{Rationale:} Surfactant protein D (SP-D) is a major immune protective molecule produced in the respiratory epithelium. SP-D binds to specific pathogen surfaces through its carbohydrate recognition (lectin) domain, that helps in their neutralization and clearance by phagocytes. SP-D also acts as an anti-inflammatory molecule. We hypothesized that SP-D plays a protective role in COVID-19.

\textbf{Methods:} Polar+, a novel quantum computing algorithm for molecular pruning, and classical \textit{in silico} modeling were used to investigate potential binding sites between SP-D and SARS CoV2. Electronegativity and topologically oriented molecular pruning, calculation of electronic forcefields and electrostatic binding combined with protein-protein docking, geometric fitting and assessment of protein glycosylation sites were employed. SP-D/- mouse lung was used to study expression of the SARS-CoV-2 viral entry molecules TMPRSS2 and ACE2. Plasma from COVID-19 patients was studied for SP-D leakage, cytokine levels and lipid mediators.

\textbf{Results:} We found that SP-D potentially binds to the same SARS CoV-2 glycoprotein (S protein) that it utilizes to bind the cellular receptor, ACE2, with high affinity. However, SP-D binds to subunit 2, instead of subunit 1 (that ACE2 utilizes). Additional studies will need to determine if SP-D binding affects S protein and ACE2 interactions. We also found that SP-D/- mice had increased expression of the TMPRSS2 gene in the lung and that both TMPRSS2 and ACE2 mRNA levels were increased during lung injury, amplified by the lack of SP-D. SP-D leakage from the lung to the circulation was significantly increased in COVID-19 patients and correlated with expression of pro-neutrophilic inflammatory markers.

\textbf{Conclusions:} We speculate that SP-D aids in the protection from SARS CoV2 infection by both acting as a potential natural decoy to prevent coronavirus entry into airway epithelial cells and by attenuating the expression of the viral entry receptor TMPRSS2. Oxidative lung injury results in SP-D leak into the circulation denoting disease severity in COVID-19 patients.

\textbf{Funding:} Chester Robbins endowment (AH)
Abstract #6 - Room 2 (11:45 a.m. - 11:55 a.m.)

Pre-recorded Presentation [CLICK HERE]

Figure 1. Protective role of SP-D in COVID-19.

(A): S protein (purple) and SP-D (blue) Polar+ pruned structural configurations and most likely binding site on SP-D (83-90 PVGPKGDN with the glycosylated Asn at 90) determined by ZDOCK, with the binding visualized as Clash/Contact sites on the UCSF Chimera (yellow rectangle).

(B): Tmprss2 and Ace2 mRNA expression in the SP-D-/- lung: Total RNA extracted from Wt and SP-D-/- (C57BL/6) lungs from naïve ctrl or ozone exposed mice. (qPCR Affymetrix chip); Median and interquartile ranges n=6-20 ***p<0.0001 vs Wt; #p<0.05; ###p<0.01 vs ctrl.

(C): SP-D measured (ELISA) in COVID-19 vs healthy plasma samples p=0.004 (n=9-30).

(D): Severely ill COVID-19 patient plasma samples were assessed after hospital admission for SP-D (ELISA) and cytokine (Luminex) and lipidomic profile.
**Rationale:** The pulmonary surfactant protein A (SP-A) plays an immune protective role by binding to the cell membrane of immune cells and by opsonizing infectious agents such as bacteria, fungi and viruses through glycoprotein and lipid binding domains. SARS-CoV-2 enters airway epithelial cells through its Spike glycoprotein (S protein) that binds the Angiotensin Converting Enzyme 2 (ACE2) on the cell surface. Using in silico methods we aimed to predict whether human SP-A could bind the S protein and whether this could interfere with SARS-CoV-2 - ACE2 binding.

**Methods:** We assessed binding sites, the relative binding affinity of the interactions, and the protein chains and residues involved using ZDOCK and a novel software “Polar+” . We then compared these binding parameters between S protein - ACE2 and S protein-SP-A binding. We hypothesized that SP-A competes for the same binding site of the S protein with ACE2. We applied the “Polar+” to determine the best binding sites between the proteins. This process involved electronegativity and topologically oriented molecular pruning, calculation of electronic force-fields and electrostatic binding combined with protein-protein docking, geometric fitting and assessment of protein glycosylation sites.

**Results:** We established the parameters of ACE2-S protein binding (left panel in figure) and found that SP-A potentially binds to the S protein with an affinity similar to that of ACE2. However, our data suggested that SP-A most likely binds to the fusion portion of the S2 chain of the S protein. This part is responsible for viral entry into the host cell (right panel in figure).

**Conclusions:** Our study supports the use of quantum computing and the Polar+ algorithm for studying protein-protein interactions. Based on our findings we speculate that SP-A while does not compete with ACE2 for S protein binding, could still interfere with viral entry to the cell through hindering the membrane fusion process. These findings are important in understanding SARS-CoV-2 biology and warrant studies in experimental biological systems.

**Funding:** Chester Robbins endowment (AH)
Figure 1. Polar+ pruned structural configurations and most likely binding sites between the S protein ACE2 (left panel) and the S protein and SP-A (right panel)

Polar+ pruned structural configurations of the SARS-CoV-2 S protein trimer [the original structure in lower left framed insert: Chain A (blue), Chain B (purple) and Chain C (pink)]. The best predicted binding sites with ACE2 (red, original structure in upper left framed insert), and SP-A (green, original monomeric configuration is shown; most common oligomeric form is an octadecamer). The top binding configuration (in the yellow rectangles) was determined by docking of the reduced/pruned structures of S protein and ACE2 and SP-A using ZDOCK, with the binding visualized (yellow) as Clash/Contact sites on the UCSF Chimera.
A Portable Sampler for Collection of Exhaled Breath Volatiles (EBV)

B. Chew¹, A. Fung¹, S. Fung¹, M. McCartney¹, C.E. Davis¹*, N. Kenyon²

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Rationale: We hypothesized volatile, exhaled biomarkers can accurately diagnose SARS-CoV-2 infection in a reagentless point-of-care device. To begin modeling and identifying biomarkers indicative of COVID-19 a device was made to repeatedly collect an accurate volume of exhaled breath sample onto sorbent packed thermal desorption tubes. These samples can then be analyzed with gas chromatography-mass spectrometry (GC-MS) to quantify the volatile chemicals present from the breath sample. In addition, the sampler could collect a background air sample to compare against the exhaled breath.

Methods: A microcontroller, sensor suite, and mechanical device were designed and built to accurately sample a set volume of gas. COVID-19 patients from UCDMC and healthy controls were asked to blow up a 5 liter Tedlar bag. The Tedlar bag was then attached to our device while 1 liter of exhaled breath from the same bag was sampled over three different sorbent bed materials. In a laboratory the samples were analyzed with GC-MS to evaluate the relative sample recovery rate of each sorbent type. In a future portion of the study, we plan to activate secondary sensors on the sampler — pre-sorbent and post-sorbent humidity sensors, and a carbon dioxide sensor to collect extra dimensions of data on the sample. This breath sample metadata collected on site will help evaluate the quality of the sample before analysis.

Results: The current device has an accurate flow rate and consistent volumetric sampling within 50mL of the target 1L. The device shows the ability to collect exhaled breath samples in an easy to transport form factor in the field, making this device useful for remote sampling. GC-MS analysis of the collected samples can be compared against a sampled room air blank taken at the same time.

Conclusion: The device is able to repeatably sample 1L of patient breath from a Tedlar bag on to the sorbent trap. Further sample collection and GC-MS analysis will reveal the optimal sorbent material for COVID-19 biomarker recovery from patent sample
Abstract #9 - Room 2 (12:15 p.m. - 12:25 p.m.)

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Devices Engineered to Collect Exhaled Breath Condensate (EBC) and their Applications

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RATIONALE  Human exhaled breath is rich in metabolomic content that represents pulmonary function and gas exchange with blood. It contains a mixture of compounds that offer insight into an individual’s state of health. Here, we present two novel non-invasive breath sampling devices for use in basic medical practice.

METHODS  The two breath samplers have a disposable mouthpiece, a set of inhale and exhale one-way flap valves to allow condensation of exhaled breath only, and a saliva filter. The housing is constructed out of Teflon®, a chemically inert material to reduce chemical absorbance. The first device condenses exhaled breath into a frozen condensate using dry ice pellets and the other is a miniaturized design that liquifies exhaled breath on a condenser surface with micropatterned features on a cooling plate. Both designs have individual strategic and analytical advantages: frozen exhaled breath condensate (EBC) has high retention of analytes and sample volume; EBC collected in liquid phase offers facilitated sample collection and device portability.

RESULTS  We investigated if breath aerosol size distribution affects the types or abundances of metabolites. We modified the geometry of the first device to redirect aerosol trajectories based on size. The trapping of larger aerosols increases with filter length, thus altering the aerosol size distribution although no significant changes in the metabolite profiles were found. With the miniaturized design, metabolite abundances were measured in a small cohort of healthy control and mild asthmatic subjects. Differences among subjects were found, as well as main differences between control and asthmatic groups. All analyses of EBC were performed with liquid chromatography – mass spectrometry. Inflammatory suppression found in asthmatic subjects can be explained by prescribed daily use of inhaled corticosteroids.

CONCLUSION  Breath collection devices can be used in intensive care units, outpatient clinics, workplaces, and at home. EBC analysis has been used to monitor asthma and chronic obstructive pulmonary disease. It can be applied to infectious respiratory diseases (e.g. influenza, COVID-19) and for monitoring environmental and occupational chemical exposures.
Glass-to-glass Fusion Bonding: Evaluation of Various Cleaning Procedures & Modes of Bonding for Optimized Bonding Quality and Strength

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Hazardous volatile organic compounds (VOCs) can have major effects to the human body. Overexposure to certain VOCs can have adverse effects that can lead to respiratory problems, causing not only lung damage but also damage the liver, kidney, or central nervous system. Due to the effects of VOC exposure, it is necessary to create a device to identify and quantify when VOCs are present.

Using micro-electrical mechanical systems (MEMS), a micro-preconcentrator chip (µPC) can be manufactured to trap volatile chemicals into a sorbent filled cavity. Usually, detection of certain chemical compounds can be challenging due to low sensitivity and selectivity levels. To enhance detection, preconcentration of chemical compounds can improve sensitivity and selectivity. This chip acts as a chemical pre-concentrator before the chip is analyzed for chemical compounds and quantification for VOC air sampling.

As the global market for MEMS electronics and sensors continue to rise, the technical requirements of MEMS-based substrates have expanded. Technical requirements of MEMS include; higher biological compatibility, optical transparency, corrosion and heat resistance, and a need to withstand high transition temperatures. Consideration of glass as the primary substrate in MEMS devices has become increasingly popular due to its unique physical properties that enable MEMS devices to meet these requirements and more. Improved glass micromachining is critical for allowing industries and researchers to investigate the full potential of glass in the integrated development of MEMS technology.

Currently, fusion bonding glass-to-glass is challenging because successful bonding requires the use of expensive and time-consuming equipment. Some glass fusion bonding procedures can last more than 10 hours with consistent failure modes in more than 70% of bonding attempts. Air bubbles, Newton rings, and cracking are also common issues during glass bonding. Increasing the understanding, simplicity, and accessibility of glass wafer bonding will undoubtedly benefit the field MEMS electronics and sensors at large.

In this work, various modes of bonding, cleaning, and prebonding procedures are explored and summarized. Borosilicate glass 4inch wafers with a thickness of 750µm were used in fusion bonding and intermediate layer-based bonding utilizing glass paste. Temperature and pressure profiles were explored to observe their effect on bonding success. Cleaning and conditioning procedures include using an Alconox detergent solution, piranha solution, and plasma activation (both alone and in combination). Wafer bonding, 1-inch by 1-inch chip bonding, and localized pressure distribution on both wafer and chip bonding were evaluated using the aforementioned cleaning procedures and bonding modes.
Work of Breathing on Proportional Assist Ventilation as a Predictor of Extubation Failure

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Rationale: Despite decades of research on predictors of extubation success, use of ventilatory support after extubation is common and 10-20% of patients require reintubation. Proportional Assist Ventilation (PAV) modes automatically calculate estimated total work of breathing (WOB\textsubscript{T}). We tested the hypothesis that a WOB\textsubscript{T} value calculated on PAV in invasively ventilated patients can predict extubation failure.

Methods: This prospective pilot study was conducted in six adult ICUs at an academic medical center. We enrolled intubated patients who completed a spontaneous breathing trial with a f/V\textsubscript{T} <105 breaths/min/L, and were deemed ready for extubation by the primary team. WOB\textsubscript{T} values were recorded at the end of a 30-minute PAV trial. Extubation failure was defined as any respiratory support, escalation in respiratory support, and/or re-intubation within 72-hours of extubation. We compared WOB\textsubscript{T} scores between groups and performance of WOB\textsubscript{T} for predicting extubation failure with receiver operating characteristic (ROC) curves.

Results: Of 61 patients enrolled, 9.8% (n=6) required re-intubation and 50.8% (n=31) required respiratory support within 72-hours of extubation. Median WOB\textsubscript{T} at 30-minutes on PAV was 0.9J/L (IQR 0.7-1.3J/L). WOB\textsubscript{T} was significantly different between patients who failed or were successfully extubated (p<0.05). The AUC was 0.71 [95%CI 0.58-0.85] for predicting any requirement of respiratory support, 0.67 [95%CI 0.52,0.82] for escalation of respiratory support and 0.85 [95%CI 0.69-1.00] for predicting re-intubation.

Conclusions: The discriminative performance of a PAV-derived WOB\textsubscript{T} value to predict re-intubation was good. If validated in additional settings, WOB\textsubscript{T} could represent an adjunctive tool for assessing extubation readiness. However, specific thresholds or threshold ranges should be considered based on the individual patients’ risk and weaning profiles.
Abstract#11 - Room 2 (12:25 p.m. - 12:35 p.m.)
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Figure. Extubation failure outcomes and total work of breathing (WOB₁) values measured at 30 minutes into proportional assist ventilation (PAV) trial in joules/liter (J/L)

Any Failure

![Boxplot showing work of breathing at 30 min for any failure.](image)

Escalation of Support

![Boxplot showing work of breathing at 30 min for escalation of support.](image)

Reintubation

![Boxplot showing work of breathing at 30 min for reintubation.](image)

Means are represented as +

*Footer: An asterisk (*) denotes the statistical performance of the Mann-Whitney U test to compare WOB₁ values between extubation outcomes groups. A single * indicates a p-value <0.05 and ** indicates a p-value < 0.01. Extubation failure outcome definitions: a) 'Any extubation failure': composite outcome defined as use of either non-invasive positive pressure ventilation (NIPPV), high flow nasal cannula (HFNC), or re-intubation within 72-hours of extubation b) 'Escalation of Support': defined as an increase in respiratory support (e.g. supplemental oxygen to HFNC, NIPPV or IMV; HFNC to NIPPV or IMV; or NIPPV to IMV); and c) re-intubation.**
Identifying the Host Targets of *M. tuberculosis* Proteases
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**Rationale:** *Mycobacterium tuberculosis* (*Mtb*) is the leading cause of death by an infectious disease worldwide. *Mtb* has the surprising ability to survive and proliferate in the harsh environment of the alveolar macrophage and establish a chronic, often lifelong, infection. Unfortunately, the molecular mechanisms underlying this ability to disrupt host defenses remain obscure. It is known, however, that many bacteria secrete proteases that cause non-specific tissue damage. The **central hypothesis** of this project is that *Mtb* secretes protease virulence factors into the host cell to specifically disrupt immune function by selectively targeting mediators of host immunity, while avoiding widespread proteolysis that would prematurely kill the host cell and expose *Mtb* to immune surveillance. Terminal amine isotopic labeling of substrates (TAILS) is a quantitative proteomic assay that allows the labeling of neo-N-termini resulting from proteolytic cleavage. The objective of this pilot study was to modify the TAILS protocol for use in human macrophages and establish a positive control in this system with the goal of characterizing the proteolytic events in an *Mtb* infected macrophage.

**Methods:** THP-1 monocytes were differentiated into macrophages by the addition of phorbol 12-myristate 13-acetate (PMA) to the culture media. After 72 hours of differentiation and a visual assessment of the macrophage-like phenotype, pyroptosis was induced using nigericin at varying doses over a series of time points from 30 minutes to 8 hours. Pyroptosis was visualized using CellEvent, a caspase 3/7 fluorescent marker and cell death was assessed via fluorescent microscopy.

**Results:** This pilot study found that the optimal conditions for nigericin-induced pyroptosis of THP-1 cells require an initial monocyte seeding density of 4x10⁶ cells/mL. After 72 hours of differentiation, the cells were stimulated with purified lipopolysaccharide at 0.3ug/mL for 4 hours followed immediately by the addition of nigericin at 10ug/mL for another 4 hours. Finally, cells were treated with bortezomib at 1ug/mL for 1 hour before staining with 2uM CellEvent for 30 minutes. Under these conditions, >90% of the cells were positive for caspase 3/7 and when compared to untreated cells a marked increase was observed.

**Conclusion:** This pilot study successfully established early modifications to the existing TAILS protocol by adapting it for use in human macrophages and establishing a reliable positive control via using drug-induced pyroptosis. Together, this will advance the overall aim of attempting to capture the dynamics of proteolysis during *Mtb* infection.
Title: Early Life Antibiotic Treatment Results in Both Transient and Persistent Changes to the Microbiome

Authors: Noah A. Siegel, Matt Ralston, Taylor Westmont, Alexa Rindy, Hitesh Deshmukh, Lisa A. Miller
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Rationale: Dysbiosis can be an unintended consequence of antibiotic treatment for respiratory infections. While the adult gut microbiome is relatively resilient to persistent changes, the impact of antibiotics on maturation of the infant gut microbiome is not well understood. We hypothesized that early life antibiotic treatment can persistently alter the infant gut microbiome. To test this hypothesis, we used the infant rhesus macaque monkey as a developmental animal model to progressively evaluate the impact of antibiotic treatment on gut microbiome populations during the first six months of life.

Methods Indoor housed infant monkeys received a daily administration of an antibiotic cocktail consisting of ampicillin, gentamicin, and vancomycin (targeting both gram positive and negative bacteria) during the first week of life. All infant monkeys were nursed and co-housed with their dams throughout the study until weaned at 5 months of age. Microbial DNA was extracted from rectal swabs collected at birth and monthly until 6 months of age. 16S rRNA sequencing was conducted on V3-V4 amplicons. Reads were analyzed to assess longitudinal effects and differences in diversity and metagenomic pathways.

Results: The infant rhesus macaque monkey gut microbiome consisted primarily of the phyla Actinobacteria, Bacteroidetes, Firmicutes, and Proteobacteria. While the proportions of Actinobacteria, Bacteroidetes, and Proteobacteria declined with age, the opposite trend was observed with Firmicutes. Relative abundance of Actinobacteria and Firmicutes showed sex-dependent alterations at 2 months of age. Alterations in both α and β diversity were observed in antibiotic-treated monkeys at 2 months of age. At 6 months of age, α and β diversity increased in antibiotic-treated monkeys relative to controls, with more prominent changes in males relative to females. Metagenomic analysis revealed an overall reduction in pathways contributing to peptidoglycan biosynthesis and maturation in antibiotic-treated monkeys. Peptidoglycan biosynthesis was increased in control males relative to females but sex-dependent differences in this pathway were not observed in association with antibiotic treatment. Linear discriminant analysis demonstrated a consistent increase in short-chain fatty acid producing bacteria from the genus lactobacillus in controls relative to antibiotic-treated monkeys at 6 months of age.

Conclusions: With antibiotic treatment, diversity of the developing gut microbiome varied at 2 and 6 months. Overall, alterations of the microbiome were more pronounced in males than females. Differences in metagenomic pathways accompanied changes in microbial abundance and diversity. Our findings suggest that early life antibiotic treatment can have lasting sex-dependent and independent effects on the developing microbiome.
Perinatal Exposure to Secondhand Smoke on Susceptibility to Viral Infection and Bacterial Challenge.

Author Block: N. Singh, A. Spediacci, C. Wu, J. A. Claude, K. E. Pinkerton; Center for Health and Environment, University of California, Davis, Davis, CA, United States.

Abstract:
Rationale: Secondhand smoke (SHS) or passive smoke exposure, is a common malady in our society. In early life, it has been estimated more than 25% of children are exposed to SHS, typically in the home. However, the impact of early life exposure to SHS on the immune response is unclear. This may be critical when the immune system is undergoing maturation. We found mice exposed to SHS during the last trimester of gestation and the first 5 weeks of postnatal life, when exposed to viral infection and bacterial challenge, is associated with a significantly higher incidence of mortality and a marked increase in pulmonary hyaline membrane formation. This study was designed to evaluate the degree of inflammation, hyaline membrane formation and the incidence of CD4 helper, and CD8 cytotoxic T-cells in the lungs of neonatal mice.

Methods: Pregnant BALB/c mice were exposed to filtered air (FA) or SHS beginning on gestational day 14. Following birth, the pups continued exposure to FA or SHS for 5 weeks. Immediately following exposure to FA or SHS, mice were randomly assigned to FA, SHS, FA+virus, SHS+virus, FA+virus+bacteria, or SHS+virus+bacteria. The virus was a murine-adapted strain of H1N1 influenza A and the bacteria was *Staphylococcus aureus*. Viral inoculation was administered 1 week prior to bacterial challenge. Immunohistochemistry (IHC) was performed on 5 μm thick paraffin sections to determine the degree of inflammation, as well as the incidence of CD4 and CD8 cells in lung tissues.

Results: Initial mortality in mice began as early as two days post-bacterial challenge, resulting by day 10 in 50% mortality in FA infected animals and 100% mortality in SHS infected animals. The increased mortality was associated with a greater incidence in the pulmonary hyaline membrane formation. CD4 and CD8 cells were found to be higher in the FA-infected and the SHS-infected mice compared with control. However, no significant difference was noted between the FA-infected and SHS-infected animals for CD4 or CD8 cells.

Conclusion: Early life exposure to SHS compared to FA was associated with significantly greater mortality in mice subsequently infected with either virus or virus+bacteria. CD4 and CD8 cells demonstrated a dramatic influx into the lungs due to viral/bacterial infection, but was not significantly altered by early life exposure to SHS.
Rationale: Antibiotics are frequently used to treat infants for respiratory infections but may be problematic long-term due to effects on the microbiome. Because the microbiome has been reported to influence pulmonary immunity and lung injury in animal models, we hypothesized that antibiotic treatment can alter normal growth and function of the postnatal respiratory tract. To test our hypothesis, we investigated the effect of early life antibiotics on measures of pulmonary function and expression of developmental genes in the respiratory tract using the infant rhesus macaque as a model of postnatal development. We also determined whether sex may influence the outcome of antibiotic use and assessed the lung for steroid hormone receptor expression.

Methods: Indoor housed infant monkeys received a daily administration of an antibiotic cocktail consisting of ampicillin, gentamicin, and vancomycin (targeting both gram positive and negative bacteria) during the first week of life. All infant monkeys were nursed and co-housed with their dams throughout the study until weaned at 5 months of age. At 6 months of age, pulmonary function testing was conducted on all animals and lung biospecimens were collected following necropsy. Saline-treated infant monkeys that were identically housed and evaluated served as controls. Developmental and steroid hormone receptor gene expression in the lung was assessed via qRT-PCR and immunohistochemistry.

Results: Vital capacity and expiratory reserve volume were reduced in antibiotic-treated animals relative to controls. When separated by sex, changes in lung volumes became more pronounced; inspiratory capacity was exclusively decreased in antibiotic-treated males. Expression of genes associated with growth and development including WNT3A, SOX2, and MMP16 were altered antibiotic-treated animals relative to controls. There were no sex-dependent differences in developmental gene expression, but males showed regionalized elevated expression of sex hormone receptors. Estrogen receptor β and androgen receptor were decreased in antibiotic-treated animals compared to controls, although androgen receptor only decreased in antibiotic-treated females and not males.

Conclusions: Antibiotic-treated monkeys showed reductions in multiple parameters of lung function at 6 months of age, with changes that were most pronounced in males. We also observed altered developmental gene expression in infant monkey airways with antibiotic treatment, however the effects were not associated with males or females. In addition, the expression profile of sex hormone receptors in the lung was associated with antibiotic treatment and sex. Collectively, our findings suggest early life antibiotic treatment can alter the normal trajectory of lung development in a sex-dependent fashion.
Role of MyD88/TLR Signaling in Combating *S. aureus* and *P. aeruginosa* Skin Infections
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**Introduction:** *Staphylococcus aureus* and *Pseudomonas aeruginosa* are two opportunistic pathogens that often result in lethal skin infections on immunocompromised individuals. The United States Centers for Disease Control and Prevention estimates that in 2017 there were more than 100,000 *S. aureus* blood stream infections out of which 20,000 were lethal. However, the prevalence of these pathogens in the environment and their ability to quickly evolve and become resistant to antibiotics poses a major public health threat. Therefore, it is important to understand the underlying mechanisms mounted by the innate immune system to combat these infections. This will guide us in engineering novel therapies to tackle bacterial infection. Here, we compare the role of TLR/MyD88 signaling in response to *S. aureus* versus *P. aeruginosa* infection on the skin and on the ability of neutrophils to contain infection and clear off bacteria.

**Materials and Methods:** Our lab employs a mouse model to characterize the mammalian immune response to *S. aureus* and *P. aeruginosa* infections. We perform 6mm dorsal wounds followed by subcutaneous inoculation of 2E6 to 1E7 CFU of bioluminescent *S. aureus* NRS384 or *P. aeruginosa* XEN41 bacteria into the wounds. Wild-type and immunodeficient MyD88/-/- mice are from a C57BL/6 background and have an EGFP reporter downstream of the LysM gene. This allows measurement of PMN trafficking into the wound as well as bacteria clearance over time. This is done via whole-animal in vivo imaging at various time points after wounding and inoculation.

**Results and Discussion:** Resolution of skin infections is dependent on MyD88 signaling. Immunodeficient MyD88/-/- mice infected with *S. aureus* succumb to sepsis 2-9 days following infection, while MyD88/-/- infected with *P. aeruginosa* survive for less than 24 hours following infection (Figure 1A). MyD88/-/- mice with *S. aureus* infection show decreased PMN recruitment and higher bacterial load compared to wild-type mice (Figure 1B and 1C). However, MyD88/-/- mice infected with *P. aeruginosa* show similar PMN numbers compared to wild-type mice but less bacteria containment (Figure 1B, 1C and 1D).

**Conclusions:** The ability of the innate immune system to combat skin infections caused by *S. aureus* and *P. aeruginosa* is dependent on the MyD88 pathway downstream of TLR activation. *P. aeruginosa* infection is more lethal and less contained on MyD88/-/- mice compared to *S. aureus*. In 2017, Falahite et al., reported that survival rates of MyD88/-/- infected with *S. aureus* on the skin improve via adoptive transfer of WT hematopoietic stem and pluripotent cells. This mechanism was shown to be dependent on IL-1B. Further research will be aimed at determining whether bacterial containment and survival rates of MyD88/-/- with *P. aeruginosa* skin infection improve via HSPC transfer as observed with *S. aureus* skin infections.
Single-cell RNA-seq analysis reveals a critical role of Tet1 in lung epithelium in house dust mite (HDM)-induced allergic airway inflammation

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Rationale
Previous studies found that Tet1, a DNA demethylase, protected against allergen-induced airway inflammation in mice and altered the lung methylome and transcriptome. Airway epithelium initiates proinflammatory responses to allergens and plays an important role in the establishment of asthma phenotypes. We aim to explore the role of Tet1 loss in individual epithelial cell types in HDM-induced lung inflammation in mice via scRNA-seq analysis.

Methods
A murine model of HDM-induced lung inflammation was established. Tet1 knockout (KO) mice and wildtype (WT) mice were challenged with Saline or HDM. Whole lungs were homogenized to generate single-cell suspension and EpCAM+ lung epithelial cells were purified by cell sorting. Libraries were generated and sequenced using Illumina Hiseq. ScRNA-seq analysis was performed using Cell Ranger Pipeline v3.10.01, and scAlign, and visualized using Seurat. Cell types were labeled using previously known markers. Gene ontology analyses were performed using Ingenuity Pathway Analysis.

Results
Nine types of lung epithelial cells were identified in our scRNA-seq dataset. Alveolar type 2 (AT2) cell was the most abundant cell type. HDM challenge increased the number of alveolar progenitor (AP), broncho alveolar stem (BAS), and goblet cells, and decreased AT2 and ciliated cells. Compare to WT+Saline, more stromal cells and fewer neuroendocrine cells were found in Tet1 KO+Saline. Furthermore, we identified marked transcriptomic changes in various types of lung epithelial cells following HDM challenge and/or Tet1 deletion. Specifically, our results suggest that the effect of Tet1 loss on HDM-induced lung inflammation was linked to a cluster of 36 genes, particularly the upregulation of Il33, Hmgb1 and Ifitm3, and the downregulation of Neat1 and Klf6 in EpCAM+ cells. At the single cell level, 30 genes (including Il33 and Hmgb1) in AT2 cells, 5 genes (including Gsp1 and Gpx2) in ciliated cells, 2 genes (Dmkn and Chia1) in AP cells, and 1 gene (Nnat) in BAS cells may contribute to the more severe allergen-induced lung inflammation in Tet1 KO mice. Additionally, our data also supported that Tet1 modulates NRF2-mediated Oxidative Stress Response and Aryl Hydrocarbon Receptor Signaling in HDM-induced inflammation in several types of lung epithelial cells, particularly AT2 and ciliated cells.

Conclusions
Collectively, different lung epithelial cells played both common and unique roles in allergic lung inflammation. Tet1 deletion altered the networks of gene expression in various lung epithelial cells, with an overall effect of promoting allergy-induced lung inflammation in mice.
Figure 1. Lung pathological alterations and airway mucus secretion in HDM-induced airway inflammation in mice. (A) HE staining. The figure demonstrates a representative view (×200) from each group. (B) PAS staining. The figure demonstrates a representative view (×200) from each group.
Title: Early Life Exposure to Aryl Hydrocarbon Receptor Agonists Results in Induction of IL-22+ ILC3 Populations in Conjunction with Increased Club Cell Secretory Protein

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Rationale: There is growing evidence that pediatric lung function decline is predictive of chronic obstructive pulmonary disease in adulthood. A known risk factor for chronic obstructive pulmonary disease is tobacco smoke exposure but the mechanism for early life pathogenesis is not well understood. Tobacco smoke is a complex mixture of over 7000 chemicals including aryl hydrocarbon receptor (AhR) agonists. AhR signaling is essential for IL-22 expression in mucosal sites and has been shown to reduce expression of club cell secretory protein (CCSP). We hypothesized that early life tobacco smoke exposure may persistently alter pediatric lung function by dysregulation of the pulmonary IL-22/IL-22Ra1 axis via AhR agonists. To test this hypothesis, we used a murine model to investigate the contribution of AhR agonists on persistent lung pathophysiology following neonatal environmental tobacco smoke exposure.

Methods: 2,3,7,8-tetrachlorodibenzodioxin (TCDD) was used as an AhR agonist for this study. C57BL/6J mice were injected intraperitoneally with 15, 0.6, or 0.005 µg/kg TCDD at postnatal day 3 while control mice received vehicle. Six week old mice were used as adult controls and given identical treatments. A separate group of mice were exposed to 1-2 mg/m³ tobacco smoke for 6 hours each day from postnatal day 3 to 7, followed by recovery in filtered air until 6 weeks of age. Following exposure and at six weeks of age, single-cell suspensions were isolated from lungs and stained for flow cytometry analysis. Lungs were also collected for mRNA extraction and qPCR analysis. A subset of mice underwent pulmonary function testing using a FlexiVent forced oscillation system.

Results: Exposure to the AhR agonist TCDD during neonatal development resulted in a dose-dependent increase in the number of IL-22⁺ ILC3 cells in lungs that was similar to that induced by tobacco smoke. In contrast, TCDD did not appear to alter numbers of IL-22⁺ ILC3 cells in treated adults. Neonatal TCDD or tobacco smoke exposure also resulted in reduced expression of lung CCSP mRNA exposure, while adult controls were refractory at identical doses. Mice exposed to neonatal tobacco smoke showed increased tissue damping as adults, relative to filtered air controls.

Conclusions: AhR agonist treatment during neonatal development elicits increased numbers of IL-22⁺ ILC3 cells and increased CCSP expression in the lung that is comparable to that observed with tobacco smoke, suggesting the AhR pathway as a central mechanism for immune modulation of the lung with this form of environmental exposure.
Lung Epithelial Cell Environment Modulates Temporal Kinase Signaling Dynamics

Nicholas DeCuzzi¹, Devan Murphy², Abhineet Ram², Kenneth Chmiel¹, Michael Pargett², Nont Kosaisawé², Amir A. Zeki¹, and John Albeck².

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Abstract:
RATIONALE: Signaling in lung epithelial cells plays a role in respiratory disease pathogenesis. Extracellular signal-regulated kinase (ERK), Nuclear factor kappa B (NFκB), and AMP activated protein kinase (AMPK) are key kinases regulating cell growth and proliferation that are implicated in airway inflammation and disease. Importantly, ERK and AMPK display heterogenous and temporally dynamic signaling activity that can be linked to cell behavior, such as inflammatory responses, but has yet to be investigated in the context of airway disease. We hypothesize that unique signatures, composed of oscillations in short-term signaling activity (minutes-hours), differentially regulate long-term (>24 hour) inflammatory responses in part via induction of the transcription factor signal transducer and activator of transcription (STATs) at the cellular level.

METHODS: Using fluorescent biosensors and live-cell imaging, we track single-cell kinase signaling activity in our Human Bronchial Epithelial (HBE1) cell line, and primary human bronchial epithelial cells (pHBE), continuously for 26 hours, both in submerged and Air-Liquid Interface (ALI) culture conditions. Computational image analysis extracts kinase signaling activity profiles in response to growth factors (EGF), and inflammatory cytokines (IL6, IL1β, and TNFα). After 24 hours of ligand exposure, cells are fixed and immunofluorescent stain for nuclear pSTAT3 is performed to measure cellular inflammatory response (Outlined in Figure 1).

RESULTS: Comparison of HBE1’s ERK signaling activity from control and ligand stimulated cells (Figure 2A/B), reveals heterogeneous and dynamic single-cell responses to inflammatory ligands relevant to both subtypes of asthma and COPD (IL-6, IL-1β, & TNFα), that is both ligand- (Figure 2C) and concentration- (Figure 2D/E) dependent. Intriguingly, pHBE cells in submerged culture display dynamic and heterogeneous ERK signaling activity, that is similarly dependent on ligand and concentration, but are unique from those seen in HBE1 cells (Figure 2F-J).

CONCLUSION: These results support our central hypothesis and demonstrate the importance of this novel and unique approach using both airway epithelial cell lines and primary bronchial epithelial cells in ALI. Ongoing work includes: 1) Finishing data collection for ERK and NFκB in pHBE cells cultured in ALI, and AMPK activity in HBE1 cells, 2) Using statistical modeling to decompose input component parameters (such as multiple signaling pathway activities) to determine their predictive contribution to pSTAT3 activation, and 3) Assessing how pharmacological agents alter activity profiles to modulate long-term inflammatory responses in these model systems. Our technique will reveal deeper knowledge about airway epithelial kinase signaling mechanisms relevant to asthma and COPD, and potentially, other lung diseases.
Abstract #19 - Room 4 (12:05 p.m. - 12:15 p.m.)

Pre-recorded Presentation

Figure 1

A

Example Images from Live Cell ERK Activity Biosensor

B

Example Images from Live Cell AMPK Activity Biosensor

Figure 1: Overview of Study Design and Approach. (A) Left Image Panel) Human Bronchial Epithelial cells (HBE1) and Primary Human Bronchial Epithelial Cells (pHBE) in response to control and inflammatory ligands. Columns are organized by treatment header and each subfigure contains 5 representative single cell ERK activity traces over time; the bottom row represents the mean of all cells and the 25th/75th quantiles of the distribution of their signals (approximately 100-300 cells per condition). Ligand stimulation occurred where indicated by the yellow triangle, with end-point control spike(s) (10 nM EGF, 500 nM PD 123025) occurring at the time point indicated by the blue star, which are used to normalize ERK signal data (outlined in legend). (A) Demonstrates baseline HBE1 cell’s ERK signaling activity and a lack of response to an empty vehicle control spike but display activation and inhibition in response to end point controls, validating reporter function. (B) HBE1’s ERK response to 10 nM EGF displays a homogenous initial activation lasting 2-6 hours, then the cells begin displaying heterogenous signaling activity, validating canonical ERK response in HBE1 cells. (C) HBE1’s ERK unique and heterogeneous response to the combination of cytokines common to forms of metabolic asthma (10 nM IL-6, IL-1β, & TNFα), (DE) HBE1’s ERK activity displaying further heterogeneity in response to IL-6 at differing concentrations (10 and 20 nM IL-6, respectively). (F) pHBE cells, ERK displays frequent, short-term activation, even in the absence of ligand stimulation. (G) Exposure to 10 nM EGF causes multi-hour ERK activation in pHBE cells that do not return to short-term oscillations seen in HBE1 cells. (H) However, at near physiological EGF ligand (0.1 nM) concentrations, ERK signaling activity in pHBE cells resembles that of HBE1 cells. (I) pHBE ERK signaling when exposed to low dose IL-6 exhibits an inverse behavior compared to EGF, with short oscillations of activity, followed by multi-hour ERK activation. (J) The amount of time pHBE ERK signaling remains oscillatory in short bursts increases with increased IL-6 concentration, suggesting ligand abundance modifies pHBE’s ERK response as well.
Excess Lung Isoprenoids Increase Airway Hyperresponsiveness in Experimental Allergic Asthma

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RATIONALE: We previously discovered that excess pools of available mevalonate pathway metabolites farnesylpyrophosphate (FPP), geranylgeranylpyrophosphate (GGPP), farnesol (FOH), and geranylgeraniol (GGOH) augmented type 2 inflammatory responses in human airway epithelial cells relevant to asthma. We hypothesized that excess GGPP and GGOH would similarly worsen allergic eosinophilic inflammation and airway hyperresponsiveness (AHR) in vivo.

METHODS: We used the house dust mite allergen (HDMA) model (Der p) to induce acute allergic inflammation. Male Balb/c (8–10 weeks old) were sensitized and exposed to 0.5 mg/kg HDMA via the intratracheal (IT) route over 4 weeks. GGPP or GGOH (20 μM) were instilled via the IT route three times per week during the HDMA exposure protocol. We collected bronchoalveolar lavage (BAL) fluid for cell counts, blood plasma, lungs for histopathology, and measured lung function via flexiVent at increasing doses of aerosol inhaled methacholine (MCh; 2.5, 5, 10, and 20 mg/mL). We plotted dynamic lung compliance and respiratory system resistance (Rrs).

RESULTS: Preliminary results showed that our model unexpectedly induced mixed eosinophilic and neutrophilic inflammation based on bronchoalveolar lavage (BAL) cell counts. There were no statistically significant differences in BAL total inflammatory cells with either GGPP or GGOH treatment in the HDMA groups, however, there was a trend of increased airway eosinophilia with both GGPP and GGOH. GGPP caused a 2- to 4.4-fold significant increase in respiratory system resistance (Rrs) from PBS baseline in the HDMA group at 10 and 20 mg/mL MCh, with a trend of reduced Rrs in the filtered air (FA) groups. GGOH caused an increase in Rrs in both the HDMA (1.5- to 4.1-fold) and FA (2.8- to 3.3-fold) groups at 10 and 20 mg/mL MCh.

CONCLUSION: The exogenous application of airway isoprenoids GGPP and GGOH had a much greater effect on AHR than airway inflammation in a mouse model of allergic asthma. Excess flux through these active mevalonate pathway metabolites may contribute to greater airflow obstruction in asthma. Strategies aimed at antagonizing the effects of GGPP and GGOH could alleviate AHR (Figure 1).
Abstract #21 - Room 4 (12:25 p.m. - 12:35 p.m.)

Type 2 Cytokines Induce the Production of Isoprenoids in Human Airway Epithelial Cells

Kamil Borkowski¹, Kenneth Chmiel², John Newman¹, Amir A. Zeki²

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RATIONALE: We previously established a method using mass spectrometry to measure the isoprenoid alcohols FOH and GGOH in cells and tissues. The isoprenoid pyrophosphate lipids and their alcohols are critical metabolites in the mevalonate (MA) pathway important in many basic biological functions. Increased MA flux in pulmonary resident and immune cells occurs in chronic inflammation and fibrosis. We hypothesized that pro-inflammatory cytokines will induce the production of FOH and/or GGOH in human airway epithelial cells in an asthma-relevant in vitro model.

METHODS: Human bronchial epithelial cells (HBE1 cell line) were grown to 100% confluence in submerged D-media w/6 factors cell culture followed by treatment with type 2 (T2) cytokine mix (T2CM) composed of IL4, IL5, IL13 (10 ng/mL) for 10-12 hours. Cells were then trypsinized and sent for FOH and GGOH measurements using ultra-performance liquid chromatography tandem mass spectrometry (UPLC-MS/MS) after protein precipitation in the presence of deuterated surrogates (i.e. d6-farnesol) and quantified against authentic standards by electrospray ionization on an API 6500 QTrap (AB Sciex, Framingham, MA, USA) (Fig. 1).

Separated residues were detected in positive mode with the following mass transitions: geraniol (137 [M+H-18] − 95; tr = 1.1); farnesol (205.2 [M+H-18] − 121.1; tr = 2.0); geranylgeraniol (273.4 [M+H-18] − 149.1; tr = 3.8); d6-farnesol (211.2 [M+H-18] − 150.1; tr = 2.0). Analytes were quantified using 6- to 10-point calibration curves and corrected for d6-farnesol responses.

RESULTS: These data validate our previously reported method for measuring the isoprenoid alcohols FOH and GGOH using cultured human airway epithelial cells. Both Figs. 1 and 2 show in different ways that stimulation of HBE1 cells with T2 cytokines causes a significant shift from predominately FOH in quiescent untreated cells (~10 nmol/million cells) to majority GGOH (~70 nmol/million cells) in treated cells with near complete catabolism of FOH. These results show for the first time that the isoprenoid arm of the MA pathway is engaged during T2 inflammation in bronchial epithelial cells, suggesting increased flux through this pathway.

CONCLUSION: Asthma-relevant T2 inflammation in human airway epithelial cells engages the MA pathway and leads to a shift in isoprenyl alcohol metabolites from FOH to GGOH. Because FOH can be converted to GGOH via the action of GGPP synthase (which converts FPP to GGPP), these data suggest the possible involvement of this enzyme in the airway epithelium. We speculate that GGPP synthase and/or its products GGPP/GGOH may play a role in asthmatic T2 airway inflammation.
Wildfire Smoke Inhalation Activates Peripheral Blood Dendritic Cells in Healthy Subjects

Melissa J. Teuber¹, Elise A. Buser¹, Maya Juarez¹, Pedro Hernandez¹, Angela L. Linderholm¹, Mariana G. Weber¹, Tina Tham¹, Somy Cho¹, Nikita Mohapatra¹, Timothy E. Albertson¹, Satya Dandekar¹, Angela Haczku¹

¹ UC Davis

RATIONALE: Wildfire smoke contains inhalable toxic gaseous and particulate matter that is associated with detrimental health effects yet there is no quantifiable outcome of wildfire smoke inhalation on the immune system available. We hypothesized that wildfire smoke exposure activates a dendritic cell response.

METHODS: Subject recruitment was coordinated by the clinical trial team of the UC Davis Lung Center. 23 age (18-65 years old) and sex matched healthy subjects were recruited from 2020 Aug 23rd- Sept 3rd (Visit 1), during the peak time of potential smoke exposures from multiple complex fires. Visit 2 took place 2 months later, when the wildfire smoke subsided. Peripheral blood was collected at both visits in an unidentifed fashion. Mononuclear cells were isolated by density gradient centrifugation and stained for Dendritic Cell (DC) membrane markers with fluorescent conjugated antibodies. To study cell activation, multicolor characterization was performed using standard flow cytometric gating strategies. FACS data was analyzed by FlowJo®. Statistical analysis was performed using Wilcoxon matched-pairs signed rank test (Prism).

RESULTS: The wildfires raging around the Sacramento area resulted in a mean AQI (air quality index) of 113 Between 2020 Aug 23rd and Sept 3rd. Although smoke exposure gradually decreased, during the time between visits (the end of Visit 1 and end of Visit 2) there was still an average AQI of 77. There was no difference in total blood cell count between visits. However, subjects in Visit 1 had a significantly decreased proportion of plasmacytoid Dendritic Cells (pDC) [CD45+ HLA-DR+ CD11c- CD123+] in the circulation, compared to their Visit 2 counts (p<0.05). Meanwhile, the numbers of myeloid lineage conventional type 1 or type 2 Dendritic Cells (cDC1, cDC2) [CD45+ HLA-DR+ CD11c+ CD141+] [CD45+ HLA-DR+ CD11c+ cDC1+ CD16-] did not change significantly. However, wildfire smoke exposure (Visit 1) significantly increased CCR4 and CD40 (but not CCR7) expression (p<0.05) on cDC2.

CONCLUSION: The increase in expression of the chemokine receptor CCR4 suggests increased migration of cDC2 cells to the peripheral tissues. cDC2 cells play a potential role in increased sensitization to allergens and could promote development of lung disease.
Abstract #22 - Room 5 (12:45 p.m. - 12:55 p.m.)
Pre-recorded Presentation [CLICK HERE]

Migratory cDC2 cell activation in peripheral blood of healthy volunteers

- pDC ([]):
  - Visit 1: Orange dots
  - Visit 2: Yellow dots

- Migratory cDC2 ({}):
  - Visit 1: Brown bars
  - Visit 2: Yellow bars

Significance:
- $P=0.0075$
- $P=0.01$
- $P=0.0002$
The role of airway epithelium in inflammation caused by exposure to an environmental pollutant

Authors: Christine Licata, MD; Angela Haczku, MD, PhD
University of California, Davis, School of Medicine, Department of Internal Medicine

Introduction: Asthma develops as a result of aberrant T-helper type 2 (Th2) and Th17-like immune responses. The underlying role of epithelial cells in the inflammatory changes is unclear. The Haczku lab has previously demonstrated that ozone induces asthma-like airway changes in a mouse model. We hypothesized that lung epithelial gene expression plays a role in environmental exposure-induced airway changes.

Methods: We utilized a model in mice exposed to ozone. The animals received ozone exposure (2 parts per million) for 2 hours. One group of n=4 were studied each 2, 6, 12, 24, 48 and 168 hours later. Total RNA was extracted from lungs and processed by an Affymetrix chip array to assess gene expression. The data were analyzed by a gene enrichment analysis.

Results: The gene enrichment data revealed time dependent expression of differentially expressed gene sets in the lungs of mice. The figure below shows the activation of IL-17-, GM-CSF-, Chemokines and cell adhesion-related, IL-1 beta- and IL-6-related signaling pathways. The time course of the changes indicates very early activation of these pathways supporting the role of airway epithelial cells in the onset of airway inflammation.

Conclusions: Through these cytokine signaling pathways airway epithelial cells can influence activation of innate lymphoid and dendritic cells which are not only central in bridging innate and adaptive immune responses, but proposed as master regulators of allergic inflammation. Activation of the IL-17 signaling pathway as the earliest one in response to ozone inhalation suggests the involvement of innate immune mechanisms in regulating airway inflammation.
Impact of Ergothioneine Pretreatment on Naphthalene Toxicity in the Mouse Lung

VJ Brown,1 L Ding,2 L Yin,2 PC Edwards,1 X Ding,2 LS Van Winkle1

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Rationale: Naphthalene (NA) is an abundant volatile aromatic hydrocarbon found in wildfire smoke and vehicle exhaust. NA causes dose-dependent Club cell toxicity in the conducting airway epithelium of mice. Despite widespread exposure, few studies have searched for protective agents to limit NA toxicity. Ergothioneine (ET) has been shown to function as a cytoprotectant against oxidative stress in several systems. It is not synthesized in the body and is bioavailable solely through the diet. The organic cation transporter novel type-1 (OCTN1), encoded by the gene SLC22A4, is responsible for the uptake of cellular ET in tissues. **Hypothesis:** ET pretreatment can reduce NA-induced lung epithelial cell damage.

Methods: Adult (2-3 months) male C57BL/6J mice (n=5/group) were fed an ET-free diet throughout gestation and the postnatal period. For five consecutive days, mice were treated with 70 mg/kg (via oral gavage, once daily) of ET, or saline (SA; vehicle). On day 8, the mice were injected i.p. with a single dose of 50 mg/kg, 100 mg/kg, or 150 mg/kg of NA in corn oil (CO) or CO alone. At 24 hours post-NA injection, the lung, and other tissues were collected for analysis.

Results: High-resolution histology of lung tissues showed that ET-treated mice were partially protected from NA-induced Club cell vacuolization and toxicity, in comparison to mice treated with NA only. ET/NA treated groups had a relatively higher gene expression of Club cell secretory protein (CCSP; Secretoglobin 1A1) than SA/NA groups. In addition, NA exposure at a low dose (50 mg/kg) caused an increase in CCSP levels (ET/NA vs ET/CO, p=0.044); but at higher doses, NA exposure appeared to cause a decrease in CCSP levels. The gene expression of SLC22A4 showed an increasing trend in groups exposed to NA. Lastly, immunohistochemistry demonstrated a parallel increase in OCTN1 protein distribution and abundance in the large airways and terminal bronchioles of NA-treated mice, compared to controls. The concentration of NA and ET present in tissues was measured using HPLC/MS.

Conclusion: It is suggested that ET not only functions as a cytoprotectant in the lungs but may also function to upregulate CCSP expression in the presence of low levels of NA induced oxidative stress. Increased expression of OCTN1 transporter may facilitate cellular uptake of ET upon exposure to NA. The results support that ET pretreatment is protective against NA induced lung damage. **Supported by NIH grant R01 ES020867 and T32 ES007059.**
A Novel Function of the MARCKS/AXL Axis in Tobacco Smoking-Mediated Macrophage Polarization and Pulmonary Fibrosis

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Rationale: Macrophages with a M2 (pro-fibrotic) phenotype have been shown to be implicated in the pulmonary fibrosis (PF) and smoking exposure. However, the mechanisms of macrophage polarization into M2 in PF remain unclear. We hypothesize that tobacco smoke exposure polarizes macrophages towards an M2 phenotype consequently driving a pro-fibrotic environment in the lung. Herein, we aimed to elucidate how macrophage polarization is regulated in response to tobacco smoke, and its functional consequence in fibrotic progression.

Methods: Both macrophage cell lines and primary macrophages were utilized for a 3D co-culture system. A phospho-receptor tyrosine kinase array screen was used to assess smoke-specific signaling pathways. Genetic manipulations and pharmacologic inhibition of the signal pathways were achieved by siRNA knockdown and target-specific inhibitors. The bio-functionality of the signaling axis in mediating macrophage polarization and promoting fibrosis was assessed through Western Blots, real-time reverse transcription polymerase chain reaction (RT-qPCR), flow cytometry, ELISA cytokine assays, immunohistochemistry, and immunofluorescent microscopy.

Results: Data from multicolor flow cytometry find that markers of M2 polarization in both human and mouse macrophages were elevated in response to tobacco smoke exposure and this elevation is concomitant with a decrease of M1 markers. Using a receptor tyrosine kinase array screen, we identify that the AXL receptor is a novel smoke-responsive molecule. Upregulated GAS6 secretion and subsequent activation of AXL receptor was noted in MARCKS-expressing cells through ELISA and RT-qPCR. We further demonstrate the GAS6-AXL-STAT3 signaling circuit was enhanced by smoke-induced MARCKS phosphorylation. Multiple primary lung fibroblast cells displayed higher levels of myofibroblast markers as well as upregulation of cell proliferation, invasion and migration in a 3D co-culture system. Targeting of this signaling axis through pharmacologic or genetic attenuation of MARCKS, attenuates the MARCKS/AXL signaling pathway and decreases M2 markers and cytokines, leading to inactivation of fibroblast cells and suppression of lung fibrosis.

Conclusion: Our findings suggest that the MARCKS/AXL signaling axis is a potential target in tackling macrophages and stopping smoke-mediated pulmonary fibrosis.

This abstract is funded by: NHLBI R01HL146802, and UCOP grants Tobacco-Related Disease Research Program (28IR-0061, T31DT1849 and 27KT-0004).
Arginine deprivation as a therapeutic approach for argininosuccinate synthase 1 (ASS1)-deficient pulmonary fibrosis

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Rationale: Metabolic reprogramming has been reported to be associated with pathogenesis of idiopathic pulmonary fibrosis (IPF). Our integrated metabolomics of the pathway alterations underlying IPF has uncovered dysregulation of the arginine pathway in IPF progression. Of these metabolic enzymes, argininosuccinate synthase 1 (ASS1) serves as a major enzyme in the biosynthesis of arginine; however, the role of ASS1 in lung fibrosis remains largely unknown. We aimed to investigate the expression of ASS1 in lung tissues and its contribution to IPF.

Methods: ASS1 expression in multiple lung fibroblasts and tissues was determined by molecular assays. Next, we genetically manipulated ASS1 to verify the functionality of ASS1 and its molecular pathways. We also tested the therapeutic potential of arginine starvation in fibroblast growth and bleomycin-induced pulmonary fibrosis.

Results: We observed a decrease of ASS1 expression at both transcriptional and translational levels in lung fibroblasts isolated from IPF patients as compared to normal lung fibroblasts. Reduced ASS1 protein expression was noted in fibrotic lung tissue specimens from IPF patients and inversely correlated with IPF status. Genetic manipulations of ASS1 studies confirm that ASS1 expression inhibited fibroblast cell proliferation, migration, and invasion. In reverse phase protein arrays complemented with a phospho-kinase array screen, we show that Met receptor was activated and acted upstream of Src and STAT3 signaling in ASS1-knockdown fibroblasts. Interestingly, arginine-free conditions using arginine-free media or arginine deiminase (ADI) were found to kill ASS1-deficient IPF fibroblasts. Both arginine-free diet and ADI treatment effectively decreased fibrotic lesions and levels of hydroxyproline in bleomycin-exposed lungs. In addition, arginine deprivation synergistically increased nintedanib efficacy in IPF lung fibroblasts.

Conclusion: These data suggest that aberrant downregulation of ASS1 is correlated with IPF in patients and arginine deprivation of ASS1-deficient fibroblasts may present a novel strategy as a therapy for pulmonary fibrosis.
Inhalation of Silver Silicate Nanoparticles Leads to Translocation from the Nose to the Olfactory Bulbs Without Significant Injury but Transient Microglial Activation

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RATIONALE: Engineered silver nanoparticles (Ag NP) are used in a wide variety of medical and consumer applications. Inhaled Ag NP has been found to translocate to the brain via the olfactory epithelium (OE) to elicit microglial activation. However, it is unclear whether inhaled 1% silver silicate (1% AgSiO₂), an encapsulated form of silver, can be translocated to the olfactory bulbs (OBs) to elicit microglial activation as noted with other Ag NP. We hypothesized that inhaled 1% AgSiO₂ will translocate to the OB through retrograde transport via OE to elicit microglial recruitment and activation.

METHODS: Male and female, nine-weeks old, Sprague-Dawley rats were exposed to filtered air (n=30) or aerosolized 1% AgSiO₂ NP (20nm) for 6 hours (n=42). Animals were examined on day 0, 1, 7, 21, and 56 post-exposure. Aerosol characterization was made by collecting air samples during the six-hour exposure period. NP concentration was determined gravimetrically, silver content by X-ray fluorescence (XRF), particle aggregate diameter by cascade impactor, and particle size by transmission electron microscopy (TEM). OBs were harvested, fixed in 4% paraformaldehyde, and embedded in paraffin in sagittal orientation. Immunohistochemistry with anti-ionized calcium binding adapter molecule-1 was done on 10-μm-thick sections, which permitted more complete visualization of microglial extending processes, to identify the site and state of microglial activation in the OBs.

RESULTS: Aerosol characterization by gravimetry demonstrated an average AgSiO₂ aerosol mass of 4.9+/−2.3 mg/m³, Ag concentration by XRF 5.6+/−2.0 mg/m³, aerosol diameter by cascade impactor 1.9+/−0.3 μm, and size distribution of AgSiO₂ by TEM from 21 to 370 nm in diameter. Morphometric analysis of microglia in the OBs demonstrated no significant difference in microglial abundance between sham control versus any post-exposure time point. There was a statistically significant change in the ratio of activated to resting microglia, with elevated ratios noted on day 1 and day 7.

CONCLUSION: Aerosol characterization indicated that 1% AgSiO₂ NP were sufficiently aerosolized with moderate agglomeration and high efficiency of deposition in the OE. Findings suggested deposited NP translocated rapidly to the brain following aerosol inhalation with prolonged retention, elicited transient microglial activation without significant microglial recruitment to the OB. The absence of significant microglial recruitment suggested a lack of robust inflammatory response in the OB and could be explained by a single inhalation exposure as well as the chemical stability of 1% AgSiO₂ NP, conferred by the silicate shell, delaying oxidation of NP.

Research support: NIEHS U01 ES027288
Pulmonary Response in Sprague Dawley Rats following Single Exposure to Aerosolized Graphene Oxide

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¹Center for Health and the Environment and ²School of Veterinary Medicine, University of California, Davis, ³Harvard University School of Public Health, Boston, MA

**Rationale:** Graphene oxide (GO) is a two-dimensional (2-D) nanomaterial which is widely used in drug delivery systems, biosensors, medical imaging, electronics, and energy storage. As a result of increased commercial use, there is a potential risk of inhalation of aerosolized materials including planar 2D nanomaterials affecting human health. GO nanoparticles have been reported to cause toxic effects by dermal, ingestion and inhalation routes of exposure. However, there are limited studies on effects of GO on human health via inhalation, which could be a primary route of exposure in the workplace. The **hypothesis** to be tested in this study is acute inhalation of 2-D GO results in an acute and transient inflammation of the respiratory tract. **Methods:** Male and female Sprague Dawley rats eight weeks of age were divided into sham control (n=12) or GO exposed groups (n=24). Exposure to aerosolized GO (400nm x 400nm) was for a single 6-hour period in a nose-only inhalation chamber to attain optimal exposure. Particle aerosol was characterized using gravimetric measurements, cascade impactor analysis and transmission electron microscopy. Animals were examined on days 1 and 7 post-exposure. Bronchoalveolar lavage (BAL) was collected from the right lung to assess protein concentration, cell number, viability, and cell differentials. The left lung was inflation-fixed, embedded and sectioned for histopathological analysis. **Results:** Average GO aerosol mass was 2.28 +/- 0.73 mg/m³. The mass median aerodynamic diameter of the aerosol was 2.37 µm. No statistically significant differences were observed for cell differentials, cell number, cell viability or protein concentration between the control and exposed animals for both male and female rats. **Conclusion:** Hence, the study suggests GO has no acute pulmonary toxicity in rats at the given concentration and duration of exposure following a single day.
TITLE: Differences in Lung Cell Type Susceptibility to Engineered Nanomaterials

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RATIONALE: Nanomaterials are widespread and diverse yet understanding of their impact on biological systems is still emerging. An on-going screening process for engineered nanomaterials (ENMs) focused on characterizing potential for toxicity via two main aerosol exposure routes: ocular and respiratory. Initial in vitro screening efforts demonstrated hexagonal boron nitride (HBN) and cadmium sulfide (CdS) are cytotoxic to corneal epithelium; this was also true for primary mouse tracheal epithelium at high doses (mTEC; 250 ug/mL). The lung has diverse cell types, however, and small particles like nanomaterials are capable of depositing deeper in the respiratory tract where alveolar epithelium dominates. Given the heterogeneity of cell types in the lung by species and region, as well as the known differences in primary cell responses and cell lines, we tested whether there are divergent responses to nanoparticles between mTEC and human lung alveolar epithelial cells (A549).

METHODS: To assess respiratory cell-type differences, mTECs were grown in vitro on air-liquid interface and exposed to HBN and CdS. A549 cells were cultured in vitro on adherent plates and exposed. ENM exposures lasted for 24 hours, followed by staining and fixation. Because assays for measuring cell number metabolically are prone to particle interference, cytotoxicity was determined by fluorescent microscopy using differential permeability to nuclear dyes and direct cell counting. For both HBN and CdS, a dose response (10, 25, 50, 100 ug/mL) was conducted in two cell types: mTECs and A549 cells. To evaluate if ENM presence is required for inhibition of wound healing, cells were exposed for the initial 2 hours of a 24-hour incubation window.

RESULTS: HBN was cytotoxic to A549 cells at 50 ug/mL and 100 ug/mL, but not to mTECs. HBN (100 ug/mL) also caused morphological changes in the A549 cells. HBN (100 ug/mL) was shown to inhibit cell migration 24-hours later, but continuous ENM presence appears to be required for the inhibitory effect. CdS did not significantly inhibit wound healing at any of the sub-cytotoxic doses.

CONCLUSIONS: Cytotoxicity results suggest A549 cells possess increased susceptibility to HBN. This was in contrast to the expected results, as A549s are an immortalized cell line, and were anticipated to be more resilient in the face of ENM exposure. Based on cellular responses, we can conclude A549 cells require a lower dose than mTECs of HBN to trigger cytotoxicity. Supported by U01ES027288 and T32HL007013.
Abstract #30 - Room 6 (1:15 p.m. - 1:25 p.m.)
Pre-recorded Presentation CLICK HERE

Feraheme Iron Oxide Magnetic Nanoparticles Inhibit Neutrophil Inflammatory Response and Recruitment.

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¹Department of Biomedical Engineering, University of California, Davis

Rationale: Magnetic nanoparticle (MNP) based therapeutics and diagnostics have seen increased research interest and clinical use. The iron oxide MNP drug Feraheme® is an FDA approved treatment for iron deficiency anemia in adult patients with chronic kidney failure. Despite increased use of MNP there is a current lack in understanding of how interactions of intravenously injected MNP with neutrophils, the most numerous leukocytes in blood circulation, could affect the carefully controlled activation and recruitment cascades that are vital for proper immune function in the microvasculature. In this study the immunomodulatory effects of Feraheme® on neutrophils was investigated.

Materials and methods: Changes in neutrophil surface expression of the adhesion molecules CD62L, CD11B, overall CD18, and high affinity (HA) CD18, in response to stimulation over a dose range with the chemokine IL-8 in the presence of Feraheme was determined through flow cytometry. The effect of Feraheme on neutrophil rolling and arrest on endothelial adhesion ligands E-Selectin and ICAM-1 was quantified using a vascular mimetic flow chamber. The fluorescent calcium indicator Fluo-4 was utilized to study the effects of Feraheme, on neutrophil calcium flux after stimulation with IL-8 (1nM).

Results and discussion: Feraheme inhibited neutrophil activation by decreasing sensitivity to IL-8 leading to a rightward shift in the IL-8 dose-response curves of the tested activation markers (Figure 1). Neutrophils rolling over a substrate of E-selectin and ICAM-1 under shear flow showed increased rolling velocities and inhibited arrest and shape change in the presence of Feraheme. Lastly, in the presence of Feraheme the rate of calcium clearance after IL-8 induced calcium flux was accelerated.

Conclusions: The results indicate that Feraheme inhibits neutrophil activation through IL-8 and recruitment under shear flow. Accelerated clearance of calcium in the presence of Feraheme is a possible mechanism for the inhibition of IL-8 induced changes in adhesion marker expression and for the inhibition of recruitment under shear flow.
Abstract #31 - Room 6 (1:25-1:35)  
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**Title:** Peripheral blood mononuclear cell mitochondrial bioenergetics is associated with aerobic capacity and muscle performance in patients with chronic kidney disease

**Authors:** Armin Ahmadi\textsuperscript{1}, Jennifer E. Norman\textsuperscript{1}, Gwenaelle Begue\textsuperscript{3}, Chenoa R. Vargas\textsuperscript{1}, Usman Rehman\textsuperscript{1}, Tae Youn Kim\textsuperscript{1}, Henning Langer\textsuperscript{1}, Thomas Jue\textsuperscript{1}, Jorge Gamboa\textsuperscript{2}, Baback Roshanravan\textsuperscript{1}

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**Rational**

Patients with CKD suffer from skeletal muscle dysfunction and impaired physical performance. Impaired muscle and systemic mitochondrial metabolism are central candidate mechanisms of skeletal muscle impairment in CKD. Live peripheral blood mononuclear cells (PBMC) mitochondrial bioenergetics may link altered metabolism in CKD with exercise intolerance. The association of PBMC bioenergetics with physical performance in CKD is unknown.

**Methods**

We recruited 13 participants with eGFR<60 ml/min/1.73m\textsuperscript{2}. Cardiopulmonary fitness (absolute VO\textsubscript{2} peak), total work performed, and work efficiency were measured using COSMED K5 wearable metabolic system during cycle ergometry testing. PBMC bioenergetics analysis were performed using the high resolution respirometry (Oroboros O2k). PBMC oxygen consumption rate was measured with sequential additions of pyruvate, oligomycin, FCCP, and antimycin A. We estimated basal, maximal uncoupled respiration (MUR) and spare respiratory capacity (SRC). SRC was defined as the difference between basal respiration and MUR. Pearson correlation coefficient was used to assess correlation of PBMC bioenergetics with muscle performance.

**Results**

The mean age of participants was 60.6 +/- 9.5 years, eGFR was 35 +/- 12.5 ml/min/1.73m\textsuperscript{2} and 53% were females. PBMC MUR correlated with total work (r=0.57, \textit{P}-value=0.041) and efficiency (absolute) (r=0.58, \textit{P}-value=0.034). PBMC SRC correlated with total work (r=0.58, \textit{P}-value=0.036) and efficiency (r=0.60, \textit{P}-value=0.029). VO\textsubscript{2} peak correlated with PBMC basal respiration (r=0.56, \textit{P}-value=0.044), MUR (r=0.69, \textit{P}-value=0.007), and SRC (r=0.71, \textit{P}-value=0.006).

**Conclusion**

These results suggest that PBMC respiration is strongly associated with exercise capacity and efficiency. Further studies are needed to investigate biologic determinants of PBMC bioenergetic health and its validity as a surrogate marker of skeletal muscle metabolic health in CKD.
Figure 1. Association of PBMC reserve capacity with A) total work efficiency and B) cardiorespiratory function (VO2 peak).

A

Work efficiency (KJ/mL/min)

r=0.6036
p value=0.0282

Reserve Capacity (pmol/min/million cells)

B

VO2 Peak (mL/min)

r=0.7101
p value=0.0065

Reserve capacity (pmol/min/million cells)
Abstract #32 - Room 7 (12:45 p.m. - 12:55 p.m.)

Pre-recorded Presentation CLICK HERE

Title: Identifying Opportunities in COPD Disease Management

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Department of Pulmonary and Critical Care Medicine
University of California Davis

Background: Therapies beyond inhaled medications have been demonstrated beneficial in patients with advanced COPD with history of exacerbations. Pulmonary rehabilitation (PR) benefits COPD patient healthcare quality-of-life and dyspnea, while PDE-4 inhibitors (e.g. roflumilast) and prophylactic macrolide (most commonly azithromycin) use may offer benefits in reducing exacerbations. Typically, referrals to PR or additions of PDE-4 inhibitors and macrolides are considered and implemented by pulmonologists and/or those with subspecialty expertise in the treatment and management of COPD.

Methods: Three clinicians retrospectively reviewed the electronic medical record of 96 patients hospitalized in 2019 with a diagnosis of COPD. Reviewers wanted to glean whether there were opportunities to improve care in patients who were primarily hospitalized for an exacerbation of their COPD. Reviewers evaluated hospital admission diagnosis, diagnostic testing, and therapies aimed at reducing exacerbations, and referrals or visits to outpatient pulmonary clinics, PR, and Palliative Care. Exemption was obtained through the University of California, Davis IRB #1759094-1.

Results: Of the 96 reviewed charts, 19 were excluded (20%) due to an incorrect COPD diagnosis and 12 (12%) were excluded due not having a PCP within the health system, leaving an N=65. Of these 65 patients, 28 (45%) had a least one COPD related admission in 2019. Two (7%) of the 28 COPD related admission patients had been prescribed roflumilast or chronic azithromycin treatments for the prevention of exacerbations. Four (14%) of the patients had been previously referred to PR, with 3 (11%) of them reaching and completing the program. Nine (32%) patients had at least one pulmonary outpatient visit in 2019. None of the hospitalized patients had been referred to palliative care.

Conclusions: Multiple opportunities for exacerbation prevention were missed in our cohort of the highest risk COPD patients. Guidelines recommend timely interventions to reduce the risk of COPD disease progression and exacerbations, but these often do not penetrate the sickest population of patients frequently admitted. Referrals to programs and disease specific clinics should be considered early in COPD but especially in patients with history of exacerbations.
Abstract #33 - Room 7 (12:55 p.m. - 1:05 p.m.)
Pre-recorded Presentation CLICK HERE

Asthma-COPD Overlap (ACO): Key Differences Between Smokers and Non-Smokers

AUTHORS: A.S. Matthys¹, J.T. Zumba¹, M. Wilson², A.A. Zeki¹

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ABSTRACT

Rationale: Asthma-COPD overlap (ACO) is an important clinical entity that has implications for patient management. Despite what has been published, we know very little about the subgroup of ACO patients without a significant prior smoking history. We hypothesize that among ACO smokers versus non-smokers, there would be a significant difference with respect to clinical presentation and objective parameters such as lung function, biomarkers, and response to therapy.

Methods: This project was approved by the U.C. Davis IRB. From a sample of 426 patient seen at UC Davis's Asthma and COPD clinics, we performed a cross-sectional analysis of 158 ACO patients defined based on GINA criteria and who met study criteria. Of the 158 subjects, 100 were classified as smokers and 58 as non-smokers (smoking defined as >10 pack-year history). We collected data including demographics (ethnicity, BMI, age, sex, etc.), comorbidities, inhaled controller medication use, prednisone use, frequency of corticosteroid use, biologics use, ACT and CAT scores, fraction of exhaled nitric oxide (FeNO), PFT, biomarkers (IgE, RAST, hCRP, IL-6, peripheral blood eosinophil counts, fasting lipids, ANA positivity), and radiographic findings. For qualitative and quantitative variables we used both the Fisher’s exact test and mean t-test analysis, respectively. For multiple variables analyses we used a Benjamini and Hochberg analysis to adjust for repeated testing and false discovery rates.

Results: ACO smokers had significantly greater emphysema on imaging, greater degree of mild to moderate hyperinflation (TLC) or air-trapping (RV), and significantly lower diffusion capacity (DLCO, DLVA) than non-smokers. However, ACO non-smokers had a significantly higher prevalence of biologic medication use than ACO smokers (2.9-fold, raw p-value 0.006). Interestingly, there was a trend of higher prevalence of autoimmune disease in ACO non-smokers than smokers (2.4-fold higher, raw p-value 0.07). Fasting lipid levels (total cholesterol and LDL-cholesterol) were greater for ACO smokers than non-smokers (raw p-value p=0.04 and p=0.05, respectively). There were no statistically significant differences in co-morbidities, markers of type 2 (T2) inflammation (FeNO, peripheral eosinophilia) or other biomarkers, symptom control scores (ACT, CAT), or prednisone use. Table 1 below summarizes our key findings.

Conclusions: Our study revealed that ACO non-smokers are more likely to be treated with a biologic medication than ACO smokers. The greater prevalence of biologic medications use in ACO non-smokers indicates that these patients may be more severe than ACO smokers or that they are more likely to have T2 inflammation. Since the indications for biologic medication use include patients who require frequent oral prednisone therapy, have continued severe symptoms despite inhaler therapy or prednisone use, and/or who have two or more acute exacerbations per year, the non-smoking population may be a more severe group. The trend of a higher prevalence of autoimmune disease in ACO non-smokers is very interesting and may indicate a unique disease phenotype. Further research is needed to investigate this possibility further.
### Abstract #33 - Room 7 (12:55 p.m. - 1:05 p.m.)

**Pre-recorded Presentation** [CLICK HERE](#)

<table>
<thead>
<tr>
<th></th>
<th>ACO Non-smoker (Mean ± SD or %)</th>
<th>ACO Smoker (Mean ± SD or %)</th>
<th>Raw p-value</th>
<th>Adaptive FDR</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age</strong></td>
<td>67.10 ± 14.06</td>
<td>70.47 ± 9.81</td>
<td>0.08</td>
<td>0.22</td>
</tr>
<tr>
<td><strong>CAD</strong></td>
<td>29.31%</td>
<td>43.00%</td>
<td>0.09</td>
<td>0.24</td>
</tr>
<tr>
<td><strong>Autoimmune</strong></td>
<td>18.97%</td>
<td>8.00%</td>
<td>0.07</td>
<td>0.22</td>
</tr>
<tr>
<td><strong>Emphysema</strong></td>
<td>23.53%</td>
<td>48.39%</td>
<td>0.004*</td>
<td>0.03*</td>
</tr>
<tr>
<td><strong>Biologic</strong></td>
<td>25.86%</td>
<td>9.00%</td>
<td>0.006*</td>
<td>0.03*</td>
</tr>
<tr>
<td><strong>FEV1 Pre-Bronchodilator (BD) (L) (%)</strong></td>
<td>1.66 ± 0.63 (60.26%)</td>
<td>1.51 ± 0.68 (57.31%)</td>
<td>0.18; 0.39</td>
<td>0.34; 0.46</td>
</tr>
<tr>
<td><strong>FEV1 Post-BD (L) (%)</strong></td>
<td>1.83 ± 0.62 (66.62%)</td>
<td>1.62 ± 0.64 (63.01%)</td>
<td>0.08; 0.36</td>
<td>0.22; 0.46</td>
</tr>
<tr>
<td><strong>FVC Pre-BD (L)</strong></td>
<td>2.99 ± 1.01</td>
<td>2.90 ± 1.00</td>
<td>0.60</td>
<td>0.63</td>
</tr>
<tr>
<td><strong>FEV1/FVC Pre-BD (%)</strong></td>
<td>58.15% ± 13.53</td>
<td>53.96% ± 16.80</td>
<td>0.11</td>
<td>0.28</td>
</tr>
<tr>
<td><strong>TLC (L) (%)</strong></td>
<td>5.39 ± 1.50 (95.32%)</td>
<td>5.99 ± 1.44 (104.05%)</td>
<td>0.02*; 0.004*</td>
<td>0.10; 0.03*</td>
</tr>
<tr>
<td><strong>RV (L) (%)</strong></td>
<td>2.42 ± 0.83 (119.69%)</td>
<td>2.90 ± 1.04 (136.42%)</td>
<td>0.006*; 0.03*</td>
<td>0.04*; 0.16</td>
</tr>
<tr>
<td><strong>DLCO (mL/mmHg/min) (%)</strong></td>
<td>17.36 ± 4.98 (69.27%)</td>
<td>15.27 ± 5.95 (64.78%)</td>
<td>0.04*; 0.14</td>
<td>0.16; 0.3</td>
</tr>
<tr>
<td><strong>DLVA (mL/mmHg/min/L) (%)</strong></td>
<td>4.20 ± 0.65 (96.13%)</td>
<td>3.62 ± 1.02 (84.71%)</td>
<td>0.0004*; 0.004*</td>
<td>0.008*; 0.03*</td>
</tr>
<tr>
<td><strong>Total Cholesterol</strong></td>
<td>176.58 ± 49.03</td>
<td>193.15 ± 44.53</td>
<td>0.05*</td>
<td>0.16</td>
</tr>
<tr>
<td><strong>LDL Cholesterol</strong></td>
<td>99.35 ± 36.34</td>
<td>113.57 ± 38.64</td>
<td>0.04*</td>
<td>0.16</td>
</tr>
</tbody>
</table>
Title: Clinical Registry Infrastructure and Early Data from UCD Comprehensive COPD Clinic

Authors:
Daniel Tompkins, CCRP, Krystal Craddock, RRT, MS, Tina Tham, CCRP, Maya Juarez, CCRP, Jimmy Nguyen, RRT, Marcy Dolan, RRT, Michael Dirks, RRT, Karla Ramirez, BS, Michael Schivo, MD, MAS, Brooks Kuhn, MD, MAS

Rationale:
Chronic obstructive pulmonary disease (COPD) is a leading cause of morbidity and mortality worldwide. Efforts to improve outcomes in this population have been uneven and inadequate compared to other prevalent diseases, in part due to the numerous and heterogeneous manifestations of COPD. Precisely identifying the endotype and phenotype is paramount to guide personalized treatment and conduct research on subcohorts likely to benefit from targeted interventions. At the founding of the UC Davis Comprehensive COPD Clinic (CCC)--a part of the ROAD program--, a clinical registry was developed with robust clinical staff and database infrastructure support. We present our early data describing the UCD CCC cohort and our clinical registry infrastructure.

Methods:
IRB approval obtained for clinical registry (IRB #1759094). Beginning January 2018, patients presenting to the CCC were enrolled in our registry. Consented participants' data was extracted by clinical research coordinators through review of standardized physician notes (e.g., defined data definitions of all elements, standardized responses), which allowed for higher fidelity data input for non-discrete data. Our REDCap database was populated with demographics, therapies, exposures, comorbidities, and spirometry data.

Results:
A total of 326 patients were enrolled in the COPD registry with 240 currently in the REDCap database. Our population identifies 46.9% female. Severity is high as expected: on initial visit, 14.2% were listed GOLD group A, 41.3% Group B, 3.6% group C, and 35.1% Group D with symptom burden assessment showing CAT score: mean 20.39 (+/- 8.56) and mMRC score distribution was 5.3% 0, 24.0% 1, 26.2% 2, 22.7% 3, 21.8% 4. Phenotypes include 51% predominantly emphysema without exacerbations, 39.5% ACOS, and 23.7% chronic bronchitis. Mean of 1.42 exacerbations per year (SD 1.93) with 67.2% of patients having prior exacerbations, of which 81.4% required hospitalization or emergency department visit and 8.3% intubated. Alpha-1 phenotype is known for 91.7% of our cohort. Regarding controller medications, 58.4% were initially in ICS, 77.1% on LABA, 70.3% on LAMA. Only 13.4% of patients previously on PDE4 inhibitor, 12.5% on chronic oral steroids, 9.5% on chronic macrolides. A total of 37.5% on supplemental oxygen. A total of 195 pulmonary function tests are available, with mean FEV1 57.6 (SD +/- 22.1), RV 133% (SD +/- 51.6), and DLCO 54.7 (SD +/- 18.7).

Conclusions:
We have developed a robust process combining our clinical efforts to personalize care with a clinical registry including endotype and phenotype data. The physician note structure allows dependable translation of clinical data into the registry. The REDCap database allows superior security, ability to share deidentified data, and limits errors in data entry. We are in the process of automating discrete data export from the electronic health record into our database (and that of a division-wide data mart). We invite collaboration with local basic science and clinical researchers to leverage this defined population.

Disclosures: None
Function of glucocorticoid receptor in airway epithelial cells during oxidative stress
Pedro A. Hernandez*, Duane Kim*, Chioma Enweasor*, Zhilong Jiang, Cameron H. Flayer, Angela Linderholm, Lisa Franzi, Angela Haczku
University of California, Davis
*Equally contributed

RATIONALE: Glucocorticoid insensitivity may be elicited by oxidative stress such as the one caused by exposure to the air pollutant, ozone (O₃). We previously showed that expression of both pro-inflammatory and antioxidant genes is altered with O₃-induced airway hyperreactivity in mice. Although the relationship of oxidative stress to the anti-inflammatory effects of glucocorticoids is unclear.

METHODS: Glucocorticoid receptor (GR) protein levels and GR DNA binding was studied in A549 and HBE cells. A549 cells were incubated in serum-free media for 6 hours and to mimic the effects of O₃, cells were treated with 0.05mM tert-butyl hydroperoxide (TBHP) and treated with 0, 10, or 100nM dexamethasone for 2hrs. TBHP was then replaced with serum-free media with 0, 10, or 100nM dexamethasone for 1 hour to complete dexamethasone treatment. Expression of Eotaxin3, Sod1, and Sod2 mRNA were measured by qPCR.

RESULTS: GR and Eotaxin3 expression showed circadian oscillation in both A549 and HBE cells. Eotaxin3 negatively correlated with the GR DNA binding activity (r=-0.75, p<0.05 n=10). Dexamethasone inhibited Eotaxin3 expression in a dose dependent fashion. TBHP did not increase A549 cell death at the concentrations studied but induced expression of Eotaxin3 mRNA. TBHP (0.05mM) abolished the inhibitory effect of dexamethasone on Eotaxin3 in A549 cells. Interestingly, dexamethasone increased Sod1 and Sod2 gene expression when under oxidative stress.

CONCLUSIONS: Our data suggest that glucocorticoid inhibition of Eotaxin3 gene expression may be counteracted by the presence of oxidative stress. The mechanism of Sod1 and Sod2 mRNA regulation by the GR receptor requires further study.
The Statin Drugs Potentiate β2-Agonist-Induced Relaxation of Human Airway Smooth Muscle

Niccole Schaible, Kenneth Chmielek, Ramaswamy Krishnan, Amir A. Zeki

Beth Israel Deaconess Medical Center, Dept of Emergency Medicine, Harvard University
U.C. Davis, Dept of Internal Medicine, Division of Pulmonary, Critical Care, and Sleep Medicine, U.C. Davis Lung Center

RATIONALE: We have recently discovered a role for the statin drugs in causing airway smooth muscle (ASM) relaxation via inhibition of the mevalonate pathway and ASM cytoskeletal apparatus. Given that β2-agonists are well-established drugs used to relax ASM and bronchodilate airways, we wondered (1) which statin has the best relaxing effect, and (2) does this statin effect further enhance the existing β2-agonist effect. We hypothesized that statins further enhance β2-agonist-induced ASM relaxation and that select statins rather than the entire drug class would have the most potent effect.

METHODS: Primary human ASM (hASM) cells from one non-asthmatic donor were plated upon collagen-coated, 3 kPa still gels in 96-well plates. The cells were plated initially in 10% FBS containing medium (2 hrs) and then incubated in serum-free medium (72 hrs). Cells were pre-stimulated with 10 μM histamine (0.5 hr) followed by drug treatments (n = number of wells per condition). Precision-cut lung slices (PCLS) derived from one human donor were pre-exposed to (i) sequentially increasing doses of histamine (0.1, 0.1, 1 and 10 μM, at 15 min. each) followed by isoproterenol (ISO) (30 μM, at 15 min.) (Control, n=3 airways), or (ii) with additional Pitavastatin (Pita) pre-treatment (5 μM, 24 hr pre-treatment) (5 μM Pitavastatin, n=5 airways). VEH (drug vehicle).

RESULTS: Using Traction Force Microscopy (TFM), ‘strain energy’ (SE) was plotted on the Y-axis (a metric of ASM contraction) normalized to histamine pre-treatment. Compared to ISO alone, co-treatment with either 0.03, 0.3, or 3 μM Pita prolonged ASM relaxation (Fig. A). Using PCLS, we observed that Pita pre-treated airways constricted less in response to histamine and dilated more in response to ISO. The differences between groups did not reach statistical significance; repeat experiments are ongoing (Fig. B). A variety of statins were screened for their ability to relax hASM cells measured using TFM (Table 1). hASM cells were exposed to different statins (10 nM to 10 μM) and SE was tracked over 24 hrs. Comparing the dose response (EC50) at 24 hrs (highlighted green) or the extent of relaxation for the highest dose, 10 μM, at 24 hrs (highlighted blue) reveals a subset of statins which potently relax hASM cells. Some statins (highlighted yellow) acutely relax hASM cells after only 1 hr of exposure (n=4 wells/group). These results reveal unique properties of each statin to ultimately effect hASM cell relaxation.

CONCLUSION: The statins have differential effects on hASM relaxation, revealing a subset of more potent and shorter-acting statins. Some of these select statins further potentiate the ASM-relaxing effects of the β2-agonist isoproterenol in a dose-dependent manner beginning at 4 hrs post treatment and persisting at 24 hrs even after the isoproterenol effect has worn off. The statin effects on histamine-induced bronchoconstriction using PCLS ex vivo requires additional investigation. Statins may have therapeutic benefit as a bronchodilator.
Philip Thai Memorial Award and Research Presentation

Best Clinical Science Abstract

Screening for Interstitial Lung Disease using Natural Language Processing

Gabrielle Echt, Janelle Vu Pugashetti, Justin M. Oldham
Benjamin Davis Memorial Award and Research Presentation

Best Basic Science Abstract

Reduced *Aspergillus fumigatus* Ingestion and Clearance by Cystic Fibrosis Macrophages Promotes Aspergillus Invasion Post-Airway Transplant

Wayland Chiu, Efthymia Iliana Matthaiou, Carol Conrad, Joe Hsu