such as IAV. It enhanced severity of inflammation and lung injury. This novel data is consistent with our previous findings in the pig model and underscore the importance of investigating the impact of $H_2S$ on respiratory tract infections.

### 4597 *In Vitro* Assessment of Radiation Exposure on Primary Human Bronchial Epithelial Cells

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While radiation therapy is an effective treatment for lung cancer, there are known secondary complications that stem from irradiation of the surrounding healthy tissue, including radiation-induced pneumonitis, radiation-induced fibrosis, and other effects associated with cell toxicity. These conditions can significantly impact the quality of life of patients, so it is important to improve our understanding of the effects of radiation on human lung tissue, especially since a model to do so has not yet been developed. Here, we use a simplified lung model to achieve this. Simplified lung cultures were created by seeding Normal Human Bronchial Epithelial (NHBE) cells on 96-well plates. The simplified lung cultures were exposed to gamma radiation at varying doses ranging from 0.5 Gy to 250 Gy. Analyses were conducted with both confluent and sub-confluent cultures and included DNA double-strand break assays as well as cell proliferation studies. Initial results show that the optimal dose to observe radiation damage without significant changes in cell proliferation and cell morphology is 2 Gy, which is clinically relevant. Radiation exposure induces DNA double strand breaks and decreases the ability of cells to proliferate. In continuing work, we will investigate the ability of Amifostine, an FDA approved radioprotectant, to mitigate the effects of damage due to radiation exposure. There has been minimal investigation of the mechanism of Amifostine *in vitro* and its ability to protect against the toxic effects of gamma irradiation. Additionally, we plan to improve upon these lung models by creating 3D models to better mimic the native lung microenvironment. Radiation exposure will also be modified to mimic clinical radiation regimens with fractionated exposure as opposed to single-dose radiation. We anticipate developing a 3D model for studying radiation damage in human lung tissue and to better understand the mechanism and capabilities of a radioprotectant in this system.

### 4598 The Role of Plk1 in Pulmonary Fibrosis

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Idiopathic pulmonary fibrosis (IPF), the most common and aggressive form of interstitial pneumonia, stems from a unknown causes that results in respiratory failure within two to five years of diagnosis. Increasing evidence suggests that the alveolar epithelium may be uniquely vulnerable to pathogenesis of pulmonary fibrosis (PF); however, the mechanism behind pathogenesis is not fully understood. The TGFβ-1 pathway is considered the major mechanism for several fibrotic diseases, including ones resulting from both environmental exposures and genetic factors. Therefore, our objective is to discern how TGFβ1 exposure leads to activation of alveolar epithelial type 2 (AT2) and/or fibroblast differentiation and eventually progression of IPF by utilizing Polo-like Kinase 1 (Plk1) inhibition. Plk1 plays many roles in cell cycle and mitotic progression, where Plk1 activity in is abolished upon DNA damage and acts as a transcriptional target of p53 to cause cell cycle arrest. This interaction with p53 inhibits its apoptotic function and drives stress responses. Plk1 also plays a major role in homologous recombination which contributes to it anti-apoptotic activity. Overexpression of Plk1 is well established in several cancers where it contributes to tumorigenesis and metastasis via abnormal cell cycle regulation. Several recent studies have established efficacy of Plk1 inhibition as a cancer therapeutic with clinical trials ongoing. I hypothesize that that polo-like kinase 1 (Plk1) acts as a critical molecule to link ROS, NFκB, and p62/Nrf2 signaling, and that this kinase can be activated by TGFβ1 exposure as it would it be human IPF. This hypothesis has been formulated on a key observation made by the Liu lab, that Plk1 activates the p62/Nrf2 signaling via direct phosphorylation of p62 and Nrf2. Therefore, Plk1 may be a novel and efficient therapeutic target to prevent aberrant AT2 differentiation and fibroblast and accumulation that leads to ECM (extracellular matrix) deposition characteristic of IPF. Additionally, by utilizing Plk1 inhibition/activation, a unique opportunity exists to investigate the contribution of aberrant cell cycle progression of AT2 cells to impact disease development mechanistically. Based on this knowledge, experiments with human alveolar epithelial and lung fibroblasts, a 3-D murine lung organoid, and novel mouse model of bleomycin-induced PF, Spc-Cre;Plk1-KO/KI, will be utilized alongside a model of familial IPF, SpcI73T;Plk1-KO/KI, and compared. Using western blotting, immunofluorescence, qPCR, flow cytometry, and immunohistochemistry, these models will be evaluated after induction of pulmonary fibrosis, for *in vivo*/*ex vivo* experiments. Preliminary results in a bleomycin-induced model of WT mice yielded significantly reduced hydroxyproline and Ashcroft scoring with Plk1 inhibition by GSK461364, supporting the TGFB1-induced pro-fibrotic state seen in human BEAS-2B and normal human lung fibroblasts. The rationale for this research is that, once the mechanism of Plk1-associated kinase contribution to disease progression is established, the Plk1 can be manipulated pharmacologically, offering a novel multi-mechanism drug target that may overcome limitations of single-mechanism therapeutics for this disease.

### 4599 Human Cell–Based *In Vitro* Systems to Assess Respiratory Toxicity: A Case Study Using Silanes

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Silanes are widely used as reducing and coupling agents with applications in surface modifications. Because of their reactivity and rapid hydrolyzation, occupational exposure to silanes is possible in the production line. In this study, called the INSPiRE Initiative (*IN vitro* System to Predict REspiratory toxicity), human bronchial epithelial cell line (BEAS-2B) and a reconstructed tissue model (MucilAir™) were exposed to triethoxysilane (TES) and trimethoxysilane (TMS) as vapors, to predict the ability of these chemicals to cause portal-of-entry effects on the human respiratory tract. Three concentrations were tested for each silane in BEAS-2B cell line (TES: 1, 50, and 150 ppm, and TMS: 1, 25, and 85ppm) and MucilAir™ tissues (TES: 75, 150, and 300 ppm, and TMS: 25, 100, and 300 ppm). All exposures were performed for 30 minutes at the air-liquid interface (ALI) using a VITROCELL® 6/4 system and appropriate negative (sodium chloride, incubator control, or nitrogen gas) and positive (nitrogen dioxide) controls were used. Endpoints were assessed 19-24 hours (BEAS-2B and MucilAir™) or seven days (MucilAir™) after exposure and included cell viability (Prestoblue™ assay), cytotoxicity (lactate dehydrogenase assay), and secretion of inflammatory markers (electrochemiluminescence immunoassay). For MucilAir™ tissues, histology (hematoxylin and eosin staining), barrier integrity (transepithelial electrical resistance (TEER)), and cilia beating frequency (CBF) and average active area (AAA) (SAVA system) were also included. In BEAS-2B cells, a dose-dependent response was observed for all endpoints for both silanes. 19-24 hours after exposure of MucilAir™ tissues, the results show decreased cell viability, TEER, and AAA, and an increase in cytotoxicity, inflammatory response, and CBF for all concentrations of both silanes. Seven days after exposure, a further decrease in cell viability and AAA was observed and inflammatory response and CBF remained elevated indicating that the cellular damage due to exposure to silanes was substantial. Interestingly, barrier integrity was restored back to pre-exposure values. The results from both *in vitro* systems indicate that TMS is more toxic than TES, which is expected based on chemical properties and existing data. Studies are underway to assess additional test chemicals and to compare ALI exposure to direct pipetting in both systems. The results of this project can be used to better understand the usefulness of *in vitro* systems in assessing the impact of chemicals on the human respiratory tract and inform regulatory decision-making.

### 4600 Acute Pulmonary Response and Lung Burden following Solid Surface Composite Dust Inhalation

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Pulmonary exposure to emissions from manipulating solid surface composite (SSC) materials with power tools has been associated with adverse health effects in humans and laboratory animals. Previous *in vitro* and *in vivo* investigations of SSC toxicity have been limited by particle delivery methods that do not fully recapitulate the workplace environment. To represent a real-world particle exposure more accurately, our group constructed a chamber for simultaneous particle generation, characterization, and animal exposure. In order to determine lung deposition and clearance, 6-week-old male C57BL/6 mice were exposed to SSC particles for 4 hours (n = 9) or filtered air control in the exposure chamber. The mice were sacrificed immediately after the exposure as well as 24 hours post-exposure and divided into two subsets. In one subset, (n = 6), whole lungs were collected and analyzed for aluminum content using inductively coupled plasma atomic emission spectroscopy. In the other subset, (n = 3), in the right lobe was neutral buffered formalin for histopathology, while the left lobe was or snap frozen and kept at -80ºC for later molecular analyses. The exposure apparatus was successfully able to generate and aerosolize particles and maintain them at the target concentration of 20 ± 1.7 mg/m3) for the full exposure period. The aerosol count median aerodynamic diameter was 820 nm, while the geometric standard deviation was 2.884. Inductively coupled plasma-atomic emission spectroscopy analyses determined the lung deposition of 19.13±5.03 µg/g elemental aluminum, or approximately 60 µg/g SSC dust, based on the relative proportion of Al in SSC bulk material. No significant clearance after 24 hours was observed. No acute pulmonary inflammation or toxicity was observed in any histology section. Lung 4-hydroxynonenal was elevated at the 0-hour timepoint, while superoxide dismutase was depressed at the 24-hour timepoint, compared to control, suggesting an oxidative stress response. Lung tissue IL-6 was significantly increased at 0 hours compared to control and returned to baseline at 24 hours. IL1-β, IL-2, IL-5, TNFα, and IFNγ were all decreased at 0 hour post-exposure and all except for TNFα remained decreased at the 24-hour time point. In conclusion, our system was able to reliably generate a consistent

and reproducible real time SSC aerosol with a portion of that aerosol inhaled by the mice. A single 4-hour exposure to 20 mg/m$^3$ SSC dust elicited changes in inflammatory markers in lung tissue, likely driven by an increase in oxidative stress. *The findings and conclusions in this report/presentation have not been formally disseminated by the National Institute for Occupational Safety and Health, Centers for Disease Control and Prevention and should not be construed to represent any agency determination or policy.*

### 4601 Inhibition of the Volume-Regulating Anion Channel LRRC8 Attenuates Contraction and Migration in Human Airway Smooth Muscle Cells

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During proliferation and migration, cells rely on cell surface ion channels like volume-regulated anion channels (VRACs), involved in ion balance and osmolyte transport. The channel leucine-rich repeat-containing protein 8 (LRRC8) is the dominant VRAC present in human airway smooth muscle cells (HASMCs), which have a crucial role in airway contraction and remodeling in asthma. However, the role of LRRC8 in HASMC function and asthma pathogenesis is poorly understood. To elucidate the role of LRRC8, we hypothesized that LRRC8 activation increases HASMC contraction and remodeling. Non-asthma lung donor-derived HASMCs were pretreated with DCPIB (0.1-10μM, 1h), a reversible inhibitor of LRRC8, or transfected with LRRC8-targeting siRNA. To assess the effects of LRRC8 on HASMC physiology, magnetic twisting cytometry (MTC) measured HASMC stiffness, while scratch-wound assay measured migration. Cells were exposed to transforming growth factor-beta1 (TGF-β1) (4ng/mL, 18h), carbachol (Cch) (25μM, 5-60min), histamine (2.5μM or 10μM, 5-60min), or isoproterenol (10μM, 5min) and measured for myosin light chain (MLC), cytosolic $Ca^{2+}$, Myosin phosphatase target subunit-1 (MYPT1), and protein kinase B (Akt). DCPIB pretreatment attenuated agonist-induced HASMC maximum and steady-state stiffness (CCh: vehicle: 1.12±0.019, DCPIB: 1.03±0.015, p<0.05; histamine: vehicle: 1.17±0.024, DCPIB: 1.00±0.016, p<0.0001; n=100-163 cells) and in a concentration-dependent manner (histamine: vehicle: 1.50±0.038, DCPIB 10μM: 1.18±0.019, p<0.001; n=112-134 cells). DCPIB pretreatment reduced mitogen-induced migration in HASMCs (0.497±0.045 of control, p<0.0001; n=3 donors). Surprisingly, in parallel, DCPIB significantly attenuated agonist-induced MLC phosphorylation, a key mediator in excitation-contraction (EC) coupling (0.733±0.121 of Cch, p<0.05, n=6 donors; 0.687±0.025 of histamine, p<0.005, n=4 donors). DCPIB attenuated TGF-β1 (0.706±0.061 of control, p<0.005, n=7 donors) and Cch-induced MLC phosphorylation (0.550±0.109 of control, p<0.005, n=8 donors). SiRNA-mediated knockdown of LRRC8 reduced histamine-induced MLC phosphorylation (0.600±0.053 of control, p<0.01, n=4 donors). These findings were unassociated with agonist-induced cytosolic $Ca^{2+}$. However, DCPIB concentration-dependently attenuated Cch-induced Akt (0.639±0.053 of control, p<0.01, n=6 donors) and MYPT1 phosphorylation (0.499±0.082 of control, p<0.01, n=4 donors). Our findings suggest inhibition of LRRC8 activity attenuated the contractile and migratory responses in HASMCs, complementing reductions in agonist-induced MLC phosphorylation. This introduces a novel mechanistic link between LRRC8 activity and asthma progression through altering the function of HASMCs and EC coupling. *Supported by NIH P01 HL114471(RAP), UL1 TR003017, and 2T32ES007148-36.*

### 4602 Establishing a Continuous Aerosol Exposure Method for Evaluating the Respiratory Toxicity of Ortho-Phthalaldehyde

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Ortho-phthalaldehyde (OPA) is an FDA-cleared high-level disinfectant for heat-sensitive medical devices. However, its inhalation exposure limit has not been determined due to the lack of comprehensive respiratory toxicity data. To fill this data gap, we repeatedly exposed a human *in vitro* air-liquid-interface (ALI) airway tissue model to a wide range of OPA aerosol concentrations (i.e., 0.31 to 5.94 μg/cm$^2$) 4 hours per day for 5 days, followed by a 4-week recovery phase to assess the reversibility of the tissue responses. A VITROCELL® BioAerosol Nebulizing Generator and a 24/48 Exposure Module were used to generate and deliver a continuous flow of OPA aerosols to the apical surface of the ALI cultures. OPA aerosols slightly increased tissue permeability after 3 repeated exposures as measured by lactate dehydrogenase release. At the end of the 5-day exposure, tissue permeability in the treated cultures was decreased, possibly due to morphological changes induced by OPA aerosols. Functional tissue responses, such as ciliary response and mucin secretion, were modulated by OPA aerosols in a concentration- and time-dependent manner. Cilia beat frequency (CBF) was significantly suppressed by a single OPA exposure at concentrations above 0.87 μg/cm$^2$; this inhibitory effect was exacerbated with an increasing number of exposures. The inhibition of CBF was accompanied by the downregulation of key ciliary proteins (i.e., acetylated α-tubulin and DNAI1), suggesting that ciliary structures may have been compromised in the treated cultures. OPA aerosols generally decreased the secretion of mucins at all timepoints, with the exception of MUC5AC secretion, which was induced after 5 repeated exposures. Quantification of cytokine/chemokine release in the basolateral medium revealed the induction of IL-1RA, IL-9, and MIP-1β secretion and reduction of VEGF secretion. From the *in vitro* concentration-response data generated on these endpoints, we are currently establishing a panel of toxicological points-of-departure to predict an OPA-specific safe limit of exposure in humans.

### 4603 Acat-1 Inhibition Limits Pulmonary Fibrosis

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The enzyme Acat-1/*Soat1* catalyzes the conversion of cholesterol to cholesterol esters, a critical step leading to intracellular lipid droplet formation. In previous studies of acute lung injury, we have observed large, lipid-laden cells, which correlate with an increase in pro-inflammatory alveolar macrophages (AM) and mature, pro-fibrotic interstitial macrophages (IM). This macrophage phenotype was reduced with the administration of the Acat-1 inhibitor K-604 *in vivo*. As persistent activation of macrophages is known to contribute to fibrotic processes, we hypothesized that limiting cholesterol esterification through Acat-1 inhibition would reduce the persistence of activated macrophages and limit the progression of pulmonary fibrosis. To induce fibrotic changes in the lung, male and female wild-type mice (C57BL6/J, n = 6-7/group) received an intraperitoneal (IP) injection of bleomycin (IPB, 0.1 U/200 μL) every 3 days from d0-15, with corresponding saline control group (IP, 200 μL). Intratracheal saline or K-604 (10 mg/kg) was administered every 3 days from d17-34 with sacrifice on day 40. IPB significantly blunted weight gain compared to control (9.6±1.3%* vs 16.0±2.5%) and gain was maintained with K-604 administration post-IPB (14.3±1.4%$^{\dagger}$). No differences in cell count or protein concentration in the bronchoalveolar lavage were observed across groups. Magnetically-selected CD45+ cells from digested lung tissue were immunostained for flow cytometry. IM (CD45+/Viable/Siglec F-/F4/80+/CD11b+) were assessed for expression of markers related to classical (Ly6c+) and alternative (CD206+) activation. Greater proportions of mature IM (CD11c+) were observed with IPB compared to control (61.1±3.7%* vs. 45.4±2.5%) which was not reduced by K-604 administration (62.7±2.7%). The proportion of CD206-expressing IM was also increased with IPB (57.8±4.8* vs. 39.3±3.2%) with no changes in Ly6c positivity across groups. Hematoxylin & eosin stained sections were scored for cellularity and epithelial thickening post-treatment, indicating significant IPB-mediated histological changes. Fibrotic endpoints were assessed via trichrome stain and hydroxyproline quantitation. Improved body weight maintenance and histological appearance with K-604 treatment, but no significant change in the progression to a pro-fibrotic IM phenotype, highlights the effects of K-604 on non-macrophage cell types in the lung. (*,$^{\dagger}$=p<0.05). *Supported by NIH HL086621 and 4T32ES007148-30.*

### 4604 Macrophage-Specific ARNT Signaling Mediates Acrolein-Associated Acute Lung Injury

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Acrolein is a potent immunomodulatory respiratory toxicant that promotes acute lung injury (ALI), however, the mechanisms remain to be fully characterized and no treatments currently exist. Pulmonary macrophages are essential for initiating and resolving lung inflammation and are thought to be key drivers of ALI. Additionally, the aryl hydrocarbon receptor nuclear translocator (ARNT) is a crucial modulator of immune homeostasis via obligate cooperation with aryl hydrocarbon receptor (AhR), hypoxia inducible factor (HIF), and NF-kB transcription factors. Accordingly, macrophage specific ARNT signaling could dictate the intensity and duration of the immune response by altering cytokine production and immune cell recruitment, and therefore severity of ALI, after acute acrolein exposure. Interestingly, ARNT is alternatively spliced into isoform 1 and isoform 3 to promote pro-or anti-inflammatory responses, respectively, and presents a potential target for therapies to treat acrolein-induced ALI. To investigate the impact of the ARNT isoforms on the initiation of the inflammatory response after acrolein exposure, 16-week-old male and female transgenic mice that overexpress *Arnt-a* (murine homolog of human ARNT isoform 1) within macrophage (*Arnt-a* Tg), were dosed intratracheally with 2 mg/kg acrolein and sacrificed 24 hours later. Transcriptomic changes attributed to sex, genotype, and treatment were assessed in isolated BAL macrophages, and the inflammatory response was further characterized using differential BAL cell counts, lung histology, and BioPlex cytokine arrays. Transcriptomic analysis revealed sex- and genotype-specific differences in NF-kB and HIF target gene expression after acrolein exposure, which was further confirmed by alterations in pro- and anti-inflammatory cytokines, chemoattractant proteins, and overall immune cell landscape. Markers of ALI were then measured in the lungs of age matched animals 5 days post-exposure to assess ARNT isoform specific effects on inflammatory resolution. Interestingly, BAL cell counts in female *Arnt-a* Tg mice reveal persistent neutrophil and lymphocyte numbers that correspond with higher ALI scores relative to non-carrier (NC) controls. Neutrophil and lymphocyte populations were not observed in male *Arnt-a* Tg or NC mice on day 5, however, *Arnt-a* Tg males had lower ALI scores relative to NC. Taken together, these results support


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