Mesenchymal Stem Cells in OVA-Sensitized and -Challenged Mice Produce Immunomodulatory Cytokines

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Rationale: Recent studies have indicated the presence of mesenchymal stem cells (MSCs) in human lung diseases. Excess airway smooth muscle, myofibroblasts and activated fibroblasts have been noted in asthma, suggesting that mesenchymal progenitor cells are involved in asthma pathogenesis. We therefore sought to determine whether MSCs are present in ovalbumin (OVA)-sensitized and challenged mice.

Methods: Balb/c mice were sensitized and challenged with PBS or OVA over a 25 day period. Flow cytometry, clonogenicity, and differentiation potential, were used to analyze the emergence of MSCs.

Results: A CD45-negative subset of cells expressed MSC antigens including Stro-1, Sca-1, CD73 and CD105. Selection for these and against CD45 yielded a pluripotent population of cells. Lungs from OVA-treated mice demonstrated a significantly greater average colony forming unit-fibroblast (CFU-F) than control mice. Sorted cells differed significantly from total lung adherent cell populations, exhibiting a pattern of gene expression nearly identical to bone marrow-derived MSCs. The sorted lung cells expressed significantly more osteopontin and proliferin than total lung adherent cell fibroblasts. Cultured MSCs also produced significant levels of IL-6, MCP-1/CCL2, KC/CXCL1, RANTES/CCL5 and eotaxin/CCL11, and co-culture experiments showed that MSCs increased the production of these cytokines by splenocytes from OVA-treated mice. Finally, we isolated cells from the BAL of a human asthma patient with identical patterns of cell surface markers and pluripotentiality.

Conclusions: Allergen sensitization and challenge is accompanied by an influx of MSCs into the lungs that may regulate inflammatory and fibrotic responses.

SCH527123, a Novel Treatment Option for Severe Neutrophilic Asthma

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Rationale: There are currently no effective treatments for patients with severe asthma associated with neutrophilic bronchial inflammation. CXCR2 is highly expressed on neutrophils and mediates chemotaxis to inflammatory sites. We investigated the use of SCH527123, a selective CXCR2 antagonist, inhibiting neutrophil migration in patients with severe neutrophilic asthma (SNA).

Design: In a randomized, double blind, parallel study, 34 patients with severe asthma (GINA 2008) and sputum neutrophils > 40% were randomized to SCH527123, 30 mg daily PO or placebo for 4 weeks. Primary endpoints were safety and changes in blood neutrophil counts, secondary endpoints were changes in sputum neutrophils, ACQ score, minor and major exacerbations and lung function.

Results: 22 patients received active treatment (13 female, mean age 48.7, median ICS dose 1738mcg, median OCS dose 11.7mg/day, mean FEV1% pred 67.9) and 12 patients received placebo (7 female, mean age 53.9, mean ICS dose 2740 mcg, median OCS dose 13.8mg/day, mean FEV1% pred 60.4) for 4 weeks. On average, over the 4 weeks there was a 27.7% (p=0.02) decline in blood neutrophils in the active group, which was significant; however, values were quite variable in time and at week 4 the difference between the groups did not reach statistical significance. Sputum neutrophils on the other hand were significantly reduced in the active group by 57% averaged over the 4 weeks (p=0.01). There were no differences in the overall rates of adverse events among the groups although there was an increase in the rate of nasopharyngitis and gastrointestinal side effects which were generally mild. Importantly, there were fewer mild exacerbations (1.3 vs 2.25, p=0.05) and there was a trend for fewer severe exacerbations in the SCH527123 group. ACQ score was improved by 0.42 points in the active group (p=0.053) although the difference did not reach clinical significance and no changes were observed in FEV1.

Conclusions: SCH527123 seems to be a safe treatment option that reduces sputum neutrophils in SNA and shows promise of clinical benefit. Larger studies are needed to evaluate clinical efficacy.

Parent-Initiated Prednisolone for Acute Asthma in School Aged Children: A Randomised Clinical Trial

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Background: Evidence from systematic reviews demonstrates the effectiveness of oral corticosteroids in the treatment of acute asthma in school-aged children when administered following physician review. As a result, the use of parent initiated corticosteroids is becoming a more widely accepted practice. There is a paucity of published evidence to support this practice.

Objective: To evaluate the efficacy of a short course of parent-initiated oral prednisolone for acute asthma in school-aged children.

Methods: A population-based sampling strategy was used to recruit children aged 5-12 years with a history of four or more episodes of acute asthma in the preceding year. Episodes of acute asthma, rather than participants, were randomised to receive a short course of parent-initiated prednisolone (1mg/kg/day) or placebo. Parents initiated a course of treatment if they felt, from previous experience, the episode to be a more severe attack, or if the symptoms were not improving after 6 to 8 hours with regular use of reliever medication. The primary end point: a validated 7-day daytime symptom score (DTSS). Other end points: a validated 7-night night time symptom score (NTSS), health resource utilisation (HRU), and school absenteeism.

Results 230 children were enrolled in the study. Over a 3 year period 131 participants contributed 155 episodes of acute asthma randomised to parent-initiated treatment with prednisolone and 153 episodes randomised to treatment with placebo. Treatment with prednisolone reduced the mean DTSS by 15% (95% CI 2% to 26%, p=0.022) and the mean NTSS by 16% (95% CI 0% to 30%, p=0.050). The number of episodes requiring a HRU was 48 (31%) in those treated with prednisolone and 69 (45%) in those treated with placebo (OR 0.54. 95%CI 0.34,0.86) The number of episodes needed to treat to prevent an HRU was 7.1, (95% CI 4.0 to 30.3). School absenteeism was reduced by 0.4 days (95% CI -0.8 to –0.0 days, p=0.045).

Conclusion A short course of oral prednisolone initiated by parents when their child suffers an episode of acute asthma may reduce asthma symptoms, HRU, and school absenteeism. Parent-initiated prednisolone is an appropriate strategy for the management of more severe episodes of acute asthma in school-aged children. The modest benefits of this strategy must be balanced against potential side effects of repeated short courses of oral corticosteroid.

Asthma and Migration: Unmasking Asthma Potential. The International Study of Asthma and Allergy in Childhood (ISAAC) Phase Three

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Rationale: Previous studies of immigrants in USA, Australia, New Zealand and Sweden have suggested that migration to a country with a high prevalence of asthma is a risk factor for developing asthma. ISAAC Phase Three (2001-2005) provided an opportunity to examine the effect of immigration on the prevalence of asthma, eczema and rhinoconjunctivitis worldwide.

Methods: ISAAC Phase Three was a questionnaire based survey of 6-7 year old children and 13-14 year old adolescents worldwide to determine the prevalence of asthma, eczema and rhinoconjunctivitis. A questionnaire was included which contained questions about immigration and duration of residence in the adopted country. Analysis was limited to those centers with at least 5% immigration and those subjects with complete covariate data. Odds ratios and 95% CI were calculated, adjusted for region of the world, gender, GNI and language.

Results: Complete data were available for 43,143 children from 15 centers in 8 countries and 89,482 adolescents from 33 centers in 22 countries. Average immigration rate was 8.9% for children and 15.9% for adolescents. Overall, immigrants had fewer symptoms of asthma and eczema in children and for asthma and rhinoconjunctivitis in adolescents (Table 1).

Odds Ratios (95%CI) for risk of asthma, rhinitis and eczema if not born in country of residence

	6-7 year old n = 43,143	13-14 year old n = 89,482
Current wheeze	0.73 (0.65,0.83)	0.81 (0.73,0.76)
Asthma ever	0.66 (0.58,0.74)	0.69 (0.64,0.75)
Current rhinoconjunctivitis	0.88 (0.76,1.01)	0.85 (0.79,0.93)
Hayfever ever	1.06 (0.93,1.22)	0.93 (0.85,1.00)
Current eczema	0.71 (0.62,0.81)	0.94 (0.85,1.04)
Eczema ever	0.57 (0.51,0.64)	0.79 (0.71,0.87)

The effect of immigration for current wheeze was greater in Oceania [0.51 (0.41,0.63)] and Asia-Pacific [0.51 (0.32, 0.82) for children and Oceania [0.62 (0.0.51,0.75)] and North America [0.61 (0.44,0.83)] for adolescents. Age at immigration had no effect for the 6-7 y.o. children. For adolescents, the protective effect for current wheeze was greater for those who immigrated at age 2 years or less [0.0.69 (0.57,0.83)] than those who migrated after the age of 2 years [0.81 (0.65,1.01)]. When analysis was restricted to those countries with the highest quartile for symptoms, the protective effect was greater for both children [0.59(0.50,0.71)] and adolescents [0.78 (0.69,0.89)].

Conclusion: In this global survey, immigration had a significant protective effect on the prevalence of the symptoms of asthma and eczema in children and asthma and rhinoconjunctivitis in adolescents. The effect diminished with increasing duration of residence in the adopted country.

TLR3 Signaling Is Required for Human Rhinovirus (HRV)-Induced Airways Neutrophilic Inflammation and Hyperresponsiveness But Not Viral Clearance in Experimentally-Infected Mice

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Introduction: Human rhinovirus (HRV), a single-stranded (ss) RNA virus, causes exacerbation of chronic lower airway diseases including asthma. HRV infection triggers innate immune responses, including the production of interferons (IFNs) and pro-inflammatory chemokines.

Methods: We investigated the requirement of Toll-like receptor (TLR)-3, which recognizes viral double-stranded RNA formed upon viral replication, for HRV-induced airway responses. Wild type (wt) B6129SF2/J and TLR3-deficient (TLR3-/-) mice were inoculated intranasally with HRV1B, a minor group virus which replicates in mouse lungs.

Results: One day after infection with HRV1B, TLR3 -/- mice showed significantly decreased expression of the neutrophil chemoattractants KC/CXCL1 and MIP-2/CXCL2, and decreased whole lung neutrophil counts compared to HRV-infected wt mice. TLR3-/- mice also displayed significantly decreased airway cholinergic responsiveness after HRV infection. However, compared to wt mice, TLR3 -/- mice showed no significant differences in viral titer, viral RNA or type I IFN expression. TLR3 -/- mice showed partial reductions in type II and III IFN expression.

Conclusions: We conclude that TLR3 is required for HRV-induced airways neutrophilic inflammation and hyperresponsiveness but not viral clearance in B6129SF2/J mice. In other words, in this model. TLR3-driven innate immune responses to HRV are paradoxically maladaptive following experimental infection.

Efficacy and Safety of Omalizumab in Patients With Moderate-to-Severe Persistent Asthma Poorly Controlled on High-Dose Inhaled Corticosteroids and Long-Acting Beta-Agonists—Results of a Phase IIIb Randomized Controlled Trial

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Rationale: Omalizumab is an injectable monoclonal antibody that binds to IgE and is indicated for the treatment of patients 12 years and older with moderate to severe allergic asthma. In this study, patients with moderate-to-severe asthma poorly controlled on high-dose inhaled corticosteroids (ICS) and long-acting beta-agonists (LABAs) ± other controller medications, were treated with either omalizumab or placebo for 48 weeks.

Methods: 850 patients, 12-75 years old with poorly controlled moderate-to-severe asthma (defined as symptomatic with at least one asthma exacerbation in the preceding year despite taking high-dose ICS equivalent to ≥500 mcg fluticasone BID and a LABA) were enrolled. Omalizumab (n=427) or placebo (n=423) was added to existing medications for 48 weeks. The primary endpoint was the rate of protocol defined asthma exacerbations over the study period. Secondary efficacy endpoints included the change from baseline to Week 48 in mean daily number of puffs of albuterol, mean total asthma symptom score, and mean overall asthma quality of life as measured by overall standardized asthma quality of life score (AQLQs).

Results: The mean % predicted FEV1 (SD) at baseline was 64.9% (14.6) and patients had an average of 2 asthma exacerbations requiring systemic steroids, in the preceding year. Seven percent of patients had past history of intubation due to asthma exacerbations. Patients required a mean (SD) of 4.06 (3.02) puffs of albuterol per day, had mean total asthma symptom score of 3.89 (1.77) and an overall AQLQs of 3.92 (1.10). Approximately, 79% of patients completed the study. Patients receiving omalizumab had a significant reduction in the rate of asthma exacerbations at 48 weeks compared to patients receiving placebo (.66 vs .88, p=0.006). The change in mean number of puffs of albuterol per day (-1.58 vs -1.31, p=0.090) and mean total asthma score (-1.56 vs -1.30 and p=0.038) favored omalizumab vs placebo but did not achieve statistical significance at 48 weeks after adjusting for multiple comparisons. There was a statistically significant improvement in mean overall AQLQs score (1.15 vs 0.92, and p=0.005). Similar percentages of AEs (80.4% vs 79.5) and SAEs (9.3 vs 10.5%) were observed in the omalizumab versus placebo groups, respectively.

Conclusions: Our results demonstrate that long-term treatment with omalizumab is safe and can significantly reduce the rate of asthma exacerbations by 25% in patients with poorly controlled moderate-to-severe allergic asthma, despite receiving aggressive asthma controller therapy (high dose ICS + LABA).

Integrating Palliative and Critical Care: Results of a Cluster Randomized Trial

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Context: Because of the high mortality and morbidity in the ICU, palliative care is an important component of intensive care. There is currently debate over whether the best approach to improving ICU palliative care is to educate ICU clinicians, incorporate palliative care consultants, or both.

Objective: Evaluate the effectiveness of a multi-faceted quality-improvement intervention to improve the quality of palliative care in the ICU by educating and supporting ICU clinicians.

Design: A cluster-randomized trial of 12 hospitals assigned to intervention or usual care.

Intervention: The intervention targeted ICU clinicians with 5 components: clinician education, local champions, academic detailing, clinician feedback, and system support.

Outcomes and Analysis: Outcomes were assessed surveying family members and nurses of patients who died in the ICU or within 30 hours of transfer from the ICU. Families completed Quality of Dying and Death (QODD) and family satisfaction surveys. Nurses completed the QODD. Data were collected before and after the intervention/control period at each hospital. We used robust linear and Cox regression to test for intervention effects, controlling for site, patient, family, and nurse variables.

Results: There were 2318 patient deaths. The patient-based survey completion rate for families was 43% (822/1924) and for nurses was 50% (636/1269). The primary outcome, family-QODD, showed no change with the intervention (p=0.23). There was also no change in family satisfaction (p=0.64) or the nurse-QODD (p=0.81). There was a reduction in ICU days prior to death associated with the intervention, but this was not statistically significant (HR=0.86; p=0.07). Among those patients who underwent withdrawal of life support, the intervention was not associated with a significant reduction in time from ICU admission to withdrawal (HR=0.93, p=0.49). Palliative care consultation was not available in 5 hospitals and in early development in 6 hospitals; only 6% of patients dying in the ICU received palliative care consultation and there was no effect of the intervention on palliative care consultation.

Conclusions: We found no improvement in family- or nurse-assessed quality of dying or family satisfaction with care associated with this ICU-clinician-focused intervention. There was a non-significant trend toward reduction in ICU length of stay prior to death but no change in time from ICU admission to withdrawal of life support. Improving patient and family outcomes may require more direct contact with patients and families. Incorporation of palliative care consultants into the ICU may offer more opportunity for benefit than exclusively focusing on educating critical care clinicians.

Anti-Fibrogenic Effects of Endothelin-A Receptor Antagonist Ambrisentan in Mouse Pulmonary Fibrosis Model

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RATIONALE: Preclinical data have demonstrated that ET-1 may have a role in the pathogenesis of idiopathic pulmonary fibrosis (IPF). Ambrisentan is an endothelin receptor antagonist (ERA) selective for ETA that is currently being evaluated in a clinical trial for IPF [ClinicalTrials.gov Identifier: NCT00768300]. Data suggest that selective ETA more so than ETB antagonism may be important in modulation of fibroblast proliferation and contractile activity of lung fibroblasts (Shi-Wen X et al. Mol Biol Cell 15: 2707-19, 2004; Gallelli L et al. J Cell Biochem 96: 858-68,2005). This study further evaluates the role and significance of selective ETA antagonism in IPF and the mechanism of action of ambrisentan on cell damage and repair processes and related signaling pathways involved in epithelial-mesenchymal transition (EMT) in a mouse IPF model.

METHODS: To evaluate the efficacy of ETA antagonism in a clinically relevant model, we examined the effect on fibrosis of delayed administration of ambrisentan after resolution of the early inflammatory response to bleomycin in C57BL/6J mice.

RESULTS: Ambrisentan (30 mg/kg/d twice daily orally) given from day 14-28 after intranasal administration of 0.08 units bleomycin significantly reduced the severity of pulmonary fibrosis around the airways and alveoli as assessed by Masson's trichrome staining for collagen and also decreased parencyhmal inflammation on day 28. Although ambrisentan treatment did not affect the increased lung collagen induced by bleomycin as determined by hydroxyproline content, it reduced fibrosis in the lung parenchyma (P = 0.059) as quantified with the well-validated Ashcroft grading system using a predetermined 0-8 scale of severity. Ambrisentan also decreased the bleomycin induction of a-smooth muscle actin (a-SMA) and DNA strand breaks [i.e., terminal deoxynucleotidyltransferase dUTP nick end labeling (TUNEL)] reactivity in the airways consistent with inhibition by ambrisentan of the key profibrogenic responses EMT and apoptosis respectively. Laser capture microdissection for isolation of airway epithelial cells is underway to determine which critical genes involved in pulmonary fibrosis in the airways are differentially regulated by ambrisentan.

CONCLUSIONS: Since no effective treatment for IPF exists, selective antagonism of ETA suggests a potential novel therapeutic approach for pulmonary fibrosis.

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Neural crest cell origin and signals for intrinsic neurogenesis in the mammalian respiratory tract

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Rationale: Our study investigates the innervation of the respiratory tract during mouse embryonic development with a focus on identification of cell origin and essential developmental signals for the resident, or intrinsic, neurons.

Methods: We characterize the intrinsic neurogenesis in the respiratory tract using lineage tracing and mouse mutants.

Results: We show that these intrinsic neurons are derived from neural crest cells and cluster to form ganglia that reside in the dorsal trachea and bronchi with diminishing frequency. Comparison of intrinsic neurogenesis between wildtype, *GDNF-/-*, *NRTR-/-* and *Ret-/-* embryos, in combination with lung organ cultures, has identified that Ret signaling, redundantly activated by GDNF and neurturin, is required for intrinsic neurogenesis in the trachea and primary bronchi. In contrast, Ret deficiency has no effect on the innervation of the rest of the respiratory tract, suggesting that the majority of respiratory innervation is controlled independent of Ret signaling by neurons whose cell bodies are located outside of the lung, so-called extrinsic neurons. Furthermore, although the trachea and the esophagus and their intrinsic neurons share foregut endoderm and neural crest cell origins, respectively, signals required for their intrinsic neurogenesis are divergent.

Conclusion: Together, our results not only establish the neural crest lineage of intrinsic neurons in the pulmonary tract, but also identify regional differences in the abundance and developmental signals of intrinsic neurons along the pulmonary tract and in the esophagus.

GENOME-WIDE ANALYSIS OF TRANSLATIONAL-REGULATED GEGE in Hypoxic Alveolar Epithelial Cells

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Introduction: Hypoxia poses a major stress in many diseases including cystic fibrosis and chronic obstructive pulmonary disease. The hypoxic response of alveolar epithelial cells (AEC) is important for homeostasis. We investigated the effect of acute hypoxia on the transcriptional and translational landscape in adult rat AEC and sought to identify translationally regulated transcripts and the putative miRNAs controlling them.

Methods: MP48 adult rat AEC at 75% confluence were exposed to normoxia or hypoxia (1% O2) for 24 h. Polyribosome fractions were prepared and the heavy fractions (actively translated mRNA) were pooled. RNA was extracted from the total RNA and heavy polyribosome fractions and mRNA expression was evaluated genome-wide with Affymetrix microarray technology. The modified PERL package GO::TermFinder was used to analyze the enrichment of miRNA targets and known RNA elements in the translationally regulated transcripts

Results: With hypoxia the heavy polyribosome fraction decreased compared to the

normoxic heavy fraction, indicating decreased global translation. There were 768 genes showing significant changes in translation during hypoxia ($P<0.05$). After correction for changes in transcription and using a 1.5 fold change cutoff, 190 genes had significant changes in translation. Of these 190, 78 genes were more actively translated and 112 genes were less actively translated. Several miRNA targets (miRNA-190, miRNA-224, miRNA-96/1271, miRNA-133, miRNA-182, miRNA-124/506, miRNA-1/206, and miRNA-376/376ab/376b-3p) and one known mRNA element (cytoplasmic polyadenylation) were enriched in the translationally regulated transcripts.

Conclusions: Hypoxia significantly alters translation and transcription of the adult rat AEC genes in complex patterns. Further studies are needed to test which of the putative miRNA actually mediate regulation of mRNA translation with hypoxic stress.

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Global DNA Methylation Analysis of Bronchial Epithelial Cells from Patients with Asthma, COPD With and Without Lung Cancer

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INTRODUCTION: Emerging evidence suggests that aberrant epigenetic regulation is involved in the development and progression of malignant and non-malignant respiratory diseases. Although asthma, chronic obstructive pulmonary disease (COPD) and lung cancer have distinct phenotypes, patients with COPD may have a significant reversible component to their airflow obstruction. Also, patients with COPD have increased risk for lung cancer. Little is known about the global pattern of DNA methylation in bronchial epithelial cells associated with asthma, COPD and lung cancer. We hypothesize that changes to DNA methylation in airway epithelial cells play a key role in the pathogenesis of these respiratory diseases. We conducted a pilot study of the global DNA methylation changes in bronchial epithelial cells of patients with these three disorders to provide a better understanding of the molecular alterations that drive these diseases. As changes to methylation have been shown to be dependent on smoking history; we also evaluate DNA methylation changes associated with active smoking in current (CS) versus former (FS) smokers.

METHODS: Bronchial epithelial cells were obtained from brushings of small airways (< 2mm diameter) during bronchoscopy from FS with asthma (n=5), COPD (n=8) and patients with COPD as well as previous surgical resection of Stage I NSCLC (n=6 FS, n=12 CS). Illumina's Infinium Methylation (HM27) assay was used to assess DNA methylation status of 27,000 CpG sites associated with 14,000 genes.

RESULTS: COPD and Asthma can be distinguished based on their methylation profiles. Genes differentially methylated between COPD and asthma include: key modulators of inflammation and chronic inflammatory disease (matrix metalloproteinases, pro-inflammatory cytokines and chemokines) and pathogen recognition and innate immunity activation (toll like receptors). Additionally, a comparison of the airway epithelia methylation levels in CS and FS with NSCLC, identified genes involved in free radical savaging, xenobiotic metabolism, detoxification and immune signalling. These preliminary results imply a possible role for DNA methylation in the deregulation of previously identified disease-related genes.

CONCLUSION: We provide the first global DNA methylation analysis of airway epithelia in asthma, COPD and NSCLC patients. Knowledge of DNA methylation disruption in respiratory disease of both current and former smokers will provide rationale for further study of existing epigenetic drugs in treatment and prevention of these diseases.

Crucial role for Protein kinase C alpha and arginase activation in the induction of pulmonary endothelial hyperpermeability by pneumolysin

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***Rationale**:* Infections with *Streptococcus pneumoniae* are accompanied by a pulmonary endothelial hyperpermeability. Death in pneumonia patients can occur when tissues are sterile and correlates with the presence of bacterial virulence factors, the most important one of which is the pore-forming toxin pneumolysin (PLY). Unfortunately, to date mechanisms of PLY-induced pulmonary endothelial hyperpermeability, as well as potential therapeutic options to tackle its activity, remain elusive. To investigate the mechanism of PLY-induced endothelial dysfunction and to identify novel therapeutic targets and treatment options.

***Methods**:* We have evaluated PLY-induced endothelial hyperpermeability in an *in vitro* culture system, using monolayers of human lung microvascular endothelial cells, as well as in a 6h *in vivo* mouse model, using intratracheal PLY instillation.

***Results**:* PLY induces an endothelial hyperpermeability in human lung microvascular endothelial cells *in vitro*, an event which is preceded by a RhoA/Rac1 imbalance and an increased myosin light chain (MLC) phosphorylation. The PKC-α/β inhibitor GÖ6976 inhibits the permeability increasing effect of PLY and the toxin moreover induces a time-dependent activation of PKC-α, indicating an implication of this PKC isoform. PLY moreover leads to an increased arginase activity in the endothelial cells, which, upon inducing eNOS uncoupling leads to a reduced NO and an increased ROS generation. Intratracheal PLY instillation causes a significantly increased endothelial permeability in mice *in vivo*, as assessed by Evans Blue incorporation. The lectin-like domain of TNF, which can be mimicked by the 17 amino acid TIP peptide, is able to inhibit the PLY-induced PKC-a activation, arginase activity and endothelial hyperpermeability.

***Conclusion**:* These results identify PKC-a as a potential upstream and arginase as a potential downstream therapeutic target during G^+-associated pulmonary hyperpermeability and moreover indicate that the TNF-derived TIP peptide is able to blunt its activation.

Loss of caveolin-1 expression leads to decreased calcium flux in freshly isolated airway smooth muscle cells

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Rationale: Caveolin-1 (cav-1) is the marker protein of caveolae, which are a specialized subset of lipid rafts. Cav-1 is highly expressed within the lung in epithelial cells, endothelial cells, various immune cells, and airway smooth muscle (ASM). Caveolae are thought of as membrane platforms that can concentrate and organize signaling proteins, leading to more rapid and effective activation of the signaling cascade. Additionally, it has been shown that cav-1 directly participates in various signaling pathways. We have previously reported (Brown et al., Am. J. Respir. Crit. Care Med., Apr 2009; 179: A5093) that the caveolin-1 deficient (cav-$1^{-/-}$) mice exposed to aerosolized lipopolysaccharide (LPS) have decreased airway hyperresponsiveness (AHR) to methacholine challenge as compared to wild-type (WT) mice. We also showed that this decrease does not directly correlate with the inflammatory response, as inflammatory cytokine and chemokine levels were increased in the bronchoalveolar lavage fluid of cav-$1^{-/-}$ mice compared to WT mice. The reduced AHR was instead attributed to decreased contraction of ASM observed in an ex vivo challenge of isolated bronchial rings. In order to investigate the mechanism behind this decreased contraction, we measured cholinergic agonist-induced calcium responses in freshly isolated ASM cells and muscarinic receptor expression in lungs from cav-$1^{-/-}$ mice and their littermate WT controls.

Methods: Intact tracheas and lungs were removed from untreated mice. The tracheas were cleaned of surrounding tissue, epithelial cells were brushed off, and the remaining tissue was minced and digested. The resulting cell suspension was plated and cells were allowed to adhere in culture. The lungs were homogenized and analyzed by Western blot.

Results: Cav-$1^{-/-}$ ASM had a 60% decrease in carbachol induced calcium responses when compared to WT ASM. The expression level of the carbachol receptor, muscarinic receptor 3 (M3), was similar in whole lung homogenates from WT and cav-$1^{-/-}$ mice.

Conclusions: Loss of cav-1 expression in ASM results in decreased calcium flux which is not a result of reduced M3 receptor expression. Taken together, our combined data suggest that cav-1 plays an integral role in airway responsiveness, and that its mechanism of action lies downstream of the M3 receptor in ASM. In addition, cav-1 polymorphisms, which have been reported in human studies, may have the potential to influence the susceptibility to inflammatory lung diseases and the severity of AHR. Funded in part by HL-30923, HL-68071, and HL-84917.

Airway regeneration is delayed by prior depletion of bone marrow Clara cell secretory protein-expressing cells

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Rationale: The contribution of bone marrow (BM) to repair of lung injury is controversial, in part due to the robust restorative capacity of endogenous lung progenitor cells. We have previously shown that a subpopulation of bone marrow cells express Clara cell secretory protein (CCSP); a marker of airway progenitor cells. To study how endogenous bone marrow CCSP-expressing cells affect local progenitor regeneration, we used the CCtk transgenic mouse model in which the Herpes simplex thymidine kinase suicide gene is expressed under the control of the CCSP promoter. Ganciclovir treatment results in elimination of CCSP-expressing cells.

Methods: Wild type female mice were lethally irradiated and transplanted with male BM cells from either CCtk or wild type mice. After two months to establish bone marrow engraftment, animals were treated with ganciclovir 7 mg/day for 20 days to eliminate bone marrow CCSP-expressing cells in the CCtk BM group with no effects in lung. Mice were treated with naphthalene 200 mg/kg to injure airway epithelium and were sacrificed after 2, 5 and 10 days of naphthalene treatment. Airway recovery was assessed using CCSP immunofluorescence staining. Inflammatory cells were estimated by total and differential cell counts of bronchoalveolar lavage (BAL) cells. Quantitative real-time PCR analysis was done to compare expression of epithelial and secretory cell markers. Arterial blood gases (PaO2/FIO2 ratio) were analyzed for each time point.

Results: Mice showed significant differences between groups only at day 5. The CCtk BM group showed less CCSP+ cells lining the airway epithelium (score of 2.75±0.17 vs. 4±0; $p<0.001$), more inflammatory cells in BAL ($6.09 \pm 1.10 \times 105$ vs. $3.03 \pm 1.93 \times 105$; $p=0.023$), lower PaO2/FIO2 ratio (497.6 ± 43.03 vs. 666.6 ± 96.7; $p=0.0315$) and down regulation of secretory cell markers CCSP, Cyp2f2, Pon1, Aox3 and Fmo3 genes ($p \leq 0.01$), compared to animals with wild-type bone marrow.

Conclusion: Bone marrow CCSP-expressing cells play a beneficial role in airway regeneration after naphthalene injury.

Involvement of Autophagy in Cigarette Smoking Extract-induced Cellular Senescence in Human Bronchial Epithelial Cells

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Introduction: Accelerated cellular senescence has been implicated in the pathogenesis of COPD and cigarette smoke, an abundant source of oxidative stress, has been demonstrated to induce cellular senescence partly via post-translational modification of cellular proteins. Autophagy, a tightly regulated degradation process of cytoplasmic proteins, plays an important role in the elimination of abnormal proteins. Accumulation of damaged and dysfunctional proteins is a characteristic feature of cellular senescence, indicating the possible association between autophagy and accelerated cellular senescence by cigarette smoke for COPD development. Indeed increased autophagy has been reported in COPD lung tissues in association with apoptosis induction and, in in vitro model, cigarette smoking extract (CSE) induced both autophagy and apoptosis in airway epithelial cells. The purpose of this study was to elucidate the protective role of increased autophagy in CSE-induced accelerated cellular senescence in human bronchial epithelial cells (HBEC).

Methods: Airways were collected from lobectomy specimens from resections performed for primary lung cancer. HBEC isolation was performed with protease treatment. To characterize autophagy, fluorescence microscopic detection of LC3-EGFP dot formation, RT-PCR, and western blotting of LC3 were performed. Autophagy was inhibited by 3-methyladenine (MA), a specific inhibitor of autophagy, treatment or knock down of LC3 by siRNA tranfection. Rapamycin and torin1, mammalian target of rapamycin inhibitors, were used to induce autophagy. Senescence associated beta-galactosidase (SA-Î²-gal) staining, a representative marker of cellular senescence and western blotting of p21 were performed to evaluate cellular senescence.

Results: CSE induced cellular senescence demonstrated by increased SA-Î²-gal staining and p21 expression levels, and while CSE also induced autophagy shown by formation of LC3-EGFP puncta and increased conversion from LC3-I to -II, it was not sufficient to inhibit cellular senescence in HBEC. However, induction of autophagy by torin1 significantly suppressed CSE-induced senescence, while inhibition by knock down with LC3 siRNA and 3MA treatment further increased CSE -induced senescence.

Conclusions: These results suggested a potential inhibitory role of autophagy in CSE-induced accelerated cellular senescence by preventing the accumulation of damaged and dysfunctional proteins, indicating the novel therapeutic potential by regulation of autophagy in COPD, which remains to be determined in the future study.

Insulin-Like Growth Factor-I Modulates Bcl-2 Expression in Hyperplastic Mucous Cells

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Bcl-2 expression sustains airway epithelial cell proliferation and mucous cell metaplasia following lipopolysaccharide (LPS)-inflammatory responses. The present study employed two approaches to identify the inflammatory mediators that modulate Bcl-2 expression in airway epithelial cells. In one approach, bronchoalveolar lavage fluid (BALF) collected from LPS-instilled rats at 3, 10, and 24 h post instillation were assessed in the naïve rat airway organ explant culture system. Bcl-2-positive mucous cells were highest in explants treated with BALF from 10 h post LPS instillation. Because Bcl-2 positivity among mucous cells was enhanced upon the depletion of polymorphonuclear neutrophils (PMNs) in previous *in vivo* studies, BALF was harvested from LPS-instilled rats treated with anti-PMN antibody or control IgG 10 h post LPS-instillation and analyzed in the airway organ culture system. Bcl-2-positive mucous cells were increased in explants treated with BALF from PMN-depleted compared to IgG-treated rats. Cytokine analysis showed that upon LPS instillation IL-6 and TNF-a were decreased, VEGF was increased, and IL-1β and IL-9 levels were unchanged in anti-PMN- compared to IgG-treated controls suggesting that IL-6, TNF-α, IL-1β, IL-9, and VEGF may be involved in modulating Bcl-2 expression. The second approach used microarray analysis of RNA isolated from epithelia microdissected by laser capture microscopy from rats 2 d post LPS-instillation and non-instilled controls. Of the 800 differentially expressed genes, a significant 6- to7-fold induction in the *IGF-1*and *IL-1β* mRNA levels was observed and validated by quantitative real-time RT-PCR. The inflammatory mediators selected by these two approaches were then tested in various airway epithelial cell lines for the affect on Bcl-2 expression. IL-6, TNF-α, IL-1β, IL-9, and VEGF increased Bcl-2 mRNA but not protein levels. In contrast, exogenous IGF-1 treatment consistently showed induction of Bcl-2 mRNA and protein levels. In addition, Bcl-2 mRNA half-life was increased in cells treated with IGF-1 and the P2 region within the 5′-UTR appeared to play a role in the IGF-1-induced slowing of the mRNA decay. Thus, the present data suggests that IGF-1 is one of the key inflammatory mediators that modulate Bcl-2 expression in the airway epithelium during LPS-induced mucus cell metaplasia. These findings may help to identify inflammatory factors that are responsible for Bcl-2-mediated increase in mucous hypersecretion in cystic fibrosis patients.

Traditional and high tidal volumes are associated with prolonged mechanical ventilation and organ failure after cardiac surgery

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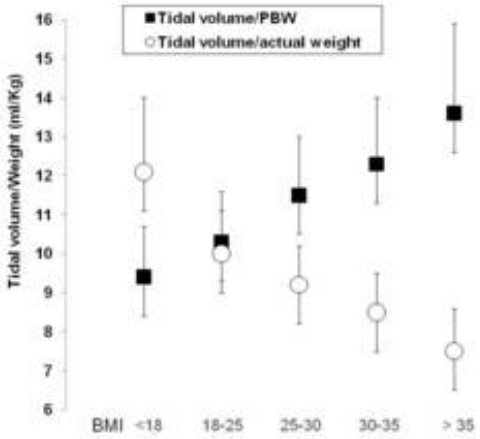
Rationale: High tidal volumes in mechanically ventilated patients with ARDS lead to baro/bio-trauma and increase mortality. Also, it was recently shown that ventilation with high tidal volumes is a risk factor for "acquired ARDS" in a medical population. We evaluated the impact of high tidal volumes after cardiac surgery.

Method: We analysed the prospectively recorded data of 3434 consecutive patients who underwent cardiac surgery from 2004 to 2006. We predefined 3 groups of patients based on the tidal volume delivered immediately after surgery: (i) Low: 7 to 9.9 (ii) "traditional": 10 to 12.9 (iii) High: above 13 ml/Kg of predicted body weight (PBW). We assessed the risk factors for organ dysfunction (prolonged mechanical ventilation, hypoxemia, hemodynamic failure and renal failure) by univariate and multivariate analysis, including the tidal volume at ICU admission in the models.

Results: Mean tidal volume/actual weight and mean tidal volume/PBW was 9.2±1.3 and 11.1±1.5 in men ($P<0.0001$), 9.1±1.4 and 12.5±2.2 in women ($P<0.0001$). 411 patients (12%) were ventilated with low tidal volumes, 2194 (63.9%) with "traditional" TV and 829 (24.1%) with high TV. The mean body mass index in the 3 groups was 23.8±4.0, 27.0±4.1 and 31.5±5.4 Kg/m^2 respectively ($P<0.0001$). With increasing BMI, the tidal volume/actual weight decreased while the tidal volume/PBW increased (Figure). The percentage of women was 12.2, 20.8 and 52.2% respectively for low, "traditional" and high TV ($P<0.0001$). Traditional and high tidal volumes were associated with prolonged intubation (>48h) (1.5% vs. 2.7% vs. 4.3%, P=0.009), hypoxemia (2.7% vs. 3.6% vs. 5.3%, P=0.03), renal failure (8.8% vs. 9.9% vs. 13.5%, P=0.007) and prolonged use of inotropes/vasopressors (5.8% vs. 7.4% vs. 11.3%, P=0.004). In a multivariate analysis, use of high tidal volumes was an independent risk factor for mechanical ventilation > 48 hours (traditional vs. low: OR: 2.2 [0.8-6.0], P=0.13 and high vs. low: OR: 3.1 [1.1-8.6], P=0.04) and prolonged use of inotropes/vasopressors (traditional vs. low: OR: 1.7 [0.9-3.0], P=0.11 and high vs. low: OR: 2.4 [1.2-4.5], P=0.01).
Figure: Tidal volume/predicted body weight and tidal volume/actual weight for different BMI.



Conclusion: Traditional and high tidal volumes are associated with prolonged mechanical ventilation and organ dysfunction after cardiac surgery and use of high tidal volumes is an independant risk factor. “Prophylactic” protective ventilatory strategy should be provided in this population with inflammatory state at risk to develop ventilator induced pulmonary edema. Women and patients with high BMI are more at risk to be ventilated with injurious tidal volumes.

Talactoferrin alfa reduces mortality in severe sepsis: Results of a Phase 2 randomized, placebo-controlled, double-blind study

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Introduction: Severe sepsis affects approximately 750,000 people in the USA annually, is increasing in incidence, and causes an estimated ICU mortality of 30% (1). Talactoferrin is an orally administered dendritic cell recruiter and activator with gut barrier protection properties as demonstrated by a reduction in indomethacin-mediated gut permeability (2).

Methods: In this multicenter, randomized, placebo-controlled double blind study, patients with severe sepsis (N=190) were randomized to receive talactoferrin 1.5 g or placebo every eight hours for up to 28 days. The primary endpoint was 28 day all cause mortality. A sample size of 95 patients per arm in this exploratory study provided 80% power to detect a ≥43% reduction in 28-day mortality (from 30% to 17%) in the talactoferrin-arm with a 1-sided α of <0.1. Patients were stratified by clinical site and by the presence or absence of cardiovascular dysfunction (hypotension refractory to ≥ 20 ml/kg crystalloid administration). Eligible patients had onset of severe sepsis within 24 hours prior to randomization. All potentially eligible patients were centrally screened prior to randomization. The quality control process during the conduct of the trial identified errors in drug labeling and randomization that altered drug assignment for some patients. Results as to the primary endpoint were therefore analyzed using logistic regression in a modified intent-to-treat (MITT) population using “as treated” instead of “as randomized” for group assignment. The labeling errors were random and did not effect blinding. A sensitivity analysis taking into account the labeling errors was conducted and supported the findings in the MITT analysis.

Results: The baseline characteristics were similar in both groups.

Baseline Characteristics		
Parameter	Talacteroferrin N=96	Placebo N=94
Mean age	58.1	60.9
Sex (females%)	45.8	50
Positive cultures (%)	46.9	52.1
Site of Infection (%) Lung	45.8	52.1

Blood Urine Intra abdominal Skin	38.5 20.8 13.5 14.6	27.7 22.3 14.9 14.9
Cardiovascular dysfunction (%)	60.4	67
Time from first organ dysfunction to randomization (mean hours)	17.6	17.9
Mean APACHE II score	23.1	23.3
Patients with APACHE II >25 (%)	44.2	42.2
Mean SOFA score at baseline	8.7	9.0
Corticosteroid use (%)	57.3	59.6
Drotrecogin alfa activated use (%)	9.4	5.3

28-Day All Cause Mortality

Parameter	Talactoferrin	Placebo	p Value (2-tailed)	p Value (1-tailed)	Odds Ratio
28 day all cause mortality (N=190)	14.6%	26.6%	0.043	0.021	0.47
28 day all cause mortality (N=190) adjusted for cardiovascular dysfunction	14.6%	26.6%	0.057	0.029	0.49
28 day all cause mortality in those without cardiovascular dysfunction (N=69)	2.6%	22.6%	0.031	0.015	0.093
28 day all cause mortality in those with cardiovascular dysfunction (N=121)	22.4%	28.6%	0.44	0.22	0.72

The incidence of grade 3 to 5 adverse events was similar in both groups.

Conclusions: Using a MITT analysis, talactoferrin reduced 28 day mortality in patients with severe sepsis and met the a priori mortality reduction target per protocol. It was very well tolerated. Further study of talactoferrin is warranted in patients with severe sepsis.

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IL-17A and TNF-α Exert Synergistic Effects on Expression of CXCL5 by Alveolar Type II cells in Vivo and in Vitro

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RATIONALE: Acute lung inflammation is a critical component of Acute Lung Injury (ALI), and may follow both systemic and local pulmonary insults. The mechanisms by which neutrophils are mobilized to the lung in response to systemic insults remain incompletely understood. We hypothesized that the alveolar type II (AE II) cell, poised at the junction between air and interstitial space, would integrate multiple signals, sense the direction of stimulus, respond differentially.

METHODS: Explants of human fetal lung (16-20wk gestation) were cultured 4 days in dexamethasone (10 nM) + 8-Br-cAMP (0.1 mM) + isobutylmethylxanthine (0.1 mM) (DCI) to induce a type II cell phenotype. The human AE II cells were cultured on transwell filters. Transepithelial resistance (TER) was measured at greater than 300 ohm/cm2 prior to stimulation of the cells. C57BL/6 mice for intrateacheal administration were divided into four groups as follows: 1) PBS alone; 2) rmIL-17A (625ng/mouse) alone; 3) rmTNF-alpha (500ng/mouse) alone; 4) rmIL-17A + rmTNF-alpha. All treatments were administered in a volume of 50 microliter.

RESULTS: Differentiated human fetal AE II cells were cultured in DCI media, and CXCL5 expression measured by ELISA and real-time PCR. TNF-alpha induced Cxcl5 mRNA and protein was dramatically increased in the presence of IL-17A, due to effects on both Cxcl5 transcription and stabilization of Cxcl5 transcripts. TNF-alpha, but not IL-17A activated NF-kappaB signaling which appeared necessary for CXCL5 expression. In vivo, intratracheal (i.t.) administration of TNF-alpha in C57BL/6 mice induced neutrophil accumulation that was dramatically synergized by IL-17A. High level production of CXCL1 and CXCL5 was observed in the bronchoalveolar lavage fluid at 24 hours. Furthermore, CXCL5 was critical in neutrophil accumulation, as neutrophil accumulation in Cxcl5-/- mice was greatly attenuated as compared to wild-type mice. To address localization of signal, human fetal AE II cells cultured on transwell inserts were stimulated with IL-17A and TNF-alpha. Apical delivery of stimulus provoked apical secretion of CXCL5, while basolateral co-stimulation by IL-17A and TNF-alpha led to both apical and basolateral secretion of CXCL5.

CONCLUSIONS: AE II cells integrate multiple pro-inflammatory stimuli, increasing CXCL5 expression in vivo and in vitro in response to TNF-alpha and IL-17A. Both transcriptional and post-transcriptional mechanisms are involved. Furthermore, AE II cells respond differently to apical vs. basolateral stimulation and secrete CXCL5 in a polarized fashion. We suggest these findings may represent a mechanism to recruit neutrophils to the lung in a fashion that distinguishes the site of initial injury.

The Role of IL-17A & Panton-Valentine-Leukocidin in the Pathogenesis of Staphylococcus aureus-Induced Skin Infection

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Background: Recently there has been a large increase in *Staphylococcus aureus* skin infections that is attributed to a community-associated strain of methicillin resistant *Staphylococcus aureus* (CA-MRSA). Patients with *Staphylococcus aureus* skin infection typically develop purulent skin lesions and abscesses. Strain USA300 is the most prevalent strain of CA-MRSA in the US. Panton-Valentine-Leukocidin (PVL), a SA exotoxin, has been linked epidemiologically to the pathogenesis of these skin infections. Interleukin-17A (IL-17A), which is an important mediator of neutrophil (PMN) recruitment and activation, has been shown to be an important determinant of PMN recruitment and abscess formation in response to *Staphylococcus*. However, the role of IL-17A in the pathogenesis of CA-MRSA-induced necrotizing skin infections has not been investigated.

Objective: We sought to elucidate the effect of IL-17A on the immune response to CA-MRSA-induced skin infection in a mouse model of necrotizing fasciitis.

Methods: *Il17ra* KO mice and their wild type controls were sub-cutaneously infected with SA bacterial strains USA300 (LAC) and its isogenic mutant strain delta-PVL (LAC/d-PVL). Skin lesions and cellular inflammation were evaluated up to 17 days after infection.

Results: Strain USA300 was found to be significantly more virulent in the wild-type mice compared to *Il17ra* KO mice resulting in a more significant weight loss (p-value of 0.004 on day 7 and 0.0256 on day 17) and larger skin lesions (p-value <0.05). Consistent with that, wild-type mice, as compared to *Il17ra* KO mice, displayed greater levels of total white blood cell count and neutrophil count. On the other hand, SA strain LAC/d-PVL was equally virulent in the wild-type mice and *Il17ra* KO mice resulting in comparable weight loss and skin lesions. Interestingly, PVL in the presence of IL-17A drove weight loss in mice on day 7 during lesion formation, however, in the absence of IL-17A, PVL was protective and promoted wound healing.

Conclusion: IL-17A plays an important role in inflammation, neutrophil recruitment, skin lesion formation and healing in mice sub-cutaneously infected with strain USA300. Importantly, PVL was found to have a beneficial role in skin infections by limiting lesion size and promoting healing. This role varied by the presence or absence of IL-17A and is likely mediated by its cytolytic effect on neutrophils recruited in response to IL-17. Identification of IL-17A as an important mediator of CA-MRSA-induced skin infection may pave the way for the development of better treatments for this disfiguring and potentially deadly infection.

Breastfeeding, aeroallergen sensitization and environmental exposures during infancy are determinants of childhood allergic rhinitis at age three

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Rationale: Early life predictors of childhood allergic rhinitis (AR) are not well defined. Few studies have addressed host characteristics and environmental exposure determinants of AR in three year old children. The objective of this study is to identify host characteristics, indoor and outdoor environmental exposures during infancy at age one that predict AR at age three in a longitudinal birth cohort.

Methods: High risk children from Greater Cincinnati in the Cincinnati Childhood Allergy and Air Pollution Study (CCAAPS) were followed annually in a large birth cohort study from birth to age three. The primary outcome, allergic rhinitis (AR) was defined by parental report of sneezing, runny or blocked nose in the prior 12 months and a positive skin prick test (SPT) to at least one of 15 aeroallergens. AR children were compared to non-atopic, non-symptomatic children. Environmental and standardized medical questionnaires determined exposures and clinical outcomes. Primary activity area dust samples were analyzed for house dust endotoxin (HDE) and (1-3)-β-D-glucan. Fine particulate matter ($PM_{2.5}$) sampled at 27 monitoring stations were used to estimate personal elemental carbon attributable to traffic (ECAT) exposure by land use regression model.

Results: Of 361 children in this analysis, 116 had AR and 245 were non-atopic, non-symptomatic. Prolonged breastfeeding in African-American children (aOR 0.8; 95% CI 0.6-0.9) and multiple children in the home during infancy were protective of AR (aOR 0.4; 95% CI 0.2- 0.8). Food SPT positivity and tree SPT positivity at age one increased the risk of AR at age three (aOR 4.4; 95% CI 2.1- 9.2) and (aOR 6.8; 95% CI 2.5- 18.7), respectively. HDE exposure was associated with AR, with low and high HDE exposure being protective, (aOR, 0.5; 95% CI, 0.3-0.8) and (aOR, 0.002; 95% CI, <0.001-0.1), respectively. In contrast, medium HDE exposure was associated with an increased risk of AR (aOR, 6.3; 95% CI, 2.3-17.2). ECAT and ETS exposure showed no effect on AR.

Conclusions: Prolonged breastfeeding in African-Americans and multiple children in the home during infancy reduced the risk of AR at age three whereas percutaneous reactivity to food and tree allergens enhanced risk. HDE exposure modified the risk of AR bi-directionally depending on the level of HDE exposure.

Higher toxic potential of 2-stroke scooter exhaust emissions compared to 4-stroke scooter and diesel car emissions

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Rationale: A growing number of scooters (small two-wheeled vehicles, maximum speed of 45 km/h, engine capacity of 50 cm^3) produce an increasing amount of potentially harmful emissions. Comparisons between cars (Euro 3 standard) and various scooters show that two-stroke scooters can emit 10-23 times more carbon monoxide (CO), 10-171 times more total hydrocarbons (HC) and 3-8 times more nitrogen oxides (NO_x), depending on scooter type. Further high emissions of polycyclic aromatic HCs and very high amounts of particulate matter in the nanoscale range are typical for scooter exhaust emissions. Therefore, scooters are significant contributors to air pollution and may play a role in the development of pulmonary and cardiovascular diseases. For that reason, the toxicity of exhaust emissions was tested, by exposing cell cultures to it.

Methods: The toxicity of exhaust emissions was evaluated using a newly developed exposure system: Triple cell co-cultures, simulating the human epithelial airway barrier, were directly exposed at the air-liquid interface to exhaust emissions (2h exposure, dilution of 1:100, constant conditions of 5% CO_2, 37°C and 80% relative humidity). Parallel to the exposure chamber, a reference chamber was also treated equally, however with ultra clean air only. Particle number, total particle surface area, mean particle diameter, CO, HC and NO_x concentrations were measured (Table 1). Cellular responses were assessed by measuring the cytotoxicity (lactate dehydrogenase assay) and (pro-) inflammatory responses (Tumor necrosis factor α, Interleukin 8) after 8 and 24h post-exposure time. For comparison, the cellular responses were summarized assessing each significant difference between control and exposure as ++ and each tendency as + (Table 1).

Results: After comparison of the overall toxic potential (Table 1), it was found that carburetor worst case (dummy muffler, Army oil, unleaded fuel) provokes the strongest cellular reactions (13+), followed by two-stroke direct injection (TSDI) worst case (8+) and four-stroke scooter (7+). TSDI absolute best case (prototype of particle filter, oxi catalyst, aspen fuel, 50% of oil ratio), diesel car without particle filter (both 5+), carburetor absolute best case and the diesel car with particle filter (both 3+) showed weaker reactions. For indentifying the relevant exhaust components, further analysis of more vehicle types and conditions are necessary.

Conclusions: Two-stroke scooter exhaust emissions have stronger adverse impacts on lung cell cultures than exhaust emissions of 4-stroke scooters or diesel cars. Technical implementations, such as particle filters, catalysts, and better fuel and oil, can reduce this impact and should be introduced.

Table 1. Physical characterization of the exhaust emissions and biological reactions.

	two-stroke direct injection		carburetor		four-stroke direct injection	diesel car	
	worst case	absolute best case	worst case	absolute best case		with particle filter	without particle filter
particle # (10-400nm) [counts/cm^3]	$6.23 \cdot 10^6 \pm 2.02 \cdot 10^4$	$2.32 \cdot 10^5 \pm 1.10 \cdot 10^5$	$5.51 \cdot 10^6 \pm 1.17 \cdot 10^6$	$9.21 \cdot 10^4 \pm 1.72 \cdot 10^4$	$1.33 \cdot 10^3 \pm 1.96 \cdot 10^3$	13.3 ± 16.6	$4.08 \cdot 10^5 \pm 4.89 \cdot 10^4$
total active surface area [μm^2/cm^3]	$6.73 \cdot 10^4 \pm 6.05 \cdot 10^3$	$2.36 \cdot 10^2 \pm 41.7$	$5.79 \cdot 10^4 \pm 1.38 \cdot 10^4$	not available	2.69 ± 1.86	0.783 ± 0.0787	$2.65 \cdot 10^3 \pm 3.81 \cdot 10^2$
carbon monoxide [ppm]	16.72 ± 1.95	0.64 ± 0.01	77.05 ± 44.99	1.19 ± 0.26	204.96 ± 35.91	0.66 ± 0.00	3.55 ± 4.97
hydrocarbons [ppm]	235 ± 12.5	8.32 ± 1.22	922 ± 7.43	29.7 ± 3.79	111 ± 12.9	2.05 ± 0.212	8.83 ± 0.382
nitrogen oxide [ppm]	30.9 ± 2.34	13.6 ± 1.79	5.47 ± 0.167	3.08 ± 0.0234	7.21 ± 0.311	11.3 ± 0.103	12.5 ± 0.249
ldh	+	0	++++	0	+++	++	0
tnfa	++++	+	++++++	+	++	+	+
il8	+++	++++	+++	++	++	0	++++
total biological reaction	**8+**	**5+**	**13+**	**3+**	**7+**	**3+**	**5+**

Health benefits from large scale ozone reduction in the United States

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Rationale
Tropospheric ozone is one of six "criteria" air pollutants for which the United States Environmental Protection Agency (EPA) sets a health-based National Ambient Air Quality Standard (NAAQS). The EPA monitors ozone levels across the United States (US) through a network of air quality monitors. Increased levels of ozone have been correlated with increased risk of adverse health effects, including premature mortality and related cardiopulmonary and respiratory morbidity. In 2008, the US Environmental Protection Agency (EPA) lowered the ozone NAAQS from 84 ppb to 75ppb, expressed as the maximum 8-hr average over a 24-hr period. Based on current monitoring data, ozone levels in numerous locations across the U.S. exceed this standard.

Methods
The avoided potential adverse health impacts were quantified among the U.S. population associated with attaining the current and two alternative ozone NAAQS levels. We estimated the avoided health impacts occurring after "rolling back" ozone values to meet three 8-hr ozone NAAQS: 75, 70, and 60 ppb (a range under current consideration for a revised ozone standard). The two lower alternatives reflect the upper and lower-bound range of EPA Clean Air Scientific Advisory Committee recommended values. The EPA Environmental Benefits Mapping and Analysis Program (BenMAP) was used to project the number of avoided cases of premature mortality and morbidity for an analysis year of 2007.

Results/Conclusions
Using a suite of short-term ozone mortality studies (Bell et al, 2005 and Levy et al, 2005) the avoided incidences from current ozone-related all-cause premature mortality range from 290-410 at 75ppb, 580-820 at 70ppb, and 1,890-2,660 at 60ppb (Table 1). We also found that attaining the 75ppb standard results in prevention of 290 emergency room visits (respiratory) and 250 hospital admissions (respiratory), with a reduction of 550,250 acute respiratory symptoms and 237,100 lost school days. Attainment of the 70 and 60 ppb 8-hr maximum levels yielded substantial additional health benefits, with about 1.1 and 3.5 million acute respiratory symptoms and 510 and 1,680 hospital admissions (respiratory) prevented at the 70 and 60ppb rollbacks, respectively. Mapping of all scenario results display regional variation in health benefits (Figure 1), and reporting by metropolitan statistical areas (MSAs) show the greatest health benefits to be in the New York, Los Angeles, Philadelphia, and Riverside, California MSAs.

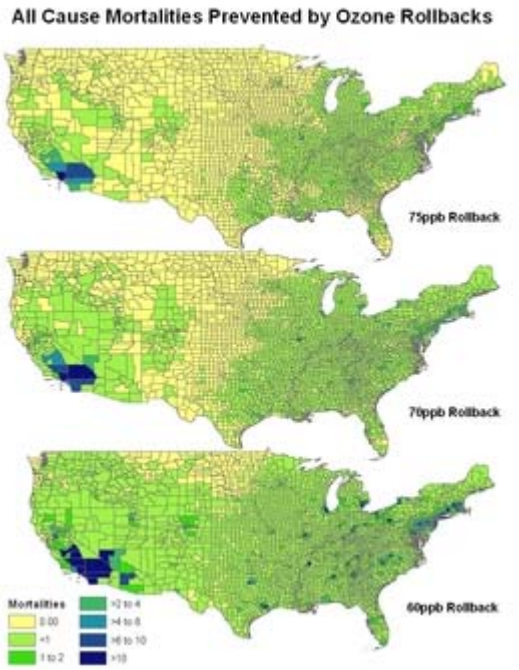

Figure 1. The geographic distribution of prevented all cause mortalities (using Bell et al., 05) following ozone rollbacks to the 75ppb standard, and further rollbacks to 70 and 60ppb.

Table 1. Nationwide reductions in mortality and morbidities resulting from ozone rollbacks to the 75ppb standard, and further rollbacks to 70 and 60ppb.

Endpoint	**Author**	**75ppb Rollback**	**70ppb Rollback**	**60ppb Rollback**
Mortality (Non-Accidental)	Ito et al (2005)	280	560	1,810
	Schwartz (2005)	140	270	890
	Bell et al (2004)	90	180	580
Mortality (All Causes)	Levy et al (2005)	410	820	2,660
	Bell et al (2005)	290	580	1,890
Mortality (Cardiopulmonary)	Huang et al (2005)	150	300	970
Emergency Room Visits	3 pooled studies	290	590	1,920
Hospital Admissions (Respiratory)	6 pooled studies	250	510	1,680
School Loss Days	2 pooled studies	207,790	410,220	1,299,790
Acute Respiratory Symptoms	Ostro and Rothschild (1989)	550,250	1,090,690	3,469,640

Surface tension of upper airway mucosal lining liquid in Down syndrome patients

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RATIONALE: The prevalence of sleep-disordered breathing in people with Down syndrome is high, reportedly due to anatomical features such as macroglossia, midface hypoplasia and obesity. A very common clinical observation is excessive oral route breathing, which has been shown to increase the surface tension of upper airway mucosal lining liquid, further predisposing to reduced upper airway patency. The aim of the present study was to compare the surface tension (γ) of saliva between subjects with Down syndrome and non-apneic healthy controls.

METHODS: We studied 64 patients with Down syndrome (25 men, 39 women; age 39.1 ± 13.7 yrs) and 45 non-apneic healthy subjects (control group; 23 men, 22 women; 36.1 ± 13.2 yrs). We obtained ~20ml samples of saliva from under the tongue via a small polyethylene tube. The g of saliva samples were then determined using the pull-off force technique. Analysis of variance (ANOVA) was used to examine group mean difference. $P<0.05$ was considered statistically significant.

RESULTS: The γ of saliva was significantly lower (55.3 ± 6.8 mN/m) in Down syndrome than in the control group (61.4 ± 4.5 mN/m; $p<0.05$). There was a significant difference between men and women in Down syndrome (57.9 ± 5.6 mN/m vs 53.6 ± 7.1 mN/m; $p<0.05$) but no significant difference between men and women in control group (60.3 ± 4.7mN/m vs 62.5 ± 4.1 mN/m).

CONCLUSIONS: The lower surface tension (γ) of saliva in Down syndrome patients may be a response compensate for an anatomical predisposition of increased upper airway collapsibility. We speculate that alterations in phospholipid and surfactant proteins (B or C) in saliva may contribute the reduced surface tension in Down syndrome patients.

Fibrotic Nanofiber Scaffolds Mimic In Vivo Lung Microenvironment in Pulmonary Fibrosis

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Rationale: Idiopathic pulmonary fibrosis (IPF) is a devastating interstitial lung disease of unknown origin that is characterized by irreversible scar tissue formation within the lungs. Collagen deposition, myofibroblast expansion, and the development of fibroblastic foci are hallmark pathological events that contribute to the scarring in IPF. The origin and mechanism of recruitment of myofibroblasts, the key cell leading to fibroblastic foci is unknown. We hypothesize that the fibrotic lung microenvironment causes the differentiation of bone marrow cells into myofibroblasts. In order to test this hypothesis, we have developed a novel method of studying the effects of the fibrotic microenvironment on various cell types through the utilization of nanofiber scaffolds.

Methods: Poly (e-caprolactone) nanofiber scaffolds were electrospun and coated with lung extracts from bleomycin or PBS-treated mouse lungs. Bone marrow cells were harvested from wild-type mice, plated on the nanofiber scaffolds, and allowed to expand. After various time-points, cells were observed by scanning electron microscopy and changes in fibrotic gene expression were determined by real-time PCR.

Results: Wild-type mouse bone marrow cells plated on the matrices coated with bleomycin- treated lung extract were observed by scanning electron microscopy to be secreting matrix materials and appearing more fibroblast-like after 8 and 14 days. These cells also had a significant increase in expression of the hallmark myofibroblast genes, type-I collagen and alpha-smooth muscle actin, as well as a significant increase in expression of connective tissue growth factor and tenascin-C.

Conclusions: These data underscore the importance of bone marrow derived cells in mediating pulmonary fibrosis. This *ex vivo* system recapitulates the three-dimensional fibrotic lung microenvironment, which will allow us to determine the cellular myofibroblast precursor and factors that that regulate its differentiation, thus providing targets for promising new therapies.

The Effect of Exercise Intensity on Airway and Systemic Inflammation in Patients with COPD

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Rationale: Airway and systemic inflammation are characteristic features of chronic obstructive pulmonary disease (COPD). A single bout of exercise increases systemic inflammation in patients with COPD, but no study has documented the airway inflammatory response, the anti-inflammatory response, or how different intensities of aerobic exercise influence the inflammatory processes.
Objective: To investigate the effect of exercise intensity on airway and systemic inflammation in patients with COPD.

Methods: Ten patients with moderate-to-severe smoking-related COPD (FEV1/FVC<0.7, FEV1 30-80% pred) and ten healthy age- and activity-matched controls were recruited for the study. Following pulmonary function and cardiopulmonary exercise tests at an initial visit, sputum and blood samples were collected ~48 hours before, and 0 (blood only) and 2 hours after a high intensity (HIGH) or low intensity (LOW) cycle exercise trial. The order of exercise was randomized and trials were equated for total work. HIGH consisted of eight intervals of 1 min at 100% workload maximum (Wmax) with 2 min at 30% Wmax, while LOW consisted of ~32 min of exercise at 40% Wmax. Sputum and blood samples were assessed for differential cell count, as well as interleukin (IL) 6, 8 and 10.

Results: Sputum differential cell counts were obtained at all time points in eight patients and six controls while blood samples were obtained in 10 patients and nine controls. Patients with COPD demonstrated higher absolute sputum neutrophils at all time points. Sputum neutrophils were reduced after LOW in patients with COPD ($p=0.028$), and this reduction was greater than with HIGH (-14.7 vs. -0.8% change in LOW and HIGH respectively, $p=0.036$). This finding was not observed in controls. A decrease in sputum IL-8 occurred after HIGH in the COPD group ($p=0.045$) but not in controls. Systemically, IL-8 was elevated and IL-10 was reduced in the COPD compared to controls at all time points ($p<0.05$). After HIGH, controls had an increase in systemic IL-10 from baseline to immediately after exercise (45.6 vs. 73.6 pg/ml, respectively, $p=0.028$), while the COPD group had no significant increase in IL-10 at any time point after either exercise trial.

Conclusions: This is the first study to investigate the airway inflammatory response to exercise in patients with COPD and demonstrates that high and low intensity exercise result in differing airway inflammatory responses. Systemically, there is a blunted anti-inflammatory response to high-intensity exercise in patients with COPD, which may have important implications for appropriately prescribing exercise for this population.

Dabigatran etexilate, an oral direct thrombin inhibitor, represses fibrotic changes in a murine model of pulmonary fibrosis

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RATIONALE: Activation of the coagulation cascade and generation of thrombin has been extensively documented in pulmonary fibrosis, both in acute and chronic lung injury, and in animal models of lung injury and fibrosis. The oral direct thrombin inhibitor (DTI), dabigatran etexilate, modulates the coagulation cascade and inhibits thrombin-induced profibrotic signaling in lung fibroblasts. This study tested whether thrombin inhibition by dabigatran etexilate attenuates bleomycin-induced pulmonary fibrosis in a murine model of lung injury.

METHODS: Lung injury was induced in 6-8 week old female C57BL/6 mice by intratracheal instillation of bleomycin. Dabigatran etexilate was given as supplemented chow (10 mg/g chow) or as matching placebo beginning on day 8 following bleomycin. Two and three weeks after bleomycin instillation mice were euthanized, and lungs, bronchoalveolar lavage fluid (BALF) and plasma were collected. Lung collagen was measured by hydroxyproline assays; dabigatran concentration by LC-MS/MS; thrombin activity by fluorometric assays; TGF-β1 concentrations by ELISA; connective tissue growth factor (CTGF) and smooth muscle α-actin (α-SMA) were assessed by immunoblotting. The association between hydroxyproline levels in lung tissue and dabigatran concentration in plasma was tested using Spearman rank correlation test.

RESULTS: In BALF we observed significant reduction of active thrombin and TGF-β1 from 46.0519.4ng/ml and 54.9±6.1pg/ml in bleomycin-placebo-treated mice to 11.95±4.4ng/ml ($p< 0.001$) and 31.144±8.7pg/ml ($p<0.01$) respectively in bleomycin-dabigatran etexilate-treated mice. Dabigatran treatment was also associated with two-fold decrease in the absolute number of cells in BALF of bleomycin-treated mice. A quantitative evaluation of histopathology by Ashcroft scale demonstrated a significant decrease in fibrosis of dabigatran-treated mice (5.76±1.64 vs. 2.98±0.88, $p<0.05$). A strong negative correlation between hydroxyproline levels in lung tissue and dabigatran concentration in plasma was observed in mice with bleomycin-induced lung fibrosis ($R=-0.96$, $p=0.0005$). There was no correlation between hydroxyproline and dabigatran in control sham-injured mice. Additionally, dabigatran reduced CTGF 9-fold and α-SMA 2.5-fold in mice with bleomycin-induced lung fibrosis, whereas it did not interfere with basal levels of the proteins.

CONCLUSIONS : Inhibition of thrombin using the oral DTI dabigatran etexilate has marked anti-fibrotic effects in a bleomycin-induced mouse model of pulmonary fibrosis. Dabigatran etexilate treatment reduces collagens, TGF-β1, CTGF, and α-SMA induced

by tissue injury, while not interfering with basal levels of these proteins in normal lung tissue. Our data suggest that dabigatran etexilate may be beneficial in the treatment of fibrosing lung diseases, e.g. scleroderma lung disease and idiopathic pulmonary fibrosis.

Effect of Vitamin E and Selenium on Incidence of Physician-Diagnosed COPD: The Selenium and Vitamin E Cancer Prevention Trial (SELECT)

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Rationale: Vitamin E and selenium play a role in antioxidant defenses and may decrease oxidant damage to tissues and thereby reduce the risk of COPD. Prior observational epidemiologic studies support this hypothesis, but no large randomized trials have assessed supplementation with selenium, and one trial (Heart Protection Study) found no effect of combined vitamins E, C and beta-carotene on COPD hospitalizations. The effect of selenium, vitamin E or both on COPD risk was assessed in the Selenium and Vitamin E Cancer Prevention Trial (SELECT).

Methods: SELECT was a double-blind, randomized, placebo-controlled trial of 35,533 men (median age 62.4 yrs) at 427 sites in the US, Canada, and Puerto Rico testing the single or joint effects of vitamin E (400 IU/day *all rac*-α-tocopherol acetate) and selenium (200 μg/d selenomethionine) in a 2x2 factorial design. As of 10/23/08, the end of supplementation in SELECT, the median length of follow-up was 5.46 years (range 4.17-7.33 years). The endpoint for the current study was assessed by participant report on bi-annual medical history questionnaires of incident physician-diagnosed emphysema, COPD and/or chronic bronchitis in 32,371 eligible men [excluding men with inadequate data (2,094) or with prevalent lung disease at baseline (1,068)].

Results: Hazard ratios [95% confidence intervals (CIs)] for COPD were 1.08 (95% CI 0.91, 1.28) for vitamin E, 1.01 (95% CI 0.85, 1.20) for selenium, and 0.94 (95% CI 0.79, 1.12) for combined vitamin E and selenium, all versus placebo/placebo. Findings were unchanged in models that included participants with a baseline report of prevalent lung disease, and Kaplan-Meier curves show no separation over time in probability of event by treatment group. The cumulative incidence of COPD was 3.27% over the follow-up period, and current smokers were at increased risk compared to never smokers (HR 7.22; 95% CI 5.99, 8.72).

Conclusion: Selenium or vitamin E, alone or in combination, did not prevent COPD in a population of relatively healthy men. Although it is possible that effects may have been missed due to the short duration of treatment and suboptimal endpoint ascertainment, this

study is a strong test of the hypothesis that antioxidants decrease the risk of physician-diagnosed COPD. Statistical power to test for subgroup effects in smokers is limited, although such a test follows directly from the study hypothesis. A spirometry endpoint, currently being evaluated, may give further insight into these questions in a subgroup enriched with smokers.

The Role of TLR signaling in the development of emphysema in ApoE-deficient mice

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Rationale: Emphysema and atherosclerosis are the leading causes of mortality in the United States. We have demonstrated that hypercholesterolemic Apoe-/- mice develop emphysematous changes in their lung. Recent studies have shown that Apoe-/- mice exhibit abnormal cholesterol efflux with activation of the TLR pathway in atherosclerotic lesions. The present study was conducted to examine the macrophages from the Apoe-/- mice and determine if TLR signaling was playing a role in the development of emphysema in these mice.

Methods: 8-week-old female Apoe-/- mice (n=6) were subjected to a Western diet for 10 weeks and compared to Apoe+/+controls (n=6). After sacrifice, their lungs were fixed for histological analysis. Total lung RNA was prepared for quantitative RT-PCR probed for MMP-9. Lung lysates were subjected to Western Blot analysis. Peritoneal macrophages were obtained from Apoe-/- and Apoe+/+mice and cultured in DMEM for 24 hours. Subsequently, they were treated with various TLR ligands: TLR2 [peptidoglycan (PGN), 2Î¼g/ml], TLR3 [polyinosine-polycytidylic acid (poly(I:C), 2.5Î¼g/ml], and TLR4 [lipid A, the active component of lipopolysaccharide, 100ng/ml].

Results: Elevated expression of Matrix Metalloproteinase-9 (MMP-9), which is a potent protease contributing to emphysema, was observed in the lungs of the Apoe-/- mice. In addition, the up regulation of IRAK1 and G-CSF, downstream targets of the TLR pathway was demonstrated in the lungs of Apoe-/- mice compared to Apoe+/+ mice, suggesting that TLR signaling is activated in the lungs of Apoe-/- mice. TLR4 ligand increased mRNA expression of MMP-9 by 8-fold in Apoe-/- macrophages and by 4-fold in wild-type macrophages. Interestingly, TLR2 and TLR3 ligands did not exhibit any effect on MMP-9 expression in Apoe-/- and wild-type macrophages. These data suggest that up regulation of MMP-9 observed in the lungs of Apoe-/- mice is TLR4 specific.

Conclusion: Augmented expression of downstream targets of TLR signaling in the lungs of Apoe-/- mice suggests that activation of the TLR pathway plays an important role in the pathogenesis of emphysema in these mice, possibly through up regulation of macrophage MMP-9 downstream of the TLR4 pathway.

Evaluation of Lung Clearance Index in Children with Cystic Fibrosis Compared to Canadian Controls

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Rationale: Groups in Sweden and the UK have demonstrated that the lung clearance index (LCI), a marker of overall ventilation inhomogeneity within the lung derived from the Multiple Breath Washout (MBW) test, has a narrow range of values in reference populations that is almost identical across studies, and seems to be independent of subject age, sex, height and weight. These groups have also shown that LCI is a sensitive tool for detecting cystic fibrosis (CF) lung disease in children with normal spirometry. While the findings from these clinical studies are promising, data confirming these results from controls and patients in North America are lacking.

Objective: Our objective was to compare our own paediatric normative data with that of the groups from Sweden and the UK, and to assess if LCI detected abnormalities in our CF patients with mild lung disease.

Methods: We measured baseline LCI values derived from a group of 28 healthy children aged 6-18 years and an age-matched cohort of 19 children with CF with a FEV1 > 80 % predicted. Our equipment and set-up were the same as described by the groups in Sweden and the UK. LCI was calculated as the number of lung volume turnovers (TO) needed to lower the end-tidal concentration of inert gas to $1/40^{th}$ of its starting concentration. The TO was calculated at each breath as the cumulative expired volume (CEV) divided by the FRC.

Results: Mean LCI values for our group of healthy children was 6.13 (standard deviation (SD): 0.41, 95% CI: 5.97 to 6.29), which is congruent with published normative data from Sweden (mean LCI: 6.33, SD: 0.43) and from the UK (mean LCI: 6.45, SD: 0.49). LCI was significantly higher in our cohort of children with CF compared to our healthy controls (mean difference 2.77 (95% CI 1.84 to 3.7), $p<0.001$). Furthermore, LCI detected 13 (68%) of the children with CF as having an abnormal LCI, defined as a value above +1.96SD of the mean LCI from our healthy control group.

Conclusions: The normative data from our group of healthy controls confirms the findings of groups in Sweden and the UK that LCI is maintains a narrow normal range of values that is consistent across research locations. The LCI was able to detect abnormalities in a high proportion of children with mild CF disease, suggesting that LCI may be a more sensitive assessment than spirometry in this disease.

Stat3 signal is required during the tumorgenesis in the lung

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Rationale: Stat3 is constitutively activated in various cancers, including lung adenocarcinoma. Because the activation of Stat3 in cancer cell has been reported to promote survival and proliferation of the cell in a cell-autonomous manner and suppress the anti-cancer inflammation in the tumor microenvironment, Stat3 signal pathway is the attractive therapeutic target for cancer. To understand the role of Stat3 in the mouse model of lung adenocarcinoma is of considerable relevance for developing the therapy targeting Stat3 signal pathway for human lung adenocarcinoma.

Methods: Stat3 was conditionally deleted from pulmonary epithelial cells of transgenic mice *in vivo*, producing *Stat3*$^{\hat{a}\hat{}\dagger/\hat{a}\hat{}\dagger}$ mice. *Stat3*$^{\hat{a}\hat{}\dagger/\hat{a}\hat{}\dagger}$ and control (*Stat3*$^{flox/flox}$) mice were injected once intraperitoneally with 1mg/g body weight urethane. The total volume of lung tumor was measured by microCT after 4 months. Histological analysis and bronchoalveolar lavage (BAL) were performed after 6 months. Tumors were microdissected from the lung for RNA or protein extraction. Microarray analysis, RT-PCR and Western blot were performed.

Results: The total tumor volume (TV) in *Stat3*$^{\hat{a}\hat{}\dagger/\hat{a}\hat{}\dagger}$ (2.9mm^3/mouse) was significantly smaller compared to that of *Stat3*$^{flox/flox}$ (6.5mm^3/mouse). Immunohistochemistry (IHC) for phospho-Stat3 showed that Stat3 is activated in the inflammatory cells which surrounded the tumor in *Stat3*$^{\hat{a}\hat{}\dagger/\hat{a}\hat{}\dagger}$. IHC for both surfactant protein-C and clara cell secretory protein indicated that the origin of the tumor cell was type II alveolar epithelial cell. Total cell count in BAL (TC) increased in proportion to TV in both mice. Howerver, the ratio of TC to TV (TC/TV) was significantly higher in *Stat3*$^{\hat{a}\hat{}\dagger/\hat{a}\hat{}\dagger}$ compared to that of *Stat3*$^{flox/flox}$. Microarray analysis showed that the genes regulating inflammatory response were significantly up-regulated in the tumor of *Stat3*$^{\hat{a}\hat{}\dagger/\hat{a}\hat{}\dagger}$.

Conclusions: Stat3 signal is required during the tumorigenesis of the lung. The abscence of Stat3 signal in tumor cell induced the increased inflammation in the lung. The target genes of Stat3 which affect survival, proliferation and immune-regulation of the lung tumor cell should be searched.

Airway Basal Cells of Healthy Smokers and Human Lung Adenocarcinomas Share a Common Embryonic Stem Cell-like Transcriptional Program

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Rationale: Various epithelial cancers, including lung adenocarcinomas, exhibit activation of a human embryonic stem cell (hESC)-like transcriptional program in association with their aggressiveness. We hypothesized that under chronic exposure to the pro-carcinogenic stress of cigarette smoking, airway basal cells, the stem/progenitor cell compartment of the large airway epithelium (LAE), acquire "cancerous" hESC-like molecular phenotype as an early step toward malignant tissue derangement.

Methods: Expression of 40 hESC-specific genes previously identified in a meta-analysis of the hESC transcriptome was assessed in freshly isolated LAE of healthy nonsmokers (n=21) and healthy smokers (n=31) and in the pure basal cell (BC) populations obtained from these samples (n=4 in both groups) using Affymetrix microarrays. BC transcriptomes were additionally analyzed by massive parallel sequencing (RNA-Seq). Expression patterns were then compared to those in primary human lung adenocarcinoma samples (n=193) and pure human lung adenocarcinoma cells propagated as xenografts in NOD/SCID/IL2R-null immunodeficient mice (n=4).

Results: Microarray analysis revealed low-level constitutive expression of a subset of hESC-specific genes in the normal healthy LAE, with higher expression in the BC population. Smoking dramatically and selectively up-regulated hESC-specific gene expression in BC. Remarkably, 35% of known hESC-specific genes were found up-regulated in BC of healthy smokers by both microarrays and RNA-Seq. The majority of these genes (71%) were not detected in nonsmokers' BC indicative of their *de novo* induction by smoking. Strikingly, 82% of hESC-specific genes induced in BC of healthy smokers were also significantly up-regulated in both primary human lung adenocarcinomas and xenograft-derived human lung adenocarcinoma cells. Clustering analysis identified a subgroup of lung adenocarcinoma samples (n=32; 6%) among 193 primary lung adenocarcinoma samples based on the high expression of hESC-specific signature found up-regulated in BC of healthy smokers. This subset of adenocarcinoma subjects exhibited distinct clinical/pathological characteristics, such as longer smoking history ($p<0.0002$), lower lung function parameters FEV1 and DLCO (both $p<0.001$), higher COPD co-morbidity (44% *vs* 14% among other adenocarcinoma subjects), larger tumor size ($p<0.0002$), more advanced tumor stage, poorer differentiation grade, and

higher frequency of *TP53* gene mutations (50% *vs* 23% among other adenocarcinoma subjects).

Conclusions: Cigarette smoking reprograms airway BC *in vivo* toward a lung cancer-associated hESC-like molecular state prior to any clinical manifestation of lung cancer. The subset of lung adenocarcinomas highly expressing smoking-induced BC-hESC-like gene signature have a distinct, aggressive clinical phenotype.

A Genome-wide Admixture Scan for Ancestry-linked Genes Predisposing to Sarcoidosis in African Americans

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RATIONALE: A genetic predisposition to sarcoidosis, a multi-organ granulomatous inflammatory disease, has long been posited with genome-wide linkage and association studies having uncovered disease-associated variants. African Americans are more commonly affected by sarcoidosis, implying that African ancestry may influence disease pathogenesis.

METHODS: We conducted the first sarcoidosis genome-wide ancestry scan using a map of 1,384 highly ancestry informative single nucleotide polymorphisms genotyped on 2,060 self-identified African American subjects, consisting of 1,357 sarcoidosis cases and 703 unaffected controls drawn from two family studies and a case-control study. Using the admixture mapping program ADMIXMAP, we analyzed a subset of unrelated 1,026 cases and 316 controls.

RESULTS: We estimated African ancestry proportions to be 82.9% in the cases and 81.5% in the controls (p=0.03). The most significant ancestry association was at marker rs11966463 on chromosome 6p22.3 (ancestry association odds ratio (aOR)= 1.90; p=0.0002). Eight other chromosomal regions met our statistical criterion for suggestive ancestry association, including marker rs1462906 on chromosome 8p12 which was the most significant signal associated with European ancestry (aOR=0.65; p=0.002), and chromosomes 5p13 (aOR=1.46; p=0.005) and 5q31 (aOR=0.67; p=0.005), which correspond to two chromosome 5 regions we previously identified through sib pair linkage analyses. Sex-stratified analyses showed that sarcoidosis ancestry associations were often sex-specific. Analyses of chest radiographic phenotypes yielded the most significant ancestry association for Scadding stage IV cases to marker rs7919137 on chromosome 10p11.22 (aOR=0.27; $p=2x10^{-5}$), a region not associated with disease susceptibility.

CONCLUSION: Using admixture mapping, we have identified several putative candidate loci for sarcoidosis that could lead to identification of novel risk variants for this disease.

Staphylococcal Activation Of EGFR Facilitates Invasion Across The Pulmonary Mucosal Barrier

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RATIONALE: *Staphylococcus aureus* are an important cause of severe pneumonia especially in hospital settings and as a potentially fatal complication of influenza. Nasal colonization with *S. aureus* increases the likelihood of infection, yet is unclear how mucosal organisms once aspirated, cross an intact epithelial barrier. The staphylococcal surface protein, protein A (SpA), binds EGFR and activates several signaling cascades. EGFR has a central role in cell development and motility and is linked to both actin and E-cadherin. EGFR initiated MAPK activity is linked to activation of Rho/ROCK/ MLC induced actinomyosin contraction and to epithelial proteases.

METHODS: We postulated that staphylococci could stimulate contraction of the epithelial cytoskeleton as well as cleavage of junctional proteins, such as E-cadherin and occludin, thus creating a gap in the paracellular junctions sufficient to enable staphylococcal translocation.

RESULTS: Using strain Newman and the epidemic USA300 MRSA strain of *S. aureus* and isogenic *spa* null mutants, we demonstrate disruption of the actin cytoskeleton in polarized human airway monolayers in response to *S. aureus* strains expressing *spa*, but not the mutants unless complemented with the SpA peptide. Bacterial translocation and permeability to 10k MW fluorescent dextran similarly was limited to SpA+ strains and correlated with SpA+ induced calpain activity. SpA- exposed monolayers had activated RhoA and phosphorylated MLC. The SpA+ strains also induced cleavage of the extracellular portions of E-cadherin and occludin that span the paracellular junctions. Monolayers treated with EGFR inhibitor BPDQ, the ERK inhibitor U0126, the ROCK inhibitor Y27632 or the calpain inhibitor calpeptin had significantly ($p<0.05$) decreased staphylococcal transmigration; by confocal imaging these monolayers were found to be protected from staphylococcal induced disruption of the cytoskeleton. In a mouse model of staphylococcal pneumonia, mice pretreated with the EGFR inhibitor AG14578 had significantly less bacteremia than did the untreated controls ($p=0.017$).

CONCLUSION: These studies suggest that activation of the EGFR- ERK/RhoA/MLC and EGFR-ERK calpain pathways by *S. aureus* protein A enable these non-motile organisms to pass through the paracellular junctions and gain access to the subepithelia space and disseminate into the bloodstream. Thus in addition to the other potent virulence factors of *S. aureus*, the protein A- EGFR interaction facilitates staphylococcal invasion through epithelial and likely through endothelial barriers as well.

Tumor-Suppressor Protein p53 binds to RSV RNA and Increases RSV Replication by Enhancement of Viral Transcriptional

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Respiratory syncytial virus (RSV) infection is the major cause of childhood lower respiratory illness and has been associated with the induction and exacerbation of asthma. Previously, we reported that RSV infection induced TGF-b expression that caused cell cycle arrest and subsequently enhanced RSV replication. In the current study, we demonstrate that a downstream target of TGF-b signaling, namely P53, is a pivotal transcription factor for RSV replication. Over-expression of p53 protein in H1299 and A549 human epithelial cells enhanced RSV replication by 9 folds. Accordingly, knockdown of p53 using siRNA significantly reduced RSV replication in both cell lines. Stabilization of p53 with nutlin-3 enhanced RSV replication by 8 folds in primary human lung epithelial (PHBE) cells. We hypothesized that a plausible mechanism of enhanced RSV replication by p53 was through direct binding of p53 to RSV RNA. A search of RSV genome revealed several putative p53-responsive elements. The direct interaction of p53 and RSV RNA was shown by EMSA analysis using RSV-specific RNA oligonucleotides. Taken together, we have identified a novel role for p53 as a cis-acting enhancer in regulation of RSV replication. These findings suggest that enhanced p53 expression through lung injury, smoking or air pollution could facilitate a significant increase in RSV replication leading to more severe respiratory pathologies.

Quercetin Inhibits rhinovirus replication and subsequent chemokine response in Airway Epithelial Cells

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Rationale: Rhinovirus (RV), a single-stranded RNA virus from the *picornaviridae* family, is responsible for the majority of common colds. RV is an important trigger of COPD, asthma and CF exacerbations. Flavonoids with antioxidant and anti-inflammatory properties may be beneficial in the treatment of viral infections particularly in patients with underlying chronic lung diseases. Quercetin (3, 3', 4', 5, 7-pentahydroxyflavone) has potent antioxidant effects and inhibits various protein kinases by competing for ATP binding site. RV induces PI3 kinase-dependent chemkine responses and oxidative stress-dependent disruption of barrier function in polarized airway epithelial cells. Therefore, we hypothesized that quercetin reduces RV-induced pro-inflammatory response and reduction in transepithelial resistence of polarized airway epithelial cells.

Method: Polarized 16HBE14o- cells were infected with major or minor group RV or replication-deficient UV-irradiated virus (UV-RV) and incubated for 1 hour at 33°C to allow binding and endocytosis of RV. Infection media was then replaced with fresh media containing either quercetin; LY294002, a chemical inhibitor of PI3-kinase, or diphenyleneimidonium (DPI), an inhibitor of NADPH oxidase. After incubation for 8 or 24 hours, TER was measured and media was collected for IL-8 and IL-29 (IFN-l1) analysis, and the cells harvested for Western blot analysis and determination of viral titer.

Results: Quercetin inhibited RV-induced reduction in TER, decreased RV-stimulated IL-8 expression. Surprisingly, we also observed reduction in interferon response, viral titer and complete abrogation of RV-triggered cleavage of eIF4GI, which is required for efficient translation of viral polypeptide. On the other hand, LY294002 although affected IL-8 response, had no effect on IL-29 response, viral titer or RV-induced cleavage of eIF4GI. DPI partially reduced viral titer and RV-induced effects in airway epithelial cells

Conclusions: Our results suggest that quercetin may reduce RV-triggered cytokine response, and disruption of barrier function by inhibiting viral replication and virus induced cleavage of eIF4GI. Further, the observed effects of quercetin on viral replication may be attributed to its antioxidant effects and not on its effect on PI3-kinase activity. Therefore, quercetin may be beneficial in the treatment of viral infections, particularly in patients with underlying chronic lung disease.

The Multiple Dimensions of Cardiopulmonary Dyspnea

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Objectives: We investigated the qualitative components of dyspnea in cardiopulmonary disease, and their relevance for clinical diagnosis.

Methods: A respiratory symptom checklist incorporating 61 spontaneously reported descriptors of dyspnea was administered to 396 patients with dyspnea due to a variety of cardiopulmonary disease. Symptom factors measuring different qualitative components of dyspnea were derived by a principal component analysis. The separation of patient groups in terms of symptom factors was achieved by a variance analysis.

Results: Seven symptom factors measured four elements of dyspnea: sensory qualities (difficulty breathing and phase of respiration, depth and frequency of breathing, urge to breathe, wheeze), affective aspect (chest tightness), behavioral impact (withdrawal from physical activity), and emotional response (anxiety). The other two factors were tingling and cough. A variance analysis with Duncan grouping on these factors separated clearly different types of patients with dyspnea. The factor of difficulty breathing and phase of respiration occurred more often in patients with COPD and asthma, with the highest mean score in COPD (R^2 = 0.12). The factor of urge to breathe was unique for patients with medically unexplained dyspnea, and clearly distinguished those patients from other patients with dyspnea. (R^2 = 0.12). The factor of wheeze showed up most frequently in asthma, followed by COPD and congestive heart failure (R^2=0.17). Chest tightness was primarily linked to the diagnosis of medically unexplained dyspnea and, to a lesser extent, to asthma (R^2 = 0.04). The factor of withdrawal from physical activity appeared more often in congestive heart failure, pulmonary vascular disease, and COPD (R^2 = 0.15). Anxiety (R^2 = 0.08) and tingling (R^2 = 0.05) characterized patients with medically unexplained dyspnea. The score of cough was the highest in COPD and diffuse parenchymal lung disease, followed by asthma (R^2 = 0.20).

Conclusions: Multiple dimensions of dyspnea were found in patients with cardiopulmonary dyspnea. These elements of dyspnea allowed separation of different types of patients with dyspnea.

Homeostatic Role for Nuclear Factor-KappaB in the Lung

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Rationale: Recent mice studies demonstrate that inhibition of basal activity of the transcription factor, Nuclear Factor-KappaB (NF-kB) in skin, gut, and liver results in severe inflammation. Furthermore, NF-kB related disruption of homeostasis has been implicated in incontinentia pigmenti and colitis in humans. The purpose of this study was to determine whether constitutive NF-kB is essential to lung homeostasis.

Methods: Sheep were instrumented to permit measurement of arterial oxygenation (paO2), pulmonary vascular resistance (PVR), peripheral blood neutrophil (PMN) count, and pulmonary endothelial permeability (lung lymph protein clearance, Clp). Sheep were given intravenously the pharmacologic inhibitor of IkBa phosphorylation Bay11 (6 mg/kg) or, on a separate day 1 week apart, vehicle and measures made for 5 hours. After Bay11, animals were euthanized and lung wet:dry weight ratio (W/D) determined. Statistical analysis used repeated measure ANOVA with post hoc Tukeys.

Results: Bay11 inhibition of NF-kB caused marked pulmonary edema whereas infusion of the control vehicle caused no physiologic changes. With Bay 11, paO2 decreased significantly ($p<0.001$) after 2 hours and remained low (5 hour paO2 decreased by 34.6 ± 3.6(SD) torr, n=5). Clp increased significantly ($p<0.001$) from baseline at 3 hours and remained high (baseline=3.0 ± 0.75; 5hour=25.5 ± 7.0, (n=3). W/D ratio was 60% higher than control values (PBS =5.46 ± 0.44; Bay 11=8.78 ± 0.49). PVR increased slightly in the last two hours of the experiment. PMN count did not change. Protein rich fluid filled the airways postmortem (protein concentration control = 0.0115 mg/ml; Bay11= 0.5924 mg/ml). A cell permeable form of mutant IkBa which cannot be degraded and which inhibits NF-kB in cells and in sheep lungs (EMSA) caused a 15 torr decrease in paO2 and a 10-fold increase in Clp, confirming the Bay11 results. Results were also confirmed in swine where Bay 11 produced pulmonary edema that was dose related and severe at higher doses (10 mg/kg) without any increase in PVR or circulating IL-6, TNFa, or neutrophil count indicating a non-inflammatory endothelial permeability defect. In cultured human pulmonary endothelial cells, constitutive NF-kB activation was completely inhibited by Bay 11 and microarray analysis showed alterations in integrin receptor gene expression.

Conclusion: Inhibition of NF-kB causes increased permeability pulmonary edema without inflammation. We speculate that constitutive NF-kB activity is essential for maintaining pulmonary endothelial barrier function. The dual role of NF-kB in maintaining lung fluid homeostasis and as a mediator of the inflammatory response should dictate caution in targeting its inhibition as potential therapy for inflammatory diseases.

Risk factors for post fiberoptic-bronchoscopy fever in immunocompetent children with recurrent pneumonia

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RATIONALE: Flexible bronchoscopy (FOB) with bronchoalveolar lavage (BAL) is useful in children with recurrent pneumonia to discriminate among different aetiologies. Fever post-FOB and BAL has reported in these children but its origin is subject to debate.

METHODS: We examined risk factors associated with fever in immunocompetent children with recurrent pneumonia undergoing FOB with BAL. Before the procedure all children performed routine blood tests, total and specific IgE dosages, Chest X-ray. All BAL specimens were tested for total and differential cell counts, interleukin (IL-8), eosinophil cationic protein (ECP), tumor necrosis factor (TNF), bacterial growth and respiratory viruses (reverse transcription-polymerase chain reaction). The cut-off for fever was 38°C in the 12 hours after the procedure.

RESULTS: The study population included 81 children (46 girls) aged between 7 and 215 months (mean age 64 months). Fever post FOB appeared in 29 of these patients (35.8%). There was a trend for age to be lower in children with fever than in those without fever (mean: 53 vs 70 months; p=0.06). Post FOB fever was present in 54.5% of children aged<36 months, in 38.6% of children aged between 36 and 84 months and in 7.7% of children aged>84 months. Among the different parameters examined, white blood cell count before the procedure was significantly higher in the group of patients with fever than without fever (mean=8777/mm^3 vs 7349/mm^3; p=0.04). Children with post FOB fever had higher basal CRP values than those without fever (mean 6.3±7.9 vs 3.3±0.5; p=0.015). CRP values within the normal range (0-6 mg/L) showed a specificity of 97.7% in predicting the absence of fever. None of the parameters examined in BAL was able to differentiate children with fever from those without fever.

CONCLUSIONS: Younger age was a substantial risk factor for appearance of post FOB fever in our population. Basal levels of WBC and CRP were also significantly associated with fever, suggesting they could be of prognostic value.

Table 1 – Clinical and laboratory findings in children with and without post FOB fever (data are presented as mean ± SD unless otherwise indicated)

Clinical and lab findings	**Fever**	**No Fever**	**p value**
Patients, N.	29	52	-
Gender, F/M	16/13	30/22	-
Age, months	53±19	70±41	0.06
White blood cells/mm^3	8777±2981	7349±1978	0.04

CRP mg/L	6.3±7.9	3.3±0.5	0.015
BAL findings			
Cell count, 10^6 /mL	4.1±5.1	4.2±4.1	0.34
Alveolar Macrophages %	56.6±22.5	58.6±24.6	0.66
Neutrophils %	34.2±23.9	33±26.2	0.69
Eosinophils %	0.4±0.9	1±2	0.10
Lymphocytes%	9.1±9	7.5±5	0.92
ECP ug/L	31±37.1	44.8±64.4	0.91
IL-8 pg/mL	1020±1047	1087±1660	0.29
TNF ng/L	17.2±30.7	23.4±63.8	0.62

Real-time dynamic CO2 administration: a novel treatment strategy for periodic breathing

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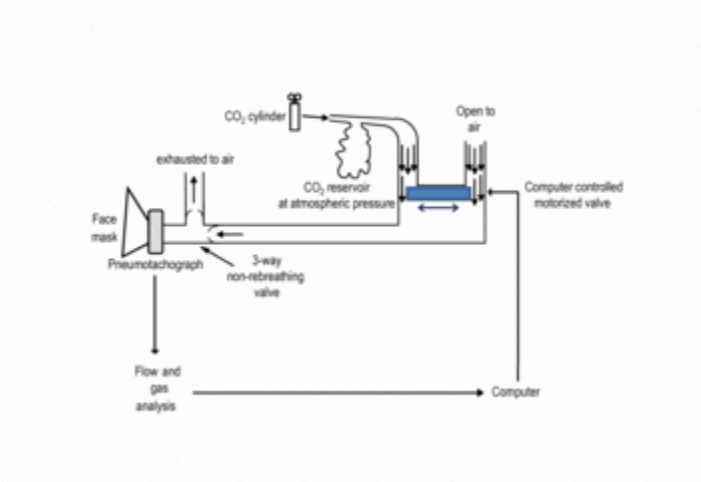
Objectives and background: Oscillations in end-tidal carbon dioxide (et-CO_2) drive the characteristic ventilatory oscillations of periodic breathing (PB) and Cheyne-Stokes respiration (CSR) in heart failure (HF). Exogenous CO_2 administration (constant concentration, constant flow) successfully avoids apnoeas but is associated with significantly increased et-CO_2 which may contribute to sympathetic hyperactivity.

We aimed to minimise the quantity of CO_2 delivered whilst still stabilising ventilation, by specifically targeting the hyperventilation phase, which drives down CO_2 levels. By filling in theses troughs in et-CO_2 with small doses of CO_2, we aimed to establish whether et-CO_2 oscillations may be attenuated, stabilizing PB without significantly increasing et-CO_2 and ventilation.

Methods: Seven heart failure patients with spontaneous daytime PB (four had apneas, EF 18.5±7.4, cardiac output 4.1±1.2 L/min) underwent dynamic CO_2 administration delivered by an automated algorithm that monitors ventilation but not CO_2. Starting from a fixed concentration of CO_2 in a reservoir and using a custom motorized valve, CO_2 was delivered in a sinusoidal profile with the peak concentration (2%) timed to be coincident with peak ventilation for up to half the ventilatory cycle. The coefficient of variation and mean et-CO_2 and ventilation, for baseline PB, dynamic therapy and static CO2 administration (2%, constant flow throughout the ventilatory cycle) were assessed.

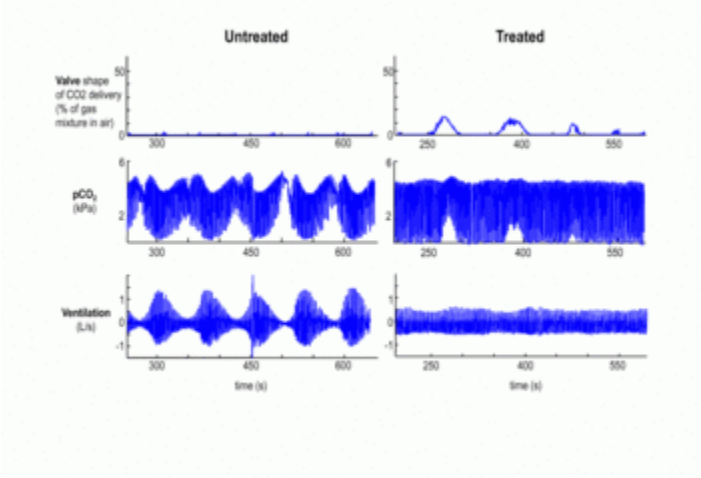
Overview of the delivery system



Representation of the system used to dynamically deliver CO2 to the subject.

Results: Et-CO_2 oscillations were 52% smaller (sd/mean = 0.072±0.03 untreated versus 0.041±0.023 treated CO_2, p‹0.01) following dynamic CO_2 administration, without significantly increasing mean et-CO_2 (4.7±0.4 versus 5.0±0.3 kPa, p=0.06). This significant attenuation of end-tidal CO_2 oscillations resulted in dramatic attenuation of the ventilatory oscillations by 68% (sd/mean of ventilation from 0.43±0.19 untreated to 0.13±0.09 treated, p=0.01 but critically there was no increase in mean ventilation with dynamic administration (0.14±0.03 versus 0.16±0.05 L/s, p=0.12). Application of static CO_2 rather than dynamic CO_2 also stabilised breathing (sd/mean of ventilation 0.14±0.06 and sd/mean CO_2 0.026±0.006). However static CO_2 significantly increased end-tidal CO_2 (5.2±0.3 versus 4.7±0.4 kPa, p=0.03) and ventilation (0.20±0.07 versus 0.14±0.03 L/s, p=0.03). There was a non-significant trend toward reduced oscillations in BP (sd/mean = 0.14±0.11 versus 0.08±0.03, p=0.15when with dynamic CO_2 administration.

Dynamic CO2 administration in one patient with Cheyne-Stokes respiration



Example of one patient with heart failure and daytime Cheyne-Stokes respiration efficaciously treated with dynamic CO2. The delivery of 2% CO2 (peak dose) at 0° with an angle width of delivery ranging from -90° to +140° was able to abolish not only the oscillation in end-tidal CO2, but also the fluctuation in ventilation, without increasing their average values.

Conclusions: This study demonstrates that dynamic CO_2 administration, when given at the right time, almost abolishes the oscillations in end-tidal CO_2 that drive PB. We have demonstrated a simple algorithm that can control delivery in real time, using only a ventilatory signal to guide it. Dynamic CO_2 offers an opportunity to develop therapies for PB, whilst avoiding some pitfalls of static CO_2 therapy.

Feasibility of Hypoglossal Nerve Stimulation Therapy to Treat Obstructive Sleep Apnea

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RATIONALE: Reduced upper airway muscle activity is fundamental to obstructive sleep apnea (OSA) pathogenesis. Prior research has demonstrated that hypoglossal nerve stimulation has the potential to reduce OSA severity without causing arousal from sleep. The objective of this study was to examine the safety and effectiveness of a novel hypoglossal nerve stimulation (HGNS™, Apnex Medical, Inc.) system as a potential treatment alternative for OSA.

METHODS: Eighteen subjects with OSA but unable to tolerate continuous positive airway pressure (CPAP) underwent surgical implantation of the HGNS system in a feasibility study. The HGNS system is designed to stimulate the hypoglossal nerve during sleep, activating the genioglossus muscle synchronous with inspiration, which is sensed by changes in thoracic impedance. Apnea-hypopnea index (AHI) during laboratory polysomnography was used to measure OSA severity pre-implant and three months post-implant, which was two months after therapy initiation. Symptomatic response was assessed using Epworth Sleepiness Scale (ESS), Functional Outcomes of Sleep Questionnaire (FOSQ), Calgary Short Sleep Apnea Quality of Life Index (SAQLI), Pittsburgh Sleep Quality Index (PSQI), and Beck Depression Inventory (BDI). Paired t-tests were used to assess changes from baseline. Mean nightly use (mean hours of use over all patient-nights) was calculated using at-home therapy utilization data stored by the HGNS system. Therapy adherence was defined as percent of subjects with mean nightly use $\geq$ 4 hours/night.

RESULTS: Clinical data at 3 months post-implant are available in twelve (12) subjects (8 male), age 55.4±10.5 yrs (mean±SD); baseline and three-month data are presented in

the table below. Enrollment and follow-up is ongoing. Three months after implant, there was a 56% reduction in mean AHI (49.3/hr to 21.6/hr), with eight subjects (67%) experiencing an AHI reduction of 50% or more (mean AHI 53.6/hr to 16.8/hr). In addition, there was improvement in symptoms based on the ESS, FOSQ, SAQLI, PSQI, and BDI scores. Adherence was 92% (11/12). Excluding the one subject who discontinued HGNS use, mean nightly use of the HGNS system was 6.5±1.1 hours/night over an average of 113±59 nights that therapy was active. One system was explanted due to infection after the three-month visit was completed. There were no device failures and no unanticipated adverse device effects.

Clinical Results Three Months Post Implant

	Baseline (n=12)	**3-Month (n=12)**	**p-value**
AHI (events/hr)	49.3±18.1	21.6±11.7	<0.001
BMI (kg/m^2)	32.4±3.4	32.3±3.5	0.883
ESS	13.3±3.7	7.9±4.6	0.005
FOSQ	14.0±2.0	17.5±2.1	<0.001
SAQLI	2.8±1.0	4.8±1.5	0.001
PSQI	9.4±2.8	7.1±3.6	0.038
BDI	15.1±9.6	6.8±7.7	0.027

CONCLUSIONS: In a substantial majority of patients in which it has been used to date, HGNS therapy significantly decreases OSA severity, measured by AHI, with associated improvement in symptoms. Therapy is well-tolerated with high mean nightly use. Data collection is ongoing.

Notch 3 receptor is localized and activated from an intracellular compartment in embryonic lung vascular smooth muscle cells

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Background: Notch receptor signaling plays a fundamental role in development by regulating cell fates. In canonical Notch signaling, the receptor is activated on the cell surface after engagement with ligands expressed on homotypic or heterotypic cells. Recently in *Drosophila*, however, intracellular activation of Notch receptor has been demonstrated to occur in ligand dependent and independent mechanisms. Notch 3 signaling is implicated in arterial vascular smooth muscle cell (VSMCs) proliferation, differentiation, and cell identity, and may play an important role in the pathogenesis of pulmonary hypertension. Here we investigated the expression and activation dynamics of the Notch 3 receptor during pulmonary artery development.

Methods and Results: Immunohistochemical studies showed that the Notch 3 receptor is expressed exclusively in lung vascular, but not bronchial smooth muscle cells. Flow cytometry analysis indicated that Notch 3 is localized solely in the cytoplasm of VSMCs, and not on the cell surface. Confocal microscopy confirmed this finding and revealed that Notch3 is localized within endosomes. To determine the dynamics of Notch 3 signaling in lung VSMCs, the activated cleaved form of the Notch 3 receptor (NICD) and the induction of Notch 3 down-stream target genes HRT-1 and HRT-2 were determined by immunohistochemistry (IHC) and real time PCR (qPRC), respectively. These data show that Notch 3 signaling is active at E18.5 and continues to around post-natal day 5. These findings raise the possibility that Notch 3 receptor activation occurs in the interior of VSMCs. To determine the possible Notch 3 receptor ligand in lung VSMCs, qPCR and IHC was performed. We found that Jagged 1 is the predominant Notch ligand expressed in embryonic VSMCs. Confocal microscopy demonstrated that Jagged 1 colocalizes with the Notch 3 receptor inside the cell, and immunoprecipitation followed by western blot analysis confirmed a physical association between Jagged 1 and Notch 3 receptor. To validate the ligand-dependent activation of Notch 3 in lung VSMCs, Jagged 1 was selectively knocked-out (KO) in smooth muscle cells. In Jagged 1 KO mice, a down regulation of the Notch 3 down-stream target genes HRT-1 and HRT-2 was observed, consistent with suppression in Notch 3 signaling in VSMCs.

Conclusion: This report demonstrates for the first time an intracellular ligand dependent Notch receptor activation in a mammalian cell. We speculate that this signaling system plays an important role in the maturation of the pulmonary artery.

Non-invasive evaluation of the effects of endothelin receptor antagonists on pulmonary blood flow in patients with pulmonary arterial hypertension at rest and under exercise

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RATIONALE

The pulmonary vascular system is characterized by a high ability to adapt to changing pressures within pulmonary circulation. In patients with pulmonary arterial hypertension (PAH) this ability decreases due to the chronically increased vascular resistance. This leads to the impairment of the pulmonary blood flow (PBF) and the cardiac output at rest and under exercise. Up to the present an effective monitoring of the effects of common PAH therapies on the PBF was only possible by right heart catheterisation. The invasiveness of this method makes it inapplicable as a close monitoring tool. Therefore, we assessed the haemodynamic effects of endothelin receptor antagonists (ERA) in PAH patients by measuring the PBF by the non-invasive measurement device Innocor (Innovision; Denmark) at rest and after physical activity.

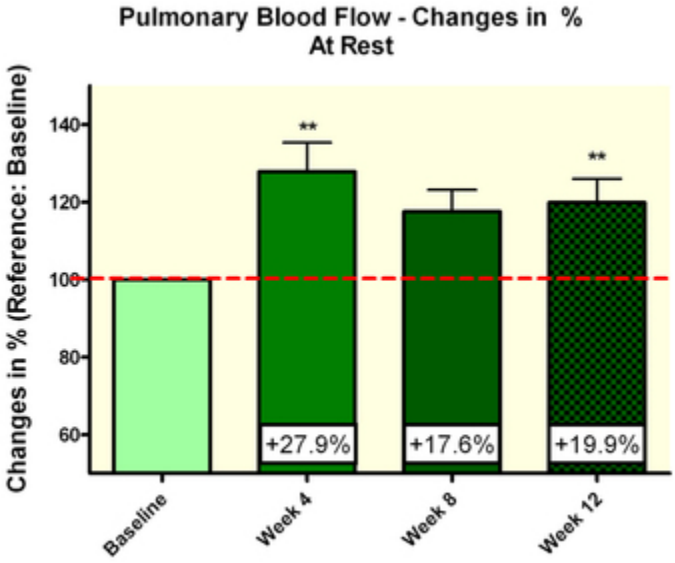
METHODS

15 PAH patients (NYHA III and IV; males:females=6:9; mean age 66) underwent routine non-invasive hemodynamic assessment with the Innocor device (inert-gas rebreathing technique) at rest and after exercise (6-minute walk test; 6MWT). These procedures were performed at baseline and every 4 weeks for 3 months after the start of ERA therapy.

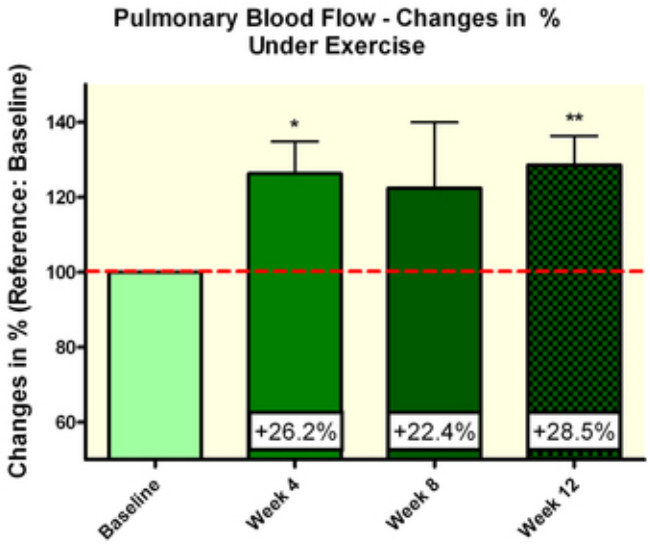
RESULTS

The directly measured parameter by the Innocor is the PBF. PBF at rest was 3.10.8 l/min at baseline, 3.9±1.1 l/min at week 4 (+27.9%; p=0.006), 4.3±1.3 l/min at week 8 (+17.6%) and 3.6±0.8 l/min at week 12 (+19.9%; p=0.008). PBF values after exercise showed an initial increase of blood flow of 26.2% after 4 weeks (p=0.023), which remained stable over the entire observational period (22.4% at week 8; 28.5% with p=0.004 at week 12). The walking distance assessed by the 6MWT showed an increase of 9% at week 4, 15% at week 8 and 14% at week 12 (p=0.0007). Also, a change of NT-proBNP levels was detected and showed an anti-parallel behaviour to the assessed PBF values.

Changes of PBF at rest



Changes of PBF under exercise



CONCLUSION

We used for the first time the device Innocor to evaluate at close intervals changes of the PBF in patients with PAH under ERA therapy. Our data show a significant increase of PBF both at rest and after exercise consistent with the simultaneous reduction of NT-proBNP levels and the improvement of the walking distance. These data support the efficacy of this new device in PAH monitoring.

Riociguat for Chronic Thromboembolic Pulmonary Hypertension and Pulmonary Arterial Hypertension: First Long-term Extension Data from a Phase II Study

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Rationale

Although management of pulmonary hypertension has improved in recent years, it remains a devastating, life-threatening disease. Riociguat is a novel oral stimulator of soluble guanylate cyclase, a key enzyme in the nitric oxide signaling pathway. Riociguat has demonstrated efficacy and a favorable safety profile in treating chronic thromboembolic pulmonary hypertension (CTEPH) and pulmonary arterial hypertension (PAH) in a 12-week trial, but no long-term data have yet been presented. We investigated the long-term safety, tolerability and efficacy of riociguat in patients with CTEPH and PAH.

Methods

Patients with CTEPH or PAH who had enrolled in a 12-week multicenter uncontrolled trial of riociguat (WHO functional class [FC] II/III, mean pulmonary vascular resistance > 300 dyn.s/cm^5 and mean pulmonary arterial pressure > 25 mmHg at baseline) were invited to enter a long-term extension phase (LTE). Riociguat doses were titrated from a starting dose of 1 mg three times daily (t.i.d.) according to systolic blood pressure (range: 0.5-2.5 mg t.i.d.). Assessments (6-minute walking distance [6MWD], FC and safety parameters) were subsequently performed at 3-month intervals.

Results

Of the 78 patients in the 12-week trial, 68 entered the LTE (CTEPH, 41; PAH, 27). At the cut-off date for this analysis, the mean LTE duration was 14.0 ± 6.3 months, 65 patients remained alive and 54 patients remained on riociguat. Riociguat dose during the LTE was 2.5 mg t.i.d. in 73.5-75.0% of patients. In addition to conventional background therapy, 26 patients took endothelin receptor antagonists and 1 patient took iloprost (6 patients took endothelin receptor antagonists at baseline). Peripheral edema (n=12) and nasopharyngitis (n=12) were the most common adverse events, followed by hypotension (n=10), respiratory tract infection (n=8) and syncope (n=7; 3 regarded as drug-related).

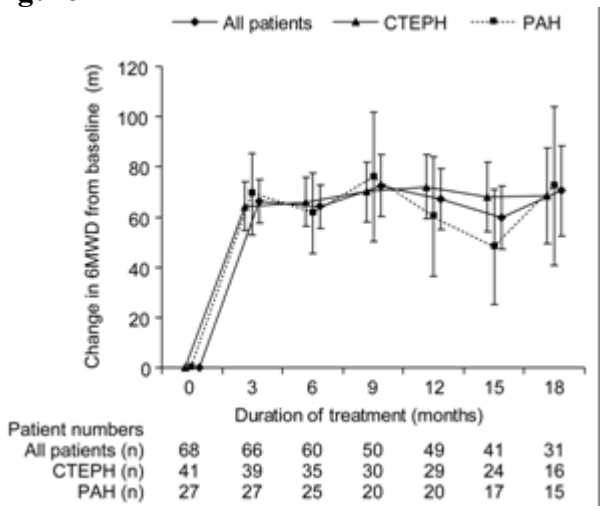
Mean 6MWD improved in the 12-week trial (baseline, 365 m; 12 weeks, 431 m) and was sustained for at least 15 months thereafter (430 m; Figure). The proportion of patients in FC I/II increased from 20.6% at baseline to 47.0% at 12 weeks, and was maintained at 53.2-58.1% during the next 15 months.

Conclusions

This study presents the first long-term data for riociguat. In patients with CTEPH and PAH, riociguat was generally well tolerated, had a favorable safety profile, and caused sustained improvement in 6MWD and FC for at least 15 months. Phase III trials in patients with CTEPH and PAH are ongoing.

This abstract is funded by Bayer HealthCare AG.

Figure 1



Mean improvement in 6MWD by subtype of pulmonary hypertension. Error bars show standard error of the mean. Two patients did not have 6MWD data at 3 months but continued into the extension phase, hence data are available for 66 patients at 3 months. 6MWD, 6-minute walking distance; CTEPH, chronic thromboembolic pulmonary hypertension; PAH, pulmonary arterial hypertension.

Hypoxia-Induced Changes in the Murine Pulmonary Circulation are Attenuated by Treatment with sRAGE

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Rationale

The receptor for advanced glycation end-products (RAGE) is a 45 kDa protein of the immunoglobulin super-family that has been recently implicated in the pathogenesis of pulmonary hypertension; a progressive disease of the small pulmonary arteries characterised by vascular remodeling and narrowing, increased pulmonary vascular resistance and a sustained increase in pulmonary pressure. Activation of RAGE by the RAGE ligand S100A4/MTS1, which is exocytosed by pulmonary endothelial and smooth muscle cells, appears to be key to the proliferatory and migratory responses of these cells to stimuli such as 5-Hydroxytriptamine (5-HT). RAGE may therefore have a role in the remodelling of pulmonary vessels and the pathogenesis pulmonary hypertension.

Methods

Male C57/BL6 mice aged 12-16 weeks were randomly assigned to either normoxia or hypoxia and were treated I.P. with either 20µg/day of the soluble form of RAGE (sRAGE) or 20µg/day murine serum albumin (MSA). Mice in the hypoxic group were placed in a hypobaric chamber and the pressure was reduced to 550mbar for a period of two weeks. At the end of this period, mice were removed from the chamber, anaesthetised and right ventricular pressure was assessed *in vivo*. After euthanasia, heart and lungs of each animal were excised and used to assess right ventricular hypertrophy (RVH). Statistical analysis was by two-way analysis of variance.

Results

Exposure to two weeks hypoxia resulted in an elevation in RVP, RVH and remodelling in animals treated with vehicle. Treatment with sRAGE prevented the increase in RVP but not in RVH.

Discussion

That treatment with sRAGE prevented hypoxia-induced increases in RVP provides further evidence of a role for RAGE and its ligands in the hypoxia induced pulmonary hypertension.