

Methods and Results The role of neutrophilic inflammation and PAR-1 was investigated in two models of murine pneumococcal pneumonia (serotype 2 (D39) and serotype 19F (EF3030)) by using the most clinically advanced PAR-1 antagonist, SCH530348. Neutrophil depletion and chemokine neutralisation studies were also performed. Samples were analysed by immunohistochemistry, cytology, flow cytometry, ELISA and microbiological techniques. Our models were characterised by evidence of intra-alveolar coagulation, increased neutrophil recruitment to areas of bacterial infection and increased PAR-1 expression (demonstrated by quantitative image-analysis). Neutrophil depletion protected mice against barrier disruption but resulted in compromised host defence. In contrast, PAR-1 antagonist treatment significantly reduced neutrophil recruitment to the bronchoalveolar space without being detrimental to host defence. Markers of alveolar leak, coagulation activation and pro-inflammatory cytokines and chemokines (IL-1 β , CXCL1, CCL2 and CCL7) were also attenuated. Neutralisation studies demonstrated that IL-1 β and CCL7, but not CXCL1 and CCL2, played a key role in neutrophil recruitment to the airspaces in this model. Translational studies were performed to examine by flow cytometry the CXC and CC chemokine receptor expression on neutrophils from blood and BALF of mechanically ventilated CAP-induced ARDS patients and controls. CXCR1 and CXCR2 expression on BALF neutrophils was higher in CAP-ARDS patients compared to controls. Additionally, chemokine expression patterns on neutrophils from CAP-ARDS patients changed within different compartments, evidenced by decreased expression of CXCR1 and increased expression of CXCR2, CCR1, CCR2 and CCR3 on neutrophils from BALF compared with blood.

Conclusion These data provide preclinical proof-of-concept that recently developed PAR-1 antagonists may offer a novel therapeutic approach for controlling or preventing alveolar barrier dysfunction and excessive neutrophilic inflammation in pneumococcal pneumonia without compromising host defence. Furthermore, they highlight a role for chemokine receptor switching in CAP-ARDS with important implications for future targeting of these chemokine receptors.

S101 SRC/BCR-ABL INHIBITION WITH DASATINIB IN STERILE AND NON-STERILE ACUTE LUNG INFLAMMATION

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Introduction and objectives Adult respiratory distress syndrome (ARDS) is a commonly fatal complication of lung infection and inflammation, with no effective treatment. It is characterised by excessive neutrophil influx and degranulation into the lungs, with alveolar leak and severe hypoxia. *Src* family tyrosine kinases are critical in integrin-dependent neutrophil degranulation. Dasatinib is a *Src/Bcr-abl* inhibitor used in chronic myeloid leukaemia. We investigated our hypothesis that extracellular neutrophil degranulation could be inhibited by dasatinib *in vitro* and would modulate the inflammatory response *in vivo* in models of infective and sterile lung injury.

Methods Whole blood and isolated blood neutrophils from healthy volunteers were pre-treated with dasatinib and treated with neutrophil stimuli or live bacteria. Degranulation was

measured by granule receptor expression and presence of extracellular granule products. Other neutrophil functions were assessed, including adhesion, L-selectin shedding, chemotaxis, phagocytosis, oxidative burst, bacterial killing and apoptosis. Neutrophilic lung inflammation was induced in mice using intratracheal *E. coli* or hydrochloric acid.

Results *In vitro*, dasatinib inhibited neutrophil degranulation in response to lipopolysaccharide derived from *E. Coli* 026:B6, fMLF and *Staphylococcus aureus* at concentrations above 100 nM, with no effect on neutrophil viability or apoptosis. Integrin-dependent functions including adhesion, chemotaxis and phagocytosis in adherent conditions were impaired, but phagocytosis was unaffected in whole blood. Intracellular oxidative burst was maintained, with normal bacterial killing, but extracellular superoxide anion release was impaired.

In vivo, dasatinib had modest effects on the pro-inflammatory response to *E. coli*, reducing%interstitial neutrophils, alveolar myeloperoxidase and TNF α at 1 mg/kg and alveolar lactoferrin at 10 mg/kg. Bacterial killing was impaired in a dose dependent fashion, with associated alveolar leak and systemic toxicity at 10 mg/kg. In sterile acid injury, 5 mg/kg dasatinib reduced%neutrophils, degranulation (interstitial CD11b/alveolar lactoferrin) and monocyte-chemotactic protein-1 (MCP-1) in the alveolar space, but induced detrimental effects at 10 mg/kg.

Conclusions The pan-*Src* kinase inhibitor dasatinib modifies multiple pro-inflammatory neutrophil functions *in vitro* and *in vivo* with an impairment in bacterial killing observed in infective lung injury. In the context of sterile inflammation, manipulation of neutrophil degranulation also alters the inflammatory environment and this approach warrants further study as a therapeutic strategy in ARDS.

S102 LIPOXIN A4 IMPROVES EFFEROCYTOSIS VIA INHIBITION OF THE HMGB1 IN HUMAN ALVEOLAR MACROPHAGES

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Introduction Effective clearance of apoptotic cells by macrophages, termed efferocytosis, is a pre-requisite for successful resolution of inflammation. High mobility group box protein 1 (HMGB1), is an alarmin that may promote inflammation as well as suppress phagocytosis. Lipoxin A₄, represents one of a unique class of lipid mediators that possess a wide spectrum of anti-inflammatory and pro-resolution actions. We hypothesised that lipoxin A₄ may promote both apoptosis in neutrophils, and stimulate macrophage efferocytosis, acting as an antagonist to HMGB-1.

Methods Neutrophils were obtained from healthy volunteers and cultured for 24 h with or without lipoxin A₄. Apoptosis of neutrophils was determined with Annexin V/SyTox staining by flow cytometry. HMGB-1 levels in Acute Respiratory Distress Syndrome (ARDS) bronchoalveolar lavage fluid (BALF) was measured by ELISA. The effects of HMGB-1 and lipoxin A₄ upon alveolar macrophage efferocytosis was assessed by measuring the ingestion of CMFDA labelled apoptotic neutrophils by flow cytometry. The PI3K (P85) protein expression was measured by western blotting.

Results Treatment of lipoxin A4 (100 nM) increased the apoptosis of neutrophils ($p = 0.0244$), and reduced the dead ($p = 0.0238$) and necrotic neutrophils ($p = 0.0358$) compared to control ($n = 8$). BALF from patients with ARDS suppressed efferocytosis of apoptotic neutrophils. The effects of BALF correlated with HMGB-1 levels in the BALF fluid. HMGB-1 decreased efferocytosis ($p < 0.05$) in a dose dependent manner, and reached a significant effect at 150 ng/ml ($p = 0.008$). Lipoxin A4 increased the efferocytosis ($p < 0.05$) of alveolar macrophages in a dose dependent manner, and reached the maximal effect at 100 nM ($p = 0.008$). Moreover, lipoxin A4 (100 nM) blocked the decreased efferocytosis response to HMGB-1 (150 ng/ml) ($p = 0.005$, $n = 8$). The lipoxin A4 beneficial effects were abrogated by ALX antagonist, BOC-2 ($p < 0.05$) and PI3K inhibitor ($p < 0.05$).

Conclusions Lipxin A₄ *in vitro* promotes the apoptosis but not necrosis of neutrophils. In tandem it stimulates efferocytosis of alveolar macrophages. Elevated HMGB-1 in ARDS BALF suppresses efferocytosis. Lipoxin A₄ can block these effects of HMGB-1. The effect of lipoxin A4 increasing efferocytosis was through ALX-PI3K signalling pathways. Lipoxin A₄ may therefore have potential as a therapeutic agent to promote the resolution of neutrophilic inflammation in ARDS.

COPD outcomes

S103 NO LOSS IN EFFICACY FOLLOWING SWITCH FROM SALMETEROL/FLUTICASONE COMBINATION TO INDACATEROL MONOTHERAPY IN PATIENTS WITH MODERATE COPD: THE INSTEAD STUDY

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Introduction Many patients with low risk of COPD exacerbations receive twice-daily (*bid*) LABA/ICS, salmeterol/fluticasone (SFC), for maintenance treatment. This study evaluated the effect of switching these patients to a once-daily (*od*) LABA, indacaterol, monotherapy.

Methods INSTEAD was a 26-week double-blind, double-dummy study in patients aged ≥ 40 years, with moderate COPD (post-bronchodilator FEV₁ 50–80% predicted) and no exacerbations in the past 12 months, who were receiving SFC 50/500 μg *bid* for ≥ 3 months prior to study entry. Patients were randomised (1:1) to continue with SFC 50/500 μg or to be switched (with

no washout) to indacaterol 150 μg . The primary objective was to demonstrate non-inferiority of indacaterol to SFC, measured by trough FEV₁ after 12 weeks (non-inferiority margin: 60 mL). Trough FEV₁ was also evaluated at 4, 8 and 26 weeks. TDI and SGRQ-C total scores were evaluated at Weeks 12 and 26; the annualised rate of exacerbations and safety were evaluated over 26 weeks.

Results A total of 581 patients were randomised (indacaterol: 293; SFC: 288); 85.4% completed the study. The primary endpoint was met, with a LSM difference in trough FEV₁ between indacaterol and SFC of -9 mL (95% CI: -45 to 26 mL; $p = 0.002$ for NI). There were no significant differences between treatments in trough FEV₁ at any of the other visits (Baseline and Weeks 4, 8 and 26). The TDI and SGRQ-C total scores and their responder rates were similar between two treatments, at both Weeks 12 and 26 (Table 1). During the 26 week treatment period, 79.5% and 74.7% of patients in the indacaterol and SFC groups, respectively, experienced no exacerbations. There was no statistically significant difference between treatments in the rate of all COPD exacerbations per year, with rates of 0.57 vs 0.67, respectively (RR 0.86 [95% CI 0.62, 1.20]; $p = 0.367$). Adverse events (AEs) and serious AEs were comparable between the treatment arms.

Conclusion Indacaterol was non-inferior to SFC in terms of bronchodilation and showed similar efficacy in terms of breathlessness, health status, and exacerbation rate indicating that this group of patients can be switched from SFC to indacaterol 150 μg with no loss in efficacy.

S104 DOUBLE-BLIND MULTI-CENTRE RANDOMISED CONTROLLED TRIAL OF VITAMIN D3 SUPPLEMENTATION IN COPD (VIDICO)

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Introduction and objectives Inadequate vitamin D status is common in patients with COPD, and it associates with susceptibility to upper respiratory infection (URI) – a major precipitant of exacerbation. Multi-centre trials of vitamin D supplementation for prevention of exacerbation and URI in COPD are lacking. We therefore conducted a multi-centre double-blind randomised placebo-controlled trial of vitamin D supplementation for the prevention of moderate/severe exacerbation and URI in adults with COPD.

Methods Two hundred and forty patients were allocated to receive a 2-monthly oral dose of 3 mg vitamin D₃ or placebo for one year. Co-primary outcomes were time to first moderate/

Abstract S103 Table 1 TDI and SGRQ scores

	Week 12			Week 26		
	Indacaterol	SFC	Treatment difference (95% CI)	Indacaterol	SFC	Treatment difference (95% CI)
TDI total score	1.89 (0.499) [†]	1.69 (0.509) [†]	0.20 (-0.32, 0.72)	2.58 (0.543) [†]	2.70 (0.552) [†]	-0.12 (-0.71, 0.48)
SGRQ-C total score	32.8 (1.58) [†]	32.9 (1.61) [†]	-0.10 (-1.9, 1.8)	33.1 (1.87) [†]	33.5 (1.93) [†]	-0.40 (-2.5, 1.6)
TDI responder (≥ 1 unit), %	61.4	61.0	0.97 (0.65, 1.46) [#]	68.7	69.4	0.88 (0.58, 1.35) [#]
SGRQ-C responder (≥ 4 unit), %	44.7	42.4	1.12 (0.76, 1.63) [#]	49.6	48.8	1.03 (0.70, 1.52) [#]

[†]Data are LSM (SE): [#]odds ratio; LSM, least square means; TDI, transition dyspnoea index; SGRQ-C, St George Respiratory Questionnaire for COPD