Abstract S97 Table 1 Patients who die within two years of oesophagectomy are more likely to have been smoking at the time of their operation, or to have developed ARDS or a surgical complication in the immediate post-op period

	Died within 2 years (n = 26)	Survived 2 years (n = 29)	P-value
ARDS – n (%)	11 (42)	3 (10)	0.007
Surgical Complication – n (%)	13 (50)	6 (21)	0.022
Current Smoker – n (%)	10 (43)	4 (15)	0.024
Median Pack Years	33	30	0.600

REFERENCES

1 Khuri *et al*. Determinants of Long-Term Survival After Major Surgery and the Adverse Effect of Postoperative Complications. *Ann Surg*. 2005 Sep;242(3):326–41

S98 A NOVEL HUMAN MODEL TO STUDY ALVEOLAR INJURY AND REPAIR

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10.1136/thoraxjnl-2014-206260.104

Introduction The development of regenerative therapies holds promise for the future treatment of parenchymal lung diseases. However, encouraging preclinical data from animal models have translated poorly in clinical trials. The cellular and molecular response to lung injury is difficult to study in man. To address this fundamental question, we have developed a novel *in vitro* human model. Precision cut lung slice (PCLS) culture is a well-established tool in airway biology and pharmacology. Here, we demonstrate lung parenchyma can be maintained and manipulated *in vitro* generating a tractable model, which allows study of lung injury and repair in man.

Methods PCLS (500 μm) were generated from agarose-inflated lung lobes from human lungs maintained *ex-vivo* by perfusion and ventilation (EVLP). The slices were cultured in serum-free medium in a rotating incubator (37°C, 5% CO_2) and analysed at days 1, 3 and 7. Cell specific immunofluorescence markers were used to identify smooth muscle, type I and type II alveolar epithelial cells (AT1, AT2), vascular endothelial cells and proliferating cells (using αSMA, Aquaporin5, ProSPC, PECAM1 and Phospho-histone H3 respectively). Slice viability was confirmed using MitoTracker, LDH and Live/Dead assays.

Results All of the expected cell types were identified in PCLS by immunofluorescence demonstrating that human PCLS maintained cellular differentiation in culture. Pro-SPC was predominant in the alveolar wall cells, particularly in the alveolar septal junctions, corresponding to known location of AT2 cells; AQ5 was distributed in thin bands lining the alveolar walls suggestive of the apical membrane of AT1 cells; αSMA was positive around airways, the known location of smooth muscle cells (SMCs); PECAM-1 was positive within alveolar walls corresponding to microvascular capillaries within alveolar septae. There was no significant cell proliferation during culture under basal conditions. Finally, cell viability studies demonstrated that PCLS can be maintained for up to 1 week in serum-free culture.

Conclusion PCLS of human lung parenchyma remain differentiated and viable for up to 7 days in serum-free culture. In future, human PCLS derived from normal and injured regions of lung from the EVLP model may provide a novel means of studying alveolar repair in human lung *in vitro*.

S99 EFFECTS OF DIFFERENTIAL TNF RECEPTOR SIGNALLING IN MODULATING NEUTROPHIL-ENDOTHELIAL INTERACTIONS IN THE PULMONARY MICROVASCULATURE

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10.1136/thoraxjnl-2014-206260.105

Neutrophil recruitment into the bronchoalveolar space is central to the pathogenesis of acute respiratory distress syndrome injury (ARDS), and occurs via interaction with the lung microvascular endothelium. Tumour Necrosis Factor (TNF) is a key mediator in these processes, activating endothelial cells and inducing changes in microvascular permeability, as well as priming neutrophils (a pre-requisite for neutrophil-mediated tissue damage) and modulating neutrophil lifespan. TNF signals through two cell surface receptors, TNFR1 and TNFR2 initiating distinct signalling pathways and cellular responses. In a human *in vivo* model of ARDS, selective TNFR1 antagonism attenuated pulmonary inflammation (O'Kane *et al*, Thorax 2013; 63:A50). Using TNF receptor specific muteins and a novel highly selective TNFR1 antagonist, we investigated the role of differential TNFR signalling on neutrophil-pulmonary microvascular endothelial cell interactions.

TNF-induced alterations in the expression of the neutrophil cell surface molecules CD11b, CD62L, TNFR1 and TNFR2 were all modulated via TNFR1. TNFR1 was also the dominant receptor mediating reactive oxygen species generation by TNF-primed, fMLP-stimulated neutrophils. We further examined the role of TNF receptors in modulating neutrophil apoptosis; whilst engagement of both TNFR1 and 2 was required to induce early neutrophil apoptosis, TNFR1 antagonism reversed TNF-induced late survival to constitutive levels of apoptosis. TNFR1 antagonsim of human pulmonary microvascular endothelial monolayers significantly reduced TNF-induced production of IL-1beta, IL-6 and IL-8 ($p < 0.05$), endothelial permeability and the release of the endothelial injury markers sICAM-1, sVCAM-1 and sE-selectin (p

Collectively, these results suggest that TNFR1 regulates multiple components of neutrophil-endothelial interactions. Selective TNFR1 antagonism may offer a novel therapeutic approach in ARDS; phase II clinical trials of this therapy are scheduled.

S100 PROTEINASE-ACTIVATED RECEPTOR 1 SIGNALLING CONTRIBUTES TO NEUTROPHILIC INFLAMMATION AND ALVEOLAR BARRIER DISRUPTION IN STREPTOCOCCUS PNEUMONIAE PNEUMONIA

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10.1136/thoraxjnl-2014-206260.106

Introduction *Streptococcus pneumoniae* is the most common cause of community-acquired pneumonia (CAP) and is associated with excessive neutrophilic inflammation. The high-affinity thrombin receptor, proteinase-activated receptor (PAR)-1, has been implicated in mediating the interplay between coagulation and inflammation. However, its role during *S. pneumoniae*-induced neutrophilic inflammation and the mechanisms for neutrophil recruitment in this context are poorly understood.

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-1β, CXCL1, CCL2 and CCL7) were also attenuated. Neutralisation studies demonstrated that IL-1β 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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10.1136/thoraxjnl-2014-206260.107

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 TNFα 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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10.1136/thoraxjnl-2014-206260.108

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 A_4, 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 A_4 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 A_4. 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 A_4 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.