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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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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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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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.