

manner; no peptide,  $2468.5 \pm \mu\text{g/ml}$ , 5 mg,  $2181.7 \pm 26.2 \text{ ng/ml}$  ( $p=0.006$ ), 10 mg,  $1576 \pm 164.7 \text{ ng/ml}$  ( $p=0.001$ ), and 20 and 30 mg completely prevented polymer formation in inclusions ( $p \leq 0.001$ ). Unrelated peptides had no effect. Elastase activity of AT in the supernatant from Z-AT cells was significantly reduced compared to M-AT cells;  $p \leq 0.001$ , in keeping with the secretory defect due to retention of Z-AT in inclusion bodies. The elastase activity (and AT concentration) in the supernatant from Z-AT cells was restored by 20 mg 4M, O.D. 405 nm, Z-AT vs Z-AT + 20  $\mu\text{g}$  4M,  $0.129 \pm 0.009$  vs  $0.788 \pm 0.054$  respectively, ( $p \leq 0.001$ ), where a higher O.D. represents higher elastase activity. Functional activity of secreted AT following treatment with 4M was confirmed by its ability to form an SDS-stable complex with elastase as shown by immunoblot. RT-PCR showed that the ER accumulation of Z-AT induced cell stress; NF- $\kappa$ B activation, expression of protein kinase RNA (PKR)-like ER kinase (PERK), and IL-6 ( $100.4 \pm 16 \text{ pg/ml}$ ) and IL-8 ( $2592.5 \pm 575 \text{ pg/ml}$ ), all of which could be abrogated effectively by 20 mg 4M (IL-6,  $45.8 \pm 28 \text{ pg/ml}$ ,  $p \leq 0.001$  and IL-8,  $184.3 \pm 29 \text{ pg/ml}$ ,  $p=0.014$ ). These findings are the first evidence that inhibitors of Z-AT polymerisation targeting s4A can prevent its cellular accumulation and deleterious effects. Importantly, this strategy was also able to improve plasma concentration of Z-AT.

## S52 ASSOCIATION OF MICROTUBULE INSTABILITY WITH DEFECTIVE PHAGOCYTOSIS IN COPD

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Acute exacerbations of COPD are the commonest cause of acute medical admissions in the UK and ~50% are associated with bacterial infection. Alveolar macrophages (AM) normally clear inhaled bacteria but defective phagocytosis may lead to chronic colonisation and increased exacerbations. Monocyte-derived macrophages (MDM), used to model AM, were obtained from COPD, smoking and healthy subjects. MDM phagocytosis of fluorescently-labelled polystyrene beads, *Haemophilus influenzae* (HI) or *Streptococcus pneumoniae* (SP) was measured by fluorimetry. MDM derived from all subjects showed equivalent ability to phagocytose beads, however, COPD and smoker MDM showed significantly reduced phagocytosis of bacteria. Phagocytosis of HI was reduced by 28% and 48% in COPD and smoker MDM respectively, compared to healthy, while SP phagocytosis was reduced by 32% and 52% in COPD and smoker MDM respectively, compared to healthy (Abstract S52 table 1). Having identified defective bacterial phagocytosis in smoker and COPD MDM, the next step was to elucidate the underlying mechanism. Cytoskeletal rearrangement was investigated, with COPD MDM showing significantly reduced phagocytosis of bacteria in comparison to healthy after pre-incubation with nocodazole (microtubule disruptor). Microtubules are involved in membrane trafficking of the phagolysosome and microtubule stability is necessary for effective phagocytosis. Tubulin is acetylated to form stable microtubules and is deacetylated by HDAC6 and Sirt2. COPD MDM showed reduced levels of acetylated tubulin compared to healthy MDM. Pre-incubation with epothilone B (10 nm) a microtubule stabiliser, improved HI phagocytosis in

Abstract S52 Table 1 Relative fluorescence values (RFU $\times 10^3$ ) for MDM phagocytosis assays at 4 h

	HI	SP
Healthy (n=21)	$11.5 \pm 0.9 \text{ RFU} \times 10^3$	$8.2 \pm 1.4 \text{ RFU} \times 10^3$
Smoker (n=20)	$4.6 \pm 0.6$ ( $p < 0.001$ )	$3.7 \pm 0.6$ ( $p < 0.001$ )
COPD (n=23)	$8.4 \pm 1$ ( $p < 0.01$ )	$5.7 \pm 0.7$ ( $p < 0.05$ )

COPD MDM by 20% ( $p < 0.05$ ) and SP phagocytosis in smoker MDM by 40%. Levels of acetylated tubulin increased on exposure to bacteria alone in healthy and smoker MDM but not in COPD MDM. Pre-incubation with epothilone B was associated with significantly increased levels of acetylated tubulin in COPD cells. No significant differences were seen in the expression of HDAC6 or Sirt2 in COPD compared to healthy cells. MDM from smoking and COPD subjects show reduced phagocytosis of common respiratory bacterial pathogens. Acetylation of microtubules appears to be reduced in COPD, whereas, increasing tubulin acetylation is associated with improvements in phagocytosis, which may allow for targeted development of future therapies to treat colonisation and prevent exacerbations of COPD.

## S53 ALARMIN IN BRONCHIOLITIS OBLITERANS SYNDROME AFTER LUNG TRANSPLANTATION

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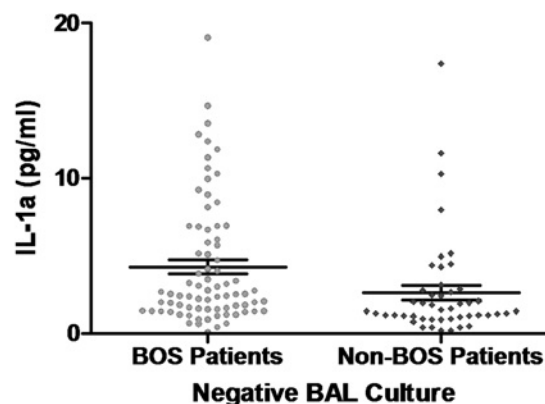
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**Introduction** Survival after lung transplantation is limited to a median of 5-year due to development of bronchiolitis obliterans syndrome (BOS). BOS is the clinical manifestation of chronic allograft dysfunction, characterised by inflammation and fibrosis of small/medium-sized airways leading to airflow obstruction. Numerous insults to the transplanted lungs have been associated with BOS development. Alarmins are cell derived danger signals released from damaged tissue which activate innate and adaptive immune responses. We hypothesised that the release of alarmins into the airway compartment after lung transplantation may contribute to BOS pathogenesis.

**Methods** A retrospective longitudinal study of 52 lung transplant recipients from 2005 to present was performed (26 recipients developed BOS; 26 remained free from BOS). All recipients had lung function and bronchoalveolar lavage (BAL) performed at 1, 3, 6 and 12 months post transplant. Further samples were taken if the diagnostic criteria for BOS were fulfilled. A total of 214 BAL samples were analysed by ELISA for the alarmins Interleukin-1a (IL-1a) and High Motility Group-Box1 (HMG-B1). Data were analysed using Mann-Whitney tests.

**Results** Both BOS and non-BOS recipients with culture positive BAL samples had significantly higher concentrations of IL-1a and

## IL-1a Assay on Culture Negative BAL Samples



Mann-Whitney U Test:  $p=0.001$

Abstract S53 Figure 1 IL-1a assay on culture negative BAL samples.

HMG-B1 than culture negative samples. Due to the confounding effect of infection, our analysis excluded 90 BAL samples with positive cultures. Concentrations of IL-1a were significantly higher in culture negative BAL from BOS patients (median 2.411, range [AJF1] 0.073–19.078 pg/ml) than from Non-BOS patients (median 1.424, range [AJF2] 1.159–17.41 pg/ml;  $p=0.001$ ). No significant difference in HMG-B1 concentrations between the two groups was observed (BOS median 58.906, range 0–197.5; Non-BOS median 76.25, range 0–211.563 ng/ml;  $p=0.2378$ ). Longitudinal measurements of IL-1a in BOS patients showed significantly higher levels 3 months before or after BOS diagnosis (median 3.935, range 1.122–13.544 pg/ml), compared to >3 months before BOS diagnosis (median 2.015, range 0.073–14.669 pg/ml;  $p=0.0153$ ). There was no such difference in HMG-B1 concentrations ( $p=0.9164$ ).

**Conclusions** An increase in the alarmin IL-1a, but not HMG-B1, is associated with BOS development. The cellular source of IL-1a requires further evaluation but may be a marker of airway epithelial injury and/or play a mechanistic role in BOS development *via* its secretion by other cell types.

## S54 POLYMERS OF Z $\alpha$ 1-ANTITRYPSIN ARE ASSOCIATED WITH PULMONARY INFECTION POST LUNG TRANSPLANTATION

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Z antitrypsin (Z-AT) polymerises in the liver and is associated with early onset emphysema. Polymers of Z-AT are not only inactivate as antiproteases, but also act as a pro-inflammatory stimulus. We studied patients with emphysema post lung transplantation, with and without AT deficiency, to examine the relationship between polymers and the presence of infection and inflammation. Bronchoalveolar Lavage Fluid (BALF) was obtained at scheduled surveillance, and when clinically indicated to assess for infection, rejection and airway injury. BALF was assessed by ELISA and immunoblot using a monoclonal antibody to polymeric AT (ATZII). BALF cell pellets were lysed, and HLE activity was used as a measure of BALF neutrophil numbers. 16 patients post-transplant were evaluated, 6 Z-AT patients (15 samples); 9 infective tracheobronchitis, 3 airway stenosis, 1 reflux, 2 normal, and 10 M-AT patients (20 samples); 7 infective tracheobronchitis, 8 rejection, 5 normal. All samples apart from one in the Z-AT group contained polymers; median (IQR) 292 (430–40.2) ng/ml. In one patient BALF was initially negative for polymers, but subsequent samples were positive. Polymers were present in association bacterial infection, colonisation, airway injury and surveillance bronchoscopy of asymptomatic patients. Airway stenosis/inflammation and bacterial tracheobronchitis was associated with a higher amount of polymers (347.35 (SEM $\pm$ 57 ng/ml) than Z-AT with normal findings (142 $\pm$ 101 ng/ml). Immunoblot confirmed the classical ladders of polymers in Z-AT group, but not in M-AT group. BALF of Z-AT group had a higher free HLE than M-AT; 139(226.5–102.75) ng/ml vs 74(105.25–46) ng/ml, respectively;  $p\leq 0.001$ . Free HLE in Z-AT was correlated with polymer concentrations in BALF;  $r^2=0.63$ . Total neutrophil numbers were higher in Z-AT compared with M-AT; OD405, 0.57 $\pm$ 0.07 vs 0.37 $\pm$ 0.04, respectively;  $p=0.033$ . BALF neutrophil numbers were significantly higher in the infected Z-AT (0.54 $\pm$ 0.1) vs infected M-AT (0.31 $\pm$ 0.1),  $p=0.026$ . We have shown that polymers of Z-AT are present in BALF of transplanted individuals. Furthermore, this was associated with excess neutrophils, and closely correlated with free HLE. The production of polymers results in further reduction of the anti-proteinase and anti-inflammatory protection in the lung and leads to neutrophil influx. This may predispose Z-AT individuals to exaggerated lung destruction and a worse outcome after lung transplantation.

## Clinical and mechanistic studies in thoracic malignancy

### S55 COST-EFFECTIVENESS AND QUALITY OF LIFE RESULTS FROM THE ASTER STUDY: ENDOBRONCHIAL AND ENDOSCOPIC ULTRASOUND VS SURGICAL STAGING IN POTENTIALLY RESECTABLE LUNG CANCER

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**Background** We recently published clinical results of ASTER, a randomised controlled trial in which endosonography, a strategy of combined endoscopic (EUS) and endobronchial (EBUS) ultrasound (followed by surgical staging if these tests were negative for malignancy), had significantly higher sensitivity and negative predictive value than surgical staging alone for mediastinal staging in NSCLC. Here we present ASTER quality of life (QoL) and cost-effectiveness outcomes.

**Methods** EuroQoL EQ-5D questionnaire was performed at baseline, end of staging, 2 and 6 months post randomisation. The UK EQ-5D social tariff was applied to calculate utility values. Quality-adjusted survival was estimated using the area under the utility curve. Full resource use information was recorded for all patients and NHS 2008–2009 Reference Costs were applied. Total expected costs over 6 months were estimated by summing the resource use multiplied by its unit cost and taking the sample average for each group.

**Results** Of 241 randomised patients, 144 (60%) provided EQ-5D data at baseline; of these 139 (97%) were followed up at the end of staging, 132 (92%) at 2 months and 124 (86%) at 6 months. At the end of staging, those randomised to endosonography had significantly better QoL than those randomised to surgical staging (utility difference=0.11, 95%CI 0.02 to 0.19). At all other time points, there was little difference between the groups, so that quality adjusted survival over the 6 months was similar (4.1 vs 4.0 months respectively). Complete resource use data were available for 172/214 (71%) patients. Other than the number of thoracotomies performed (66% of patients in the surgical staging arm and 53% in the endosonography arm) resource use did not differ between the two groups. The endosonography group had a non-significant cost saving of £746 per patient compared to the surgical staging group.

**Conclusions** Given that (a) the sensitivity of endosonography was significantly higher than that of the surgical staging arm; (b) QoL post-staging was higher in the endosonography arm and (c) there is no difference in cost between the two strategies, mediastinal staging should commence with endosonography proceeding to surgical staging if there is no evidence of malignancy.

### S56 EBUS-TBNA PREVENTS MEDIASTINOSCOPIES IN PATIENTS WITH ISOLATED MEDIASTINAL LYMPHADENOPATHY: A PROSPECTIVE CLINICAL TRIAL AND COST MINIMISATION ANALYSIS

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**Introduction** Isolated mediastinal lymphadenopathy (IML) is a common presentation to respiratory physicians and