pro-fibrotic mediator TGF- β 1, plays a critical role in the pathogenesis of Idiopathic Pulmonary Fibrosis (IPF). Both galectin-1 and galectin-3 potentiate this TGF- β 1 signaling pathway to promote fibrogenesis, although the exact mechanism is unclear. Integrins are glycoproteins thus their activity can be facilitated by glycan-mediated interactions with N-linked glycosylation the most well studied.¹

Objective To investigate whether galectin-1 and galectin-3 interact directly with α v β 1, α v β 5 and α v β 6 integrins using biophysical methods.

Methods Integrin-galectin interactions were determined by surface plasmon resonance (SPR) in the presence of divalent cations and the effect of extensive integrin deglycosylation or removal of N-linked oligosaccharides alone explored. SPR was used to assess integrin-galectin interactions in the presence of small molecule galectin inhibitors, GB1107 (galectin-3 selective inhibitor) or GB1490 (galectin-1 selective inhibitor). Binding of both galectins to N-Acetyl-D-glucosamine was assessed by isothermal titration calorimetry (ITC).

Results SPR data showed that both galectin-1 and galectin-3 bind to recombinant human α v β 1, α v β 5 and α v β 6 in a glycosylation-dependent manner. Minimal integrin-galectin binding was observed following integrin protein deglycosylation or in the presence of small molecule galectin inhibitors which act via the galectin carbohydrate binding domain (CBD). However, the removal of integrin N-linked oligosaccharides alone resulted in only a partial decrease in integrin-galectin binding. Additionally, ITC demonstrated that both galectin-1 and galectin-3 were unable to bind N-Acetyl-D-glucosamine; the α 5 β 1 terminal sugar required for α 5 β 1-fibronectin binding.

Conclusion Galectins are able to bind to integrins via their post-translational glycosylation sites. Collectively, these data suggest that the presence of both N-linked and O-linked glycan residues are essential for integrin-galectin binding, and that this binding may occur at the galectin galactoside-binding pocket. Understanding the precise role of galectins in integrin-mediated TGF- β 1 activation and IPF pathogenesis may be critical for the continued development of more effective and selective treatments for IPF patients.

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Please refer to page A190 for declarations of interest related to this abstract.

S98 DISSECTING HUMAN PLEURA AT SINGLE-CELL RESOLUTION

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The mesothelium is a serous membrane lining of the coelomic cavities, which comprise the pleura, pericardium, peritoneum, tunica vaginalis testis and tunica serosa uteri. It is a dynamic

Abstract S98 Figure 1 Human parietal pleura scRNAseq of freshly prepared cells. T-distributed stochastic neighbour embedding (tSNE) of jointly analysed single-cell transcriptomes from 12,162 cells from 2 pneumothorax patients. Vasc_Endo, vascular endothelial cells; SMC_Per, smooth muscle cells; T_ILC, T cells, innate lymphoid cells; B_pDCs, B cells, plasmacytoid dendritic cells; Lymph_Endo, lymphatic endothelial cells

structure important for tissue homeostasis by regulating inflammation and wound healing. Defects of the pleura are involved in the pathogenesis of pleural fibrosis and adhesions, and in malignant mesothelioma, an aggressive cancer associated with previous exposure to asbestos.

Currently, there is an inadequate understanding of pleural biology in health, which impedes the development of treatments for these pleural pathologies. To address this, we aimed to establish a reproducible protocol for the isolation and culture of mesothelial cells from human pleural tissue. Moreover, using single-cell RNA profiling, we explored the cellular heterogeneity of human pleura in 8 patients treated for pneumothorax (figure 1). This resulted in the generation of a comprehensive atlas composed of mesothelial, stromal and immune cells, providing a valuable resource for further pleural research.

S99 FLUORESCENCE-LIFETIME IMAGING: A NOVEL DIAGNOSTIC TOOL FOR SUSPECTED LUNG CANCER

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Introduction and Objectives Lung cancer is the commonest cause of cancer-related deaths. Early detection improves outcomes, however, the diagnostic yield of existing sampling techniques is suboptimal. Fluorescence-lifetime imaging microscopy (FLIM), an autofluorescence-based technique which measures endogenous fluorophore decay rates, may aid identification of optimal biopsy sites in suspected lung cancer. We describe the

Abstract S99 Figure 1 (a) Translational fibre-based FLIM system, with optical imaging fibre (1.42mm outer diameter) delivered via the working channel of a bronchoscope. Fluorescence lifetime images of (b) non-cancerous and (c) cancerous human lung tissue using fibre-based FLIM. (d) Corresponding haematoxylin and eosin stained sections of non-cancerous and cancerous (adenocarcinoma) lung tissue

application of a novel fibre-based FLIM system, which utilises 488nm excitation to enable fluorescence intensity and lifetime imaging, to detect changes in freshly resected lung cancer and adjacent healthy tissue. The mechanisms responsible for alterations in lung cancer fluorescence lifetime are not understood. We investigate the contributions of cancer cells and tumour stroma to fluorescence lifetime signatures using fixed unstained lung cancer and benchtop FLIM.

Methods Paired cancer and non-cancerous lung tissues were obtained from resection patients (n=21). A 488nm fibre-based FLIM platform was used to perform high-resolution fluorescence intensity and lifetime imaging (figure 1). Co-registered fixed unstained lung cancer sections were evaluated using benchtop FLIM and image analysis software.

Results Fluorescence lifetime is significantly reduced in fresh *ex vivo* lung cancer, compared with non-cancerous tissue (mean±SD, 2.15 ± 0.26 ns vs. 1.79 ± 0.40 ns, $p < 0.0001$). Fibre-based FLIM distinguishes lung cancer, from adjacent tissue, with 81.0% sensitivity and 71.4% specificity. Lifetime-based signatures are retained after fixation, as evidenced by benchtop FLIM of fixed unstained lung cancer, which demonstrates no difference in fluorescence lifetimes compared with fresh cancer ($p = 0.55$). By applying this technique on fixed tissues, we demonstrate that cancer cells have significantly longer lifetimes, compared with tumour stroma ($p = 0.027$). Cancer subtype also influences lifetime signatures (mean±SD, adenocarcinoma 1.55 ± 0.38 ns vs. squamous cell carcinoma 2.57 ± 0.47 ns, $p = 0.0005$). Mean fluorescence lifetimes of fixed unstained lung cancer positively correlate with the proportion of cancer cells within tissue section area ($r = 0.80$; $p = 0.033$).

Conclusions Our novel fibre-based FLIM system discriminates lung cancer from adjacent healthy tissue *ex vivo* with good performance characteristics. This minimally invasive technique, which is deliverable via endoscopic platforms, may permit advanced *in situ* diagnostic capabilities in lung cancer. FLIM of fixed unstained tissue enables cellular resolution lifetime characterisation, and highlights the role of cancer cells in

determining the overall fluorescence lifetime signature in lung cancer.

Please refer to page A190 for declarations of interest related to this abstract.

Ease that wheeze: managing risk in COPD

S100

RISK OF CARDIOVASCULAR MORBIDITY AND MORTALITY IN PEOPLE WITH CHRONIC OBSTRUCTIVE PULMONARY DISEASE VERSUS THOSE WITHOUT COPD: A STRUCTURED REVIEW OF THE EVIDENCE

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Introduction and Objectives Comorbid chronic obstructive pulmonary disease (COPD) and cardiovascular disease (CVD) frequently occur together and are associated with worse outcomes for patients. This study provides a synthesis of recent evidence from observational studies determining the excess risk of CVD morbidity and mortality in patients with COPD, and the impact of COPD severity on risk.

Methods A structured review of the literature was conducted, using predefined search strategies, to search Medline and Embase (August 2020) for systematic reviews and meta-analyses (last 10 years) or observational studies (last 5 years) that reported risk of CVD (heart failure, hypertension, dysrhythmia, acute myocardial infarction [AMI], hypercholesterolaemia and stroke), diabetes and CV mortality in patients with COPD compared to those without. Risks are presented as reported in identified studies. Meta-analysis results are presented where available, otherwise an individual study range is reported.