Blocking the functions of Siglec-7 and -9 on both primary monocytes and MDM with neutralising antibodies does not alter the *in vitro* inflammatory response to Mtb.

Antibody crosslinking Siglec-9, but not Siglec-7, on MDMs induces endogenous MMP inhibitor TIMP1 ($p = 0.001$, see figure 1), lowering the MMP9/TIMP1 ratio which is a predictor of proteolytic damage.

Conclusion Crosslinking Siglec-9 on MDMs has potential to reduce their net proteolytic activity in Mtb infection and may reduce harmful tissue damage.

Abstract S48 Figure 1 Siglec-9 activation upregulates TIMP1 release from Mtb-infested MDM

Idiopathic Pulmonary Fibrosis: Mechanisms

S49 THE ROLE OF PLATELET-DERIVED TGF β IN PULMONARY FIBROSIS

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Background Pulmonary Fibrosis (PF) is characterised by abnormal wound healing involving fibroblast proliferation, myofibroblast differentiation and increased extracellular matrix deposition. TGF β is an important driving force in fibrotic disease, however the source of this cytokine in PF is ill-defined. Platelets can release large amounts of TGF β , and we, and others, have shown platelet deposition in the lungs of patients with idiopathic pulmonary fibrosis (IPF), although the role of these cells in PF is unknown.

Hypothesis We propose that platelet aggregation and release of platelet-derived TGF β contributes to the aberrant wound healing in fibroproliferative lung disease.

Methods We used a double-transgenic mouse with megakaryocytic-specific deletion of TGF β (PF4-Cre⁺/Tgfb1^{fl}) and hence platelets lacking TGF β . Knockout (KO) mice and wildtype (WT) littermate controls were subjected to the experimental model of lung fibrosis induced by oropharyngeal bleomycin administration. Lung tissue and broncho-alveolar lavage fluid (BALF) were investigated at 6, 21 or 28 days post-bleomycin. Complementary *in vitro* studies were performed on isolated neutrophils to investigate the effects of platelet-derived TGF β in chemotaxis assays.

Results *In vitro*: Platelet-derived TGF β was shown to be a potent neutrophil chemoattractant with maximal effect at 1ng/ml. *In vivo*: At 6 days after bleomycin treatment, neutrophils and macrophages were significantly elevated in the lung and BALF in both WT and KO animals as measured by flow cytometric analysis. No significant difference in the percentage or total cell numbers was found between WT or KO mice. At 21 days post-bleomycin, the lungs developed large fibrotic lesions when examined by micro-CT. Bleomycin-treated KO mice exhibited an attenuated fibrotic response compared with WT animals (26.9 vs. 19.6%), although not reaching statistical significance. During the wound resolution phase at 28 days post treatment, the degree of fibrosis between WT and KO animals was very similar (9.56 vs. 9.84%) as determined by micro-CT analysis.

Conclusion Our data suggest that despite being a potent neutrophil chemoattractant *in vitro*, platelet-derived TGF β *in vivo* is not a major driving force during the inflammatory or resolution phases of our PF animal model, but may contribute to the development of fibrotic disease. This will be the subject of further study.

S50 MONOCYTES FROM IPF PATIENTS SHOW PRE-CONDITIONED PRO-REPAIR FEATURES

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Introduction The central mechanism in IPF is a dysfunctional alveolar epithelial-fibroblast interaction resulting in an aberrant repair process. This defect is influenced by other immune processes; one of these is the macrophage pathway. Macrophages are heterogeneous immune cells that can control all phases of the repair process. 'M2' or 'reparative' macrophages have anti-inflammatory and reparative phenotype, with high scavenger activities. We investigate how monocytes (precursors of monocyte-derived lung macrophages) might contribute to fibrogenesis in IPF.

Methods 35 IPF patients (25 sampled while stable and 10 with AE-IPF) diagnosed according to the 2011 ATS/ERS/JRS/ALAT guidelines, with 'definite' or 'probable' IPF and age and gender-matched healthy controls were recruited over a one-year period. Those with emphysema greater than 25%, current smokers and malignancy were excluded. Lung function and CT fibrosis score1 were performed. Phenotype and function of purified monocytes and monocyte-derived macrophages (MDMs) were determined using qPCR and multi-flow cytometry for selected M1 and M2 genes and proteins (M1 – CD64 M2 – CD163 and CD200R by FACS; and 26 M1 and M2 macrophage markers against three house keeping genes). The ability of MDMs to phagocytose

(using pHrodo method) and ROS content (using H2DCFDA assays) *ex vivo* were also examined.

Results and discussion Circulating monocyte levels were significantly higher in IPF compared to healthy controls ($p < 0.001$) and correlated negatively with lung function (FVC $r = -0.6$, $p = 0.003$) and CT fibrosis score ($r = 0.45$, $p = 0.007$). IPF monocytes displayed higher M2:M1 ratio profile compared to healthy controls – with higher *IL10*, *CD163*, *IL1R2*, *FGL2* and lower *TNFA* and *CXCL10* gene expression. When these monocytes were differentiated to macrophages (MDMs) *ex vivo*, IPF macrophages showed a significantly higher level of M2 markers and CD14 expression, reduced phagocytosis and produce lower levels of ROS –supporting M2 and pro-repair phenotype.

Conclusions Our data show that circulating monocytes in IPF are elevated compared to age-matched controls, correlate positively with disease severity, and are different from those found in age-matched healthy controls. They have pro-repair, M2-like features and differentiate to pro-repair monocyte-derived macrophages and may contribute to the aberrant repair process in IPF.

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S51 MTOR REGULATES TGF- β INDUCED PRO-FIBROTIC GENE EXPRESSION IN PRIMARY HUMAN LUNG FIBROBLASTS

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Introduction TGF- β is a major pro-fibrotic cytokine with a critical role in the pathogenesis of idiopathic pulmonary fibrosis (IPF). TGF- β drives fibroblast to myofibroblast differentiation and extracellular matrix synthesis. mTOR plays a critical role in regulating protein translation and is the catalytic subunit of two functionally distinct complexes, mTORC1 and mTORC2, which have differential sensitivities to rapamycin. The aim of this study was to delineate mTOR signalling in response to TGF- β in human lung fibroblasts and investigate the role of the mTOR pathway in TGF- β mediated myofibroblast differentiation and collagen synthesis.

Methods All human samples were obtained with informed, signed consent and with research ethics committee approval. Primary human lung fibroblasts (pHLFs) were grown from explant cultures. Cells were pre-incubated with varying concentrations of inhibitor before stimulation with TGF- β 1ng/ml. Collagen biosynthesis and α SMA expression were measured by a high-content imaging based molecular crowding assay. Gene expression was measured by qPCR. Western blots were performed to assess mTOR substrate phosphorylation.

Results TGF- β was found to stimulate the delayed and sustained induction of mTOR signalling in pHLFs and this signalling pathway was critical for mediating the late peak in TGF- β induced pro-fibrotic gene expression. Accordingly, active-site mTOR inhibition exerted pronounced inhibitory effects on pHLF collagen biosynthesis and myofibroblast differentiation. The induction of mTOR signalling in response to TGF- β was dependent on the canonical Smad pathway. In addition, potent and selective pharmacological agents demonstrated that TGF- β induced mTOR signalling was independent of PI3K/Akt activity, suggesting that

mTOR is not activated through the prototypical linear PI3K/Akt axis downstream of TGF- β . Moreover, rapamycin-resistant mTOR signalling was found to be critical for TGF- β induced pro-fibrotic gene expression in pHLFs.

Conclusion mTOR is an important pro-fibrotic signalling node downstream of TGF- β and a potential target for therapeutic intervention in IPF.

S52 SUBERANILOHYDROXAMIC ACID (SAHA) INHIBITS COLLAGEN DEPOSITION IN A TRANSFORMING GROWTH FACTOR β 1-DRIVEN PRECISION CUT LUNG SLICE (PCLS) MODEL OF PULMONARY FIBROSIS

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Introduction and objectives Idiopathic Pulmonary Fibrosis (IPF) is a chronic, progressive interstitial lung disease that is refractory to current treatment options. Transforming growth factor (TGF)- β 1 is a key pro-fibrotic cytokine that plays a crucial role in IPF pathogenesis. Our group previously demonstrated distinct epigenetic modifications involved in repression of the antifibrotic gene cyclooxygenase-2 (COX-2) in fibroblasts from IPF (F-IPF) lungs compared with fibroblasts from non-fibrotic lungs (F-NL). Epigenetic drugs capable of inhibiting DNA and histone modifications may, therefore, represent a putative novel therapy. The aim of this study was to investigate the ability of 4 epigenetic inhibitors to regulate TGF- β -driven fibrosis in *ex vivo* mouse lung.

Methods A precision-cut lung slice (PCLS) model of fibrosis was established using the previously described¹ CC10-tTS-rtTA-TGF β 1 transgenic (tgTGF- β 1) mouse. The model was first assessed by investigating PCLS overexpression of TGF- β 1 in response to stimulation of the transgene by doxycycline treatment. Gene expression of COX-2 and fibrotic markers including collagen were assessed after 4 days of treatment. The anti-fibrotic potential of 4 epigenetic inhibitors; BIX01294 (BIX, inhibitor of G9a histone methyltransferase), 3-deazaneplanocin A (DZNep, inhibitor of EZH2 histone methyltransferase), SAHA (inhibitor of histone deacetylases, HDACs) and Decitabine (DAC, DNA demethylating agent) was investigated. Viability of PCLS was assessed by MTT and Prestoblu[®] assay.

Results Treatment of PCLS from tgTGF- β 1 mice with doxycycline induced a concentration-dependent increase in global TGF- β 1, pro-fibrotic markers including collagen and pro-inflammatory COX-2, which was comparable to recombinant TGF- β 1 treatment. Treatment with three of the epigenetic inhibitors BIX01294, DZNep and DAC did not reduce the pro-fibrotic response following doxycycline treatment. However SAHA demonstrated a significant suppressive effect on COX-2 and collagen expression, while not directly affecting TGF- β 1 transgene expression.

Conclusions The data suggests that SAHA has the potential to reduce fibrosis in a TGF- β 1 driven model of pulmonary fibrosis. Further work is currently underway to assess the anti-fibrotic potential of this drug in tgTGF- β 1 animals.

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