Inhibitors of these molecules (Samuraciclib/CDK7, Dubematinib/AXL, IPR-803/UPAR) proved effective against TSC2-/- LAM-derived cells, significantly reducing proliferation in vitro. Conclusion LAF-derived ECM enhances TSC2-/- cell proliferation in vitro and may contribute to disease progression by providing a pro-proliferative microenvironment for LAM cells in vivo. A number of pro-proliferative molecules are upregulated when TSC2-/- LAM-derived cells are grown on decellularised ECM in vitro, and targeting these pathways may provide novel therapies for LAM patients with reduced response to rapamycin.

Please refer to page A285 for declarations of interest related to this abstract.

**S70 MESENCHYMAL CELL SENESCENCE INFLUENCES ATII CELL VIABILITY IN LAM**

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Background Lymphangioleiomyomatosis (LAM) is a progressive, incurable multisystem disease-causing respiratory failure, lymphatic abnormalities, and renal tumours. LAM is rare as a sporadic disease but is common in women with the autosomal genetic disease tuberous sclerosis complex. LAM cells have bi-allelic inactivation of TSC2 which codes for tuberin, a component of a multiprotein complex inhibiting the kinase mTOR. We hypothesise, due to mTOR dysregulated cells in LAM nodules become senescent, generate senescence associated secretory proteins and lipids which in turn induce senescence in adjacent cells including alveolar type II cell impairing alveolar repair mechanisms to promote parenchymal lung destruction.

Methods Using laser microdissection, we examined the transcription profile of LAM nodules compare to rest of lung and control lungs. The result from transcription validated in dual label immunohistochemistry, primary cell co-cultures and coculture of human drives ATII organoids with Primary LAM model cells.

Results Cyclin Dependent Kinase Inhibitor 1A (p21) and Cyclin Dependent Kinase Inhibitor 2A (p16) proteins were increased in LAM lung. p21 and p16 co-localised with SPC (ATII cells). In Murine model of LAM homograft, senescence associated beta-galactosidase activity increased with time and significantly higher than control animals. scRNAseq of human LAM lungs showed alterations in ATII cell regulation of cell death, apoptotic pathways, senescence and Wnt signalling. A Stat 3 / p53 dependent pathway governing apoptosis and alterations in lipolysis and ATII differentiation were present. In vitro LAM cell / LAF / human organoids ATII co-cultures showed that senescence associated genes are upregulated in an mTOR dependent manner. Interrogation of scRNAseq data from nodules and epithelial areas validates these findings and is associated with lung function and disease duration in humans.

Conclusions mTOR dysregulated LAM cells induce fibroblast and epithelial senescence and reduce ATII cell viability to impair the repair response to lung injury.

Please refer to page A285 for declarations of interest related to this abstract.

**S71 DECIPHERING THE ROLE OF γδ T CELLS IN HYPERSENSITIVITY PNEUMONITIS**

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Introduction and Objectives Hypersensitivity pneumonitis (HP) is a form of interstitial lung disease, characterized by persistent lymphocytosis and granuloma formation in the lungs, but its pathophysiology is poorly understood. Pigeon Dropping Extract (PDE) is a common antigen causing bird-related HP. IL17A-producing γδ T cells have been shown to mediate an immune response against PDE, and to be more abundant upon secondary exposure to the antigen.1 The aim of this study was to determine whether this memory-like response against PDE is antigen-specific.

Methods C57BL6/J mice (n=16) underwent a sensitization protocol to create convalescent mice, where they were administered 8 μg of PDE on days 1,3,5,8,10,12 and 15. Control group of naïve mice (n=17) were given saline on the corresponding days. Following a 2-week recovery period, all mice were exposed either to PDE, or another antigen (Saccharopolyspora rectivirgula, SR) causing HP. The numbers of tissue-
resident, circulating, and IL17A+ γδ T cells were quantified using flow cytometry. Immune cells in bronchoalveolar lavage fluid were immunostained and counted with light microscope. Immune cell infiltration was observed in H&E-stained formalin-fixed paraffin-embedded lung sections.

**Results** The numbers of tissue-resident and IL17A+ γδ T cells following PDE-exposure were higher in convalescent mice compared to control. SR caused non-significant increase in tissue-resident γδ T cells. Total cell count and density in bronchoalveolar lavage were significantly higher and there was more immune cell infiltration in H&E-stained lung sections in convalescent mice that were exposed to PDE than SR.

**Conclusions** It was demonstrated that the memory-response was specific to PDE and mediated by tissue-resident and IL17A-producing γδ T cells. However, there was cross-reactivity to SR. Potential causes included the capability of γδ T cells to detect non-MHC-bound molecules. Future studies should examine reactions to other antigens with similar immune profiles, γδ T cell-depletion and other tissue-residence markers. Better understanding pathophysiology of HP would allow for identification of diagnostic biomarkers and potential novel therapeutics.

**REFERENCE**


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**S72 SPECIFIC THORACIC CT PATTERN OF PERIHILAR CONGLOMERATION AND CONSOLIDATION IS ASSOCIATED WITH DEVELOPMENT OF LUNG FIBROSIS IN PULMONARY SARCOIDOSIS**

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**Background** Approximately 20% of patients with pulmonary sarcoidosis develop fibrosis. There is currently no predictor of fibrosis.

**Aims** We questioned if a specific radiological feature were associated with development of fibrosis in pulmonary sarcoidosis.

**Methods** In a retrospective study, n=376 sarcoidosis patients (BTS Statement 2020 diagnostic criteria) from the Oxford Sarcoidosis Clinic were included. Their first and last thoracic CTs were classified into: A. Active disease (defined as presence of nodularity, ground glass opacities, interlobular septal thickening, and/or consolidation but not perihilar) without fibrosis, B. Active disease with perihilar consolidation/conglomeration without fibrosis, C. Active disease with fibrosis, D. Fibrosis without active disease, E. hilar and/or mediastinal lymphadenopathy only. Those with fibrosis on their first CT were excluded (n=127). Of the remaining, those with CT intervals between 2 and 12 years were selected for final analyses (n=201). 67 of these patients developed fibrosis on the second CT. Starting FVC [mean (S.D.)] was 99(20)% predicted, TLCO 81(17)%; 91% were non-smoker. A multivariable Cox regression model was constructed to examine the association of age, gender, first CT pattern (A, B and E) and treatment (Prednisolone or any immunosuppressants – yes/no during CT interval), with development of fibrosis on second CT (which accommodates the variable follow up period). Kaplan-Meier survival analysis with log rank test of significance was also performed for time to fibrosis for the three CT patterns.

**Results** For the final cohort (n=201), median age was 52y, CT pattern A (n=151); B (n=15) and E (n=35). Median interval (S.D.) between the two CTs were 4.7(2.3)y and 5.7 (2.7)years for the fibrotic and the non-fibrotic groups respectively. From the multivariable Cox regression model, presence of active disease with peri-hilar conglomeration/consolidation (CT pattern B) was the strongest independent predictor for fibrosis (p < 0.001, HR – 20.45, 95% CI : 5.37 – 77.85). Active disease (pattern A) was also associated with development of fibrosis but with a lower HR (p=0.01, HR=4.70, 95% CI – 1.43–15.28). Kaplan-Meier analysis supports these findings (logrank test, p < 0.01) (figure 1).

**Abstract S72 Figure 1 KM plot for cumulative proportion of patients with fibrosis over the years**