was modified by additional use of other anti-diabetic medications, BMI or HbA1c.

**Results** 14,292 cases and 54,529 controls were included. Metformin was associated with 24% reduced odds of an exacerbation, its effect remained up to 180 days after the prescription date (OR=0.76, 95%CI 0.64–0.88) but there was no effect >180 days after metformin use (p>0.05). Using GLP-1 agonists with metformin had a multiplicative effect, associated with a 72% reduction in odds of an exacerbation (with GLP-1: OR=0.28, 95%CI 0.11–0.75). BMI, HbA1c and other anti-diabetic medications (DDP-4 and SGLT-2 inhibitors, sulfonylur- eas and insulin) were not found to influence the effect of metformin on exacerbations.

**Conclusion** Metformin reduces the risk of COPD exacerbations, but its mechanism may not be through improved systemic glycemic control or weight loss. The concurrent use of GLP-1 receptor agonists augments the effect of metformin, other anti-diabetic medication does not. Diabetes is common in COPD and metformin is relatively cheap, early use may prevent exacerbations.

**P206** IDENTIFICATION OF NOVEL SENOLYTIC CANDIDATES FOR THE TREATMENT OF CHRONIC RESPIRATORY DISEASES WITH ACCELERATING AGING

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**Background** Cellular senescence is a process that induces cells into a state of irreversible replicative arrest, whereby they are apoptosis-resistant, and release inflammatory mediators known as the senescence-associated secretory phenotype (SASP). Cellular senescence and the SASP potentially have a significant influence on the process of ageing and pathologies of accelerated premature-ageing related chronic respiratory diseases such as chronic obstructive pulmonary disease (COPD) and idiopathic pulmonary fibrosis (IPF). Senolytic agents, capable of selectively killing senescent cells by inducing apoptosis, could potentially have major implications for treating the senescence-driven accelerated ageing pathology found in COPD and IPF. The aim of this project is to identify a new class of senolytic agents.

**Methods** Compounds (0.1 and 10μM) from an FDA-approved chemical library (700 compounds) were treated 2 days after etoposide (1μM)-treated or untreated airway epithelial cells, BEAS-2B. The resazurin cell viability assay was conducted 2-days post-treatment and the ability of the compounds to eliminate senescent cells was assessed. Positive candidates were treated in double hit etoposide senescent BEAS-2B. Senescence markers p21WAF1/CIP1 and p16INK4A expression (western blotting) and positive Senescence-Associated-b-Galactosidase (SA-β-Gal) staining were assessed. SASP marker PAI (Plasminogen Activator Inhibitor)-1 was detected in supernatant using ELISA.

**Results** Single treatment with etoposide was confirmed to induce cellular senescence characterized by an increase in SA-β-Gal staining, p16INK4A and p21WAF1/CIP1 expression. After high throughput screening with this cell model, we identified 6 candidates to be able to eliminate senescent cells, but not healthy cells. Further validation revealed that Dipyridamole (DP) showed decrease in p21WAF1/CIP1 expression by 20.5% compared to etoposide control (p>0.05, n=4), where a well-established senolytic cocktail (a combination of Dasatinib and Quercetin) reduced p21WAF1/CIP1 by 27.6%. DP and Amlodipine (AL) also reduced the proportion of SA-β-Gal positive cells by 15.5% and 31.5%, respectively, compared to etoposide control. Etoposide-induced PAI-1 release was also reduced by those candidates (p>0.05) as well as DQ (57.0% reduction).

**Conclusion** DP and AL showed some potentials as senolytic agents. Further studies using primary cells obtained from patients with COPD or IPF are needed for full validation.

**P207** SENOLYTIC EFFECTS OF TELAGLENASTAT, A GLUTAMINASE INHIBITOR, ON SENESCENT AIRWAY EPITHELIAL CELLS

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**Background** COPD is characterized by pulmonary inflammation and accelerated lung aging, where elevated number of senescent cells are observed. Senescent cells may prevent lung repair and drive chronic lung inflammation. Telaglenastat, a non-competitive glutaminase inhibitor, is recently reported as a potential senolytic agent which removes senescent cells (Johmura et al., Science, 2021).

**Aim** Investigate the senolytic effects of Telaglenastat on airway epithelial cells.

**Methods** Immortalised bronchial epithelial cell line, BEAS-2B cells, were treated with known senescence inducer etoposide
either once or twice (3 days apart). 10 days post-treatment, cellular senescence was characterised by high levels of p21WAF1/CIP1 and p16INK4A expression (western blotting) and Senescence-Associated-b-Galactosidase (SA-b-gal) positive staining (Senescence detection kit, Abcam). Senescent cells were treated with Talaglenastat (0.4 μg/ml) or Dasatinib (200 nM) and Quercetin (50 μM) (D+Q) for 72 hours and changes in senescence markers detected. In addition, human induced pluripotent stem cells (iPSCs)-derived alveolar epithelial cells cultured under the air-liquid interface (ALI) condition (HiLung Inc, Japan), were treated with etoposide to induce senescence, prior to Talaglenastat treatment.

**Results** Talaglenastat treatment showed reduction of SA-b-gal staining in double etoposide treated BEAS-2B (approx. 33% reduction compared to etoposide treated cells, p<0.05, n=4). Senolytic cocktail, Dasatinib and Quercetin reduced senescent cell number, but not% SA-b-gal staining. In addition, Talaglenastat showed a concentration dependent reduction of p21WAF1/CIP1 and p16INK4A expression with IC₉₀ values of 0.725 and 0.284, respectively, in BEAS-2B cells with etoposide single treatment. Etoposide single and double hit also induced cellular senescence in iPSc ALI alveolar epithelium, characterised by an increase in p21WAF1/CIP1 and p16INK4A expression, and therapeutic treatment of Talaglenastat (0.057μg/ml) reduced the senescence marker expression.

**Conclusion** Talaglenastat displayed senolytic activities in etoposide treated senescent BEAS-2B cells and ALI cultured iPSC alveolar epithelium. This profile suggests that Talaglenastat offers the potential therapeutic treatment for patients with COPD.

Please refer to page A292 for declarations of interest related to this abstract.

**P208 EXTRACELLULAR VESICLES FROM COPD SMALL AIRWAY FIBROBLASTS SPREAD SENESCEENCE TO HEALTHY FIBROBLASTS**

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**Background** COPD is associated with cellular senescence and fibrosis. Extracellular vesicles (EVs) are membrane-derived vesicles involved in intercellular communication. EVs contain miRNAs, mRNA and proteins and have been implicated in COPD to induce senescence and the transition of fibroblast to myofibroblasts. This study examined whether EVs derived from COPD fibroblasts drive senescence in healthy recipient fibroblasts. Changes in expression of p21CIP1 and alpha-smooth muscle actin (αSMA) were chosen as markers of senescence and transition of fibroblasts to myofibroblasts respectively.

**Abstract P208 Figure 1** Effect of Large and Small EVs on p21CIP1 and αSMA Expression in NS Fibroblasts Stimulated for 48h. Healthy fibroblasts from non-smoker (NS) subjects were incubated with large and small EVs derived from healthy NS or COPD fibroblasts, derived from cells that had been cultured in the absence or presence of 100μM H₂O₂. Cells were also stimulated with media only (NT) and media containing H₂O₂ as controls. Cells were lysed after 48h (a, d) and the expression of p21CIP1 (b, e) and αSMA (c, f) was measured relative to β-actin expression and data presented as mean±SEM. Representative blots are presented in panels a and d.