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 SENESCENCE TO HEALTHY FIBROBLASTS**

J Davey, PS Fenwick, PJ Barnes, JV Devulder, LE Donnelly. National Heart and Lung Institute, Imperial College London, London, UK

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 (aSMA) 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 aSMA 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 aSMA (c, f) was measured relative to b-actin expression and data presented as mean±SEM. Representative blots are presented in panels a and d.
Methods Large EVs, and small EVs were isolated from media from non-smoker (NS) and COPD fibroblasts cultured with or without H₂O₂. EVs were labelled with pk67 and uptake measured by flow cytometry. Healthy recipient fibroblasts were cultured with EVs or EV-free media for 24h and 48h and protein expression of p21^CIP1 and αSMA measured using western blots and CXCL8 release by ELISA.

Results There was a time-dependent uptake of EVs into recipient cells with no difference between EVs from control or COPD fibroblasts with 91.8 ± 3.8% of recipient cells pk67 positive by 48h (n=4). Incubation of recipient fibroblasts (n=2–5) with large EVs from either non-smokers or COPD subjects did not alter the expression of p21^CIP1 or αSMA at 24h. Similarly large EVs from fibroblasts exposed to H₂O₂ had no effect on these markers in recipient cells. By contrast, at 48h (figure 1), small EVs from COPD cells showed a trend to increased expression of p21^CIP1 and EVs from both non-smokers and COPD subjects increased expression of αSMA. Incubation of recipient cells with large EVs from non-smoker fibroblasts that had been cultured with or without H₂O₂ increased release of CXCL8 (0.36±0.15ng/ml to 5.43±3.92ng/ml and 5.44±5.23ng/ml respectively) and small EVs from COPD fibroblasts induced CXCL8 release at 48h (0.36±0.15ng/ml to 3.75±3.16ng/ml).

Conclusions Large and small EVs tend to increase the expression of p21^CIP1 and αSMA in recipient fibroblasts. These results are confirmed by the uptake analysis showing that maximum uptake of EVs from both NS and COPD fibroblasts is reached after 48h. Altogether, these data suggest that EVs participate in COPD pathophysiology by spreading senescence in recipient fibroblasts.

Background The complexity of standard chronic obstructive pulmonary disease (COPD) therapy regimens can lead to treatment non-adherence, negatively impacting on health-related quality of life (HRQoL) and long-term outcomes. TriOptimize is the first global real-world study to evaluate HRQoL and treatment adherence in patients with poorly-controlled, moderate-to-severe COPD, treated with fixed triple therapy. Here we present the final analysis from the UK study cohort.

Methods TriOptimize-UK (NCT04355546) is a prospective, non-interventional study investigating an extrafine formulation single-inhaler triple therapy (beclometasone;BDP/formoterol:FF/glycopyrronium;G [Trimbow® pMDI 87/5/9]) on HRQoL in patients with COPD. The primary objective was change in HRQoL (measured by COPD assessment test [CAT]) after prescription of BDP/FF/G, between baseline (Visit1 [V1]) and month 6 (Visit 3 [V3]) stratified by previous COPD therapy. Secondary measures included Test of Adherence to Inhalers (TAI), change in CAT items, and CAT total score at month 3 (Visit 2 [V2]).

The plan was to recruit 3,800 patients worldwide, with 200 from the UK; UK patient recruitment and follow-up was restricted by the COVID-19 pandemic.