(0.67) vs those who did not (0.36) and AB/FF 400/12 μg significantly reduced the rate of exacerbations vs PBO ($p < 0.05$; Table 1). The overall AE frequency was similar throughout (range with ICS, 54.8–60.7%; without, 56.0–60.3%). The most common AEs across patient groups were COPD exacerbation, nasopharyngitis and headache, irrespective of ICS use.

Conclusion In this analysis, aclidinium/formoterol 400/12 μg twice daily improved bronchodilation and dyspnoea in patients independent of ICS use and reduced exacerbations in patients using ICS. Combining AB and FF along with an ICS increased bronchodilation vs either monotherapy. AE frequencies were similar between the patient groups, regardless of ICS use.

Abstract S60 Table 1 Change from baseline in morning pre-dose (trough) FEV_1 at Week 24 and rate of exacerbations by concomitant ICS use

	AB/FF 400/12 μg BID	AB/FF 400/6 μg BID	AB 400 μg BID	FF 12 μg BID	Placebo BID
LS mean change from baseline in morning pre-dose (trough) FEV_1 at Week 24 by ICS use, mL[a]					
ICS use	98***	47***	44***	27**	-47
No ICS use	85***	71***	71***	18***	-50
Rate of exacerbations per patient/year by ICS use[b]					
ICS use	0.40*	0.53	0.59	0.45	0.67
No ICS use	0.31	0.27	0.29	0.44	0.36

[a]Analyses based on the mixed model for repeated measures: treatment effects and treatment comparisons.
[b]Analysis based on the log-linear model.
*$p < 0.05$ vs placebo; **$p < 0.01$ vs placebo; ***$p < 0.0001$ vs placebo.
AB, aclidinium bromide; BID, twice daily; FEV_1, forced expiratory volume in 1 s; FF, formoterol fumarate; ICS, inhaled corticosteroid; LS, least squares.

S61 ANALYSIS OF THE EFFICACY AND SAFETY OF THE COMBINATION OF TIOTROPIUM + OLODATEROL IN PATIENTS WITH COPD BY PREVIOUS USAGE OF INHALED CORTICOSTEROIDS

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Rationale Tiotropium (T), a long-acting muscarinic antagonist, and olodaterol (O), a long-acting β_2-agonist (both administered once daily), have been studied as a once-daily combination. Two Phase III studies have demonstrated that T+O significantly improved lung function and symptoms over T and O monotherapy treatments in patients with moderate to very severe chronic obstructive pulmonary disease (COPD).[1] During these studies, patients were allowed to continue existing treatment with inhaled corticosteroids (ICS); this analysis was conducted to determine the effects of study treatment in patients receiving or not receiving ICS as reported at baseline.

Methods A total of 5162 patients were randomised to treatment with O 5 μg, T 2.5 μg, T 5 μg, T+O 2.5/5 μg or T+O 5/5 μg (Respimat® inhaler) in two 52-week, double-blind, parallel-group studies (NCT01431274 and NCT01431287). Primary efficacy end points were trough forced expiratory volume in 1 s (FEV_1) response (ie, change from baseline), FEV_1 area under the curve from 0–3 h (AUC_{0-3}) response and St George's Respiratory Questionnaire (SGRQ) total score after 24 weeks. Pooled data are presented for the patient subgroups either using or not using ICS at baseline.

Results In the overall population, all treatments resulted in clinically relevant improvements in lung function, with significant increases with both T+O doses over the individual components ($p < 0.01$).[1] These effects on lung function were observed irrespective of whether or not patients had reported concomitant use of ICS at baseline (see Table 1). In the 'ICS usage' and 'no ICS usage' subgroups, there were no statistically significant differences between the combinations and monotherapy treatments in changes in SGRQ total scores from baseline to Week 24, although SGRQ total scores were improved during this period with T+O.

Abstract S61 Table 1 Lung function responses at 24 weeks according to baseline ICS usage[a]

	Trough FEV_1, L		FEV_1 AUC_{0-3}, L	
	n	Adjusted mean (SE) change	n	Adjusted mean (SE) change
ICS usage				
O 5	497	0.046 (0.009)	503	0.129 (0.009)
T 2.5	471	0.084 (0.009)	476	0.142 (0.009)
T 5	464	0.088 (0.009)	465	0.147 (0.009)
T+O 2.5/5	489	0.114 (0.009)$^{\dagger\#*}$	492	0.246 (0.009)$^{\dagger;\#\#**}$
T+O 5/5	503	0.133 (0.009)$^{\dagger;\#\#**}$	505	0.260 (0.008)$^{\dagger\#\#**}$
No ICS usage				
O 5	510	0.067 (0.009)	514	0.139 (0.009)
T 2.5	533	0.062 (0.009)	537	0.132 (0.008)
T 5	536	0.073 (0.009)	543	0.155 (0.008)
T+O 2.5/5	511	0.122 (0.009)$^{\dagger\#\#**}$	517	0.252 (0.008)$^{\dagger\#\#**}$
T+O 5/5	500	0.149 (0.009)$^{\dagger\#\#**}$	503	0.263 (0.009)$^{\dagger\#\#**}$

$^{\dagger}p < 0.0001$ vs O 5; $^{\#\#}p < 0.0001$ vs T 2.5; **$p < 0.001$ vs T 5.
[a]Patients were not recorded as receiving LAMA or LABA at baseline in this study.
SE, standard error.

Conclusions In patients with COPD, T+O 5/5 μg significantly improved lung function over T 5 μg and O 5 μg monotherapy, irrespective of whether patients had reported ICS use at baseline.

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Mechanisms of lung injury and fibrosis remodelling on the fly

S62 USING DROSOPHILA MELANOGASTER TO STUDY PATHOGENIC MUTANTS OF SURFACTANT PROTEIN C

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Introduction and objectives Surfactant protein C (SFTPC) is secreted by type II pneumocytes to reduce alveolar lining fluid surface tension and thus prevent alveolar collapse at low lung volumes. The immature form of SFTPC must undergo proteolytic

processing before being secreted as the mature form, but several pathogenic *SFTPC* mutations associated with familial interstitial lung disease impede this process. Mutations in the C-terminal BRICHOS domain of SFTPC (ΔEx4 and L188Q) lead to retention of the protein within the endoplasmic reticulum (ER), while other mutations (e.g. I73T) cause SFTPC mis-trafficking.

Methods To study these mutants *in vivo* in a genetically tractable organism, we generated lines of *Drosophila melanogaster* expressing wild type or mutant human SFTPC. The transgenic proteins could be tagged with green fluorescent protein (GFP) to facilitate *in vivo* visualisation. These fusion proteins were expressed under the control of tissue-specific drivers. Components of the ER associated degradation (ERAD) machinery or of the autophagy pathway were depleted in those tissues by RNA interference. Lines expressing an ER stress reporter or autophagy reporter were used as readouts for these phenomena.

Results Expression of the BRICHOS mutants ΔEx4 and L188Q led to the progressive deposition of protein aggregates when expressed in the fly eye. In contrast, the I73T mutant accumulated in a more diffuse distribution. When expressed in the larval salivary gland, the BRICHOS mutants where retained within the cell, in contrast to the wild type protein that was trafficked to the cell surface. The I73T mutant showed low-level cell surface and weak intracellular fluorescence. Depletion of the ERAD E3 ubiquitin ligase Hrd1 or its associated E2 ligases failed to affect mutant protein levels arguing against an important role of ERAD in the degradation of SFTPC in this model. In contrast, inhibition of autophagy by depletion of Atg8 enhanced the accumulation of L188Q SFTPC. Accordingly, robust activation of autophagy was detected in L188Q SFTPC-expressing tissue. Interestingly, ER stress was not detected.

Conclusion In a *Drosophila* model of hSFTPC trafficking, autophagy was the major degradation pathway for L188Q mutant SFTPC.

S63 HUMAN MESENCHYMAL STROMAL CELL (HMSC) REGULATION OF HUMAN MACROPHAGES IN *IN VITRO* MODELS OF THE ACUTE RESPIRATORY DISTRESS SYNDROME (ARDS)

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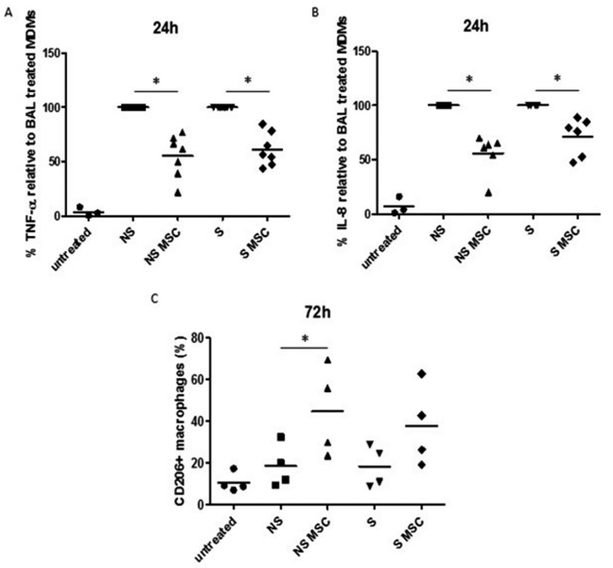
Background Currently there is no effective therapy which targets the mechanisms underlying the development of ARDS. MSCs present a promising candidate therapy and are being tested in clinical trials for ARDS however their mechanisms of effect in ARDS are not fully understood. Since the alveolar macrophage is key to orchestrating the alveolar inflammatory response, it was hypothesised that hMSCs induce an anti-inflammatory M2-like phenotype in human macrophages. The aim of this study therefore was to determine the effect of MSCs on macrophage phenotype and function and to elucidate the mechanisms of these effects.

Methods Using an *in vitro* non-contact co-culture system, human MSCs and human monocyte-derived-macrophages (MDMs) were stimulated with *E.coli* lipopolysaccharide (LPS). Cytokine and marker expression profiles were examined using ELISAs, multiplex and flow cytometry. Phagocytic capacity of MDMs was measured using fluorescent *E.coli* bioparticles by flow cytometry. For additional clinical relevance, the ARDS microenvironment was mimicked by using bronchoalveolar lavage fluid (BALF) obtained from patients with ARDS to examine the effect of MSCs.

Results MSCs suppress the production of both pro-inflammatory and anti-inflammatory cytokines by MDMs stimulated with LPS. MSCs increase expression of M2 markers CD163 and CD206 and have no effect on M1 markers CD80 and ICAM-1. Importantly, in spite of the immunosuppressive effect on macrophages, MSCs increase their phagocytic capacity. MSC effects on cytokine secretion and marker expression were maintained in the presence of BALF from patients with ARDS (Figure 1).



hMSCs suppress the pro-inflammatory response of human MDMs stimulated with BALF from patients of ARDS and induce an M2-like macrophage phenotype

Abstract S63 Figure 1 MSCs decrease secretion of pro-inflammatory cytokines TNF-α (A) and IL-8 (B) and increase expression of M2 macrophage marker CD206 (C) by MDMs stimulated with BALF from non-septic (NS) or septic (S) patients of ARDS. (A + B, n = 3–7, Kruskal Wallis *p < 0.05) (C, n = 4, ANOVA *p < 0.05)

Conclusions Human bone marrow-derived MSCs induce an M2-like phenotype and suppress cytokine secretion in primary human MDMs stimulated with LPS or ARDS patient BALF. Importantly, these effects are coupled with augmentation of macrophage phagocytosis which may be important in the clearance of bacteria and apoptotic cells. Uncovering the paracrine mechanisms responsible for the MSC effects on human macrophages remain the focus of ongoing work.

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S64 ALVEOLAR EPITHELIAL TYPE II CELL EXPRESSION OF VEGF-AXXXA IS CRITICAL FOR DEVELOPMENT OF IDIOPATHIC PULMONARY FIBROSIS (IPF): AN ANTI-FIBROTIC ROLE FOR VEGF-AXXXB ANTI-ANGIOGENIC ISOFORMS?

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