

Haemophilus influenzae and *Staphylococcus aureus*. Opsonisation with ficolin-2 promoted phagocytosis of *P aeruginosa* (PA01) by human neutrophils in a MASP-2 but not c1q dependent manner ($p<0.0001$) (Abstract T2 figure 1). On multivariable analysis chronic bacterial colonisation ($OR=3.5$; $p<0.0001$) and particularly *P aeruginosa* colonisation ($OR=2.8$, $p=0.0001$) were independently associated with ficolin-2 insufficiency. These patients also had more frequent outpatient exacerbations (mean 3.2/yr vs 2.4/yr, $p=0.01$) and unscheduled hospital admissions for exacerbations ($OR=2.3$; $p<0.0001$).

Conclusion Single nucleotide polymorphisms in the ficolin-2 gene affecting serum levels and carbohydrate binding are associated with non-CF bronchiectasis and increase susceptibility to colonisation with *P aeruginosa*.

T3 MEASURING EOSINOPHIL KINETICS IN HUMANS

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Introduction and Objectives Eosinophils are major cellular effectors of allergic inflammation and represent an important therapeutic target in asthma. While much is understood about the generation and activation of eosinophils, little is known about their intravascular kinetics and physiological fate. The purpose of this study was to image sites of eosinophil distribution and measure eosinophil kinetics in healthy individuals using autologous 111-Indium-labelled eosinophils.

Methods To determine "gold standard" kinetics of minimally-manipulated eosinophils, mixed leucocytes were isolated from the blood of healthy volunteers, labelled with 111-Indium-tropolonate and re-injected. Blood was sampled 0.75–72 h post-injection. Neutrophils and eosinophils were isolated in parallel, and cell-associated radioactivity was measured. To image sites of eosinophil margination/uptake eosinophils were purified using plasma-Percoll gradients and anti-CD16 immunomagnetic beads, labelled with 111-Indium-tropolonate and re-injected. The distribution of eosinophils was recorded by γ camera dynamic imaging (0–40 min) followed by static imaging up to 72 h.

Results Using minimally manipulated granulocytes we found that the 45min neutrophil recovery was $57\pm10\%$ ($n=7$) and the intravascular lifespan was 10.3 ± 0.1 h, in agreement with previous studies. By contrast, the 45min recovery of eosinophils was $15\pm2\%$ ($n=7$) and eosinophil lifespan was 25.2 ± 3.8 h. Moreover, eosinophils appeared to re-circulate at ~ 4 h and 9 h before mono-exponential removal. Using 111-Indium-eosinophils and γ camera imaging, we demonstrated initial retention of cells in the lungs, clearing to baseline by 40 min, with some early accumulation in the liver and progressive accumulation in the spleen ($n=3$). Simultaneous blood sampling showed that the 45 min recovery and intravascular lifespan of purified labelled eosinophils were $11\pm2\%$ and 29.3 ± 2.1 h, respectively, comparable to minimally manipulated cells. Purified cells also exhibited recirculation at ~ 6 h and 12 h. Of note, the disappearance of eosinophils from the liver at 6 h and 9 h coincided with their re-appearance in circulating blood, suggesting the liver as a possible site of transient eosinophil sequestration.

Conclusions This work provides the first in vivo measurements of eosinophil kinetics in healthy volunteers. Our data suggest that 111-In-labelled-eosinophils can be used to monitor the organ distribution and fate of eosinophils non-invasively. This technique may have an important role in assessing the therapeutic effects of eosinophil-targeted drugs.

T4 SAFETY AND EXPRESSION OF A SINGLE DOSE OF LIPID-MEDIATED CFTR GENE THERAPY TO THE UPPER AND LOWER AIRWAYS OF PATIENTS WITH CYSTIC FIBROSIS

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Introduction and Objectives We undertook a clinical trial of non-viral CFTR gene therapy assessing safety, dose and transgene expression in preparation for a Multi-dose trial (MDT) designed to assess clinical efficacy.

Methods A single nebulised and/or nasal dose of plasmid CFTR (pGM169)/GL67A was delivered to patients aged ≥ 16 years with a baseline FEV1 $>60\%$ predicted. Clinical and laboratory parameters were measured at intervals until day 28. A cohort of patients also underwent pre- and post-dosing (day 6 or 14) bronchoscopies for functional (airway potential difference (PD)) and molecular (QRT-PCR) evidence of vector-specific CFTR expression. Patients receiving a nasal dose underwent brushings for QRT-PCR and serial nasal PD measurements.

Results 35 patients received a nebulised dose of 20 ml ($n=17$), 10 ml ($n=10$) or 5 ml ($n=8$). A short-lived, dose-related drop in FEV1 was observed over the next 6 h (mean [SD]: 20 ml 25.7 [10.2]%; 10 ml 17.7 [9.9]%; 5 ml 13.0 [4.4]% of baseline). Subjects also experienced a systemic inflammatory response which was similarly dose-related and generally limited to the first 24–48 h post-dosing. A cohort of 6 patients (4@10 ml; 2@5 ml) received 4 g paracetamol over an 18-h period post-dosing; none of these patients developed a fever. Intriguingly, these subjects also appeared to have reduced systemic inflammatory responses. Molecular (mRNA) evidence of gene transfer was observed in some individuals from upper or lower airway brushings. On lower airway PD measurement, the majority of patients showed an increase towards non-CF values after nebulised gene therapy. 19 patients received a 2 ml nasal dose and 11 (58%) had some response in chloride secretion on nasal PD. In the two most positive individuals, responses were within the normal (non-CF) range and persisted to days 63 and 91, respectively.

Conclusions We consider the side effects after 20 ml nebulised dose excessive for repeated application. Those at 10 and 5 ml were more acceptable. Gene expression was confirmed in some patients, and restoration of CFTR function to the non-CF range has been observed out to 13 weeks following a single nasal dose. These data support progression of this agent to MDT.

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T5 THE KCa3.1 K⁺ CHANNEL MEDIATES WOUND HEALING IN HUMAN MYOFIBROBLASTS

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Idiopathic pulmonary fibrosis (IPF) is a common progressive interstitial lung disease and current treatments are ineffective. The Ca^{2+} -activated K^{+} channel $KCa_{3.1}$ modulates the activity of several structural and inflammatory cells which play important roles in model diseases characterised by tissue remodelling and fibrosis. We hypothesise that $KCa_{3.1}$ -dependent cell processes are a common denominator in IPF. We have therefore examined $KCa_{3.1}$ expression and function in human myofibroblasts derived from patients with IPF and non-fibrotic controls (NFC). IPF tissue was obtained from diagnostic lung biopsies, and NFC tissue from healthy lung removed

at surgery for carcinoma. Myofibroblasts grown in vitro were characterised by Western blot, immunofluorescence and RT-PCR to determine $K_{Ca3.1}$ channel expression. Patch clamp electrophysiology was used to demonstrate functional $K_{Ca3.1}$ channels. Wound healing and proliferation assays were performed using two specific $K_{Ca3.1}$ blockers (TRAM-34, ICA-17043 [Senicapoc]). Both NFC and IPF myofibroblasts expressed $K_{Ca3.1}$ channel mRNA and protein. Using the $K_{Ca3.1}$ channel opener 1-EBIO, $K_{Ca3.1}$ ion currents were elicited in 59% of NFC and 77% of IPF myofibroblasts tested ($p=0.0411$). These currents were blocked by TRAM-34 (200 nM). The 1-EBIO-induced currents were significantly larger in IPF cells compared to NFC cells ($p=0.0124$). Basic fibroblast growth factor (bFGF) (10 ng/ml) significantly increased the frequency of $K_{Ca3.1}$ currents across groups ($p=0.0046$). Similarly bFGF stimulation significantly increased myofibroblast wound healing ($p=0.002$). Following $K_{Ca3.1}$ blockade bFGF-stimulated wound healing was attenuated dose-dependently. Thus at the 48 h time-point wound healing was reduced by $22.2\pm 11.1\%$ and $27.3\pm 9.5\%$ for TRAM-34, 20 nM and 200 nM respectively ($p=0.0467$ across groups), and reduced by $16.9\pm 8.1\%$ and $24.4\pm 6.6\%$ for ICA-17043, 10 nM and 100 nM respectively ($p=0.0076$ across groups). $K_{Ca3.1}$ blockade had no effect on myofibroblast proliferation. We show for the first time that human lung myofibroblasts express the $K_{Ca3.1}$ K^+ channel. $K_{Ca3.1}$ currents are larger and more frequently present in cells from patients with IPF, and functional channel expression is increased by pro-fibrotic growth factors. $K_{Ca3.1}$ inhibition attenuates bFGF stimulated myofibroblast wound healing. These findings raise the possibility that blocking the $K_{Ca3.1}$ channel will inhibit pathological myofibroblast function in IPF, and thus offer a novel approach to IPF therapy.

T6

TRAIL IS A POTENTIAL NOVEL THERAPEUTIC TARGET IN PULMONARY ARTERIAL HYPERTENSION

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Introduction and Objectives Pulmonary Arterial Hypertension (PAH) is a life threatening disease characterised by the progressive

narrowing and occlusion of small pulmonary arteries, driven by the dysregulated growth of vascular cells. Current therapies are unable to reverse PAH and so identifying key pathways in disease pathogenesis should permit the development of more targeted therapeutics. The cytokine, Tumour Necrosis Factor (TNF)-Related Apoptosis-Inducing Ligand (TRAIL) induces EC apoptosis and SMC proliferation in the systemic circulation, but hitherto has not been studied in PAH. We recently determined that TRAIL is a mitogen for human PA-SMCs in-vitro and was associated with pulmonary vascular lesions in humans and rodent models. We thus hypothesised that TRAIL is an important mediator in the pathogenesis of PAH and now describe the potential therapeutic benefits of targeting TRAIL in-vivo using two animal models.

Methods To test whether blocking TRAIL could prevent the development of PAH, an anti-TRAIL antibody was delivered via osmotic mini-pump coincident with the induction of PAH in the MCT rat model. We also tested whether genetic deletion of TRAIL (ApoE^{-/-}/TRAIL^{-/-} mice) would confer protection to diet-induced PAH. To test whether inhibiting TRAIL could reverse established disease we again treated both models with an anti-TRAIL antibody starting from day 21 in the rat and 8 weeks in the ApoE^{-/-} mouse. Phenotyping included echocardiography, closed chest cardiac catheterisation followed by immuno-histological and biochemical analyses of the lung.

Results Antibody blockade (MCT) and genetic deletion (ApoE^{-/-}) of TRAIL prevented the development of PAH in both models. Interestingly a PAH disease phenotype was restored in ApoE^{-/-}/TRAIL^{-/-} mice by the administration of recombinant TRAIL. In rodents with established PAH, an anti-TRAIL antibody, significantly increased survival and reduced pulmonary vascular remodelling in the fatal rat MCT model ($p<0.05$ cf control). In the murine model, an anti-TRAIL antibody treatment reversed both haemodynamics (RVSP 27 mm Hg vs 88 mm Hg, $p<0.001$) and pulmonary vascular remodelling.

Conclusions Our preclinical investigations are the first to determine the importance of TRAIL to disease pathogenesis and highlight its potential as a novel and rational target to direct future translational therapies for PAH.