Abstract S53 Table 1 Primary and Secondary Efficacy Outcomes in the Intent-to-Treat Population (Change from Baseline at 90 Days post Final Treatment)

	RePneu Coil Treatment (n=23)	Control (n=24)	Between-Group Difference in Change from Baseline	P-value
	number (95% confidence interval)			
	Initial Analysis†			
Primary outcome				
Mean change in SGRQ	–9.11 (–14.59 to –3.62)	1.43 (–4.05 to 6.92)	–10.54 (–17.52 to –3.56)	0.004
Secondary outcome				
Mean change in TLC (L)	–0.36 (–0.55 to –0.18)	–0.25 (–0.43 to –0.07)	–0.11 (–0.35 to 0.12)	0.330
Mean change in RV (L)	–0.64 (0.92 to –0.37)	–0.29 (–0.57 to –0.02)	–0.35 (–0.70 to 0.00)	0.051
Mean change in 6-minute Walk Test (m)	52.98 (29.18 to 76.78)	–17.41 (–41.21 to 6.39)	70.39 (40.10 to 100.68)	<0.001‡
Mean percent change in FEV_1	14.85 (7.46 to 22.23)	2.04 (–5.35 to 9.42)	12.81 (3.41 to 22.21)	0.009‡

SGRQ denotes St. George's Respiratory Questionnaire, TLC total lung capacity, RV residual volume, and FEV_1 forced expiratory volume in 1 second.
†P-value determined by analysis of variance (ANOVA) with factors of treatment and site.
‡Statistical significance via the Hochberg adjustment for multiplicity for secondary outcomes.

Results Significant improvements in the treatment group compared to control group were observed for the primary end point mean SGRQ (Δ-10.54 points, p=0.004), as well as secondary end points mean six-minute walk distance (Δ+70.39 metres, p<0.001) and forced expiratory volume in one second (Δ+12.81%, p=0.009). Between group difference in change in mean residual volume did not reach significance (Δ-0.35 litres, p=0.051), despite a 0.64 litre reduction in the treatment group. There was a good safety profile with treatment.
Conclusions Treatment with endobronchial coils in patients with severe emphysema and hyperinflation significantly improves quality of life, exercise capacity and pulmonary function with a good safety profile. LVRCs present a novel, safe, and minimally invasive treatment option for patients with both homogenous and heterogenous emphysema, with benefits unaffected by collateral ventilation. A larger randomised controlled pivotal trial with longer follow-up is now needed. Funding shared by PneumRx and study sites.

Regulating inflammation in acute lung injury

S54 THE ROLE OF PRE-RECEPTOR GLUCOCORTICOID METABOLISM IN REGULATING THE SEVERITY AND PERSISTENCE OF MURINE LUNG INJURY

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Introduction Acute lung injury (ALI) is a major cause of respiratory failure in the critically ill patient. With a mortality rate of 40–60%, 50% of survivors left with pulmonary impairment and no current licenced treatment there is a need for novel therapies. Our current research suggests that local steroid metabolism by alveolar macrophages is defective in ALI patients. As the predominant function of these cells is phagocytosis of apoptotic neutrophils during resolution of inflammation, we sort to investigate the effect of pre-receptor glucocorticoid metabolism in a murine model of ALI
Methods Using intra-tracheal instillations of LPS (50μg), we analysed the inflammatory response in wild type (WT) mice compared to those deficient in 11β-hydroxysteroid dehydrogenase-1 (HSD-1 KO). These mice specifically lack the enzyme which converts inactive cortisone to active cortisol. Cell infiltrates and expression of several inflammatory markers within bronchial lavage fluid (BALF), as well as tissue permeability and mouse oximetry were examined to evaluate the immune response and lung damage.
Results Intra-tracheal LPS challenge in WT mice induced a significant increase in lung permeability (p=0.0153), infiltrating neutrophils (p=0.0121) and recruitment of $CD11c^+CD11b^+$ monocytes (p<0.0001), which was associated with significant hypoxia (p<0.0001) compared to PBS-treated controls 48hrs post-instillation. The severity of acute neutrophilic inflammation and epithelial barrier defects as measured by lung permeability index were similar in WT and HSD-1 KO mice.

In contrast, during resolution of LPS-induced injury (72 hrs post-instillation), HSD-1 KO mice had a significant accumulation of apoptotic neutrophils (p=0.02) and a significant increase in $CD11c^+CD11b^+$ monocytes (p=0.0007) recruited into the lung compared to WT controls. Moreover, Luminex arrays revealed a significant increase in BALF levels of IL-1β (p=0.003) and dysregulation of IL-6, TNFα and CXCL1/KC during the time course.
Conclusion Our data indicate that insufficient alveolar glucocorticoid metabolism augments the duration but not initial severity of lung injury, possibly via a dysregulation of apoptotic neutrophil clearance and suggests that therapies targeting defective macrophage HSD-1 expression may have value in promoting the resolution of ALI.

S55 THE AMINOPEPTIDASE CD13 REGULATES HOMOTYPIC AGGREGATION OF NEUTROPHILS

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Neutrophils are critical effector cells of the innate immune response and are recruited to sites of tissue injury in response to locally generated chemoattractants. Neutrophil recruitment is a highly regulated process involving complex interactions with the vascular endothelium and underlying tissue stroma. In addition to adhering to the endothelium, neutrophils can also self-associate, (a process known as homotypic aggregation - HA), which has been proposed to play a key role in disease states such as sepsis.
Aminopepetidase N or CD13 is a widely expressed membrane-bound metallopeptidase involved in the migration and invasion of cancer and endothelial cells. Neutrophils express CD13 on their cell surface, which is upregulated by TNF-α, IL-8 and fMLP We have shown that inhibition of aminopeptidase activity enhances the efficacy of TNF-α-induced neutrophil apoptosis (Cowburn et al. J Biol Chem 2006; 281:12458). Cross-linking anti-CD13 monoclonal antibodies (mAb) have been shown to induce HA of monocytic cells through PI3K activation (Mina-Osorio et al. J Leuk Biol 2006; 79:719). We hypothesised that CD13 may be involved in neutrophil migration and HA.

Using plasma-Percoll purified human neutrophils and a modified Boyden filtre assay we showed that IL-8 mediated neutrophil chemotaxis was not affected by either the CD13 mAb WM-15 or the aminopeptidase enzymatic inhibitor bestatin. In contrast, IL-8-mediated neutrophil migration through type 1 collagen gels was significantly impaired by WM-15 and MY7 mAbs, which both inhibit enzymatic activity and induce clustering of CD13. The non-clustering CD13 antibody WM-47 and bestatin had no effect. WM-15 and MY7 also

promoted neutrophil aggregation as assessed by light microscopy. Phase-contrast video-microscopy demonstrated that in WM-15 treated neutrophils, where HA was evident, the percentage of cells entering collagen 1 gels in response to IL-8 was significantly reduced (26.9% vs 71.8% in non-HA cells). WM-15 does not prime neutrophils, as assessed by superoxide production and shape change, and the cell surface expression of CD11b, CD18 and CD66b were not altered. These data suggest a novel role for CD13 in the homotypic aggregation of neutrophils, which reduces chemoattractant-induced migration through collagen 1 matrix and may predispose to neutrophil micro-aggregation within the circulation.

S56 UNRAVELLING VEGF165 SIGNALLING IN THE LUNG

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Introduction Vascular endothelial growth factor (VEGF) is a potent mitogenic, angiogenic and permeability factor that has been implicated in the development of lung injury and repair in a number of respiratory diseases such as ARDS and IPF $VEGF_{165}$a functions via VEGF receptors in particular VEGFR-2, leading to a diverse and complex network of signalling pathways including activation of both the MAPK pathway and eNOS This results in changes to cell permeability, migration and proliferation. We have investigated the downstream signalling mechanisms regulated by $VEGF_{165}$a in pulmonary and systemic endothelial cells. Understanding the signalling pathway used by $VEGF_{165}$a to regulate lung biology is critical to preferentially induce specific beneficial effects.

Methods Human Umbilical Vein Endothelial Cells (HUVEC) and Human Lung Microvascular Endothelial Cells (HUMVEC-L) were treated with 20ng/ml of $VEGF_{165}$a lysed and studied using phospho-specific antibodies which measure the phosphorylation/activation of key signalling molecules. Phosphorylation of VEGFR-2 was measured using phosphotyrosine-specific antibody to tyr^{1175} and tyr^{1214}. Phosphorylation and hence activation of MEK, MAPK and eNOS were also measured. The effects of VEGF isoforms on cell permeability in a time and dose dependent manner were measured by using a transwell system and "Electrical Cell-Substrate Impedance Sensor" (ECIS). Changes in the cellular distribution of VE-cadherin a protein known to be involved in the regulation of cell permeability was assessed by immunofluorescent labelling and confocal microscopy.

Results Phosphorylation of VEGFR-2 at tyr^{1175} and $tyr^{1214}$was induced between 5 and 10min (n=4; >5 fold increase). Activation of MEK and p44/42 MAPK (members of the MAPK pathway which regulates cell proliferation) were seen over a similar time course to that of VEGFR-2 (n=4; >5 fold increase) (Figs 1A, B). Phosphorylation of eNOS which regulates cell permeability was also observed (n=3; >2 fold) and indeed $VEGF_{165}$a increased permeability in both HUVEC and HUMVEC-L (Huvec $p<0.001$); (Humvec-l $p<0.01$) (Fig 1). Finally we showed that in both cell types VEGF induced changes in the cellular distribution of VE-cadherin.

Conclusion These results demonstrate that signalling pathways, previously suggested to induce mitogenesis or permeability are activated by VEGF 165a in HUVEC and HMVEC-l cells, identifying potential future therapeutic targets.

S57 THE ROLE OF VITAMIN D DEFICIENCY IN REGULATING THE SEVERITY AND DURATION OF MURINE LUNG INJURY

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Introduction Vitamin D has been shown to modulate both the innate and adaptive immune responses. Patients deficient have increased susceptibility to both infection and autoimmunity. Our research suggests patients with, or at risk of developing acute lung injury (ALI), are severely Vitamin D deficient/insufficient. As there are no licenced treatments for ALI, novel therapies need to be developed, therefore we investigated the effect of Vitamin D deficiency in a murine model of ALI to understand the mechanistic drivers of its action.

Methods Using a diet completely devoid of Vitamin D, we established near complete Vitamin D deficiency in otherwise wild type C57Bl/6 mice. We combined this with intra-tracheal instillations of LPS (50μg), and analysed the inflammatory response within the lungs of these mice compared to those fed on a Vitamin D sufficient diet. In addition, systemic Vitamin D supplementation was assessed by intra-peritoneal injection of cholecalciferol 48hrs prior to LPS

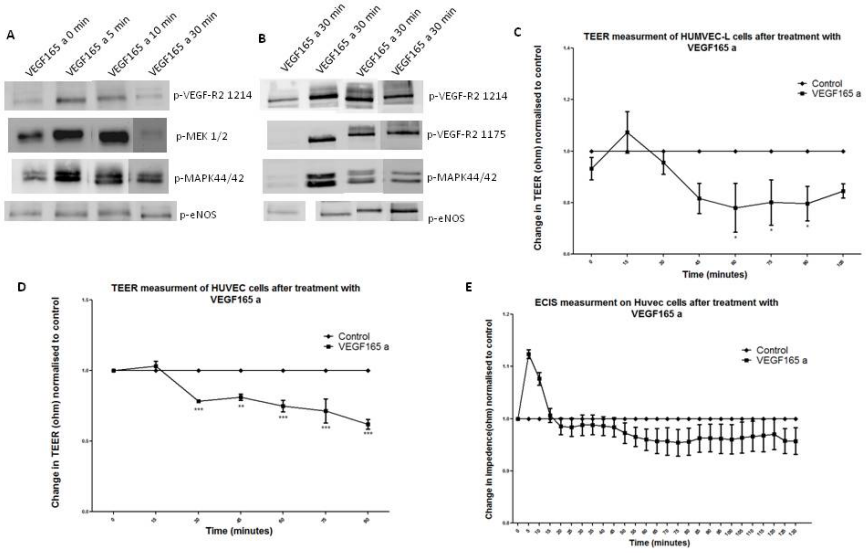

Figure1. Treatment of HUMVEC-L and HUVEC cells with VEGF solution at 20ng/ml. *A*, immunobblotting of primary HUMVEC-L treated for 0 to 30min and immunoblotted for the phosphorylation of VEGFR-2, MEK, MAPK and eNOS using phosphospecific antibodies. *B*, immunobblotting of primary HUVECs . *C*, VEGF165a reduces HUMVEC-L transendothelial electrical resistance (TEER) (increased permeability) in insert cultures monolyers.*p<0.1 (between 60 and 90min), compared with control (untreated cells). *D*, VEGF165a reduces HUVEC TEER .***p<0.001, compared with control (between 30 and 90min) . *E*, Electrical Cell-Substrate Impedance Sensor (ECIS) measurement in HUVECs using 8 well assay 8W10E+; VEGF165a reduces the resistance (increased permeability) compares to the control. Data were analysed using one-way ANOVA and Bonferroni post test analysis.

Abstract S56 Figure 1