Elk1$^{+/0}$ controls (9.40, 2.24 median relative expression, respectively n=3). We therefore performed immunohistochemical staining for αSMA in the lungs of mice aged to 1 year and demonstrated visible increases in expression of αSMA in the alveolar epithelium of *Elk1*$^{-/0}$ mice but not in *Elk1*$^{+/0}$ controls.

Conclusion These data suggest that Elk1 gene deletion result in age-related early fibrotic changes associated with the development of pulmonary fibrosis.

S119 MAPPING MOUSE MODELS OF SEVERE ASTHMA ONTO HUMAN DISEASE

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10.1136/thoraxjnl-2017-210983.125

Introduction Severe asthma represents a significant unmet need in terms of therapeutics. Drug development in asthma has been slow and expensive and one of the reasons for this is that positive findings for drugs tested in preclinical models have not readily translated into successful therapies in man.

Aims and Objectives We sought to improve the predictive power of existing models of asthma by using novel bioinformatics techniques to align these models with subsets of human asthma.

Methods We applied differential gene expression analysis to transcriptomic data from whole lung samples of 6 murine models of asthma and oxidative stress to produce gene signatures that represented each model. These signatures were then used to calculate enrichment scores (ESs) for transcriptomic data from bronchial biopsies taken from 81 asthmatic and 26 healthy subjects from the U -BIOPRED cohort using gene set variation analysis. These ESs were taken as an index of similarity between each model and each patient and were used to drive further analyses using topological data analysis and goodness of fit modelling.

Results We found that no single mouse model was aligned well with all asthmatics. We identified three clusters of patients who were represented to varying degrees by different mouse models and who displayed clinical features that aligned well with phenotypes of asthmatics identified previously by clustering analyses based upon clinical features and biological markers. Patients in cluster χ were defined by neutrophilic sputum, later onset of disease, higher incidence of sinusitis, more frequent exacerbations and more airflow limitation. Patients in cluster Y1 showed significantly lower sputum neutrophils, a trend towards higher sputum eosinophils, a significantly later onset of airways disease and a trend towards higher BMI. Patients in cluster Y2 showed a significantly higher percentage of neutrophils in the blood, a trend towards increased sputum lymphocytes and were more likely to identify aspirin as a trigger (p=0.06).

Conclusion Our evidence supports the assertion that it is possible, on a transcriptional level, to align mouse models of asthma to subsets of human asthma and that doing so may have significant implications for the expedience of drug development in asthma.

S120 GENE THERAPY FOR PULMONARY ALVEOLAR PROTEINOSIS

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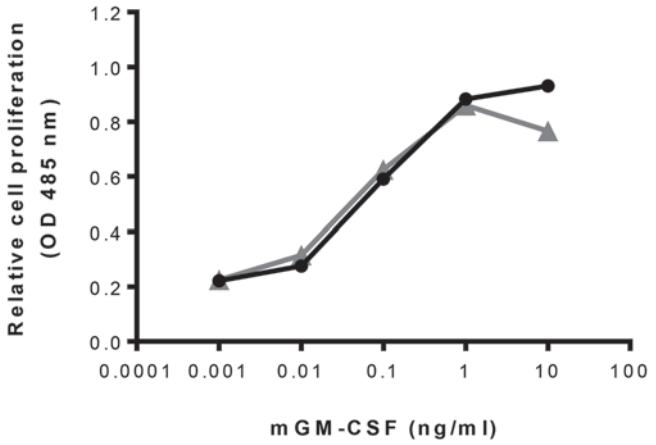
10.1136/thoraxjnl-2017-210983.126

Introduction Pulmonary alveolar proteinosis (PAP) is characterised by accumulation of surfactant in the terminal airways. Granulocyte-Macrophage Colony-Stimulating-Factor (GM-CSF) stimulates alveolar macrophages to clear surfactant. The presence of GM-CSF autoantibodies in autoimmune PAP (aPAP) leads to surfactant build-up and impaired gas exchange. This causes respiratory symptoms and can ultimately be fatal due to hypoxaemic respiratory failure. We hypothesise that lentivirus-mediated gene transfer of GM-CSF may be suitable to treat aPAP and propose to assess efficacy of GM-CSF gene transfer in GM-CSF knockout mice, which recapitulate aPAP lung disease. The murine GM-CSF (mGM-CSF) cDNA was cloned into a lentiviral vector, which was pseudotyped with the F and HN proteins from Sendai virus to enable efficient lung transduction (rSIV.F/HN-mGM-CSF).

Methods and Results To confirm if the vector produces mGM-CSF we first transduced A549 cells with multiplicity of infection (MOI) of 0.1–100 (n=6/group). 48 hours after transduction dose-related mGM-CSF expression was confirmed in the medium. We next assessed whether the mGM-CSF produced after gene transfer was biologically active by comparing the proliferation rate of FDC-P1 cells, a mGM-CSF-dependent mouse myeloid progenitor cell line, in the presence of gene therapy- produced mGM-CSF (0.001–10 ng/ml) and purchased recombinant mGM-CSF protein (n=6/group). The dose-related proliferation rates in both conditions were similar (figure 1ss). In preliminary experiments, we next assessed whether gene transfer led to GM-CSF production *in vivo*. rSIV.F/HN-mGM-CSF (1e7 transduction units (TU)/mouse) was administered to wild-type mice by nasal "sniffing". Control mice remained untransduced (n=3/group). mGM-CSF levels were quantified in lung tissue homogenate and broncho-alveolar lavage fluid (BALF) 14 days after gene transfer. mGM-CSF levels in untreated mice were below the limit of detection of the ELISA, but high levels of mGM-CFS were detectable in lung tissue (median 825 (range 460–3790) pg/mg) and BALF (median: 3330 (range 2307–7958) pg/ml).

Conclusion rSIV.F/HN-mGM-CSF produced mGM-CSF *in vitro* and *in vivo*. The biological function of the protein was confirmed *in vitro* and evaluation of mGM-CSF gene transfer efficacy in murine aPAP model is ongoing.



Abstract S120 Figure 1 Comparison of biological function of murine (m) GM-CSF produced after lentiviral-gene transfer and purchased purified protein (red: mGM-CSF produced through gene transfer, black: purchased mGM-CSF protein).

S121 CELL TRACKING IN LUNG CANCER

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10.1136/thoraxjnl-2017-210983.127

Introduction Lung cancer is the leading cause of cancer death worldwide with over 70% of patients presenting with incurable disease and few effective treatments. We previously demonstrated that mesenchymal stem cells transduced to express TNF-related apoptosis inducing ligand (TRAIL), will home to and induce apoptosis of tumour cells *in vitro* and reduce tumour growth in multiple *in vivo* models. A key unknown of cellular therapy is the location and duration of cells following intravenous delivery. ^{111}Indium-oxine is established for lymphocyte tracking but it has low sensitivity and is toxic to cells. ^{89}Zirconium-oxine is a novel PET tracer which has better sensitivity and lower toxicity. Our study aimed to label MSCTRAIL with ^{89}Zr with the aim of tracking cells in patients enrolled in the TACTICAL trial – an early phase trial delivering MSCTRAIL to patients with metastatic lung adenocarcinoma.

Methods MSC-TRAIL cells were incubated with multiple doses of ^{89}Zr-oxine and label retention measured using a gamma counter. Cells were assessed for cell viability using cell proliferation assays, TRAIL expression was determined using flow cytometry and ELISA and apoptosis was determined using co-culture experiments with luciferase expressing cancer cell lines and bioluminescent readout. DNA damage and cellular stress was assessed using western blotting. To determine whether radiolabelled cells could be detected *in vivo*, 2×10^5 ^{89}Zr-Oxine MSC-TRAIL cells were delivered intravenously and imaging was performed at multiple time points (Mediso PET-CT, AMI-X).

Results ^{89}Zr-oxine labelling at clinically relevant doses did not affect cell proliferation and therapeutic efficacy was maintained in co-culture experiments. There was no evidence of DNA damage and cell stress response and cellular phenotype was maintained. CT/PET imaging after labelling and delivery of the cells into mice showed good correlation with bioluminescent signal confirming its use a high sensitivity tracking tool.

Conclusion ^{89}Zr-oxine can be used to successfully radiolabel genetically modified stem cells without effecting cell viability or therapeutic efficacy. We are currently performing *in vivo* studies to enable further translation into a clinical trial and will ultimately track MSC-TRAIL after patient administration via radiolabelling with ^{89}Zr-oxine.

S122 A ROLE FOR THE BONE MORPHOGENETIC PROTEIN TYPE 2 RECEPTOR (BMPR2) IN DIFFERENTIATION OF THE COMMON MYELOID PROGENITOR LINEAGE IN MICE AND HUMANS

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10.1136/thoraxjnl-2017-210983.128

Rationale There is increasing evidence of a link between abnormalities in the myeloid cell lineage and pulmonary arterial hypertension (PAH). Heterozygous mutations in the gene encoding the bone morphogenetic protein type 2 receptor (*BMPR2*) are the most common genetic cause of PAH. We sought to characterise the impact of the genetic loss/reduction of *BMPR2* function in the myeloid lineage in mice and humans, and whether this altered susceptibility to PAH.

Methods Mx1-cre mice were crossed with *bmpr2*$^{flox/flox}$ mice. At approximately 8 weeks of age cre-recombinase was induced with polyinosinic-polycytidylic acid (Poly I:C). Control mice (*bmpr2*$^{flox/flox}$ mice with no cre) were also induced with Poly I:C. At approximately 16 weeks post-induction mice underwent right-heart catheterisation, exsanguination and tissue was removed for analysis. The spleens were weighed and histology was performed on the femurs. Mouse data are presented as mean ±SEM. In a large cohort of PAH patients with (n=160) and without (n=831) *BMPR2* mutations blood count indices were analysed. Data presented as median [IQR].

Results 16 weeks after induction of cre-recombinase in Mx1-cre/*bmpr2*$^{flox/flox}$mice we observed significant increases ($p<0.05$) in red blood cells ($x10^6/mm^3$) (12.7±0.9 compared with 12.1±0.2), haematocrit (%) (64.8±0.7 compared with 62.6±1) and haemoglobin (g/dl) (16±0.9 compared with 15.4 ±0.2) compared with *bmpr2*$^{flox/flox}$mice alone. A significant increase in circulating monocytes ($x10^3/mm^3$) was also observed ($p<0.05$) (0.4±0.05 compared with 0.3±0.05). In addition, we identified a significant increase ($p<0.05$) in megakaryocytes in the femurs (80±10 compared with 17±5) and a significant increase ($p<0.01$) in the ratio of spleen weight/body weight (0.003±0.0001 compared with 0.002±0.0001) in Mx1-cre/*bmpr2*$^{flox/flox}$ mice. During right heart catheterisation right ventricular systolic pressures were similar in both groups. In PAH patients significant differences ($p<0.05$) were seen in haemoglobin (*BMPR2* mutation: 162 g/L [151.75–173]) vs. no mutation: 150 g/L [135 – 163]), haematocrit (0.48 [0.45–0.52] vs. 0.44 [0.41–0.48]) and white blood cells (8.8 [7.3–10.4] vs. 8.11 [6.77–9.61]).

Conclusions we have identified a role for *bmpr2* in the differentiation of the mouse myeloid lineage, which was also confirmed in PAH patients with *BMPR2* mutations. *BMPR2* appears particularly important in the differentiation of megakaryocyte-erythrocyte lineage.