were induced with tamoxifen to genetically label KRT7 cells EGFP+. BLM-injured *KRT7*-CreERT2:*ROSA26*-mTmG ferretsdemonstrated a dynamic change of KRT7-positive lineage cells in lung during theprogression of fibrosis. KRT7-positive cells within the alveolar regions of thelung expanded significantly during early phases of injury and then laterdeclined as terminal fibrotic stages were achieved, mirroring changes in KRT7expression evaluated by snRNA-seq. Prior to injury KRT7 expression cells in thelung appeared to express both AT-1 (AGER) and AT-2 (SFTPB) cell markers, but unidentifiedcell types were also observed. We are currently identifying and validating celltype transitions of KRT7 lineage cells following BLM injury by scRNA-seq and FISH.KRT7-positive lineages in the alveolar regions of the lung may represent a stemcell compartment that facilitates repair could be a therapeutic target for gene and cell therapy for IPF.

1577. Hermansky-Pudlak Syndrome: Gene Therapy Assessment for HPS-1-Associated Pulmonary Fibrosis in a Novel Hps1 Knockout Mouse Model

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Hermansky-Pudlak syndrome type 1 (HPS-1) is a complex autosomal recessive disorder characterized by albinism and bleeding predisposition. It is part of a heterogeneous genetic syndrome with eleven types (HPS-1 to HPS-11), each associated with specific mutations in genes involved in lysosome-related organelles complex biogenesis. Current treatments, like lung transplantation, have limitations, necessitating alternative approaches. Previous work using a spontaneous murine mutant (Hps1ep/ep) elucidated disease mechanisms but lacked insights into spontaneous PF development. Gene therapy offers promise for HPS-PF treatment. This study introduces a novel Hps1 model and explores HPS1 gene augmentation. To understand HPS-1 and identify PF markers, we generated a CRISPR-Cas9-engineered knockout mouse model ($Hps1^{\Delta/\Delta}$), characterizing it at the cellular and molecular levels. AAV-based gene therapy (AAV5 and AAV8) aimed to identify predictive surrogate endpoints for HPS-PF outcomes. AAVs containing enhanced green fluorescent protein or mouse Hps1 gene were systemically delivered, with saline injections as controls. $Hps1^{\Delta/\Delta}$ mice exhibited clinical manifestations seen in HPS-1 patients, including reduced gene and protein expression, platelet abnormalities, and PF. Tropism analysis showed Hps1 expression in the lungs post-AAV treatment. AEC2 size was reduced in AAV-treated mice. AAV8-Hps1-treated mice exhibited significant pulmonary function improvements, indicating therapeutic potential. These findings highlight gene therapy advancements for HPS-1 PF, offering transformative treatment possibilities. Ongoing research is crucial to refine gene therapy, ensuring long-term safety and efficacy for HPS1-PF patients.

1578. Preclinical Optimisation of Lentiviral Delivery to Piglet Lungs for Gene Therapy of Surfactant Deficiencies

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Introduction. Surfactant protein deficiencies account for 10% of childhood interstitial lung disease and the prognosis depends on the specific protein that is deficient. Infants with severe surfactant protein B (SP-B) deficiency or double null mutations in ABCA3, a protein that transports surfactant lipids to lamellar bodies, present shortly after birth with respiratory distress and failure. Despite ventilation and treatment with synthetic surfactant, they rarely live beyond the first few months of life. An effective gene therapy for surfactant deficiencies has the potential to be transformative. **Methods**. A proprietary Simian Immunodeficiency Virus (SIV) based lentiviral vector, pseudotyped with Sendai Virus F/HN proteins and expressing EGFP, was delivered to the lungs of ventilated neonatal piglets. This was performed by multisite bronchoscopic instillation via an endotracheal tube to model clinical delivery of vector to the lungs of ventilated infants. At post-mortem, precision cut lung slices (PCLS) were prepared to assess the impact of vector dose and volume on the distribution and type of transduced cells. We used quantitative immunohistochemistry (IHC) with antibodies against EGFP as well as alveolar type I (ATI) and type II (ATII) pneumocytes. Transgene mRNA was quantified by qRT-PCR and localised by hybridisation chain reaction (HCR) assays to detect transgene mRNA, Surfactant Protein-C mRNA (ATII cells) or AGER-1 mRNA (ATI cells). **Results**. We hypothesised that delivery of a given vector dose in a larger volume can reduce the heterogeneity of transduction/transgene expression. Multisite instillation with small volumes of concentrated vector at two doses was compared with lavage-like delivery of a similarly high dose of vector in a larger volume. Multisite instillation of 5x 2e8 TU or 5x 1e9TU in small volumes (300ul per site) resulted in dose-dependent expression of EGFP but showed significant heterogeneity with approximately 50% of captured images showing no detectable EGFP, and efficiency ranging from 0 to 98% of alveolar epithelial cells in individual images. Delivery of the 5x 1e9TU dose in larger volumes (8ml per site) reduced the number of images without detectable EGFP (31% images and a range from 0 to 17% EGFP-positive cells per image). The overall average efficiency in the latter study was 4% EGFP+ alveolar epithelial cells. Increasing the dose further (5x 3e9TU) in the same volume (8ml per site) reduced the number of images with no GFP to 15% and increased the average expression to 8% of cells (Range 0-27%). The reduced heterogeneity from delivery in larger volume is supported by more consistent transgene mRNA levels in tissue samples across the lung. Expression of both mRNA and protein has been demonstrated in ATII cells. **Conclusion.** We have shown that multisite bronchoscopic instillation