Title: Gene therapy for surfactant protein B deficiency using recombinant AAV

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Surfactant Protein B (SP-B) deficiency is a rare, autosomal, recessive genetic disease of the lung that causes alveolar collapse and severe respiratory distress in neonates. The disease is invariably fatal during the first months of life. Surfactant replacement therapy in these full-term infants has been shown to be ineffective. The only treatment option is lung transplantation, which is rarely attempted as the mean age of death is 3 months. At an estimate, 1 in 1 million live births in North America and Europe are affected. To improve prognosis, we wish to develop gene therapy for SP-B deficiency using recombinant adeno-associated viral (rAAV) vectors.

Effective gene therapy for SP-B deficiency needs to target alveolar type II (ATII) cells in the lung parenchyma as they are the only cells that can produce mature SP-B. We selected AAV serotypes 5, 6.2 and 9 to compare transduction of ATII cells using eGFP as a reporter transgene. Mice (n=2-4) were dosed intranasally with 1E11 genome copies (GC). After 28 days, fixed-frozen sections of the treated lungs were analysed for cell transduction using confocal microscopy and automated imaging of whole lung sections. Immunostaining (with anti-pro-surfactant protein C antibody) was used to identify ATII cells. Recombinant AAV2/5 achieved the highest transduction rate of total murine lung cells (mean 3.06%, SD=0.96) which was significantly different from naive (Kruskal-Wallis test with Dunn's post-hoc test, P= 0.0234). Mean transduction rates with serotypes 9 and 6.2 were lower (0.32%, SD=0.16 and 0.34%, SD=0.20, respectively). The rAAV2/5 vector also achieved the highest mean transduction rate in the target ATII cells which was significantly different from naive (19.4%, SD=4.6, p=0.014 with Kruskal-Wallis test with Dunn's post-hoc test, P=0.0141) compared with rAAV2/6.2 and rAAV2/9 (4.0%, SD=1.8 and 4.5%, SD= 1.0 respectively). Furthermore, serotype 5 was the most specific for transduction of ATII cells with no other transduced cell types observed.

Serotypes were further investigated in preliminary studies using human precision cut lung slices (PCLS) of surgical lung resections. Fresh human lung tissue was cut in 500 µm sections and treated ex-vivo with 1E11 GC of rAAV2/5 expressing eGFP as reporter transgene. On day 12, PCLS (n=4) were processed in fixed-frozen sections and analysed for native eGFP fluorescence using confocal microscopy. Although serotype 5 showed the highest transduction rate in the murine lung, transduction was not observed in any of the 2-3 analysed sections per human lung slice. This stresses the importance of choosing appropriate model systems for the investigation of gene therapies. Further studies are ongoing to compare rAAV2/5 with other serotypes in human models of the lung.