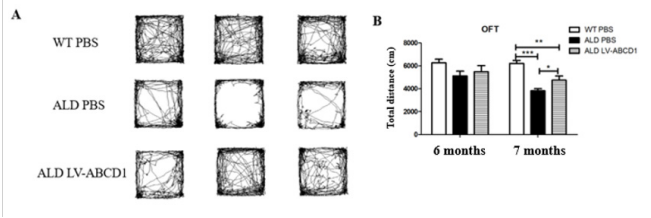
not as fully active as WT mice (6,217±577cm, P<0.01). The direct behavioral tests supported the successful establishment of an early ALD mouse model and demonstrated neurological improvement after LV-*ABCD1* injections. **Conclusion:** We have established a new X-ALD mouse model with early onset of disease phenotype. The intracerebral injection of LV-*ABCD1* demonstrated expression of *ABCD1* with partially corrected behavioral phenotype in the dl3/9*ABCD1* KO mice.



Cardiovascular and Pulmonary Diseases

523. Reversal of Cardiac Hypertrophy with an Optimized MYBPC3 Gene Therapy

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Cardiomyopathy is the number-one cause of sudden cardiac arrest in children under 18. Hypertrophic cardiomyopathy (HCM) affects 0.5 million Americans, potentially resulting in heart failure or sudden death. Loss-of-function mutations in *Myosin Binding Protein C3*, *MYBPC3*, are the most common genetic cause of HCM. The majority of *MYBPC3* mutations causative for HCM result in truncations, via nonsense, frameshift or splice-site mutations. The sarcomeric pathophysiology of the majority of HCM patients with *MYBPC3* mutations appears to be due to haploinsufficiency, as the total amount of MYBPC3 protein incorporated into sarcomeres falls significantly below normal. Decreased sarcomeric levels of MYBPC3 result in decreased myosin inhibition with more myosin heads engaged on the actin filament, resulting in hypercontractility. The clearest path to the treatment of haploinsufficiency is the restoration of the insufficient gene product; in this case wild-type MYBPC3. Thus, we have successfully engineered an AAV vector (TN-201) with superior properties for selective restoration of MYBPC3 to cardiomyocytes upon systemic delivery. Critically, we have demonstrated for the first time with AAV the ability of both a mouse surrogate and TN-201, which encodes the human gene, to reverse cardiac dysfunction and hypertrophy in a symptomatic murine model of disease. Dose-ranging efficacy studies exhibited restoration of wild-type MYBPC3 protein levels and saturation of cardiac improvement at the clinically relevant dose of 3E13 vg/kg. Further, pilot safety studies in adult and infant mice injected with >10X an efficacious dose exhibited no clinical observations, no alterations in cardiac function, and no histopathological findings. Importantly, we have determined that TN-201 produced utilizing the highly scalable Sf9 platform results in similarly potent efficacy as HEK293-produced material in a *Mybpc3*$^{-/-}$ model of disease. Finally, we have established that our observed efficacy is sufficiently meaningful for stable benefit up to one year post-injection, as well as reversal of cardiac dysfunction even in late-stage homozygote disease.

524. Gene Therapy for Autoimmune Pulmonary Alveolar Proteinosis

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Autoimmune pulmonary alveolar proteinosis (aPAP), is a rare lung disease, characterised by the accumulation of surfactant in the alveoli due to anti-granulocyte macrophage colony-stimulating factor (GM-CSF) auto-antibodies. The standard of care is whole lung lavage (WLL), an invasive procedure that only treats symptoms. Recombinant GM-CSF protein has been shown to outcompete the auto-GM-CSF antibodies and stimulates clearance of pulmonary surfactant by alveolar macrophages in some patients, but this treatment is expensive not widely available and a recent Phase 3 trial showed only modest benefit reached. We are, therefore, currently assessing whether gene therapy may be able to overcome some of the limitations of the current therapies. The UK Respiratory Gene Therapy Consortium has developed a lentiviral vector (rSIV.F/HN) designed to transduce a range of lung epithelial cells Lungs of PAP mice were transduced with rSIV/F/HN expressing GM-CSF (1e7-9.2e8 transduction units (TU)/mouse, n=4-13/group) by nasal sniffing leading to dose-related and persistent (11 months) expression of GM-CSF. In addition, we observed a dose-related reduction of disease biomarkers with doses as low as 1e6 TU/animal, which reduced SP-D levels (treated: 114.9, control 502.8 ng/mg, p<0.0001) and surfactant deposition in the lung (treated/control: PAS positive alveoli (%) 0.46/1.5 , p<0.05) (Figure 1 A-C). Biomarkers remained unchanged in mice treated with 1e5 TU, which is consistent with the lack of detectable GM-CSF expression at this dose. In mice treated with 1e7 TU/mouse all biomarkers were significantly reduced s (treated/control: BALF turbidity: 0.48/1.1 OD, p<0.001; SP-D: 20/502 ng/mg, p<0.0001; surfactant deposition: 0.1/1.5 %, p<0.05) (Figure 1 A-C). However, mice treated with doses over 1e6 TU/animal also developed dose-related side-effects over time, due to infiltration and accumulation of inflammatory cells. Our results demonstrate that a single dose of a lentivirus GM-CSF gene therapy persistently ameliorates markers of aPAP disease. The next step will be to repeat these studies in a model that expresses anti-GM-CSF autoantibodies to more closely mimic human disease.