Neonatal Airway Gene Therapy Delivery to Enable Effective Adult Dosing: A Lentiviral Vector Study

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Introduction

- Cystic fibrosis (CF) airway disease progression begins early in childhood and produces a steady deterioration in breathing and quality of life leading to early mortality.
- An effective gene therapy that inserts a properly functioning copy of the *CFTR* gene into the cells of the airway epithelium would potentially alleviate, and subsequently correct CF airway disease.
- We have previously demonstrated a gene therapy treatment for CF airway disease showing a proof of principle in nasal airways of a CF mouse model.
- Achieving consistent high and sustained levels of transgene expression is essential but remains challenging.

Aims

- To determine if delivery of a LV vector during neonatal development – when immune-privilege could establish vector components as 'self' – facilitates reliable and/or higher levels of transgene expression from subsequent adult LV vector dosing.

Methods

- The nasal airways of neonatal (3 day old) Sprague-Dawley rats were treated with 10 µl of VSV-G LV vector containing the FLuc-F2A-eGFP bicistronic cassette driven by the EF1α promoter, or a PBS sham control.
- Bioluminescence imaging (BLI) of the rats was performed 6 weeks post neonatal vector dosing to assess nasal transgene expression.
- One week following BLI assessment, the now-adult animals received an airway-conditioning treatment of 25 µl LPC followed 1 hr later by 50 µl of LV vector, delivered by bronchoscopy into the right main bronchus. BLI examination was repeated one week later to assess transgene expression.
- Blood serum samples were collected at both BLI time points for the control and treatment groups and compared to LV untreated animals for antibody responses to the Luc transgene.

Results

- Luminescence was detected 6 weeks post neonatal LV dose in the nasal airways of all animals in the treatment group, but not in controls (Fig 1).
- This Nasal luminescence persisted after LV dosing to the lungs as an adult of all neonatal LV-treated rats (Fig 1); lung luminescence was observed in greater number of animals in LPC neonatal dosing vs PBS (Fig 2). There was no immune response towards the transgene detected in the control or treatment groups when compared to animals not treated with the LV vector.

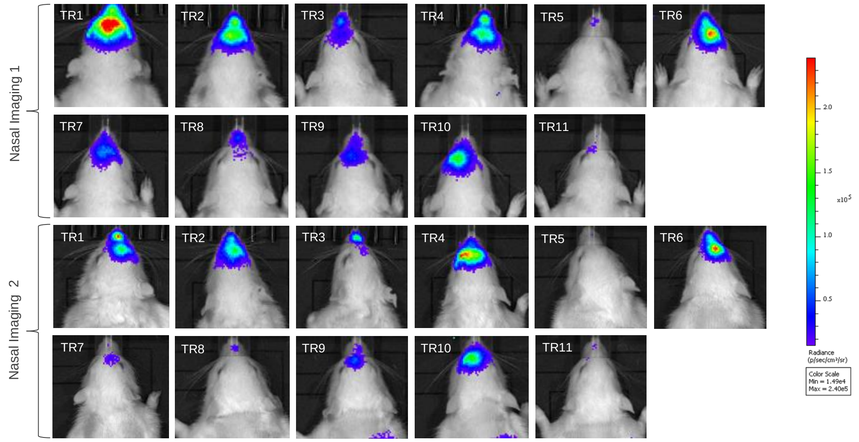

Figure 1: Luc gene expression (flux bioluminescence) in the nasal airways of rats following neonatal LV vector dosing. Luc expression was observed in all animals in the treatment group at the first imaging time point 6 weeks after dosing and not in the control group (not shown). Luc expression remained in all animals in the treatment group at the second imaging time point 8 weeks after dosing and 1 week after adult lung dosing.

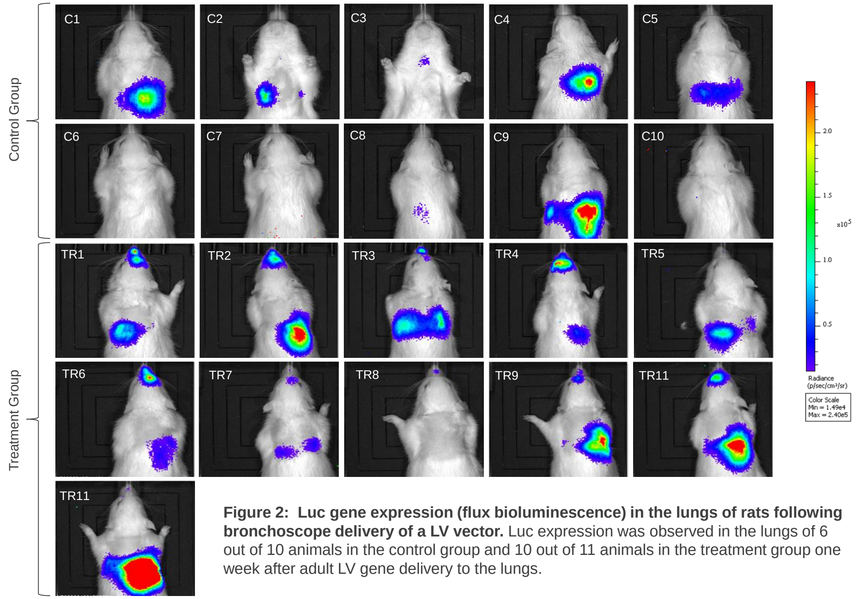

Figure 2: Luc gene expression (flux bioluminescence) in the lungs of rats following bronchoscope delivery of a LV vector. Luc expression was observed in the lungs of 6 out of 10 animals in the control group and 10 out of 11 animals in the treatment group one week after adult LV gene delivery to the lungs.

Statistics

- Statistical analysis assessed through a fitted linear mixed model to log (BLI) in R version 4.0.0 showed there was no statistical difference in the mean BLI between the control and treatment group after adult lung LV dosing (p=0.055) (Fig 3)

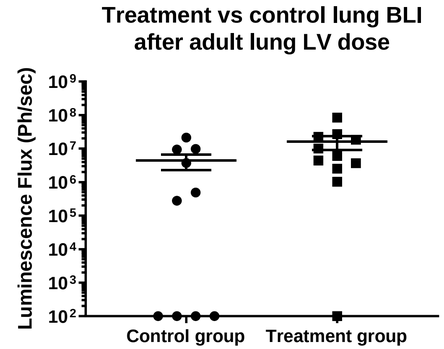

Figure 3: *In vivo* bioluminescent imaging of the lung region one week after adult LV vector lung dosing and 8 weeks after neonatal LV vector nasal dosing. There was no statistical difference between the control and treatment groups (p=0.055)

Conclusions

- Our results suggest that this form of neonatal dosing did not increase overall acute lung transgene expression levels, compared to the control group.
- The number of responders in the neonatal treatment group compared to the control group suggests that delivery of a LV vector at a neonatal age may result in a more reliable outcome of successful transgene expressing animals. Future studies with a larger sample size are required to determine this.

Acknowledgments

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