

One Year Persistence From A Single HIV-1 Lentiviral Vector Delivery

into the Marmoset Lung via a Single HIV-1 Lentiviral Vector Delivery

D Parsons^{1,3,4,5}, N Farrow^{1,3,4,5}, D Miller⁵, S Le Blanc¹, R B. Knight^{1,5}, P Cmielewski^{1,4} and D S Anson⁴

1. Respiratory and Sleep Medicine, Women's and Children's Hospital, CYWHS, Adelaide, South Australia;
2. Gene Technology Unit, SA Pathology, Women's and Children's Hospital, CYWHS, Adelaide, South Australia;
3. School of Paediatrics and Reproductive Health, University of Adelaide, South Australia;
4. Centre for Stem Cell Research, University of Adelaide, South Australia;
5. Women's and Children's Health Research Institute, Adelaide, South Australia.



Government of South Australia
SA Health



BACKGROUND:

an effective cystic fibrosis airway gene therapy, one able to produce long term transgene expression. In the lungs of the marmoset, a non-human primate, we have achieved short-term (7 day) LacZ expression. We have now asked whether the same dosing protocol could produce gene expression that would extend for at least a year, as has been produced in mouse airway by our group.

anaesthetized (Isoflurane), intubated and dosed with 350µl of lysophosphatidylcholine (LPC, 0.1%) delivered via a cannula inserted to extend from the ET tube in the distal third of the trachea. Following LPC delivery, a 500µl volume of the lentiviral vector was delivered. Lung, liver and spleen samples were examined for presence of a Gag fragment incorporated into the LacZ gene ('LacZ-Gag') via real time-PCR. Circulating antibodies to LacZ were analysed in sera via ELISA.

RESULTS: Positive controls derived from our earlier 7-day study where clear LacZ staining was present (Marmoset 1, Fig. 2) were used as a similar index of LacZ expression and gag gene presence (Fig. 3).

However after excision and processing of the lungs, the typical blue LacZ staining in the lungs were not observed, possibly due to excessive fixation following an unexpected delay in lung shipment. Compared to untreated lungs, LacZ staining was observed in both marmosets the lungs (10/18 and 11/18 slides respectively) primarily in the bronchioles. When trachea, liver and spleen samples were examined the LacZ-Gag gene was also present at levels similar to that present in Marmoset 1. Circulating antibodies to the LacZ gene was established in both marmosets by 7 days and continued up to 1 year, returning to baseline by 14 months (Fig. 5.)

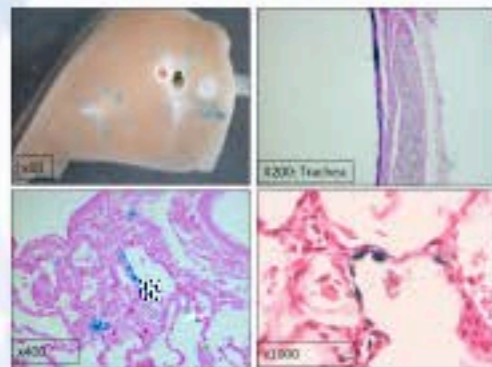


Fig. 2. LacZ staining (blue cells) in Marmoset lung and trachea from 7 day study (Marmoset 1).

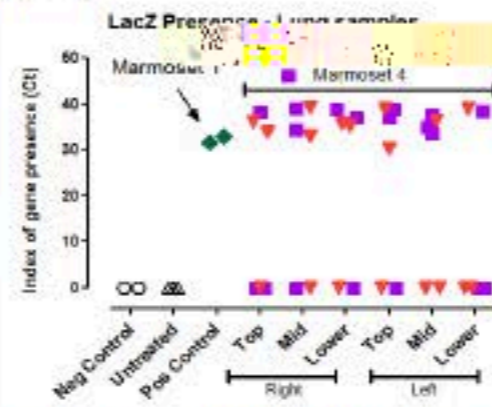


Fig. 3. Gag presence in PFA fixed Lung samples at 14 months

Fig. 4a. Gag presence in PFA fixed tracheal samples, n=3 replicates



Fig. 4c. Gag presence in frozen spleen samples= 2-3 replicates

Fig. 4b. Gag presence in frozen liver samples, n=3 replicates



Fig. 5. Circulating antibodies to LacZ

CONCLUSION: A single LV vector airway administration can introduce a transgene into a primate lung that persists for at least 14 months. The presence of vector genes in liver and spleen indicate that long term gene distribution occurs outside the lung. The presence of circulating antibodies to the transgene for 12 months may be due to long term transgene expression or an exposure to the transgene.

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