One Year Persistence From A Single HIV-1 Lentiviral Vector Delivery into Marmoset Lung: LacZ And Vector Gene Presence

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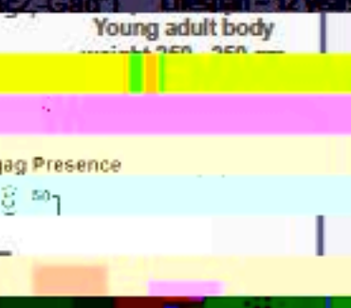
BACKGROUND: [illegible] an effective cystic fibrosis airway gene therapy, one able to produce long term transgene expression. In the lungs of the marmoset, a non-human primate, we have achieved short-term (7 [illegible] pseudotyped [illegible] asked whether the same dosing protocol could produce gene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

[illegible] anaesthetized (Isofluorane), intubated and dosed with 350µl of lysophosphatidylcho[illegible] (LPC, 0.1%) delivered via a cannula inserted to extend from the ET tube in[illegible] distal thir[illegible] LPC delivery, a 50[illegible] [illegible] expression via Xgal, LacZ genes via real time PCR in 18 samples take[illegible] spleen samples were examined for presence of a Gag fragment incorpora[illegible] 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) [illegible] showed a similar ind[illegible] and Gag gene presence. (Fig. 3.) However after excision and processing of the 14 month study, the typical blue LacZ cell staining in lungs were not observed, possibly due to excessive fixation following an unexpected delay in lung shipment. Compared to untreated lun[illegible] in both marmosets the lungs [illegible] presence (10/18 and 11/18 [illegible] es respectively) primarily [illegible] When trachea, liver and spleen samples were examined the LacZ-Gag gene was also present at levels similar to that present in Marmoset 1 [illegible] Circulating antibodies to the LacZ gene was established in both marmosets by [illegible] continued up to 1 year, returning to baseline by 14 months (Fig. 5.)

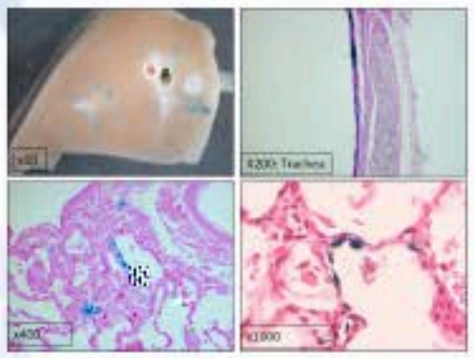


Fig. 2. LacZ staining (blue cells) in Marmoset lung and trachea from 7 day study (Marmoset 1).

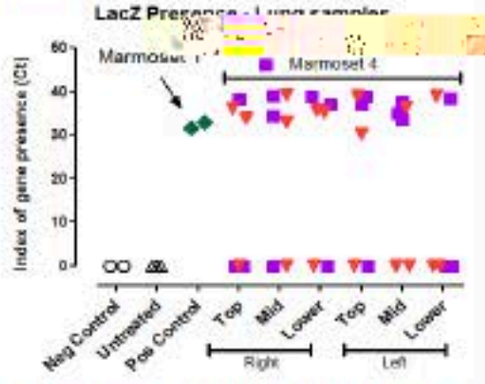


Fig. 3. Gag presence in PFA fixed Lung samples at 14 months

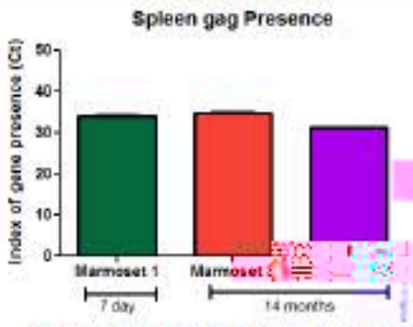


Fig. 4c. Gag presence in frozen spleen samples= 2-3 replicates

Fig. 4a. Gag presence in PFA fixed tracheal [illegible] replicates

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

Fig. 5. Circulating antibodies to L[illegible]

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 ex[illegible]

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