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

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BACKGROUND: We are developing techniques to create 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) airway gene transfer using a VSV-G pseudotyped HIV-1 based lentiviral (LV) LacZ vector. We 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.

METHODS: Two 1 year old marmosets (Marmoset 3 (Female) and Marmoset 4 (Male) Fig. 1.) were anaesthetized (Isofluorane), intubated and dosed with 350µl of lysophosphatidylcholine (LPC, 0.1%) delivered via a cannula inserted to extend from the ET tube into the distal third of the trachea. One hour after LPC delivery, a 500µl or 550µl bolus (respectively) of LV-LacZ vector was similarly introduced. Animals received normal care over the following 14 months with regular blood, waste and secretion samples taken. After humane killing (Nembutal) lungs and other tissues were removed for assessment: lung LacZ expression via Xgal; LacZ genes via real time-PCR in 18 samples taken from each lung. Trachea, Liver and spleen samples were examined for presence of a Gag fragment incorporated in the LacZ gene ("LacZ-Gag") via real time-PCR. Circulating antibodies to LacZ were analysed in sera via ELISA.

Fig. 1. Marmosets. Lifespan ~ 12 years; Young adult body weight 250 - 350 gm

RESULTS: Positive controls derived from our earlier 7-day study where clear LacZ staining was present (Marmoset 1, Fig. 2.) showed a similar index (Ct) of LacZ 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 lung tissue, in both marmosets the lungs displayed LacZ gene presence (10/18 and 11/18 samples respectively) primarily from upper lung regions (Fig. 3).

When trachea, liver and spleen samples were examined the LacZ-Gag gene was also present at levels similar to that present in Marmoset 1 (Fig. 4a-c).

Circulating antibodies to the LacZ gene was established in both marmosets by week 2, which continued up to 1 year, returning to baseline by 14 months (Fig. 5.)

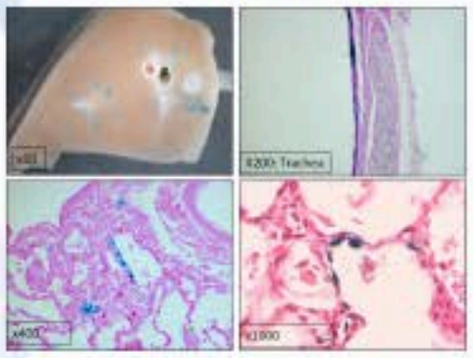

Fig. 2. LacZ staining (blue cells) in Marmoset lung and trachea from 7 day study (Marmoset 1).

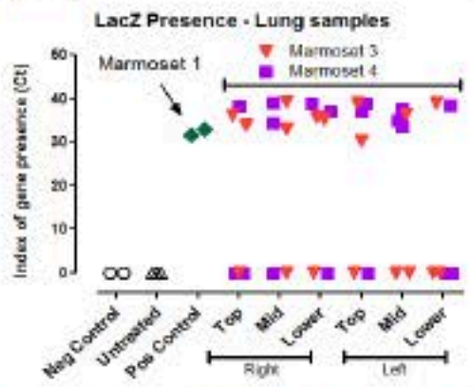

Fig. 3. Gag presence in PFA fixed Lung samples at 14 months

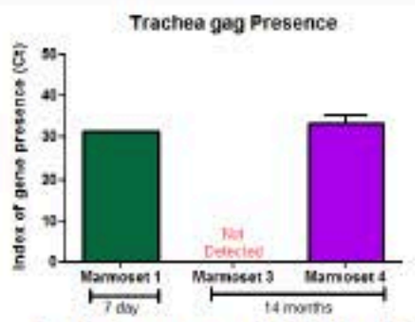

Fig. 4a. Gag presence in PFA fixed tracheal samples, n=2 replicates

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

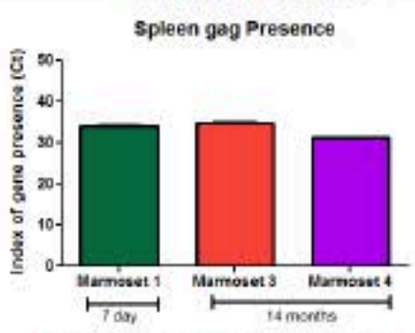

Fig. 4c. Gag presence in frozen spleen samples= 2-3 replicates

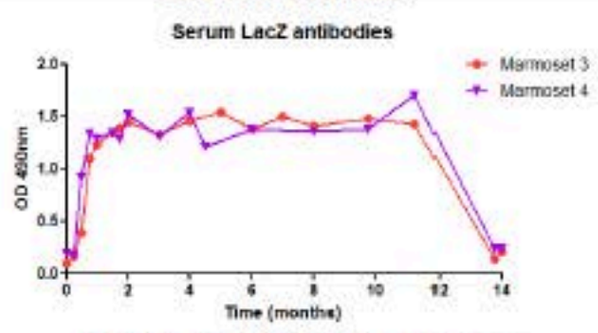

Fig. 5. Circulating antibodies to LacZ in sera

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 extended immune response to a brief transgene expression.

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