AIRWAY LENTIVIRAL GENE TRANSFER IN MARMOSETS

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BACKGROUND: Preclinical studies in non-human primates (NHP) are important for developing clinically-appropriate gene transfer protocols to treat CF airway disease. We report here initial findings from the first studies of LV lung gene transfer performed in the non-human primate the marmoset *Callithrix jacchus* (Fig 1). Marmosets typically have a lifespan of ~12 years, a body weight of ~250-350g and are an increasingly used animal model for gene manipulation studies.

(1) *Callithrix jacchus*

MATERIALS AND METHODS: LPC pre-treatment (0.1%, 200-350 µl) was followed 1 hour later by LV-LacZ vector pseudotyped with a VSV-G envelope (~ 1.2 x10^9 TU/ml, 350-500µl), each delivered via an ET tube into the mid-trachea of four anaesthetised animals (2 M, 2F). After 7 days two animals were humanely killed and trachea, lungs and other organs in two animals were examined for LacZ reporter gene expression via standard X-gal staining protocols. Regular blood samples, secretions and tissue samples were collected to examination the presence of vector particles. It is planned that the remaining two animals will be examined by the end of 2010.

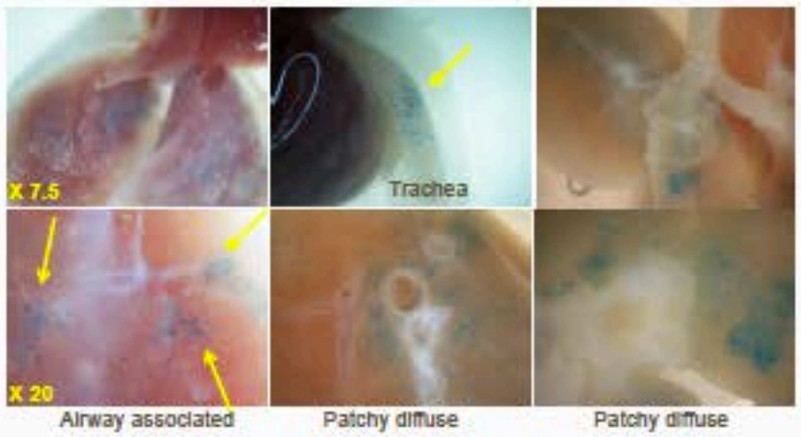
RESULTS: A rapid but transient O_2 desaturation was present in some animals after LPC administration, however all behavioural and physiological indices were normal post-procedure.

Body weights followed usual post-anaesthesia trajectories. Patchy epithelial cell LacZ gene expression was evident *en face* (Fig 2) and in cross-sections (Fig 3, 4), primarily in conducting airways.

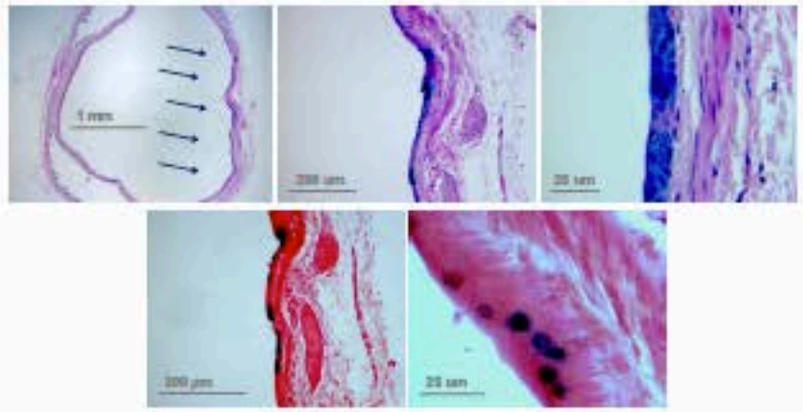
No evidence of LacZ gene expression was detected in any other organ tissue.

LV vector capsid protein levels (p24) were present in serum at Day 1 but absent from Day 2 onwards (Fig 5).

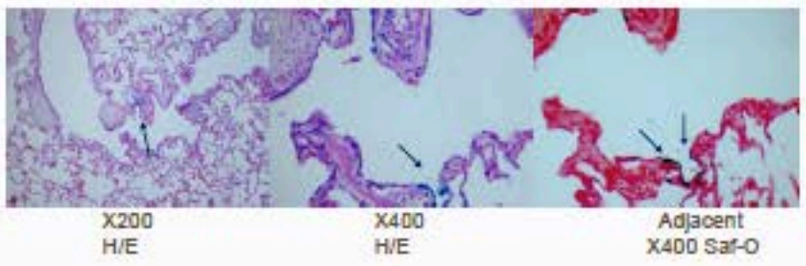
Histological, immunological and RT-PCR analyses await completion for the 2 remaining animals (Fig 6).



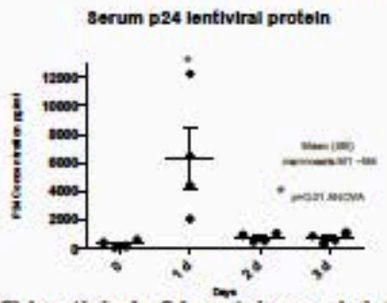
(2) *En face* examples of lung LacZ gene expression.



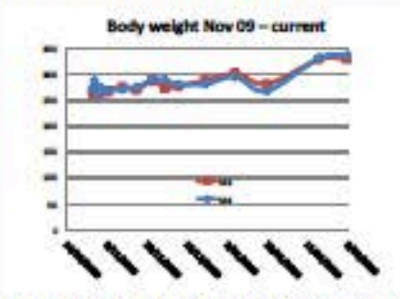
(3) Lung LacZ gene expression in cross sections. H/E and Saf-O staining. Ciliated, non-ciliated and basal cells are transduced.



(4) LacZ transduction in distal airways.



(5) Lentiviral p24 protein was briefly present in serum.



(6) Body weights of long term animals are following normal trajectory.

CONCLUSION: These initial studies suggest LPC/LV dosing procedures are well-tolerated and can produce transgene expression in this non-human primate lung. Two additional animals are being maintained for longer-term assessment of single-dose lung gene transfer. Gene vector components can reach the vascular space after airway dosing, indicating attention to host immunity and vector distribution is warranted. The marmoset appears a suitable animal model for testing airway gene transfer procedures prior to consideration of human clinical trials.

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