Airway Lentiviral Gene Transfer In Marmosets

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Introduction

Preclinical studies in non-human primates (NHP) are important in developing clinically-appropriate gene transfer protocols for treatment of 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* (Figure 1), an animal model increasing in use in gene manipulation studies.

Methods ?? c4CF logo above, CSCR also

LPC pretreatrment (0.1%, 200-350 ul) was followed 1 hour later by LV-LacZ vector pseudotyped with VSV-G (~ 1.2 x10^9 TU/ml, 350-500ul), each delivered via an ET tube into the mid-trachea of four anaesthetised animals (2 M, 2F). After 7 days 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 for examination of the presence of vector particles.

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* (Figure 2) and in cross-sections (Figure 3), primarily in conducting airways. No evidence of LacZ gene expression was detected in any other tissue. LV vector capsid protein levels (p24) were present in serum at Day 1 but absent from Day 2 onwards (Figure 4). The LV gag structural gene was present in trachea and in some tissues of one animal (Figure 6). Further histological, immunological and RT-PCR analyses await completion of the remaining two animals.

Callithrix jaccus

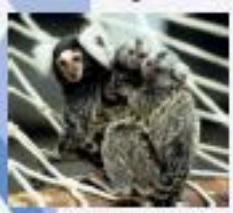

Fig 1: Marmosets. Lifespan ~ 12 years; Body wt's 250 - 350 gm

En face LacZ gene transfer

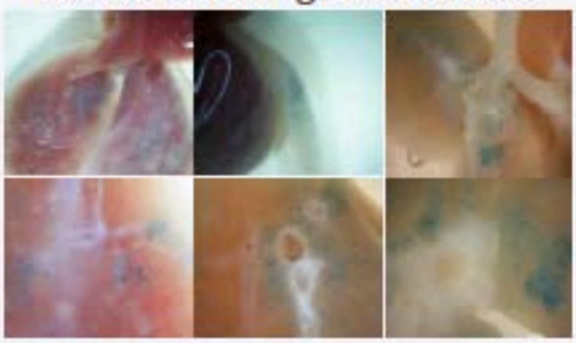

Fig2: En face examples of lung lacZ gene expression.

Xs LacZ gene transfer

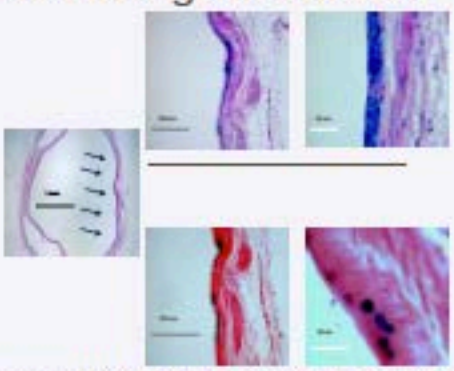

Fig3: Lung lacZ gene expression in cross sections. H/E and Saf-O staining. Ciliated, non-ciliated and basal cells are transduced.

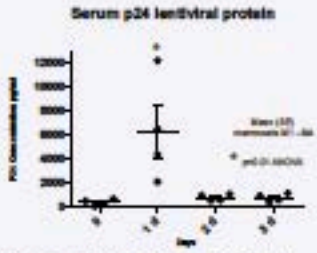

Fig4: Lentivirus P24 protein was briefly present in serum.

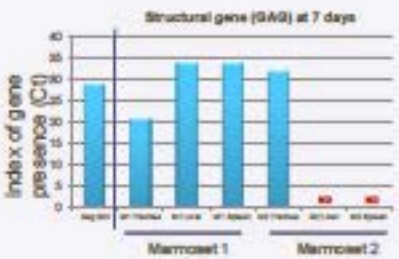

Fig5: Vector GAG structural gene was present in the trachea of both animals, but in liver and spleen of only one animal.

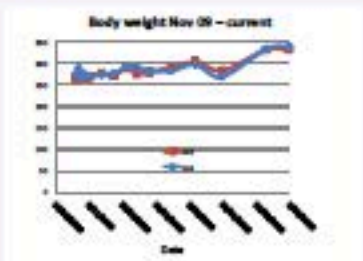

Fig6: Body weights of long term animals are following normal trajectory.

Conclusion

These initial studies suggest LPC/LV dosing procedures are well-tolerated and produces 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, suggesting attention to host immunity and vector safety issues is warranted. The marmoset appears a suitable animal model for testing airway gene transfer procedures prior to consideration of human clinical

Acknowledgements

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