GENE THERAPY FOR CF IS LONG TERM EXPRESSION A CONSEQUENCE OF TRANSDUCING CONDUCTING AIRWAY ENDOGENOUS RESPIRATORY STEM CELLS

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Introduction:

Gene therapy studies utilising a HIV-1 VSV-G pseudotyped lentiviral vector have shown long term marker and therapeutic gene expression for 24 months and 12 months respectively [1,2]. We hypothesise that long term transgene [illegible] vector LacZ reporter gene [illegible] the nasal and tracheal airways.

Methods:

The [illegible]ys of four groups [illegible] HIV-1 VSV-G pseudotyped lentiviral [illegible] gene. Two groups were used as short and long term co[illegible] groups were treated with Polidocanol to transiently ab[illegible] epithelium forcing regrowth from basal stem [illegible]. This procedure was then repeated in the trachea of four additional groups of mice in the same manner (n=11).

At the endpoint of the study all animals were humanely killed and processed to reveal the pattern of LacZ expression in the nose or trachea.

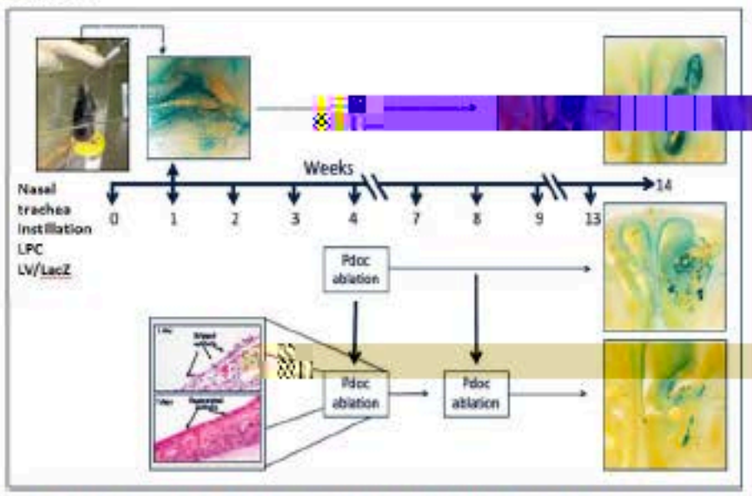

Figure 1: Schematic representation of the nitrogen mustard (Pdoc) transient injury model for detection of long-lived LacZ [illegible] transduced nasal epithelial stem/progenitor cells. Images of *en face* tissue nasal [illegible] displaying LacZ positive staining were captured by serial [illegible]. Images were then combined using Z-stacking to generate a single in-focus image of the section.

Results:

All animals in the study displayed [illegible] patterns of LacZ expressing cells observed in the ablation ablation groups (Fig 2 and 3). In the nasal and trachea[illegible] airways the pattern of LacZ expression was observed as two distinctly different clonal cluster types; spotted and linear (Fig 2 c -f).

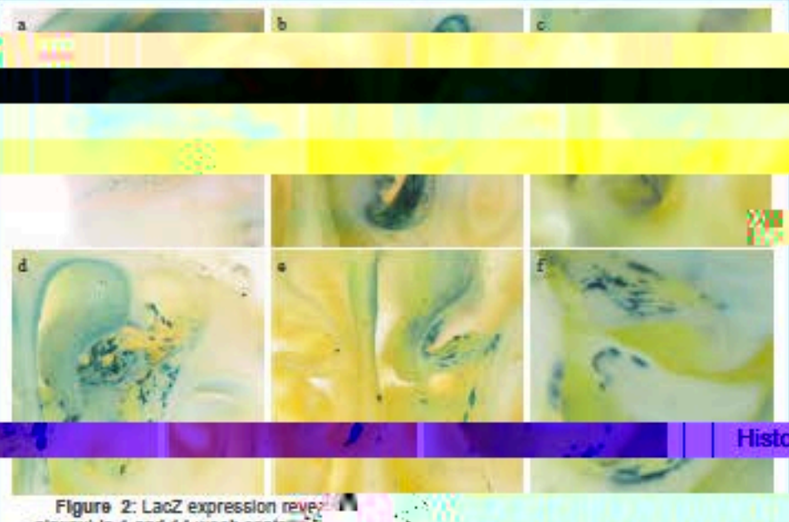

Figure 2: LacZ expression revea[illegible] airway) in 1 and 14 week contr[illegible] injury [illegible] spotted clusters and linear clusters (c-f).

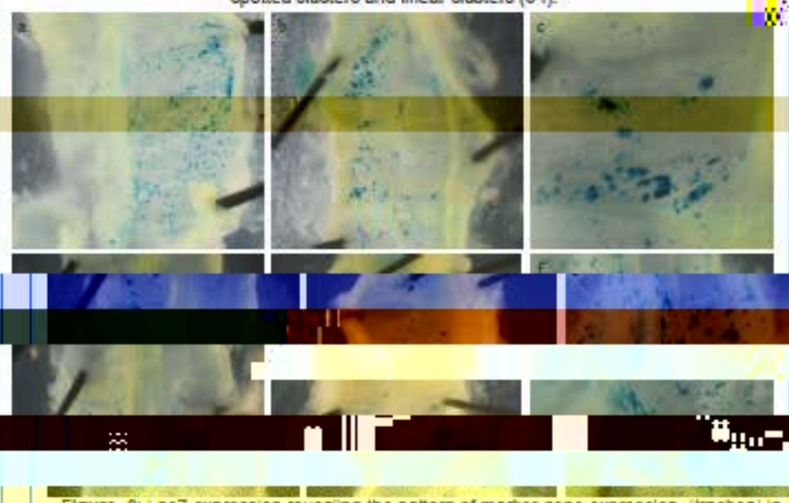

Figure 3: LacZ expression revealing the pattern of marker gene expression (trachea) in 1 and 14 week controls (a-b), and the [illegible]nal clusters in t[illegible] upon epithelial regeneration (c,[illegible] [illegible]rowth was ob[illegible] clusters and linear clusters (c-f).

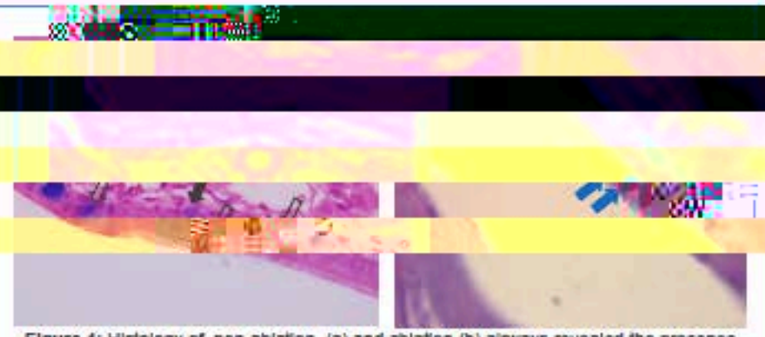

Figure 4: Histology of non-ablation (a) and ablation (b) airways revealed the presence of a maker gene expressing cell type (goblet) (blue arrows) in the regenerated airway (b) not observed in the unablated airway . Images 100x

Histological (Ab/Pas) analysis of LacZ expressing cell types revealed the presence of transdu[illegible]d ciliated and basal cell types in the no-[illegible]

Summary:

The results showed a pattern of LacZ expression consiste[illegible]nt with **clonal regrowth from transduced ba[illegible]**

These findings are consistent with the notion that transduced airway basal stem cells pass the transgene on to their progeny upon differentiation, resulting in sustained transgene expression for the life of the animal.

Acknowledgements :

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2. Limberis, M., et al., *Recovery of airway [illegible]stic fibrosis transmembrane conductance regulator function in mice with cy[illegible]rosis after single-dose lentivirus-mediated gene*

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