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 expression is a consequence of transducing airway basal stem cells. To test this hypothesis we used a forced injury model following lysophosphatidylcholine pre-treatment and HIV-1 VSV-G pseudotyped lentiviral vector LacZ reporter gene instillation in the nasal and tracheal airways.

Methods:

The nasal airways of four groups of mice (n=11) were treated with a HIV-1 VSV-G pseudotyped lentiviral vector carrying the LacZ reporter gene. Two groups were used as short and long term controls and two groups were treated with Polidocanol to transiently ablate the airway epithelium forcing regrowth from basal stem cells (Fig 1).

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.

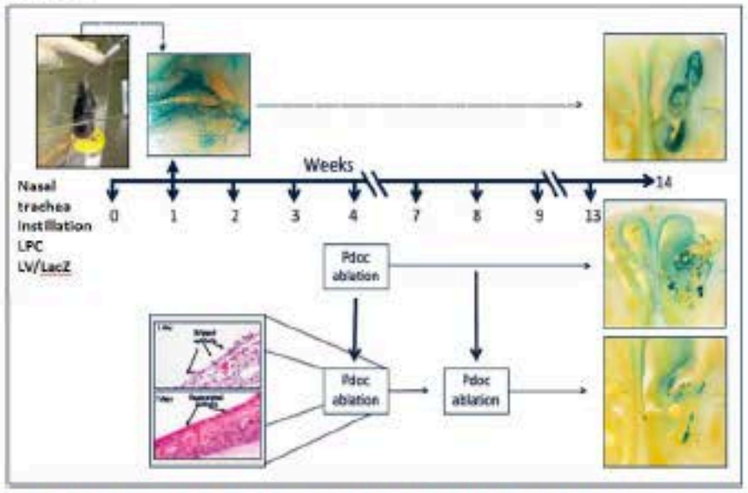

Figure 1: Schematic representation of the polidocanol (Pdoc) transient injury model for detection of long-lived LacZ-transduced nasal epithelial stem/progenitor cells. Images of *en face* mouse nasal airways displaying LacZ positive staining were captured by serial focusing. Images were then combined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

Results:

All animals in the study displayed LacZ expression with differing patterns of LacZ expressing cells observed in the ablation and no ablation groups (Fig 2 and 3). In the nasal and tracheal airways the pattern of LacZ expression was observed as two distinctly different clonal cluster types; spotted and linear (Fig 2 c -f).

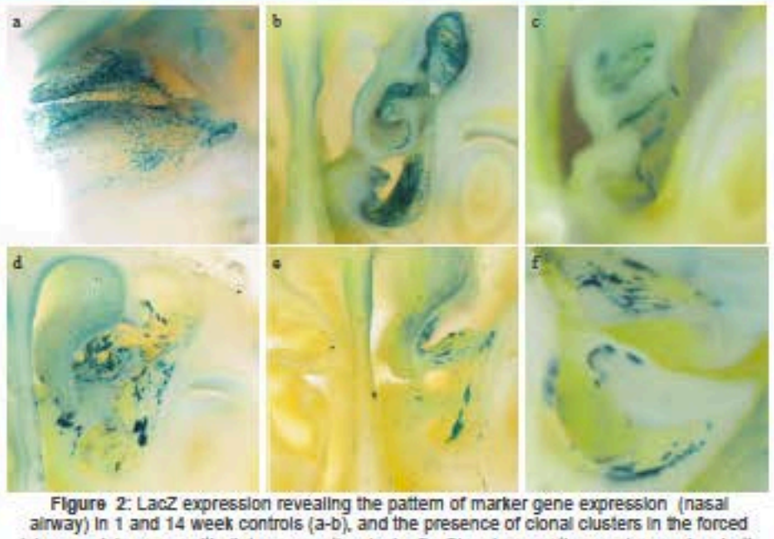

Figure 2: LacZ expression revealing the pattern of marker gene expression (nasal airway) in 1 and 14 week controls (a-b), and the presence of clonal clusters in the forced injury models upon epithelial regeneration (c,d,e,f) . Clonal regrowth was observed as both spotted clusters and linear clusters (c-f).

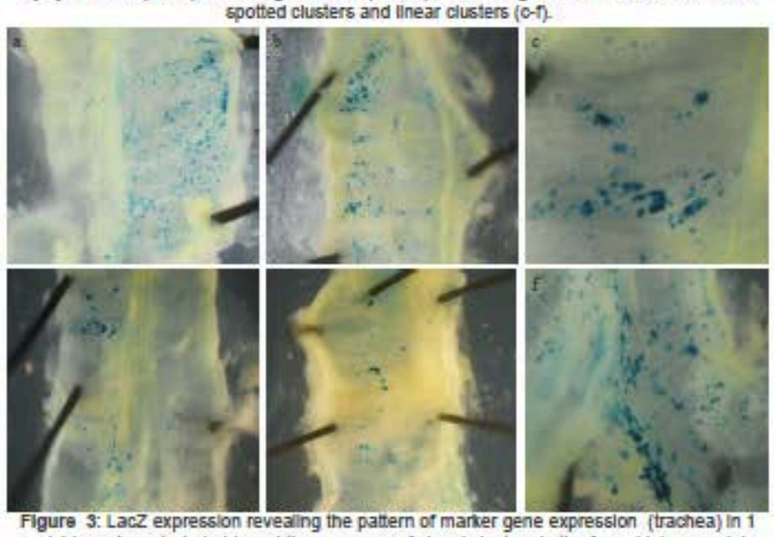

Figure 3: LacZ expression revealing the pattern of marker gene expression (trachea) in 1 and 14 week controls (a-b), and the presence of clonal clusters in the forced injury models upon epithelial regeneration (c,d,e,f) . Clonal regrowth was observed as both spotted clusters and linear clusters (c-f).

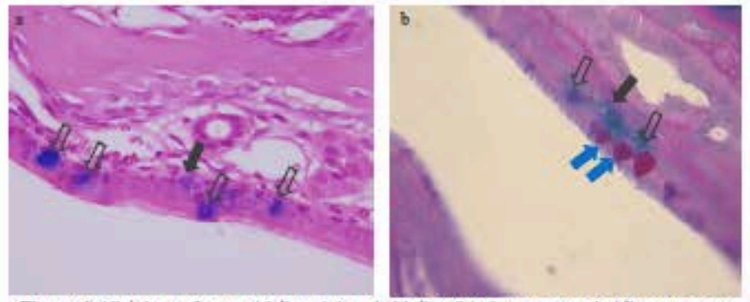

Figure 4: Histology of non-ablation (a) and ablation (b) airways revealed the presence of a maker gene expressing cell type (goblet) (blue arrows) in the regenerated airway (b) not observed in the unablated airway . Images 100x

Histological (Ab/Pas) analysis of LacZ expressing cell types revealed the presence of transduced ciliated and basal cell types in the no-ablation groups and the same cell types in the ablation groups with the addition of LacZ expressing goblet cells (Fig 4) suggesting outgrowth from transduced basal stem cells.

Summary:

The results showed a pattern of LacZ expression consistent with clonal regrowth from transduced basal stem cells.

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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References:

1. Stocker, A.G., et al., *Single-dose lentiviral gene transfer for lifetime airway gene expression.* J Gene Med, 2009. **11**(10): p. 861-7.
2. Limberis, M., et al., *Recovery of airway cystic fibrosis transmembrane conductance regulator function in mice with cystic fibrosis after single-dose lentivirus-mediated gene transfer.* Hum Gene Ther, 2002. **13**(16): p. 1961-70.