IMPROVED SURVIVAL IS BY AIRWAY LENTIVIRAL CFTR GENE TRANSFER IN A CYSTIC FIBROSIS MOUSE MODEL

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Introduction

We have shown that long term (> 12 month) partial CFTR correction can be achieved in timed-group studies in CF mice treated with a lentiviral (LV) vector.

In this gene transfer study a repeated-measures study design was used to extend our ability to examine the effects of CFTR gene correction in individual CF mice.

Methods

Male and female CF mice ($cftr^{tm1unc}$) were tested under 3 experimental conditions. Control animals were pre-treated with the airway surfactant lysophosphatidylcholine (LPC, 0.3%, 4 µl) prior to a lentiviral (20 µl) vector that contained no gene (LV-MT, n=6), or with a saline (PBS, 4 µl) pre-treatment prior to the therapeutic gene (LV-CFTR, n=6). The primary treatment group received LPC prior to LV-CFTR (n=12). Viral titre was 0.6-2.5 $x10^{10}$ TU/ml. CFTR function was assessed by nasal potential difference (PD) measurement at 1 week, 1 and 3 months, and then at 3 monthly intervals. Mouse group survival was expressed as Kaplan-Meier plots, with survival differences determined by Mantel–Cox log rank test. Outcomes were also compared to survival data from Luciferase (Luc) reporter gene studies in 3 groups of normal C57 mice treated similarly but with the Luc gene instead of the CFTR gene.

Results

- Significant and persistent functional CFTR gene transfer ($p<0.05$, ANOVA) was present in the nasal airway for up to 12 months (ΔPD_{Cl-} range 12-54% towards normal) in CF mice treated with LPC/LV-CFTR (Fig.1).
- In the two control groups the mean DPD after PBS pre-treatment or LV-MT treatment was no different to that in untreated CF mice (n.s. RM ANOVA).
- The functional nasal CFTR gene expression produced by LPC/LV-CFTR was also significantly and strongly correlated to the mean nasal Luc reporter-gene expression in this treated cohort (Fig. 2, $p<0.01$, $r^2= -0.92$).

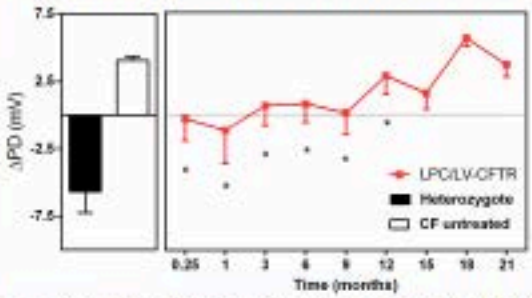

Fig. 1. Partial CFTR correction over time (*p<0.05, RM ANOVA, n=3-12).

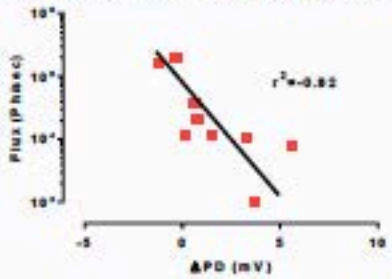

Fig. 2 Correlation of functional correction (ΔPD) response and reporter gene expression (Flux). (p<0.01, Spearman Correlation $r^2= -0.92$).

- LPC/LV-CFTR significantly extended median survival (20.1 mo), compared to either PBS/LV-CFTR (14.4 mo) and LPC/LV-MT (8.8 mo) control groups (Fig. 3).
- Survival in normal C57Bl6 mice similarly treated with the reporter gene Luc was no different between all groups with a median survival greater than 23 months and historical untreated C57 mice (Fig. 4).

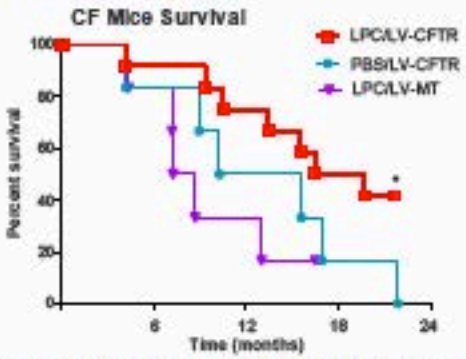

Fig. 3. Survival Curves of CF Mice (*p<0.05 Mantel-Cox log rank, n=6-12)

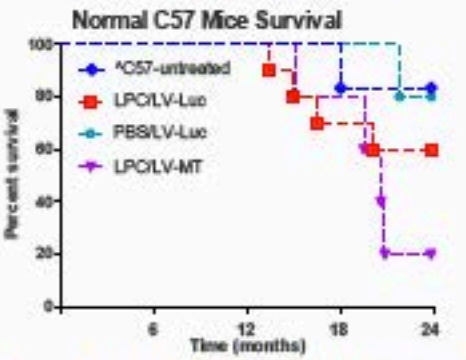

Fig. 4. Survival Curves C57 Mice , n=5-10. (^C57 Historical data).

Conclusion

These results suggest LV CFTR gene transfer targeted to the anterior CF mouse nasal airway can significantly improve lifetimes of treated animals. Some nasal dose could reach lung airways and gut via "spillover" of gene vector to potentially improve CFTR function there, with direct or indirect benefits. Further studies are essential to determine the reasons for the substantial improvement in animal survival following such limited airway gene transfer.

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www.Cure4CF.org

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