NON-VIRAL GENE EXPRESSION IN THE LUNG WITH THE MINI-CFTR PROMOTER

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Introduction

- Clinical studies are underway for the aerosol delivery of Genzyme Lipid GL67A complexed with plasmid DNA (pDNA) to the lungs of patients with cystic fibrosis (CF).
- The clinical plasmid utilises the synthetic CpG-free hCEFI (human Cytomegalovirus enhancer/elongation factor 1α) promoter to express the CFTR protein[1,2].
- This plasmid and a similar luciferase (Lux) expressing version generated persistent high levels of gene expression (> 8 weeks) following aerosol delivery of GL67A/pDNA to the mouse lung[2].
- However, we are also investigating promoters that have the potential to give tissue-specific CFTR gene expression.
- The **mini-CFTR promoter** encompasses the minimum sequence from the 5' region upstream of the CFTR gene able to provide promoter activity and confer cell-type specific expression *in vitro*[3].
- Here we investigate gene expression and CFTR function from plasmids with the mini-CFTR promoter and test the persistence of expression following delivery into mice.

Construction of mini-CFTR plasmids

- The mini-CFTR promoter was cloned into our 4th generation CpG-free plasmid backbone to create[2]:
- **Native** (promoter only) + Luciferase or CFTR (fig. 1)
- **Enhanced** (promoter + human CMV enhancer) + Luciferase or CFTR

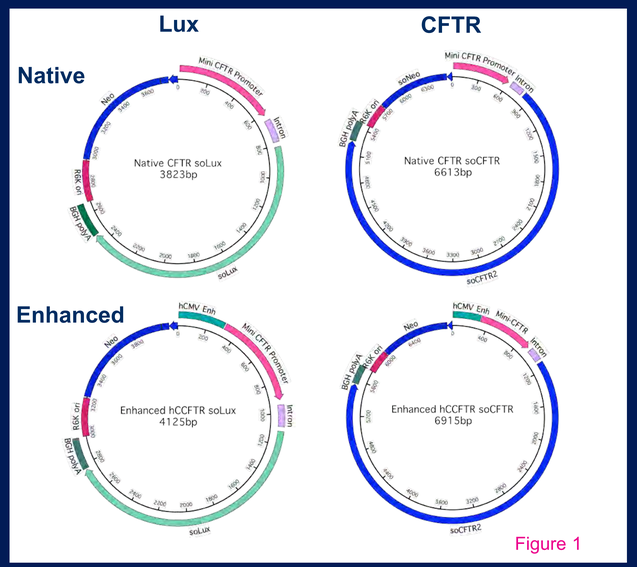

Figure 1

Gene expression in HEK293T cells

- pDNA (3µg) complexed with Jet PEI (6µg, 200µl total volume)
- Transfected HEK293T cells
- **Lux plasmids:** Harvested at 24 hours and assayed for lux
- Both express significant levels of luciferase after transfection
- Enhanced plasmid levels are - 2 fold > the Native plasmid (fig. 2)
  - 12% hCEFI promoter levels
- **CFTR plasmids:** Harvested at 48 hours, mRNA levels assayed by TaqMan
- Both CFTR plasmids express mRNA (fig. 3)
- The Native has higher levels (though not significantly different) than the Enhanced

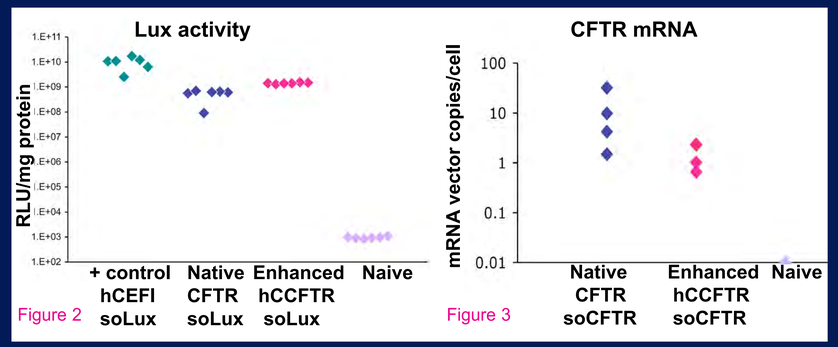

Figure 2

Figure 3

- **CFTR protein activity was confirmed by Iodide efflux**
- Both CFTR plasmids generate CFTR-dependent iodide efflux in HEK293 cells
- Native & Enhanced plasmid-dependent efflux is 11% & 20% hCEFI levels (fig. 4)

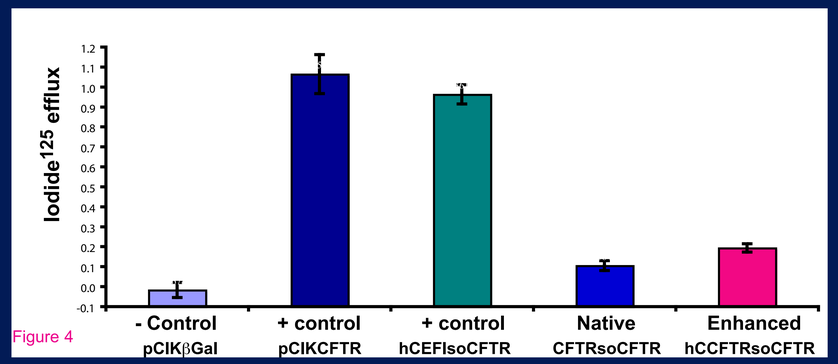

Figure 4

Lux activity in Mouse lungs

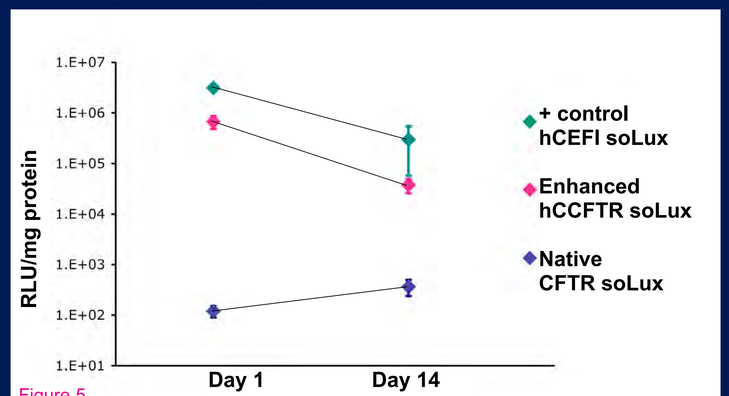

Figure 5

- Plasmid DNA (80µg/100µl) complexed with GL67A
- BALB/c mice were dosed by lung instilation
- Post-dosing lux activity was measured from lung lysates at d1 & d14
- The Enhanced plasmid gives 21% & 12% hCEFI levels on d1 & d14
- The Native plasmid gave low expression levels (fig. 5)

Conclusions

- **Incorporation of the human CMV enhancer boosts expression from the mini-CFTR promoter**
- **The Enhanced plasmid is capable of expressing at 10-20% reported hCEFI promoter levels in the lung (lux) and HEK293T cells (Iodide efflux)**

Future work

- These plasmids will be assesssed in human Air-Liquid Interface cells (MucilAir) to compare levels of expression in differentiated human respiratory epithelia.
- We shall investigate the potential for tissue specific expression from these plasmids.

References

1. Utilizing CpG-free plasmid technology developed by Cayla-InvivoGen (Toulouse, France)
2. Hyde *et al.* (2008) Nature Biotechnology, 26, 549-551
3. Chou, *et al.* (1991) Journal of Biological Chemistry, Vol 266, 24471-2447

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