Introduction
Cationic lipid-mediated gene transfer has been shown to be a potential approach to treat lung diseases such as Cystic Fibrosis (CF). However, pre-clinical and clinical studies have reported vector associated inflammatory responses [1]. This response is caused partly by the recognition of unmethylated CpG motifs contained within the plasmid DNA [2].

The inflammatory response is characterised by increased production of pro-inflammatory cytokines.

Nuclear transcription factor – κB (NF-κB) is an important transcription factor that regulates this response.

Recently the inhibition of NF-κB activation using synthetic double stranded and single stranded oligodeoxynucleotides (ODNs) had reduced pro-inflammatory cytokines successfully in conjunction with a lipid vector delivered systemically to the endothelium of the murine lung [3].

Figure 1. NF-κB signal transduction pathway

NF-κB is held inactive in the cytoplasm by the binding of an inhibitor molecule, IκB. TLR9 recognises unmethylated CpG motifs in DNA and initiates a signalling cascade, leading to the phosphorylation of the Iκκ molecule, Iκκ NF-κκ to the promoter region of target genes.

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Figure 2. Sequence of NF-κB related decoy ODNs

S and S11 are single-stranded 20-mer ODNs with a phosphothioate backbone. The underlined region in S+ indicates the consensus binding sites to the p50 subunit of NF-κκ B. ODN S11 contains a scrambled sequence with no NF-κκ B p50 binding potential and was used as a control.

Aim
To investigate the effect of NF-κB decoy ODNs on the inflammatory response following topical delivery of Genzyme lipid 67 (GL67)pDNA complexes to the airways of the mouse lung.

Figure 3. Experimental procedures

Mice were dosed with pCIKLux and ODN S+ or S11 as described in Fig 4. TNF-α and IFN-γ levels were determined in lung lysates 24hrs post-dosing using mouse cytokine ELISA kits. With topical delivery of the S+ or S11 Gl67/pDNA complexes there were 60% and 70% reductions in TNF-α (Figure 5A, ANOVA with Fishers PLSD, p<0.0001) and IFN-γ (Figure 5B, p<0.0004), respectively, compared to dosing with pCIKLux/GL67 alone.

Results
Figure 4. Co-delivery of NF-κB ODNs with GL67/pCIKLux does not affect transgene expression

The addition of synthetic ODNs did not compromise the high levels of gene expression from GL67/pCIKLux.

Figure 5. NF-κB decoy ODNs reduce TNF-α and IFN-γ levels produced by GL67/pCIKLux

The reduction in cytokine levels may allow increased duration of transgene expression.

Conclusions
1. The addition of the NF-κB ODNs did not compromise high levels of gene expression from GL67/pCIKLux.
2. Co-delivery of NF-κB decoy ODNs with GL67/pDNA complexes resulted in reduced pro-inflammatory cytokine levels.
3. Delivery of NF-κB ODNs with GL67/pDNA may reduce CpG related inflammation in the clinic.
4. Further studies should confirm whether this reduction in pro-inflammatory cytokine levels is specific to the NF-κB ODNs.
5. The reduction in cytokine levels may allow increased duration of transgene expression.

References
2. Yew et al. 2000, Molecular Therapy, 1, 255-262.