DEVELOPMENT OF ZERO CpG PLASMIDS FOR NONVIRAL LUNG GENE THERAPY

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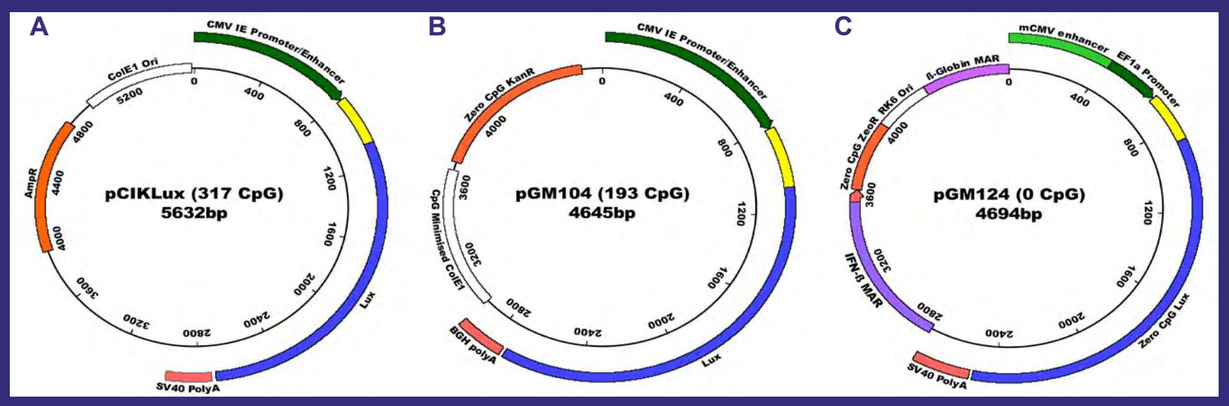
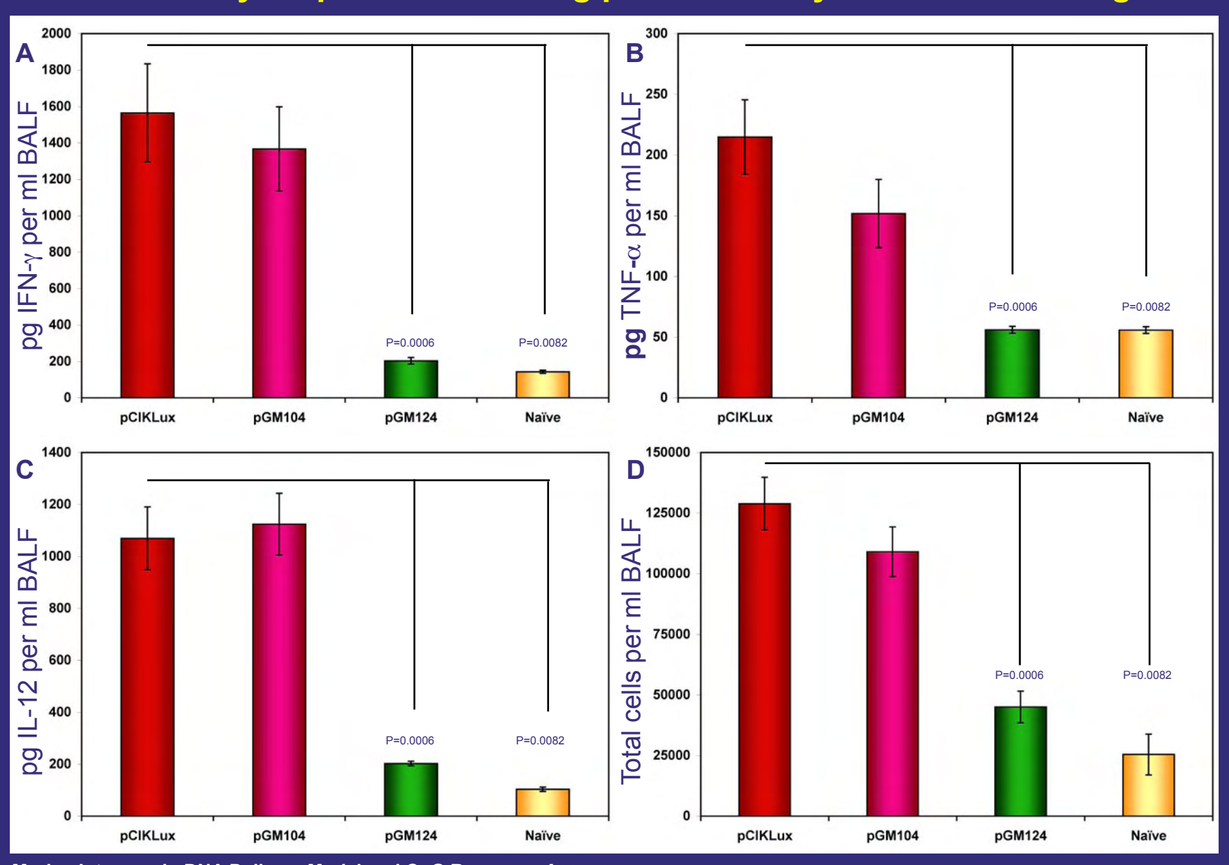
Overview of project.

- Nonviral gene therapy is being developed as a treatment for Cystic Fibrosis (CF).
- CpG motifs in plasmid DNA can cause an inflammatory response *in vivo*.
- The CpG response will limit the effectiveness of nonviral gene therapy:-
  1. It will limit the level and duration of expression.
  2. It may be harmful to patients.
- We are investigating the effects of CpG depletion in mouse models of lung gene therapy.
- Plasmids have been constructed with varying numbers of CpGs.
- The inflammatory response from these plamids has been tested *in vivo*.
- The reporter gene expression from these vectors has been tested *in vivo*.
- Clinically relevant zero CpG plasmids are being developed.

Plasmid DNA vectors used in this study.



Inflammatory responses following pDNA delivery to the mouse lung.



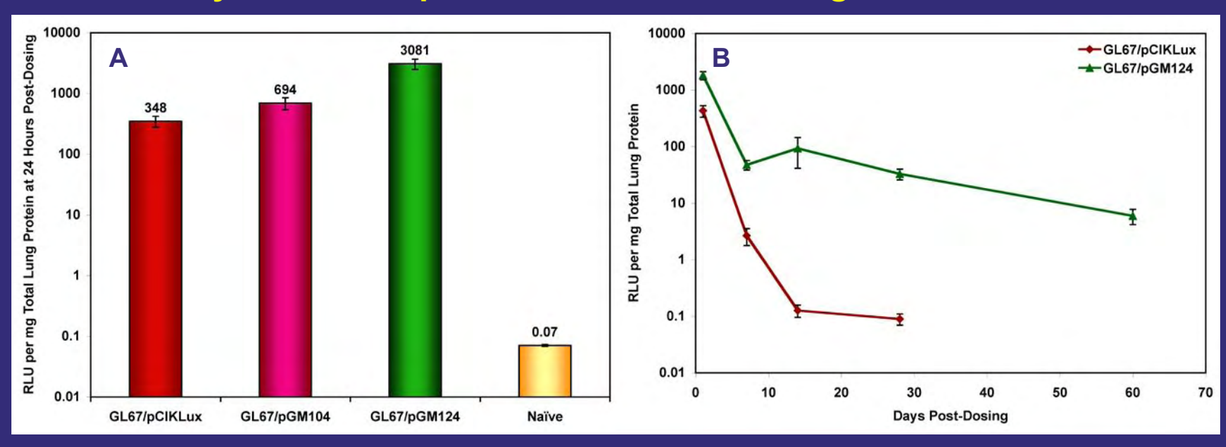
Murine Intranasal pDNA Delivery Model and CpG Response Assays.

1. Plasmid DNA was complexed with Genzyme lipid 67 (GL67) (80 µg pDNA/100 µl) (Pringle *et al.*, 2005, Gene Ther, 12, 1206-14).
2. Female BALB/c mice (n = 9 per group) were anaesthetised with metofane and dosed intranasally with 100 µl GL67/pDNA .
3. At 24 hours post-dosing 3 ml bronchoalveolar lavage fluid (BALF) was collected from the treated mice and untreated naive controls.
4. ELISA was used to determine levels of (A) IFN-γ, (B) TNF-α and (C) IL-12 in the BALF and a count (D) of total cells/ml was also determined.
5. Significan□

Results.

- Delivery of GL67/pCIKLux (317 CpGs) results in high levels of all four inflammatory markers.
- Partial reduction of pDNA CpG content (pGM104 193 CpGs) has no effect on CpG response.
- The zero CpG plasmid (pGM124) produces no inflammatory response in the mouse lung.

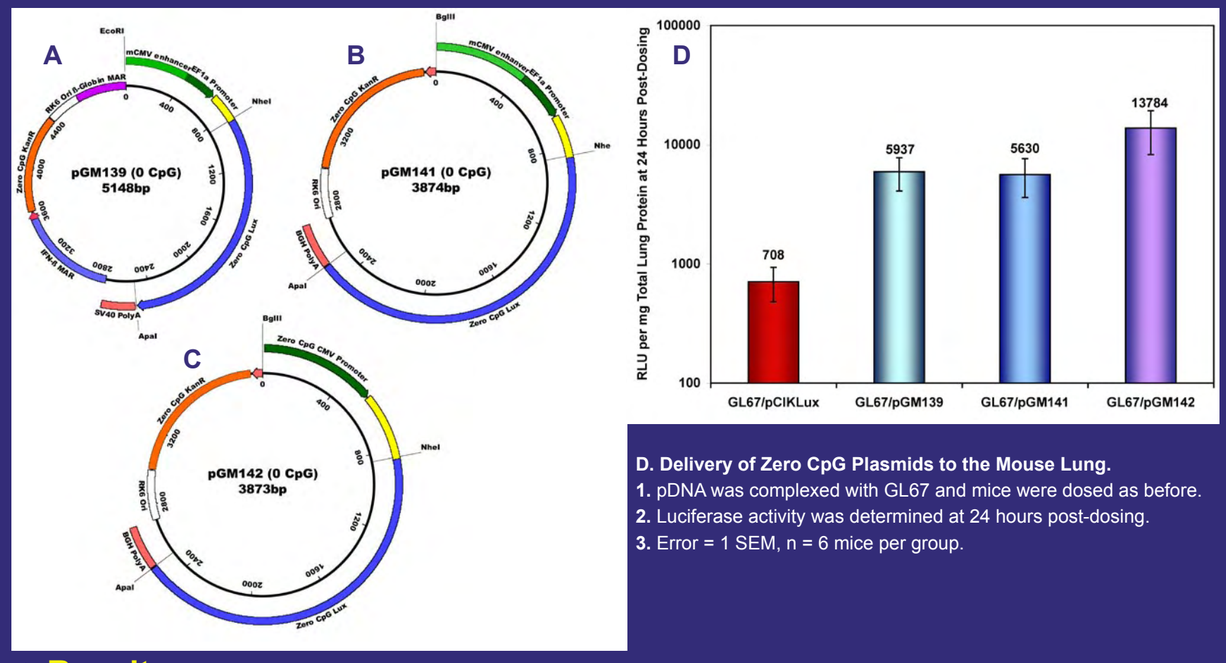
Lux activity from GL67/pGM124 in the mouse lung.



Results.

- Peak expression from pGM124 is 10-fold higher than conventional CpG-rich plasmids (A).
- Expression from pGM124 persists for at least 60 days post-dosing (B).
- High level peak expression may be due in part to the codon optimised Lux.
- No other GL67/pDNA complex has ever resulted in persistence beyond 7 days in this model.
- pGM124 is not a suitable platform for the creation of clinical zero CpG plasmids:-
  1. pGM124 has a Zeocin resistance gene instead of Kanamycin.
  2. It is not known what effect the matrix attachment regions will have.
  3. It lacks suitable restriction sites for cloning different promoters and transgenes.

Development of clinical zero CpG plasmids.



D. Delivery of Zero CpG Plasmids to the Mouse Lung.
1. pDNA was complexed with GL67 and mice were dosed as before.
2. Luciferase activity was determined at 24 hours post-dosing.
3. Error = 1 SEM, n = 6 mice per group.

Results.

- New modular zero CpG plasmids have been created (A, B & C):-
  1. Kanamycin resistance as preferred by FDA.
  2. Well defined unique restriction sites for cloning different promoters and transgenes.
  3. Optional inclusion of matrix attachment regions to determine the effect on expression.
- These novel vectors direct high levels of reporter gene expression in the mouse lung (D).
- At 24 hours post-dosing expression is 8-20 fold higher than a conventional CpG-rich plasmid.

Future Studies.

- Duration of expression from these plasmids is being tested in aerosol delivery models.
- Zero CpG clinical plasmids expressing zero CpG CFTR cDNA are being created.
- An extended range of zero CpG promoters are being investigated.

Conclusions.

- Partial CpG reduction does not lessen CpG response in mouse lungs following delivery of GL67/pDNA.
- Lung delivery of a zero CpG plasmid induces no increase in IFN-γ, TNF-α, IL-12 levels or cellular influx.
- High levels of expression were obtained with this new generation of plasmid vectors.
- The zero CpG plasmid also exhibited improved duration of expression in the mouse lung.
- Clinical zero CpG plasmids are now under development for the treatment of Cystic Fibrosis.