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.
3. We are investigating the effects of CpG depletion in mouse models of lung gene therapy.
4. Plasmids have been constructed with varying numbers of CpGs.
5. The inflammatory response from these plasmids has been tested in vivo.
6. The reporter gene expression from these vectors has been tested in vivo.
7. Clinically relevant zero CpG plasmids are being developed.

Plasmid DNA vectors used in this study.

Results.

Delivery of GL67/pDNA 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.

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.