Non-viral gene therapy is being developed as a treatment for the lung disease associated with Cystic Fibrosis (CF).

CpG motifs found in plasmid DNA (pDNA) cause a potent host inflammatory response in vivo.

The host inflammatory response may result in gene silencing and therefore transient gene expression.

We investigated the effects of CpG depletion on in vivo gene expression and the host inflammatory response.

**Inflammatory Responses Following Delivery of GL67/pDNA to the Mouse Lung**

- **Complete But Not Partial Reduction of Plasmid CpG Content Increases Transgene Expression and Eliminates The Inflammatory Response Associated With Delivery Of Non-Viral Vectors To The Lung**


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**Conclusions.**

- Partial CpG reduction does not reduce the CpG inflammatory response in mouse lungs following delivery of GL67/pDNA complexes.

- A single plasmid-derived CpG is sufficient to direct a CpG inflammatory response in mouse lungs following delivery of GL67/pDNA complexes.

- Complete CpG depletion abolishes the CpG inflammatory response in mouse lungs following delivery of GL67/pDNA complexes.

**Zero-CpG plasmids harbouring the hCEFI enhancer/promoter direct sustained lung transgene expression following aerosol delivery of GL67/pDNA complexes.**

**Expression Profile of Zero CpG pDNAs Following Aerosol Delivery To The Mouse Lung**

A variety of Fourth Generation pDNAs have been constructed containing alternative CpG-free enhancers (mouse CMV, human CMV) and promoters (human CMV, human Elongation Factor 1α). Zero CpG Fourth Generation pDNAs containing the hCEFI enhancer/promoter direct sustained lung transgene expression following aerosol-mediated non-viral gene delivery.

Fourth Generation pDNAs are suitable for use in clinical trials.