Zero CpG plasmids eliminate the inflammatory response associated with lung non-viral gene transfer
Introduction

- Cystic Fibrosis:-
  - Common fatal monogenic disorder
  - Disease affects many organs
  - Chronic lung disease is the primary cause of mortality

- Multiple phase I clinical trials have been conducted
  - Repeat administration is required
    - Viral vectors - Lack of successful repeat administration
    - Non-viral - Limited duration of expression

- UK CF Gene Therapy Consortium
  - Genzyme lipid 67 (GL67) is our lead candidate for lung trials
GL67/pDNA Lung Trial

- GL67/plasmid DNA aerosol delivery to the lungs
  - 25 % correction of CF ion transport defect
  - Mild ‘flu-like’ symptoms and inflammation

- Inflammation attributed to CG dinucleotides (CpGs) in plasmid

- Research hypothesis/challenge:-
  1. Reduction of CpGs in plasmid will reduce inflammation
  2. Eliminate CpGs without compromising expression

Cationic lipid-mediated CFTR gene transfer to the lungs and nose of patients with Cystic Fibrosis: a double-blinding placebo controlled trial.
Mouse Lung Model

1. Produced plasmids with varying CpG content

2. Plasmid DNA complexed with GL67

3. Lung instillation to BALB/c (n=10) (100 µl/80 µg)

4. Harvest lungs at 24 hours post-dosing
   - Reporter gene expression

5. Inflammatory markers in bronchoalveolar lavage fluid (BALF)
   - Total cells per ml BALF (predominantly neutrophils)
   - Inflammatory cytokines IFN-γ, IL-12 & TNF-α
Reducing CpGs to Reduce Inflammation?

Backbone - Similar To That Used In Multiple Phase I Trials In Mid 1990’s
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Backbone - Reduced CpG Content
Overall 40 % fewer CpGs
Reducing CpGs to Reduce Inflammation?

Backbone - Similar To That Used In Multiple Phase I Trials In Mid 1990’s

Backbone - Reduced CpG Content Overall 40 % fewer CpGs
Inflammation from Generation 2 Plasmids

- 40% reduction of CpGs had no effect on inflammation.

Cells

- IFN-γ
- IL-12
- TNF-α

BALB/c
n=10
GL67 Instillation

Mann Whitney
P>0.05
› Develop Zero CpG Generation 3 Plasmids

- R6K origin
- Extensive codon alteration
- Zero CpG promoters
Effect of Zero CpG on Inflammation

- Inflammation reduced to background levels by zero CpG plasmids
Do we really need Zero CpGs?

- Limits choice of promoter
- All genes have to be remade
- Less options for *E. coli* strain
Do we really need zero CpGs?
Effect of Single CpG Motif in Mouse Lung

1 CpG sufficient to induce an inflammatory response in mouse lung

IFN-γ

IL-12

TNF-α

Mann Whitney P<0.05

GL67 Instillation

BALB/c n=10
1 CpG Causes inflammation

- Generation 1 Plasmid = $8.0 \times 10^{13}$ CpG/100 µg
- Generation 2 Plasmid = $4.0 \times 10^{13}$ CpG/100 µg
- 1 CpG Plasmid = $2.5 \times 10^{11}$ CpG/100 µg

Can we use zero CpG plasmids in the clinic?
Will zero CpG compromise expression?
Develop Zero CpG Plasmids for the Clinic

- Not suitable for clinical trials
  - Removed unnecessary DNA
  - Inverted the backbone
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US FDA Docket No 96N-0400. Points to consider on Plasmid DNA Vaccines for Preventative Infectious Disease Indications
Generation 4 Clinical Plasmids

- Minimal backbone sequence
- High yields still possible
- Expression in aerosol studies
- Range of different promoters
  - EFI
  - mCEFI
  - hCEFI
  - GZB
Expression from Best Generation 1 Plasmid

RLU per mg Total Lung Protein

Days Post Dosing

Best Generation 1

BALB/c n=6 GL67 aerosol
Expression from Best Generation 2 Plasmid

BALB/c n=6
GL67 aerosol
Expression from Generation 4 Plasmids

BALB/c n=6
GL67 aerosol

RLU per mg Total Lung Protein

Days Post Dosing

Best Generation 2
Best Generation 1
Expression from Best Generation 4 Plasmid

BALB/c n=6
GL67 aerosol
Summary & Future Work

- Developed plasmids with zero CpG motifs
- Eliminated CpG response to GL67/pDNA in mouse lung
- Improve level and duration of gene expression

- Planned clinical studies in CF patients
  - Single dose pilot study Spring 2007
  - Multi-dose study 2008

Choice of plasmid has major impact of on overall performance of non-viral vectors
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