

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

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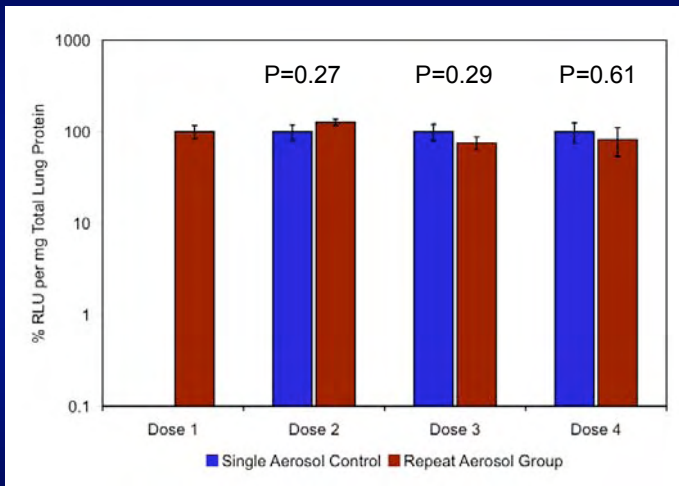


## ▶ Introduction



- ▶ 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
  - ▶ Extensive evaluation of available GTAs
  - ▶ Genzyme lipid 67 (GL67) is our lead candidate for lung trials

## ▶ GL67/pDNA can be repeat aerosolised



BALB/c n=6  
GL67/pDNA aerosol

Dosing at 7 day intervals

Single dose controls in the same exposure box

Harvest at 1 day post-dosing

Mann-Whitney

## ▶ No loss in gene expression after 4 administrations

## ▶ 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

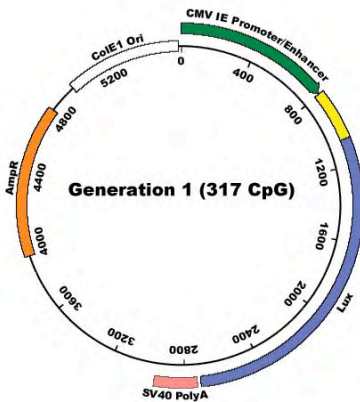
Alton *et al.*, 1999. *Lancet*, 353, 947-953.

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

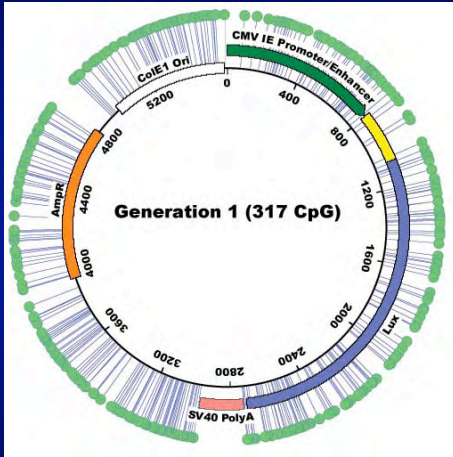
1. Produced plasmids with varying CpG content
2. Plasmid DNA complexed with GL67
3. Lung instillation to BALB/c (n=10) (100  $\mu$ l/80  $\mu$ g)
4. Harvest lungs at 24 hours post-dosing
  - ▶ Reporter gene expression
5. Inflammatory markers in bronchoalveolar lavage fluid (BALF)
  - ▶ Total cells per ml BALF (predominantly neutrophils)
  - ▶ Inflammatory cytokines IFN- $\gamma$ , IL-12 & TNF- $\alpha$

## ▶ Reducing CpGs to Reduce Inflammation?



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

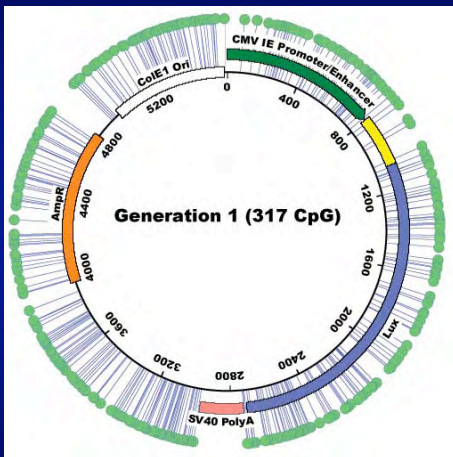
## ▶ Reducing CpGs to Reduce Inflammation?



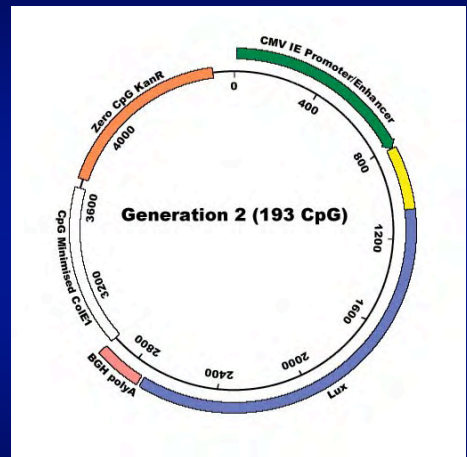
● = 1 CpG Motif

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

## ▶ Reducing CpGs to Reduce Inflammation?

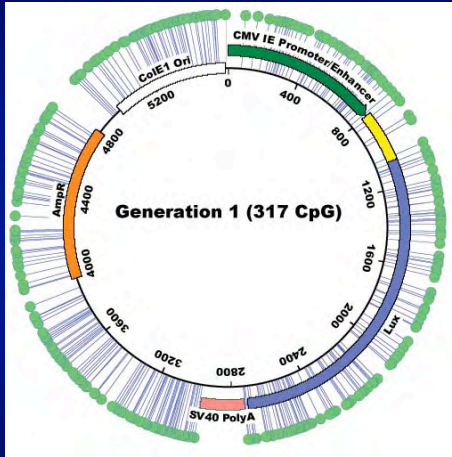


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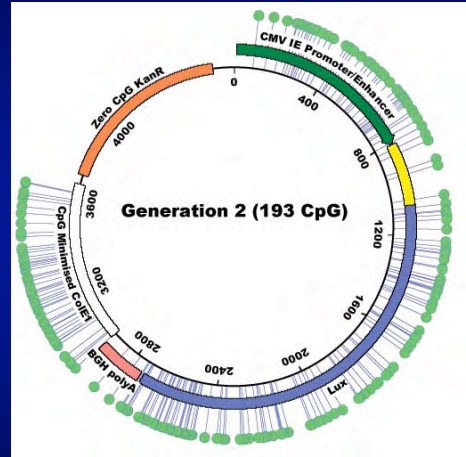


**Backbone** - Reduced CpG Content Overall 40 % fewer CpGs

## ▶ Reducing CpGs to Reduce Inflammation?

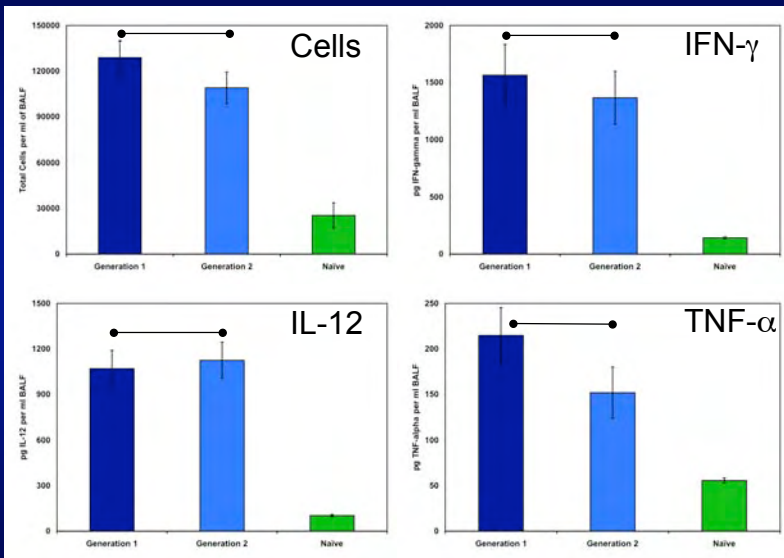


**Backbone** - Similar To That Used In Multiple Phase I Trials In Mid 1990's



**Backbone** - Reduced CpG Content Overall 40 % fewer CpGs

## ▶ Inflammation from Generation 2 Plasmids

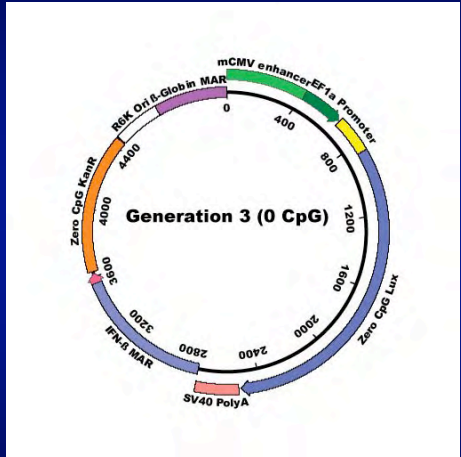
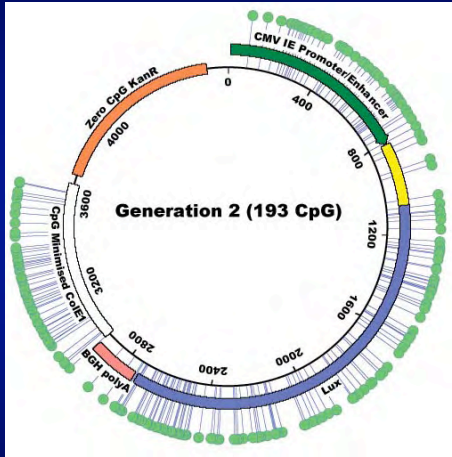


BALB/c  
n=10  
GL67  
Instillation

Mann  
Whitney  
P>0.05

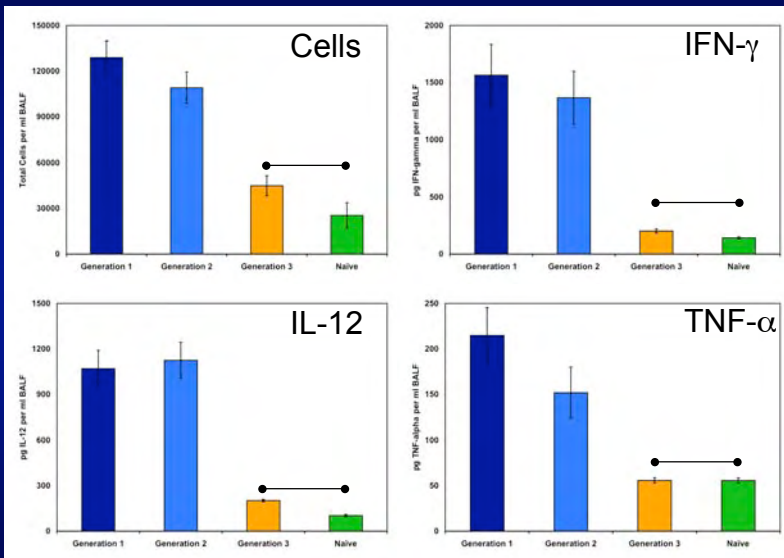
▶ 40 % reduction of CpGs had no effect on inflammation.

## ► Develop Zero CpG Generation 3 Plasmids



- R6K origin
- Extensive codon alteration
- Zero CpG promoters

## ► Effect of Zero CpG on Inflammation

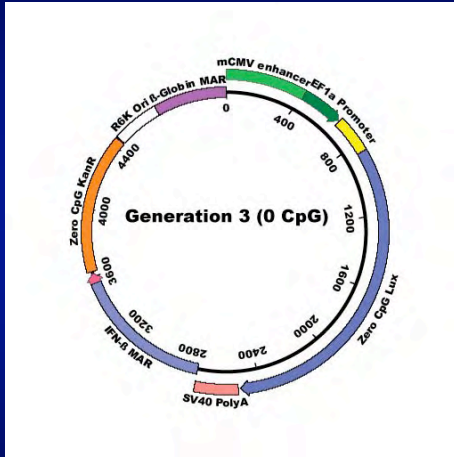


BALB/c  
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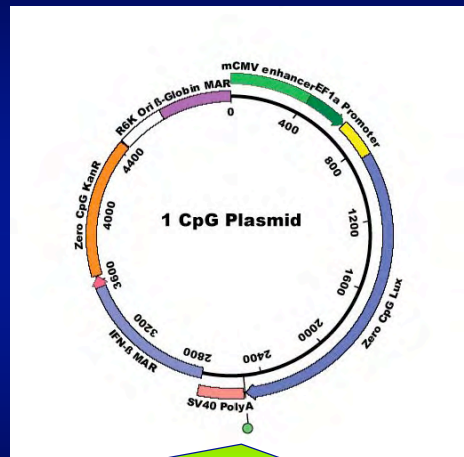
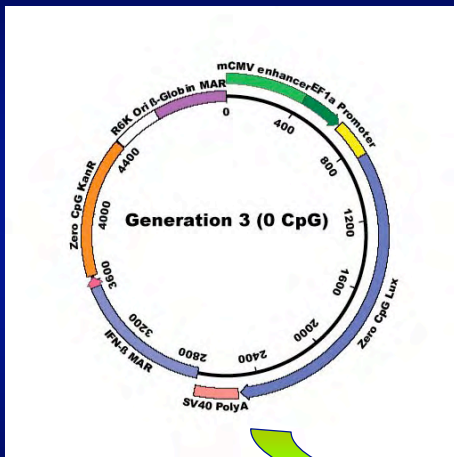
- Inflammation reduced to background levels by zero CpG plasmids

## ▶ Do we really need Zero CpGs?

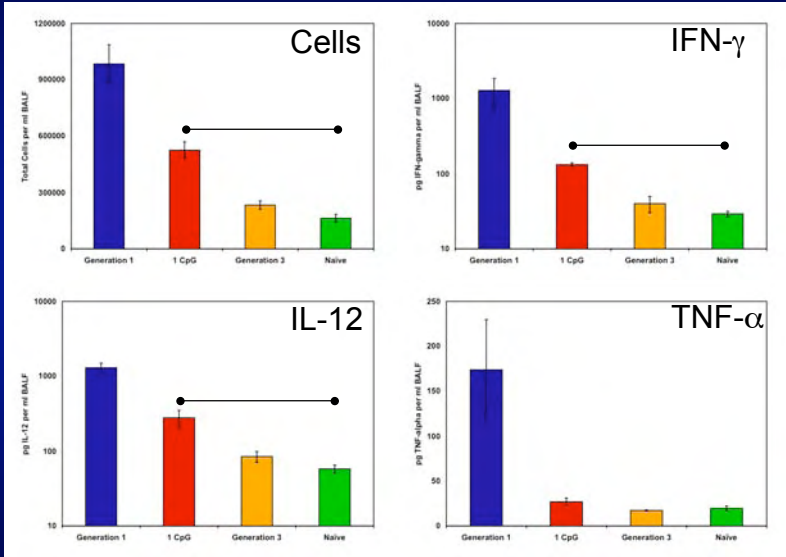


- ▶ Limits choice of promoter
- ▶ All genes have to be remade
- ▶ Less options for *E. coli* strain

## ▶ Do we really need Zero CpGs?



## ► Effect of Single CpG Motif in Mouse Lung



BALB/c  
n=10  
GL67  
Instillation

Mann  
Whitney  
P<0.05

► 1 CpG sufficient to induce an inflammatory response in mouse lung

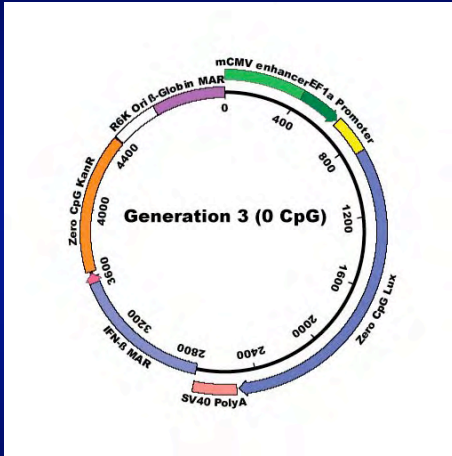
## ► 1 CpG Causes inflammation

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

- Can we use zero CpG plasmids in the clinic?
- Will zero CpG compromise expression?

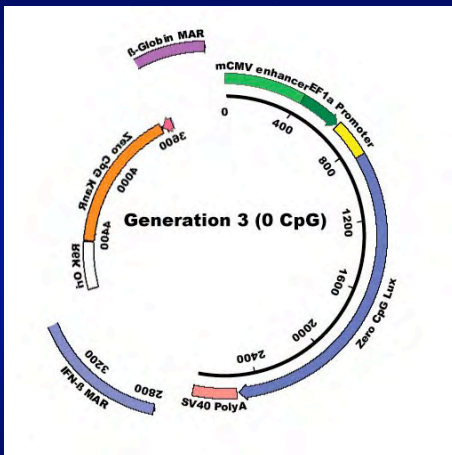


## ▶ Develop Zero CpG Plasmids for the Clinic



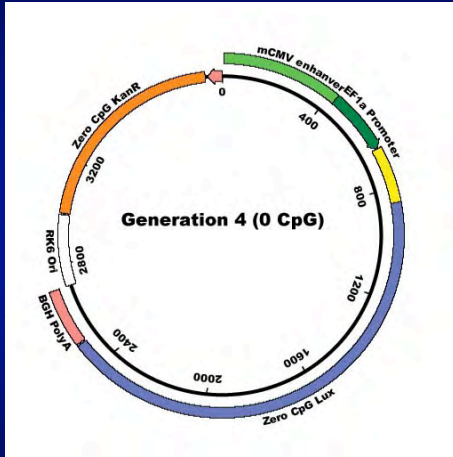
- ▶ Not suitable for clinical trials
  - ▶ Removed unnecessary DNA
  - ▶ Inverted the backbone

## ▶ Develop Zero CpG Plasmids for the Clinic



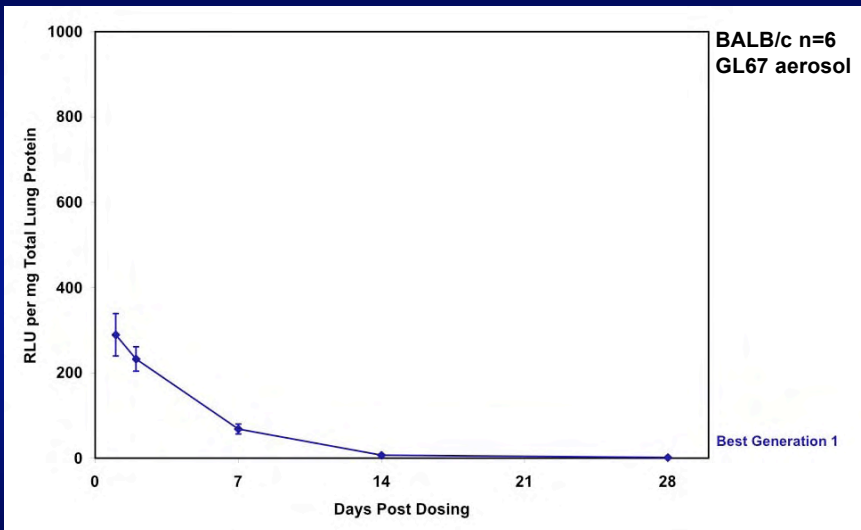
- ▶ Not suitable for clinical trials
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## ▶ Generation 4 Clinical Plasmids

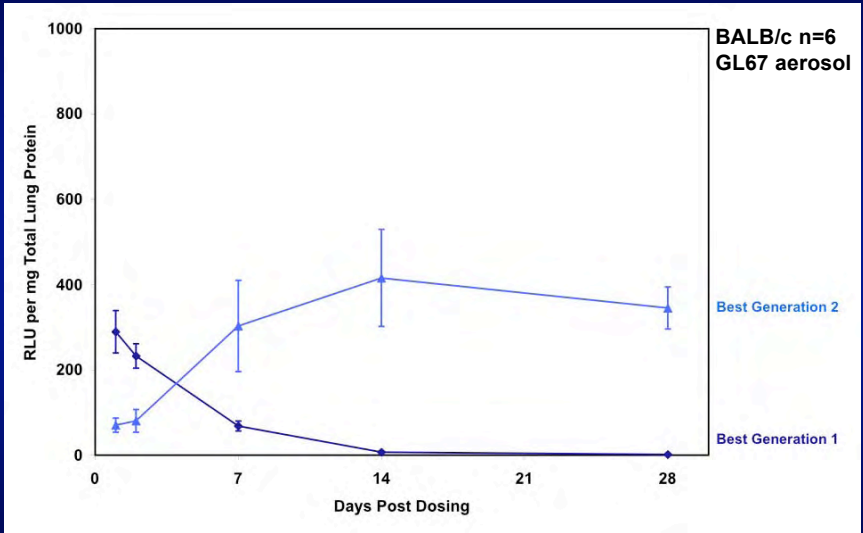


- ▶ Minimal backbone sequence
- ▶ High yields still possible
- ▶ Expression in **aerosol** studies
- ▶ Range of different promoters
  - ▶ EFI
  - ▶ mCEFI
  - ▶ hCEFI
  - ▶ GZB

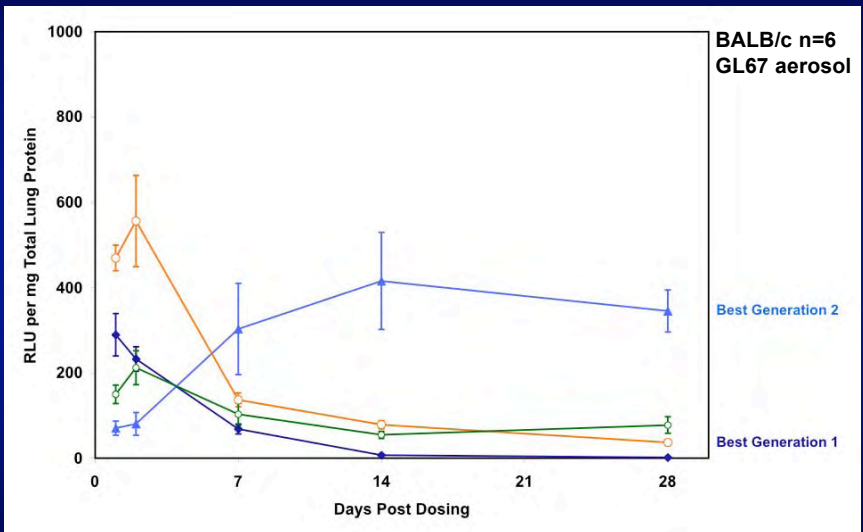
## ▶ Expression from Best Generation 1 Plasmid



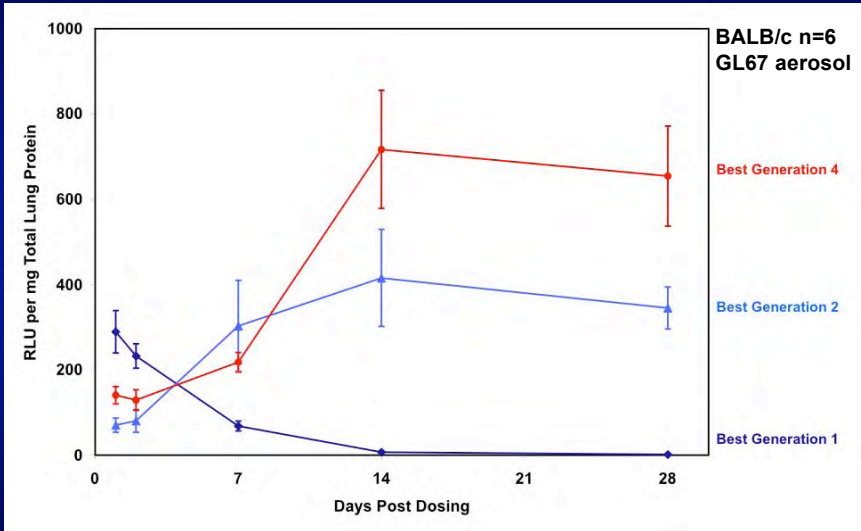
## ► Expression from Best Generation 2 Plasmid



## ► Expression from Generation 4 Plasmids



## ► Expression from Best Generation 4 Plasmid



Best generation 4 plasmid =  $\Delta$ CpG Human CMV enhancer/ $\Delta$ CpG Human EF1a promoter (patent pending)

## ► 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

## ▶ Acknowledgements

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