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# CpG Free Plasmids For Enhanced Duration & Reduced Toxicity Of Non-Viral Airway Gene Transfer

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Gene Medicine Research Group  
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## **Cystic Fibrosis - CF**

- **Most Common Serious Genetic Disease In EU**  
**In The UK: ~1:20 Carriers**  
**Worldwide: ~70,000 Affected**
- **Disease Of The Surfaces (Epithelia) Of The Body**  
**Many Organs Affected**  
**Lungs Accumulate Sticky Mucus**  
**Sticky Mucus Encourages Bacterial Infections**  
**Chronic Bacterial Infections Lead To Lung Destruction**
- **CF Gene Identified In 1989**  
**Cystic Fibrosis Transmembrane Conductance Regulator (CFTR)**  
**CFTR Controls The Movement Of Salt (& Hence Water) Across Epithelia**  
**CFTR Is An Epithelial Chloride Channel**  
**CFTR Regulates ENaC The Epithelial Sodium Channel**

## **CF Gene Therapy: Clinical Experience**

- **Multiple Clinical Trials**
  - Adenoviral Vectors**
  - Adeno-Associated Virus**
  - pDNA / Cationic Liposomes**

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- **Broadly Similar Results**
  - Modest Evidence Of Gene Transfer**
  - Transient Correction Of Some CF Related Defects**

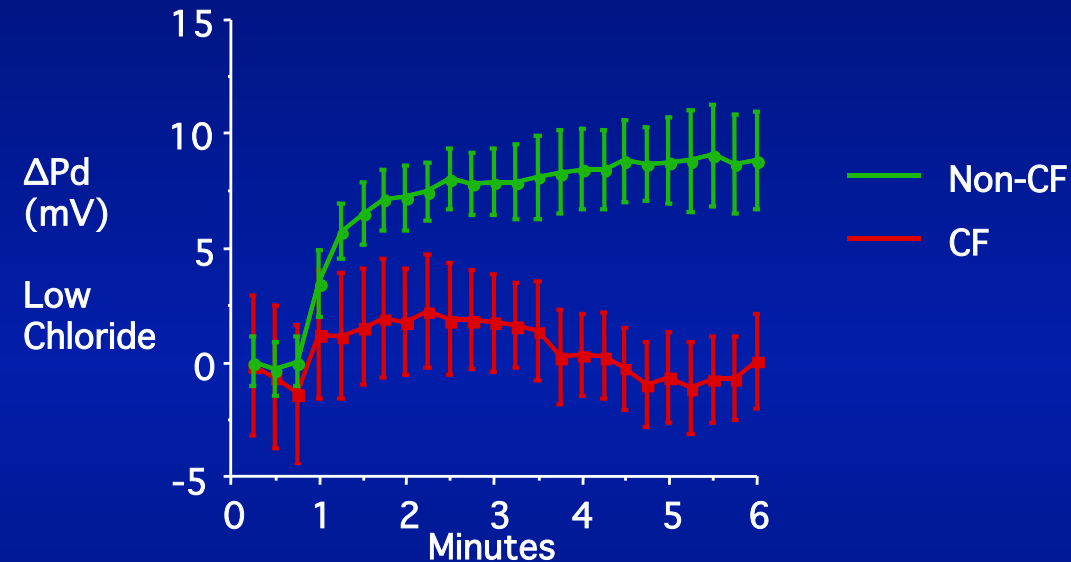
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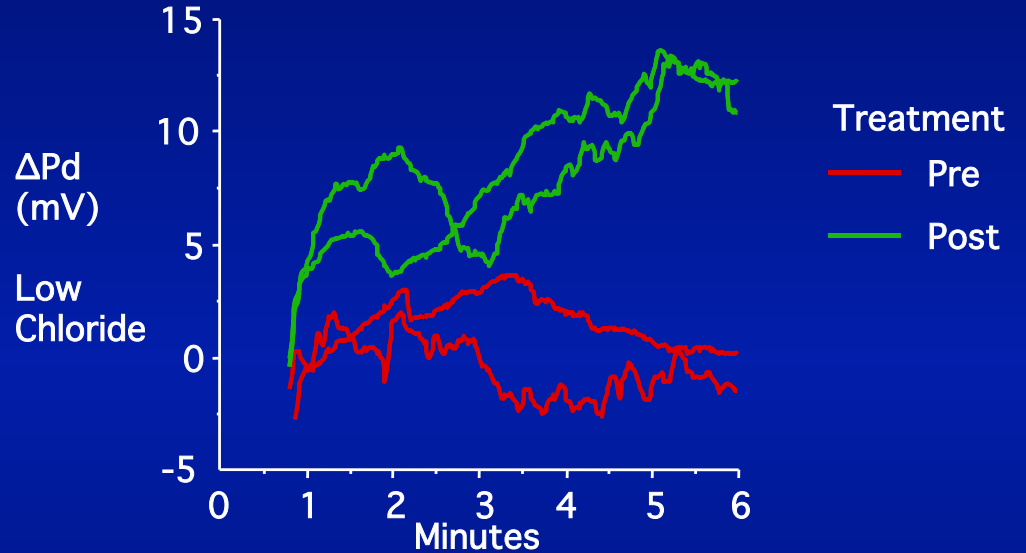
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- **Broadly Similar Results**
  - Modest Evidence Of Gene Transfer
  - Transient Correction Of Some CF Related Defects ~ **1 Week**
  - Mild To Moderate Flu-Like Symptoms**

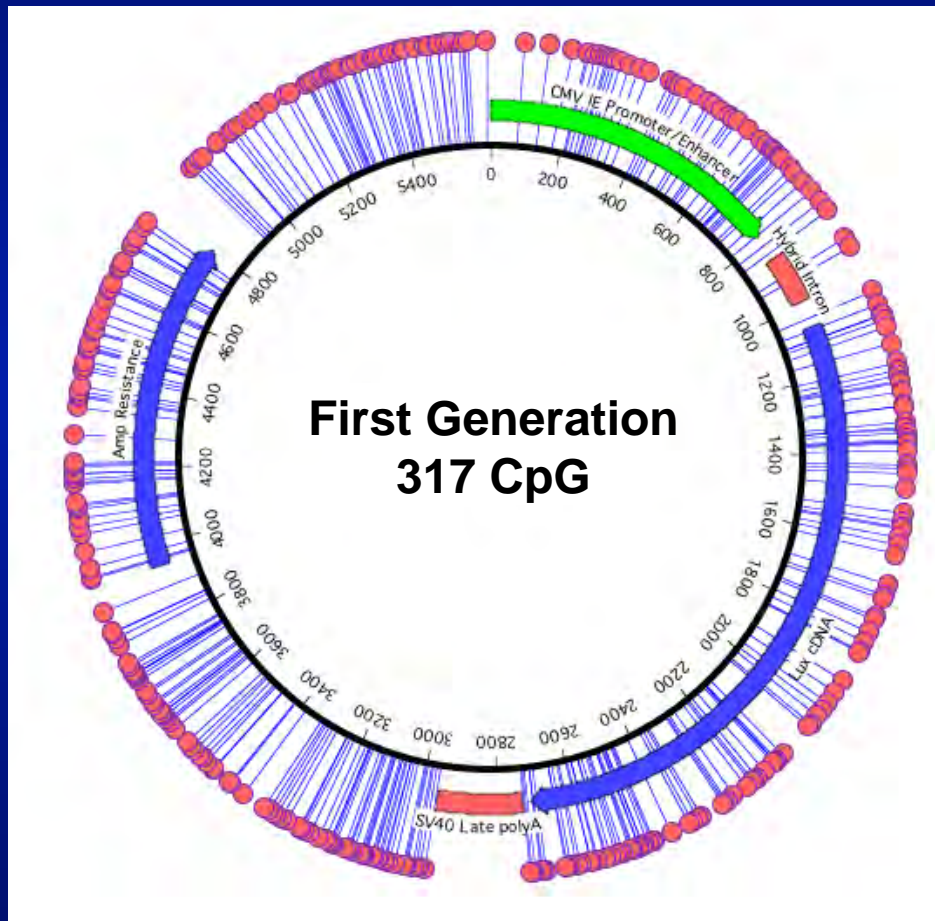


# Host Response To Non-Viral GTA: Impact Of CG Sequences Within DNA



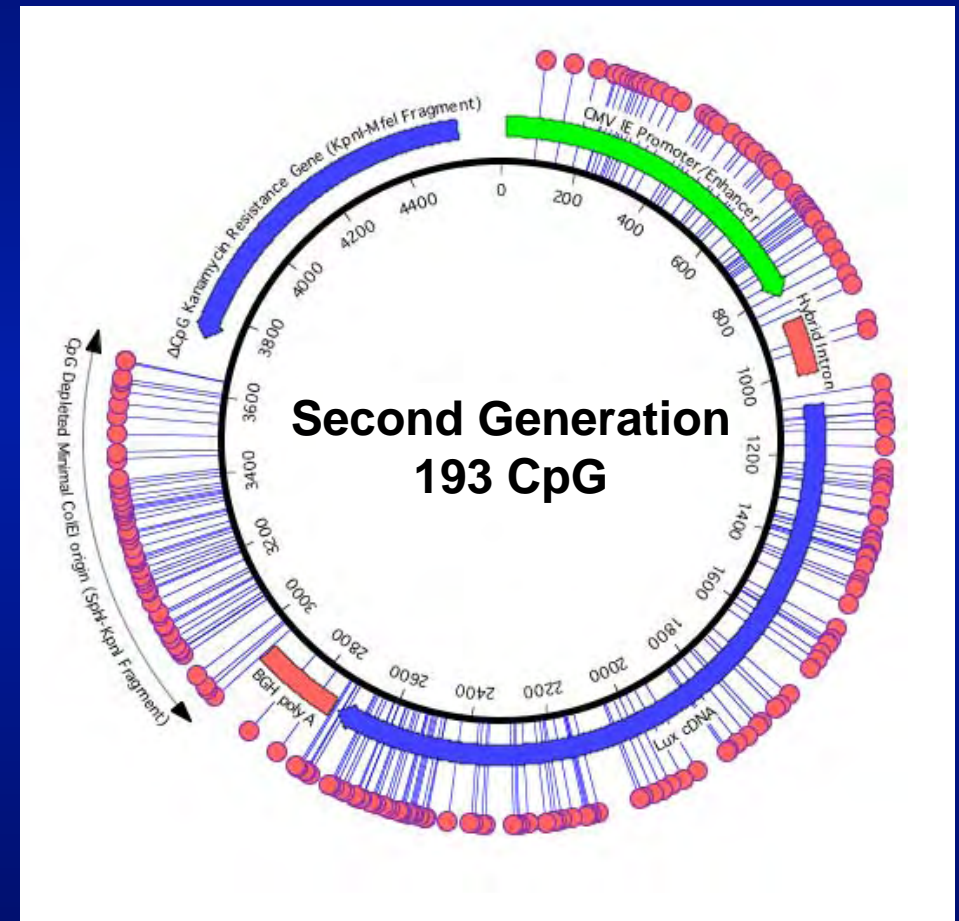
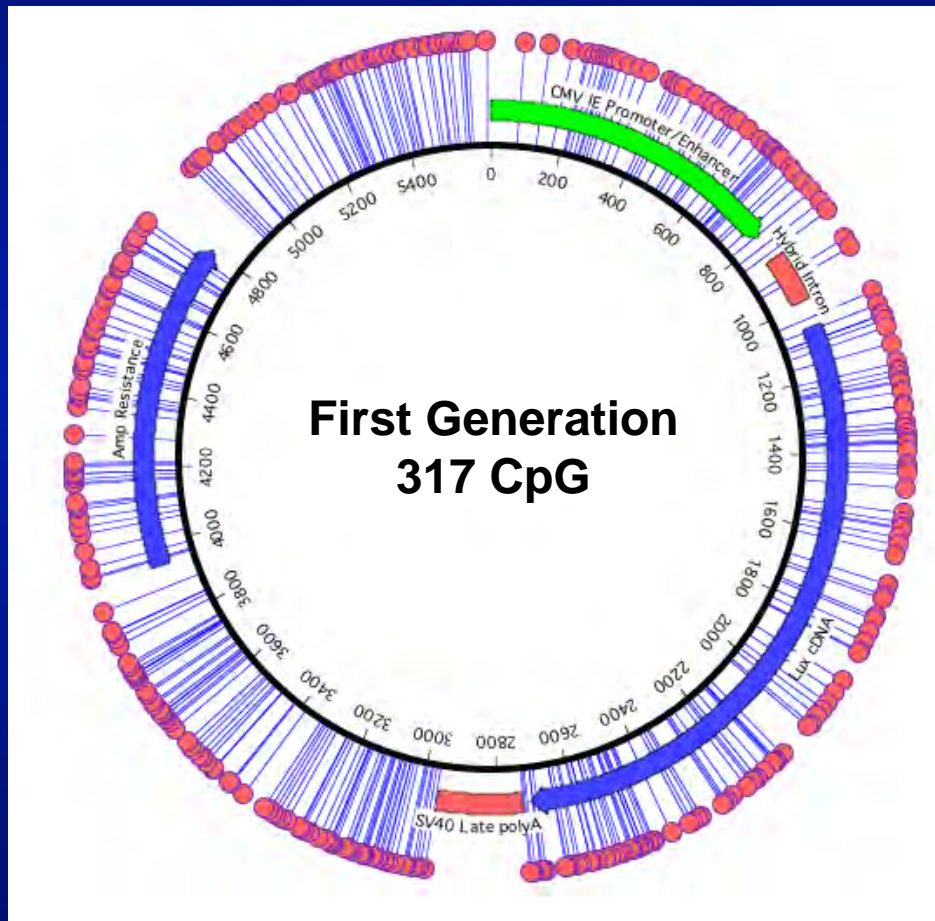
- **CpG response:**
  - CG Sequences Are Common In Bacterial DNA, Rare In Mammalian DNA**
  - Mammalian CGs Tend To Be Methylated, Bacterial CGs Are Unmethylated**
  - pDNA Used In Non-Viral Gene Transfer Contain Many Unmethylated CGs**
  - Unmethylated CGs Are Recognised In Mammalian Cells By TLR9**
  - TLR9 Activates The Innate Immune System & Induces Inflammatory Cytokines**
  
  - Exploited - In Vaccination Protocols (pDNA/Virus Prime/Boost)**
- **Hypothesis: CpG Response Causes 'Flu-Like' Symptoms On Delivery Of GL67/pDNA**
- **Strategy: Reduce pDNA CpG Content**
  - Compare Housekeeping & Viral Promoters For Sustained Expression**

# Evolution Of Non-Viral Expression Plasmids: First Generation pDNAs Have Many CpGs



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

# Evolution Of Non-Viral Expression Plasmids: Second Generation pDNAs Have Fewer CpGs



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

CpG Free Antibiotic Resistance Region  
Minimal *ColE1* Origin *Yew Mol Ther* 1:255 (2000)

# Second Generation pDNA Development: Promoter Elements



- **CMV- Like Promoters (n=2)**

- CMV Immediate Early Enhancer : Promoter / Hybrid Intron

- $\Delta$ CpG CMV Immediate Early Enhancer : Promoter / Hybrid Intron

- **UbC-Like Promoters (n=2)**

- Human UbC Promoter / 1<sup>st</sup> Intron

- $\Delta$ CpG Human UbC Promoter / 1<sup>st</sup> Intron

- **Hybrid Promoters (n=3)**

- CUBI Promoter - CMV Enhancer : UbB Promoter / 1<sup>st</sup> Intron

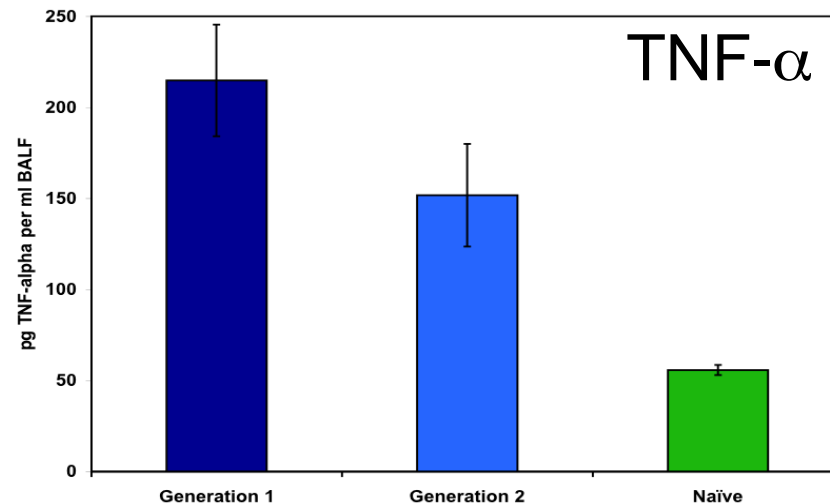
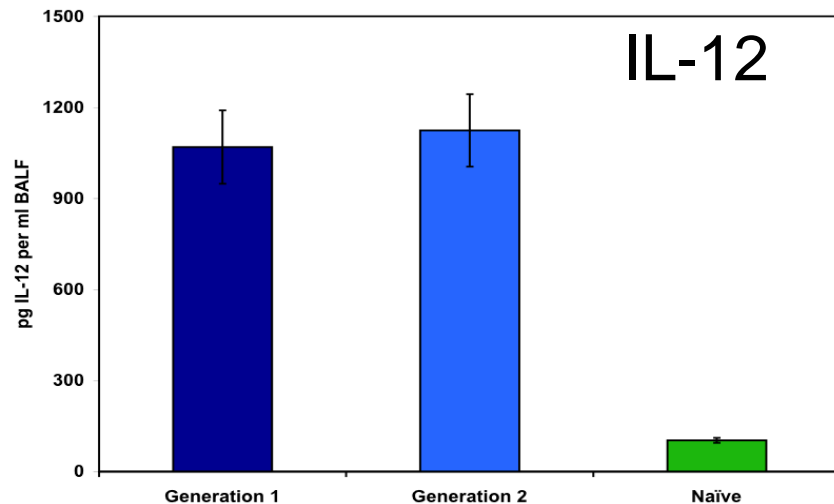
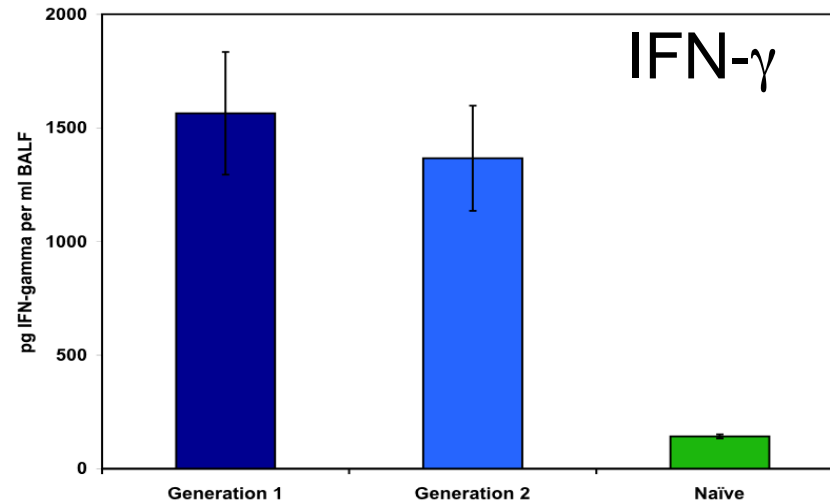
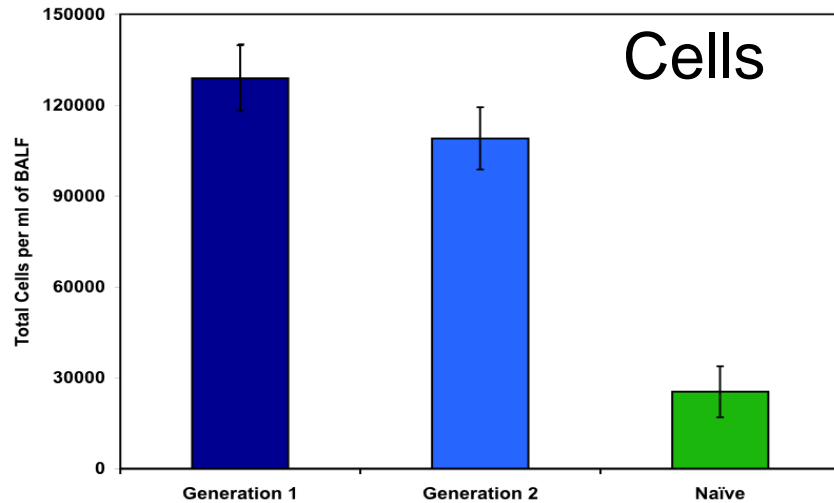
- CUCI Promoter - CMV Enhancer : UbC Promoter / 1<sup>st</sup> Intron

- $\Delta$ CpG CEFI Promoter - CMV Enhancer : EF1 $\alpha$  Promoter / Hybrid Intron

# **Second Generation pDNAs: Effect Of Promoter Choice On GL67A Aerosol Potency**



# Second Generation pDNAs Do Not Reduce GL67A Mediated Lung Inflammation



Mann  
Whitney  
P>0.05

n=10 BALB/c  
Lung Instillation  
GL67A/pDNA

pDNA <5EU/mg

# Second Generation pDNAs Do Not Reduce Lung Inflammation

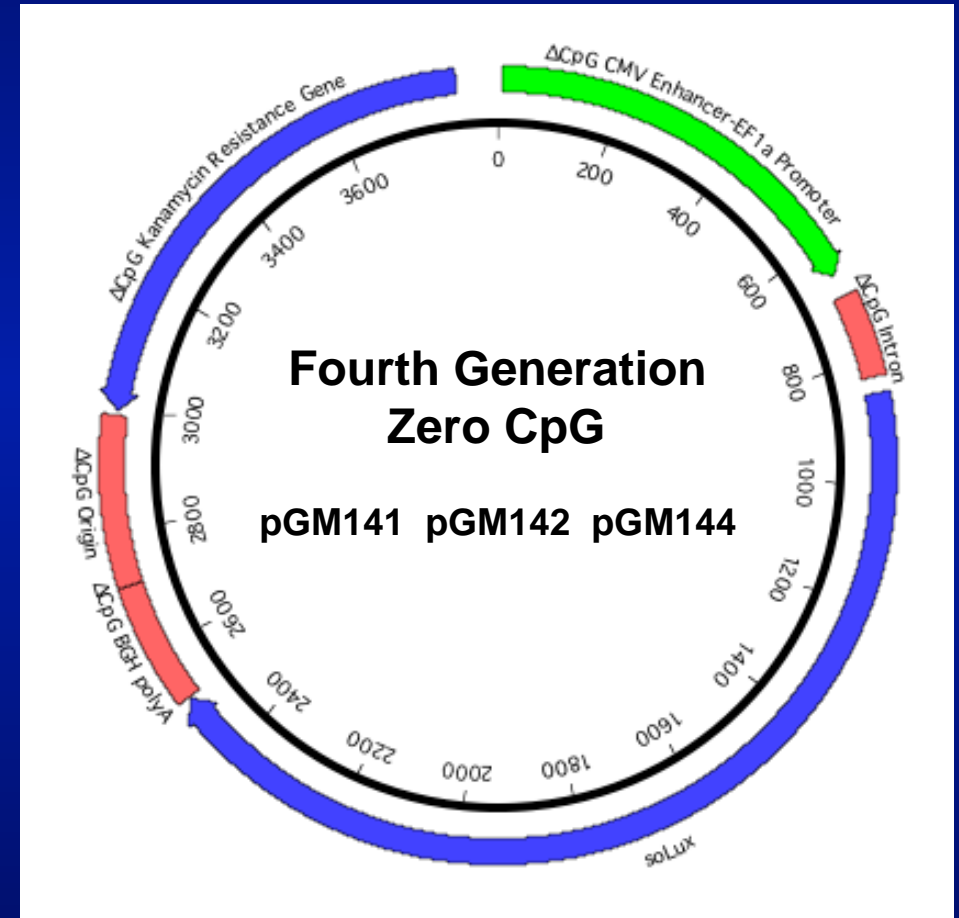
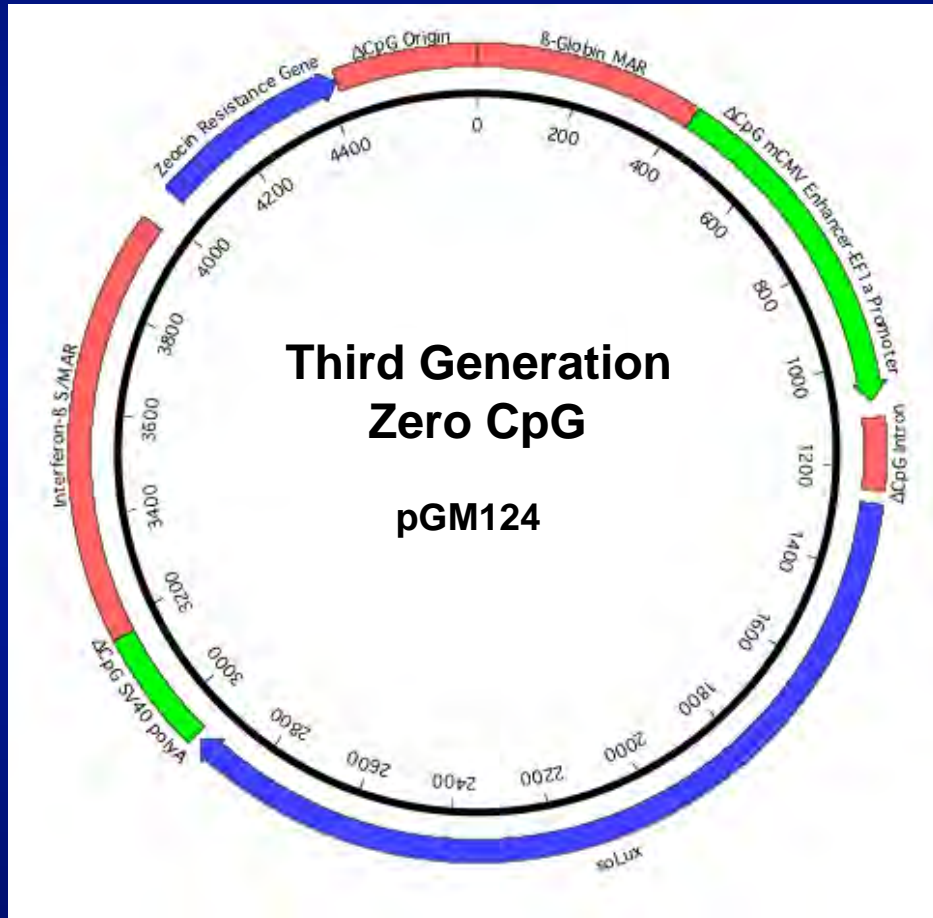


- CpG Burden

First Generation pDNA	317 CpG	$\sim 4 \times 10^{15}$ CpG's / 100 $\mu$ g
Second Generation pDNA	193 CpG	$\sim 2 \times 10^{15}$ CpG's / 100 $\mu$ g
	1 CpG	$\sim 1 \times 10^{13}$ CpGs / 100 $\mu$ g

- Eliminate CpGs From pDNA Altogether

# Evolution Of Non-Viral Expression Plasmids: Third & Fourth Generation pDNAs Have No CpGs

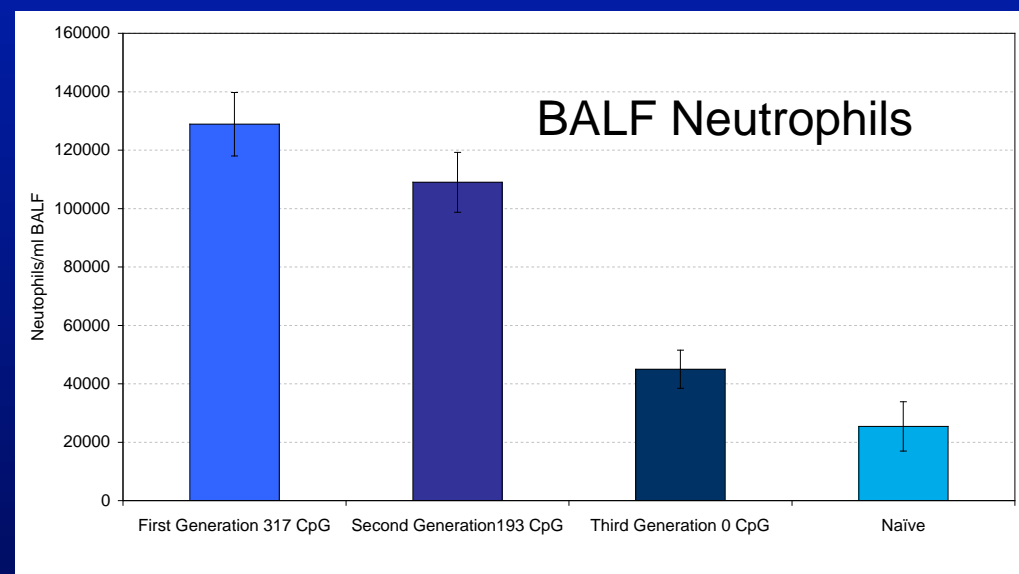
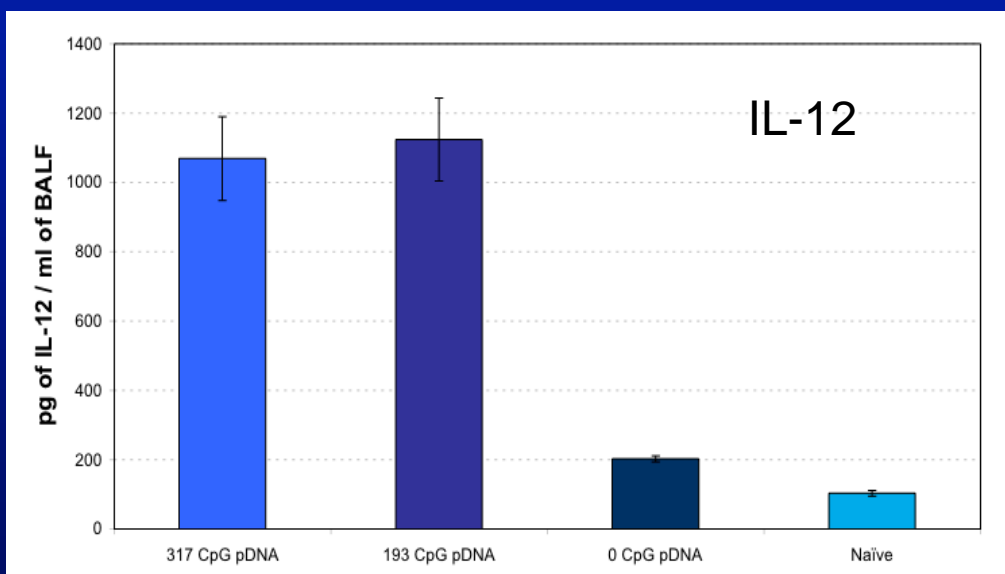
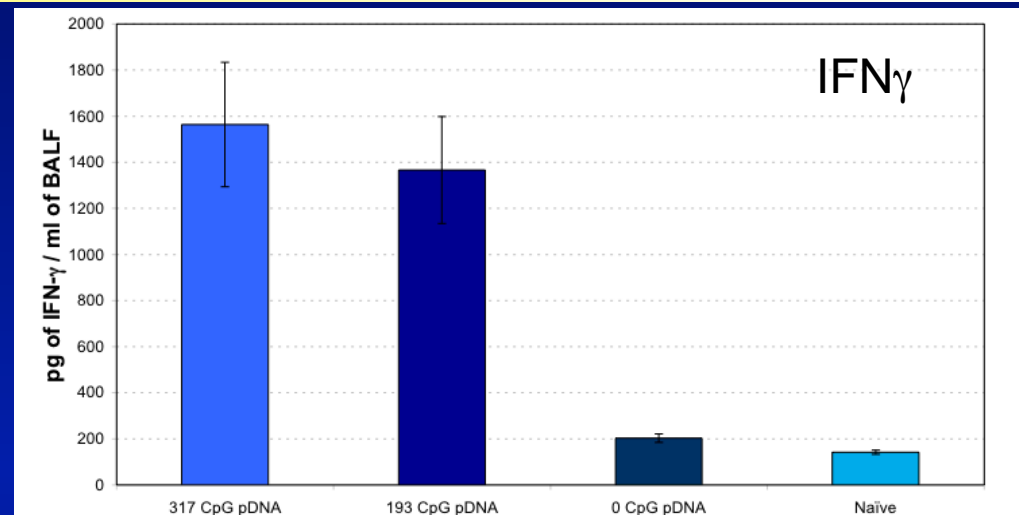
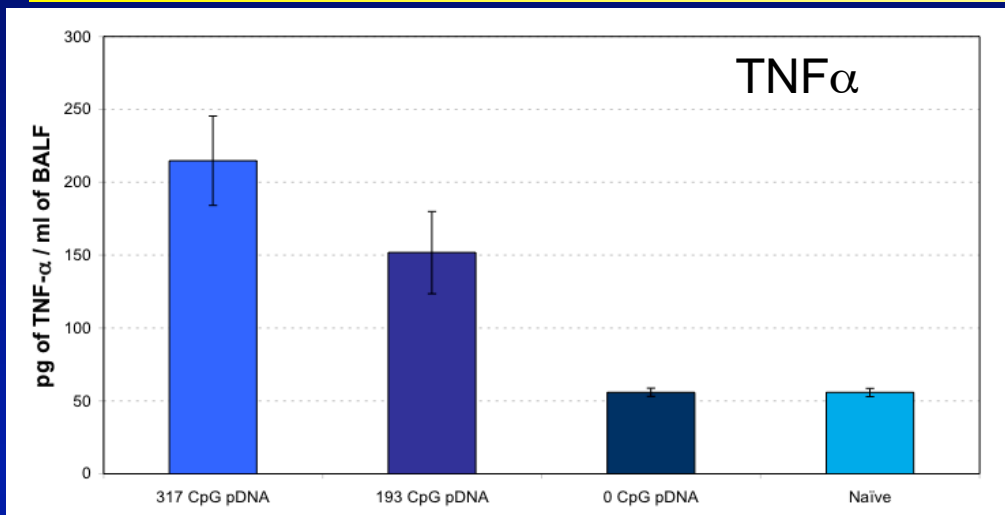


Based on InvivoGen Zero CpG Backbone  
pCpGLacZ

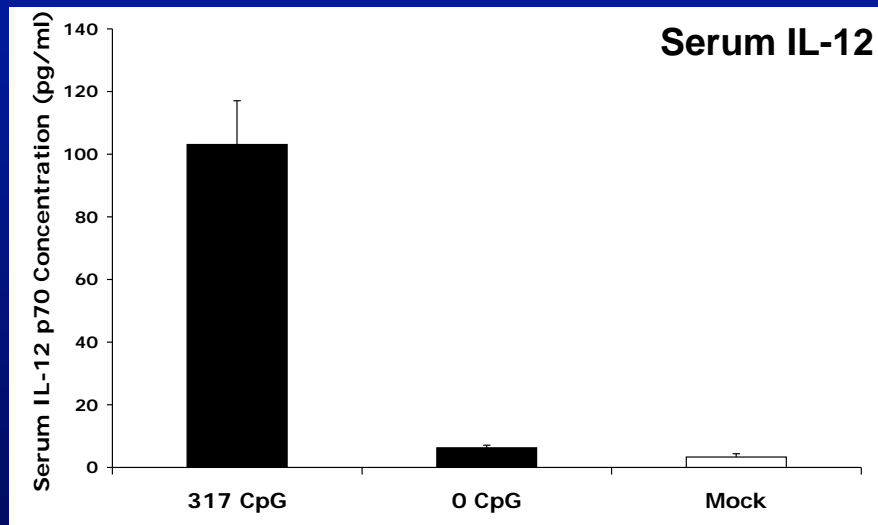
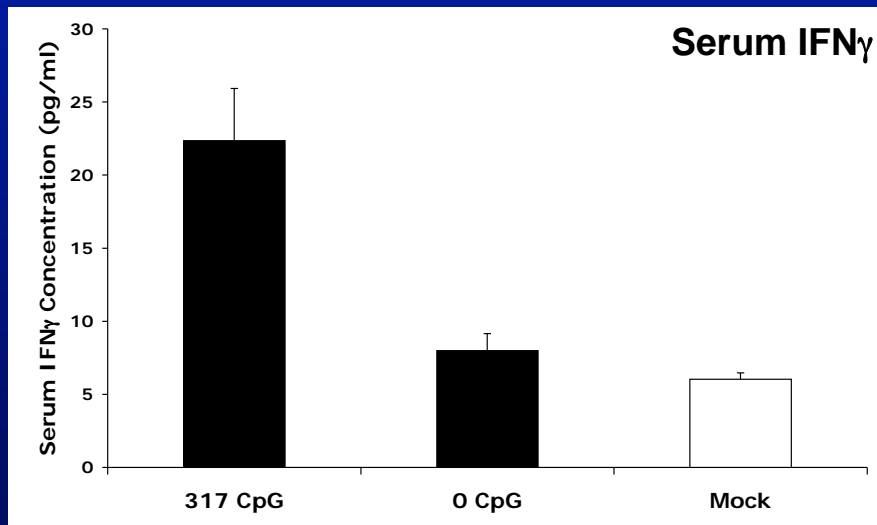
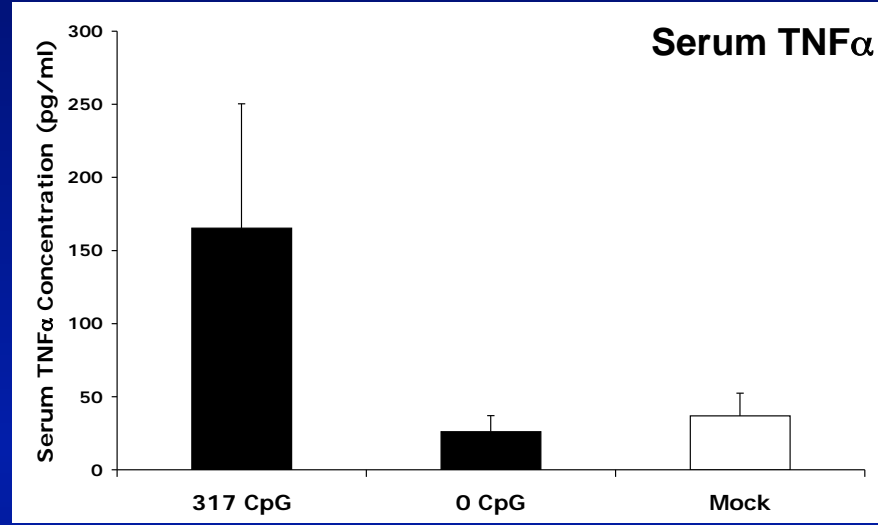
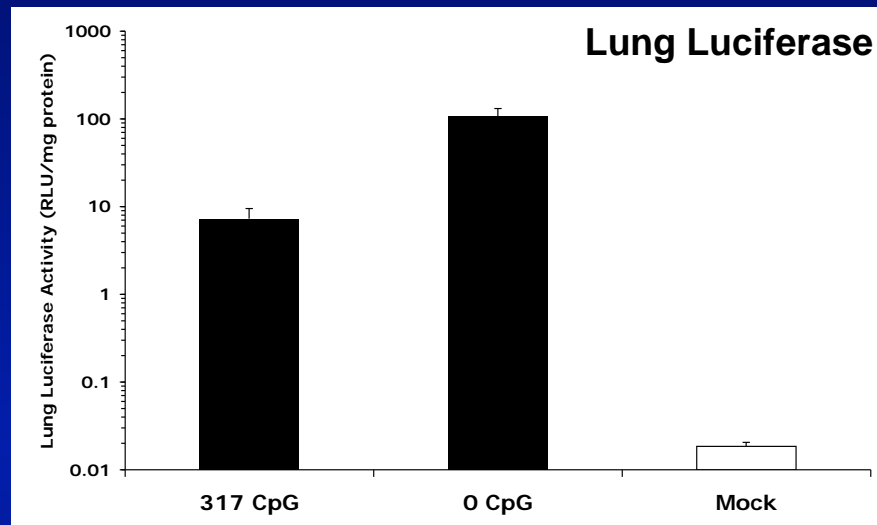
UK CF Gene Therapy Consortium Zero CpG Backbone  
**FDA Compliant** Hyde 2008 *Nature Biotechnology* 26:549



# Third & Fourth Generation pDNAs Abolish Flu-Like Symptoms And Lung Inflammation



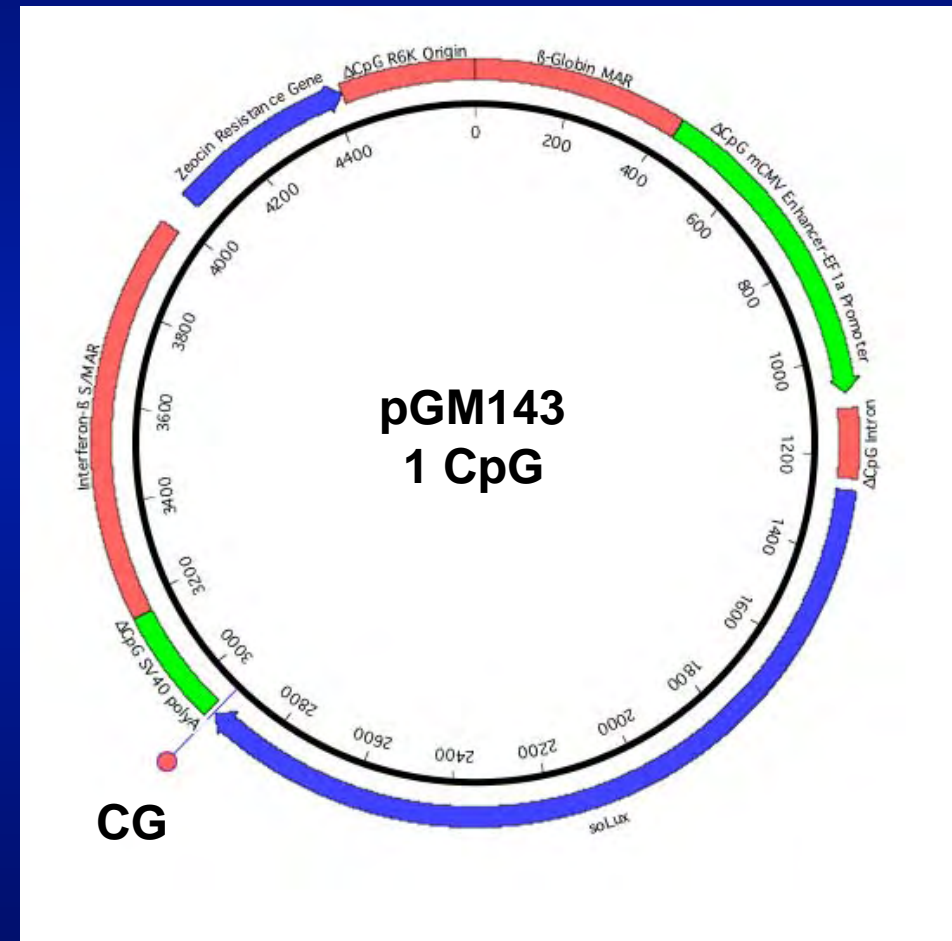
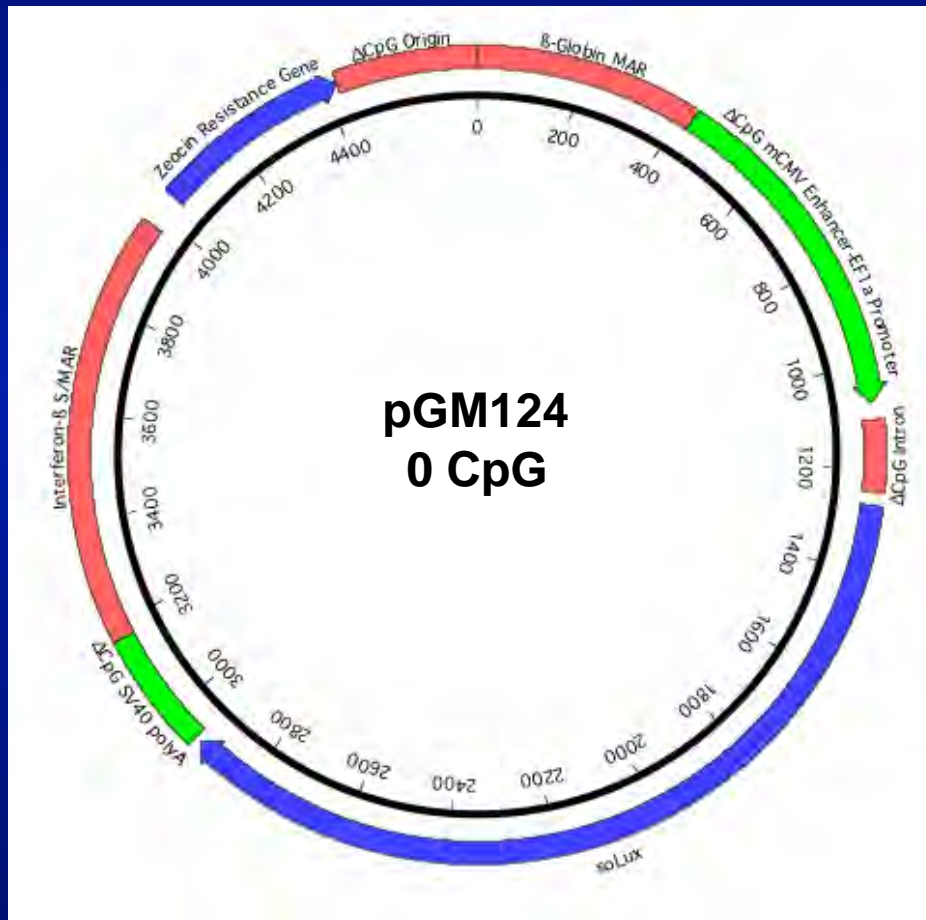
# Fourth Generation pDNAs Reduce Inflammatory Reactions After IV Delivery



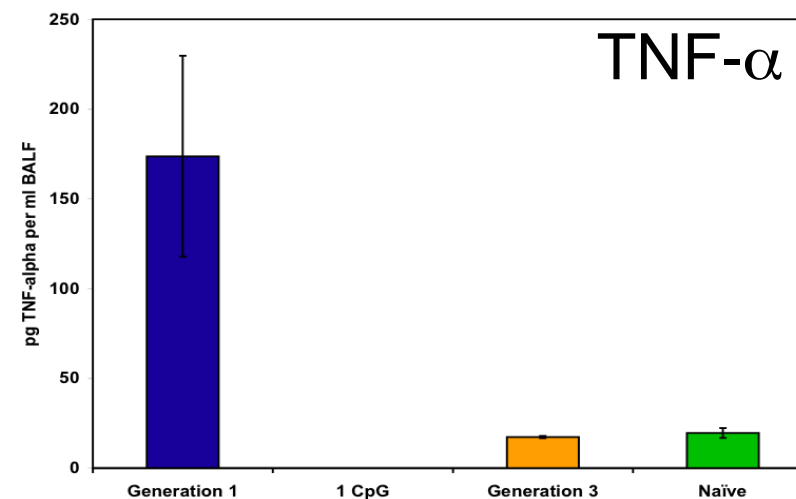
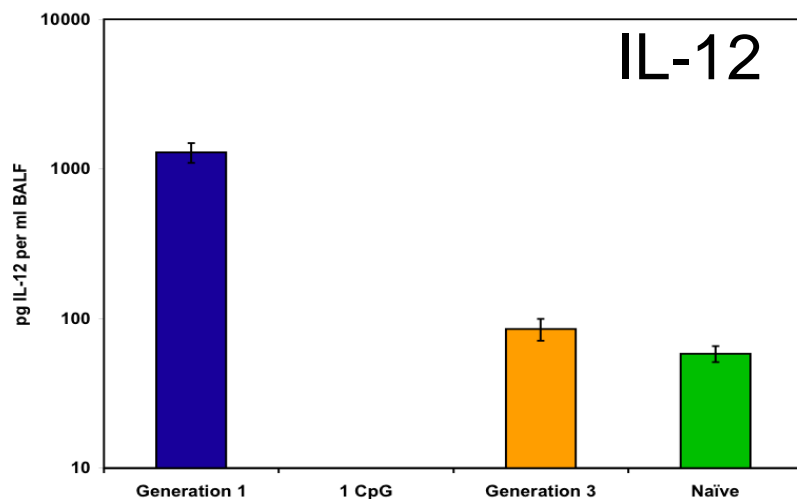
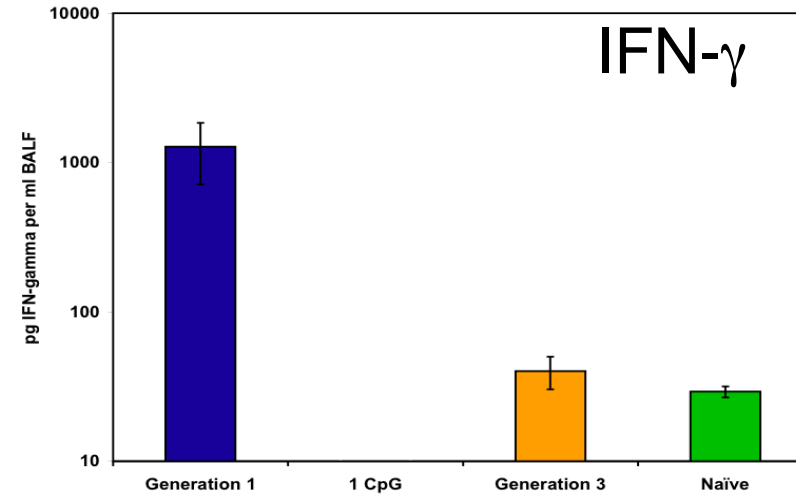
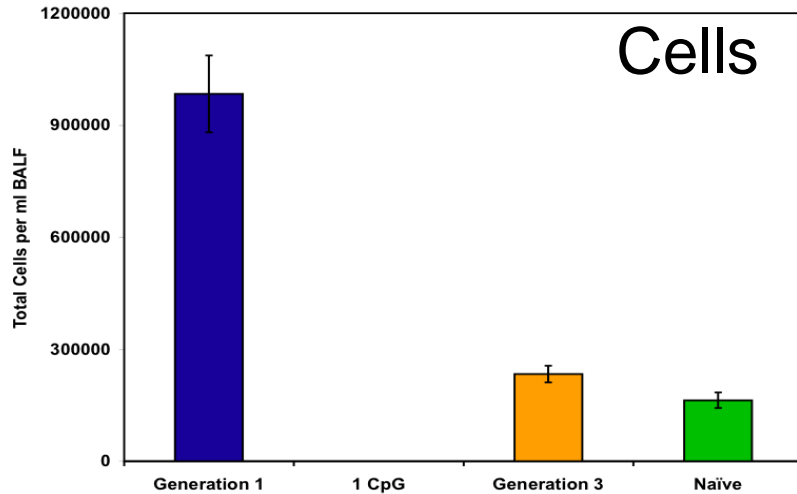
n=5-6 BALB/c  
IV Infusion  
GL67A/pDNA

pDNA <5EU/mg

# Do We Really Need Zero CpG pDNA ?

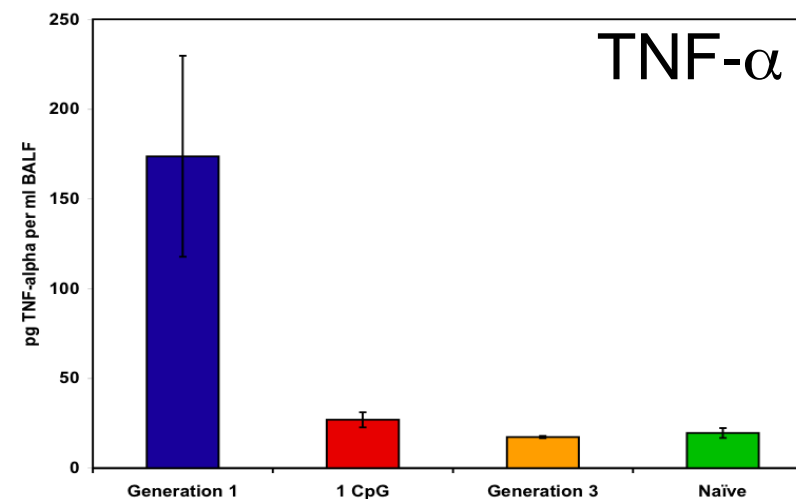
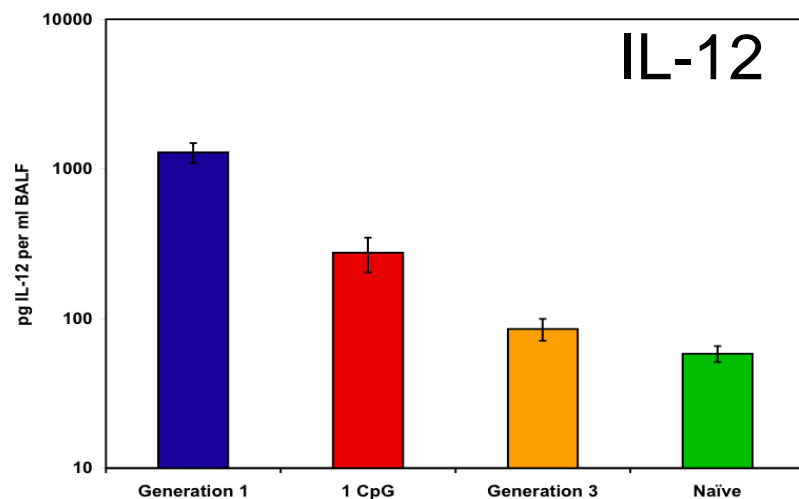
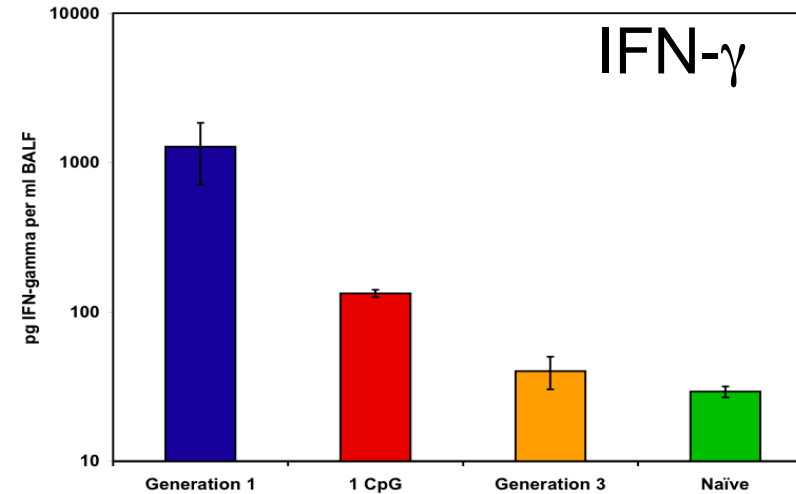
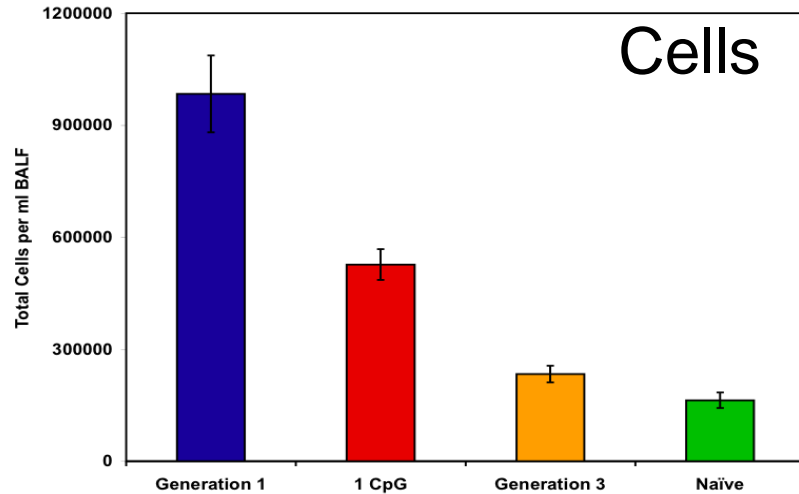


# Effect Of Single CpG In The Mouse Lung



**BALB/c**  
**n=10**  
**GL67A**  
**Instillation**

# Effect Of Single CpG In The Mouse Lung



**BALB/c**  
**n=10**  
**GL67A**  
**Instillation**

## CpG Free Enhancer / Promoters

- Enhancers & Promoters Tend To Have High CpG Density  
Promoters Regions Often Defined By CpG Islands...
- Enhancer / Promoter Selection  
Find Naturally Occurring CpG-Free Enhance / Promoter Elements (Abstract 484)  
Generate CpG-Free Versions Of Commonly Used Enhance / Promoter Elements
- CpG Depletion Strategy  
Convert CG To TG or AG
- CpG Free Enhancer / Promoter Elements
 

UbC	$\Delta$ CpG UbC3 Promoter (V Low Activity)
<b>CMV</b>	Human CMV Enhancer : Human CMV Promoter
<b>EFI</b>	: Human Elongation Factor 1a Promoter
<b>mCEFI</b>	Mouse CMV Enhancer : Human Elongation Factor 1a Promoter
<b>hCEFI</b>	Human CMV Enhancer : Human Elongation Factor 1a Promoter

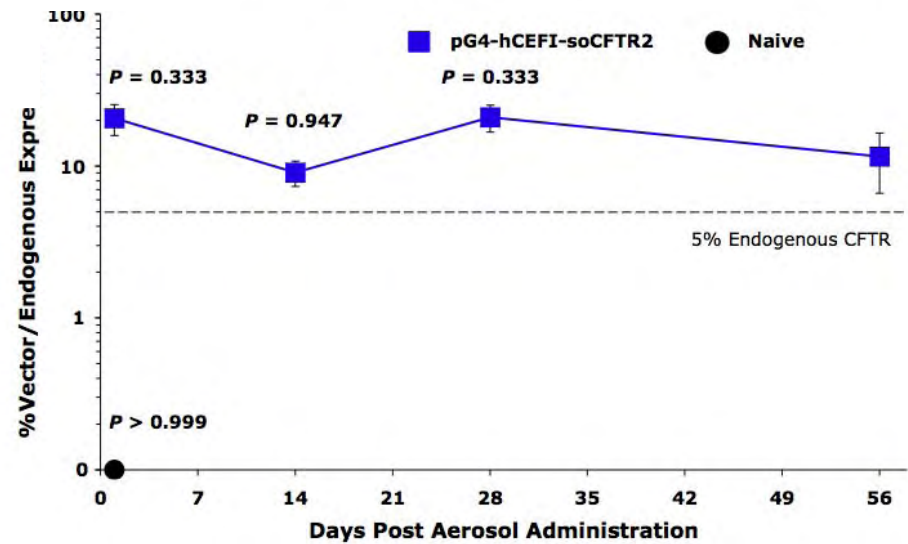
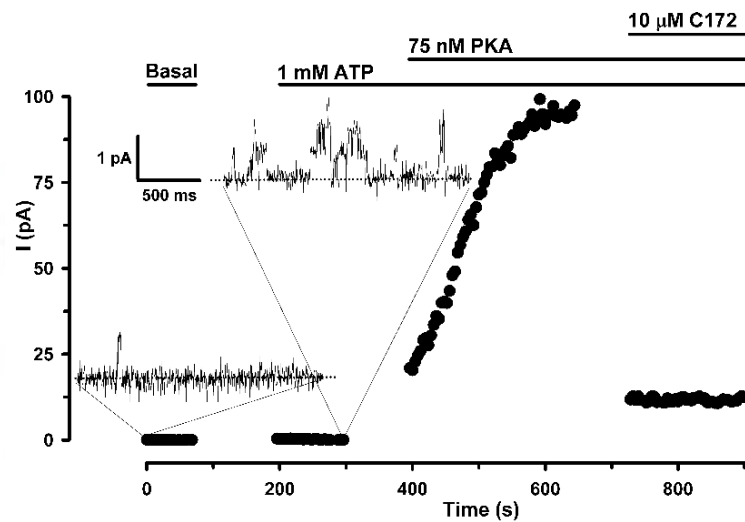
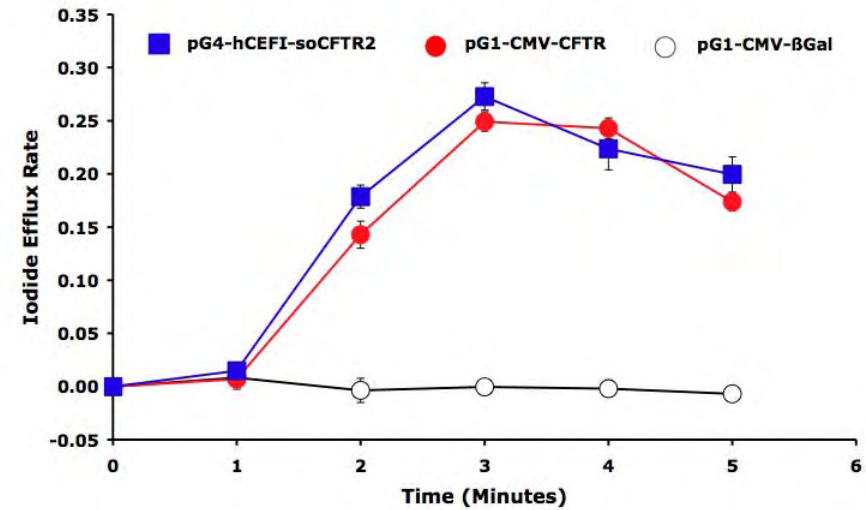
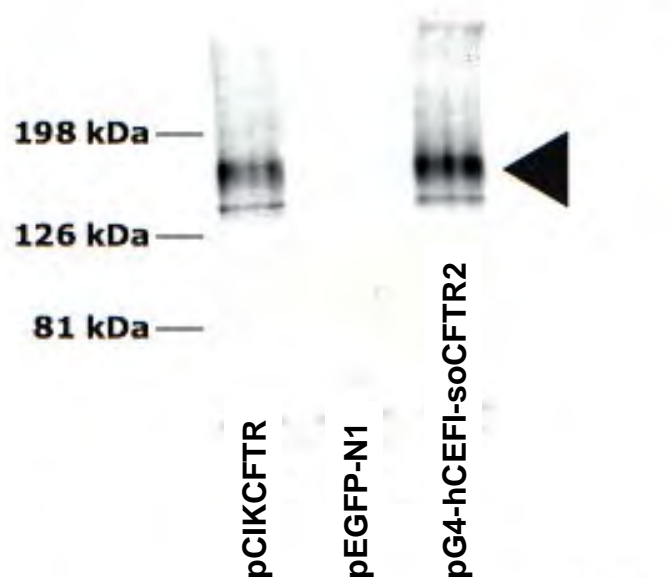
# **Fourth Generation pDNAs: Effect Of Promoter Choice On GL67A Aerosol Potency**

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# **Optimal First, Second & Fourth Generation pDNAs: GL67A Aerosol Potency**



# Fourth Generation pDNA With CpG Free CFTR cDNA Directs Lung Expression For $\geq 56$ Days

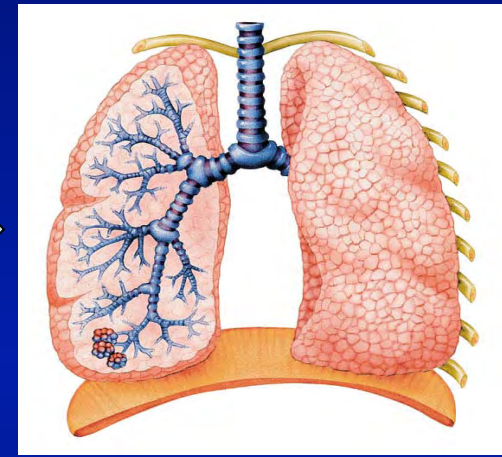
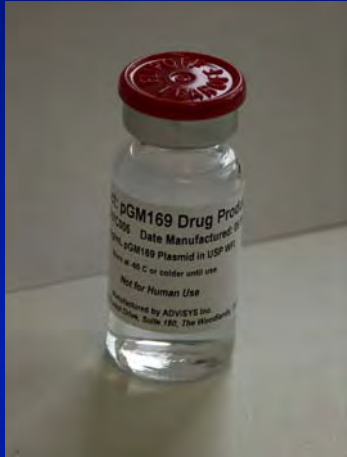


# UK CF Gene Therapy Consortium

## Single Dose Non-Viral Gene Transfer Study



- Extensive Pre-Clinical Programme Identifies Potent Non-Viral Formulation:



Zero CpG  
CFTR Plasmid

GL67A  
Liposomes

AeroEclipse II  
Nebuliser

- Single Dose Clinical Trial Starts **Q4 08**  
N=27 CF Subjects. Nose & Lung Aerosol Administration  
Pre & Post Bronchoscopic Sampling  
Safety & Duration Of Expression Endpoints
- Leading To Multi Dose Study n=50 Active n=50 Placebo **2010**



