

# CpG Depletion Results in Increased Duration of Gene Expression From Plasmid Vectors *in vivo*

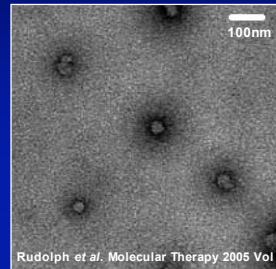
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Gene Medicine Research Group  
Oxford University

## Introduction

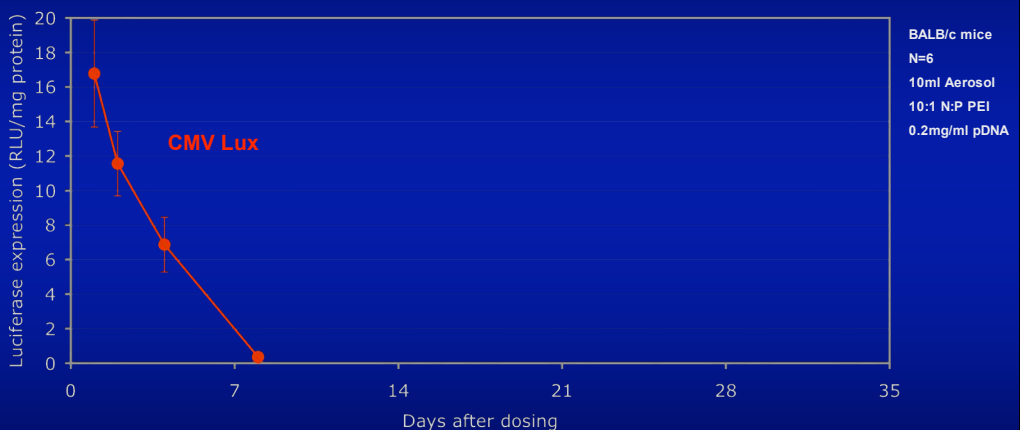
- Cystic Fibrosis
  - Common lethal genetic disorder
  - Epithelial ion transport
  - Multi-organ
  - Lung disease primary cause morbidity/mortality
- UK Cystic Fibrosis Gene Therapy consortium is committed to the testing and development of gene therapy vectors for CF clinical trials
- CF lung gene therapy vectors
  - Topical delivery by aerosol
  - Target airway epithelium
  - Repeat administration

## Polyethylenimine

- 25kDa branched polyethylenimine (PEI)
- Well known GTA
- Aerosol delivery
- Mouse lung aerosol model
- Whole body exposure
- 30 mice/aerosol
- 2mg pDNA dose
- Luciferase reporter
- Highly reproducible



## Aerosol administration of pDNA/PEI



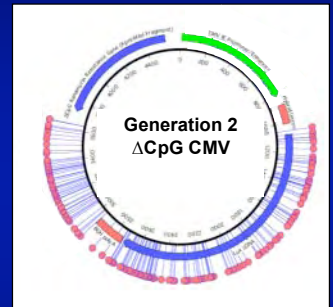
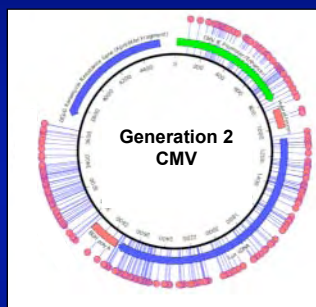
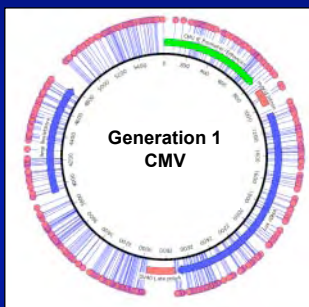
•CMV mediated lung gene expression is transient

# How to increase persistence of lung gene expression



- Persistent expression clinically desirable
- One possible strategy is to reduce CpG motifs
  - Un-methylated CG dinucleotides in pDNA
  - Associated with promoter attenuation
- CpG plasmid generations
  - Generation 1 - Standard CpG content
  - Generation 2 - CpG reduced plasmids

## CpG reduced CMV plasmids



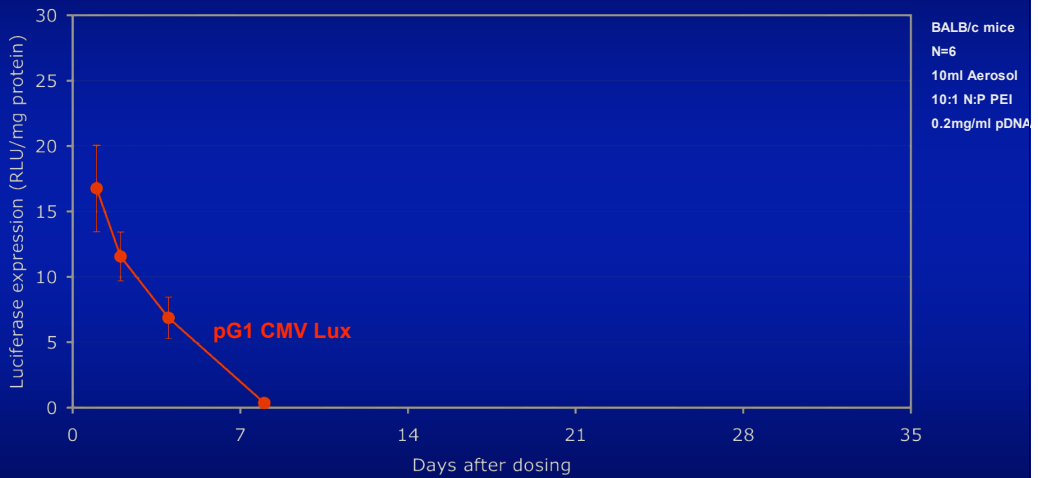
pDNA Backbone is Minimal CpG pOri-K-syn From Genzyme Yew *Mol Ther* 1:255 (2000)

pG1 CMV Lux  
317 CpGs

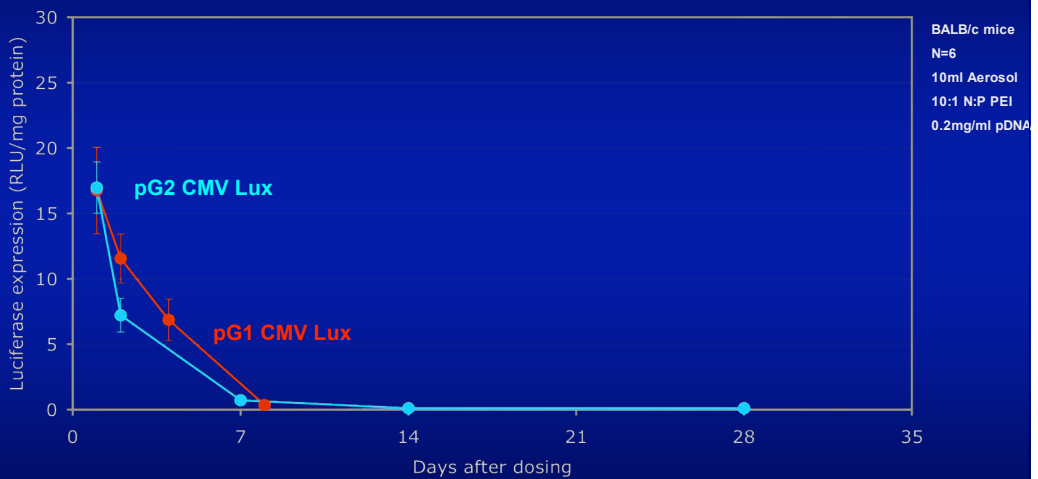
pG2 CMV Lux  
193 CpGs  
(40% reduction)

pG2 ΔCpG CMV Lux  
146 CpGs  
(55% reduction)

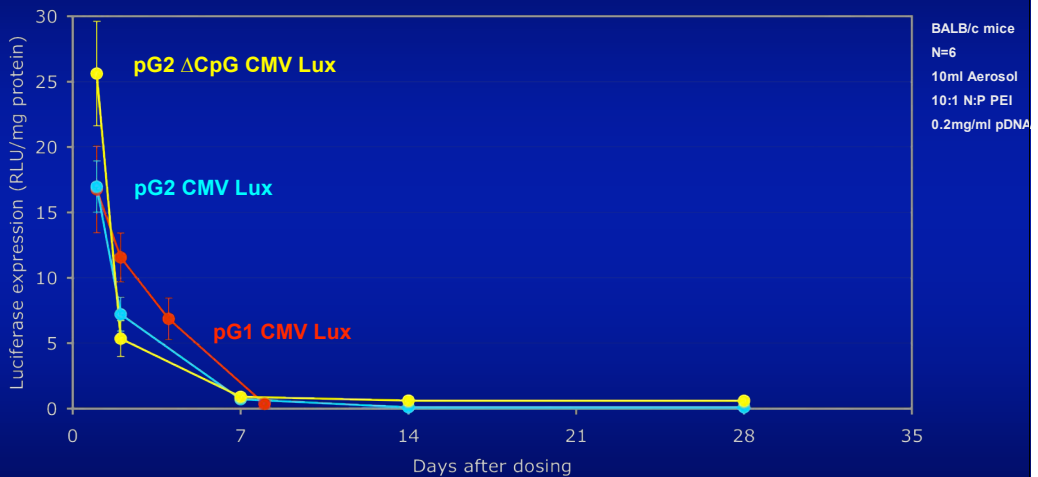
## CMV mediated lung gene expression



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## CMV mediated lung gene expression

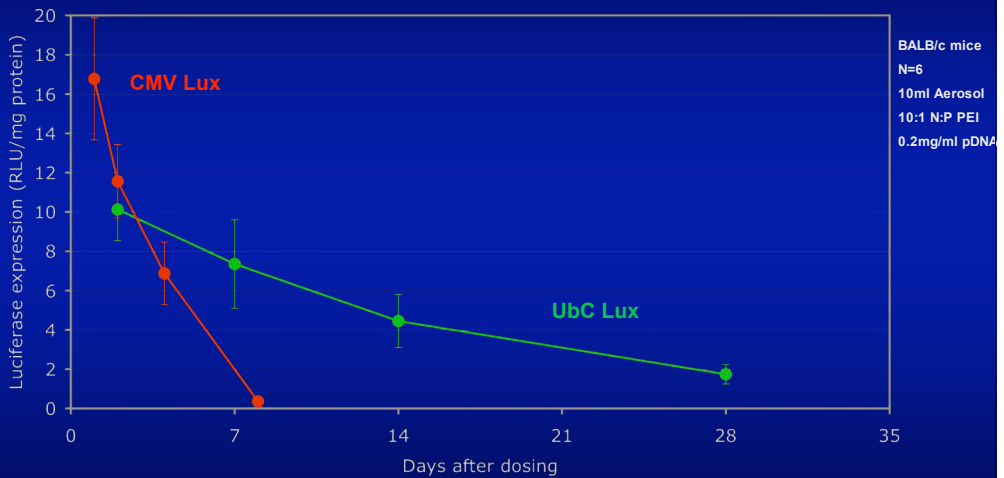


•CpG reduction - no increase in persistence of expression with CMV

## Choice of promoter

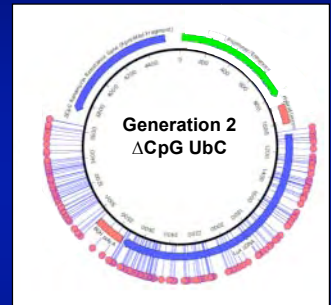
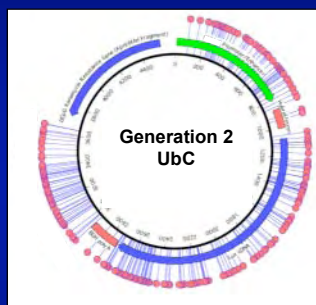
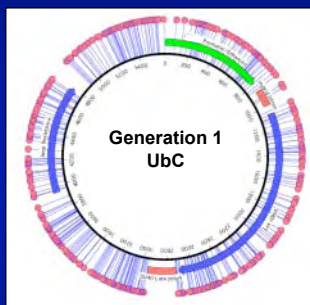
- Alternative strategy - proven successful with naked pDNA (Gill *et al.* Gene Therapy 2001)
- Remove strong viral promoter
- Human housekeeping gene promoter - Polyubiquitin C (UbC)

## Aerosol administration of UbC plasmids



•UbC promoter mediates persistent low level lung gene expression

## CpG Reduced UbC plasmids



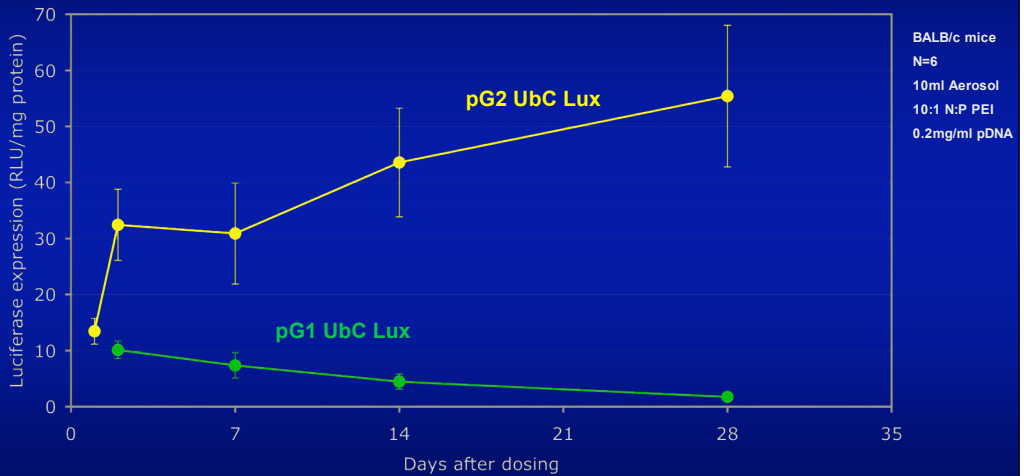
pDNA Backbone is Minimal CpG pOri-K-syn From Genzyme Yew *Mol Ther* 1:255 (2000)

pG1 UbC Lux  
369 CpGs

pG2 UbC Lux  
245 CpGs  
(33% reduction)

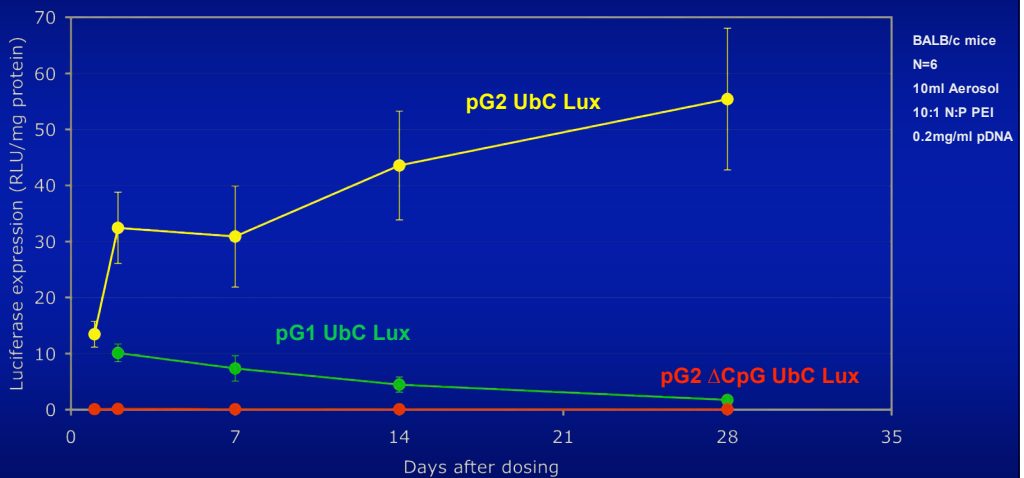
pG2 ΔCpG UbC Lux  
153 CpGs  
(60% reduction)

## UbC mediated lung gene expression



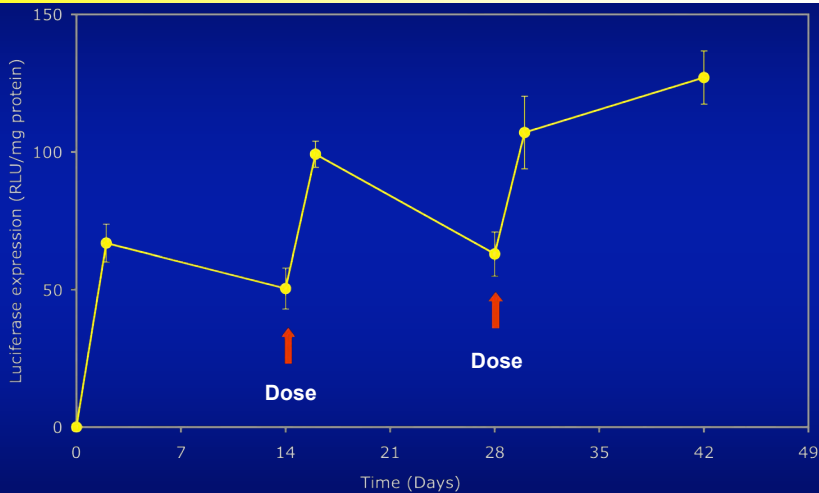
•UbC promoter and CpG reduction - increased level and duration of expression

## UbC mediated lung gene expression



•Can we further boost expression?

## Repeat aerosol administration



BALB/c mice  
N=6  
20ml Aerosol  
10:1 N:P PEI  
0.2mg/ml pDNA

- Can repeat administer pDNA/PEI complexes
- Can be used to boost expression levels

## Summary

- Mouse aerosol delivery model
  - 25kDa PEI
- Investigated 3 strategies to enhance persistence
  - CpG reduction
  - Human housekeeping gene promoter
  - Combined approach
- Increased both level and duration of lung gene expression



## Acknowledgements

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