

Persistent Gene Expression in the Ovine Lung from a Human Elongation Factor 1-alpha Promoter Plasmid following Non-Viral Gene Delivery



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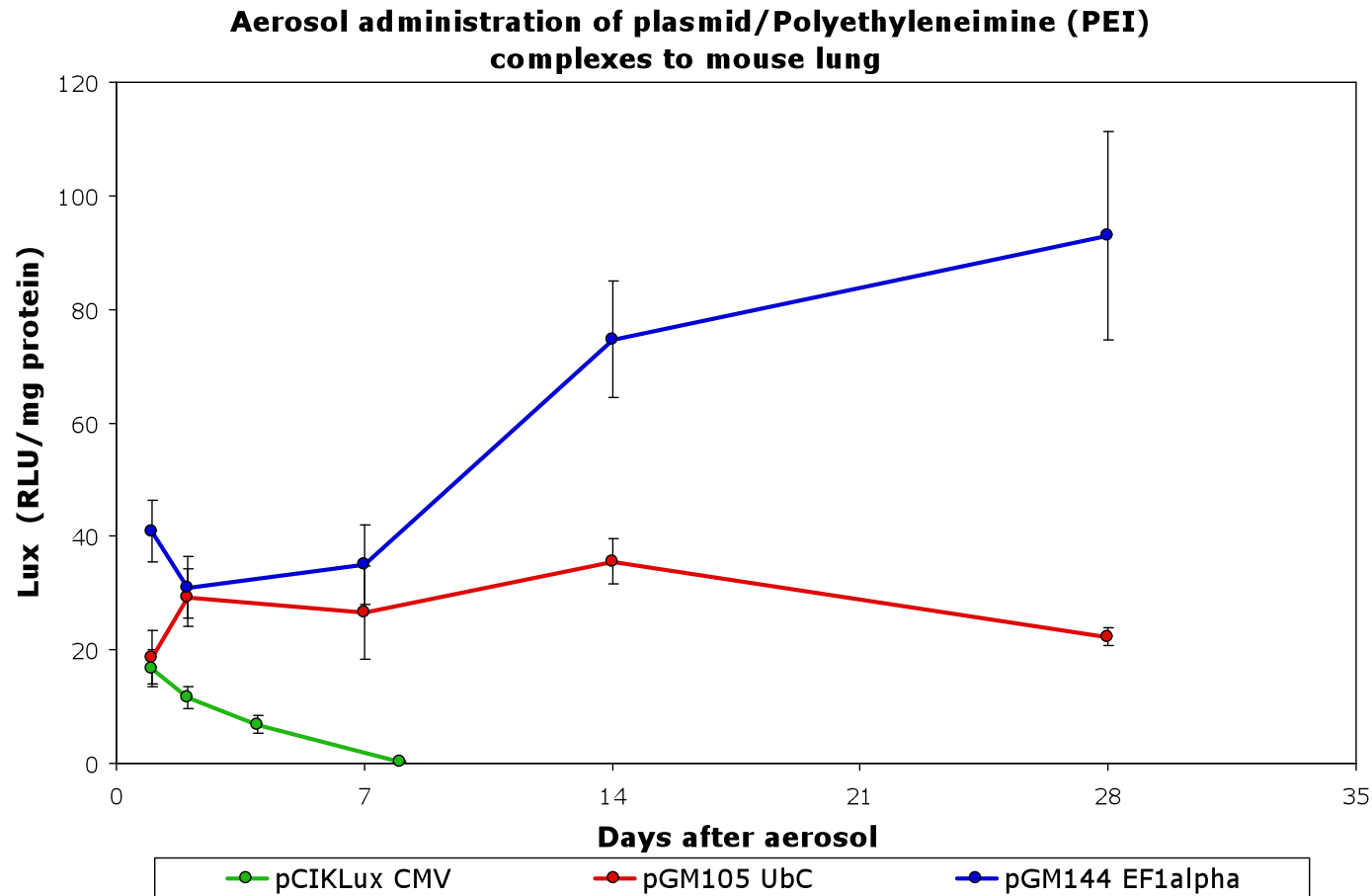
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Cystic fibrosis patients will require sustained expression of CFTR protein

- Epithelial cell turnover – diminish transgene expression over time.
- Repeat Administration
- Maximise the interval between doses by extending duration of expression (cost and practicality)
- Non-viral pDNA/GTAs - transient expression in the lung.
 - Viral promoter sequences
 - Host response to non-methylated CpG motifs.
- Recent studies of aerosol delivery of non-viral GTAs to the mouse lung
 - persistent reporter gene expression can be achieved using mammalian promoter sequences
 - human ubiquitin C (UbC) promoter or human elongation factor 1 (hCEF1) alpha promoter, with reduced or zero CpG content.

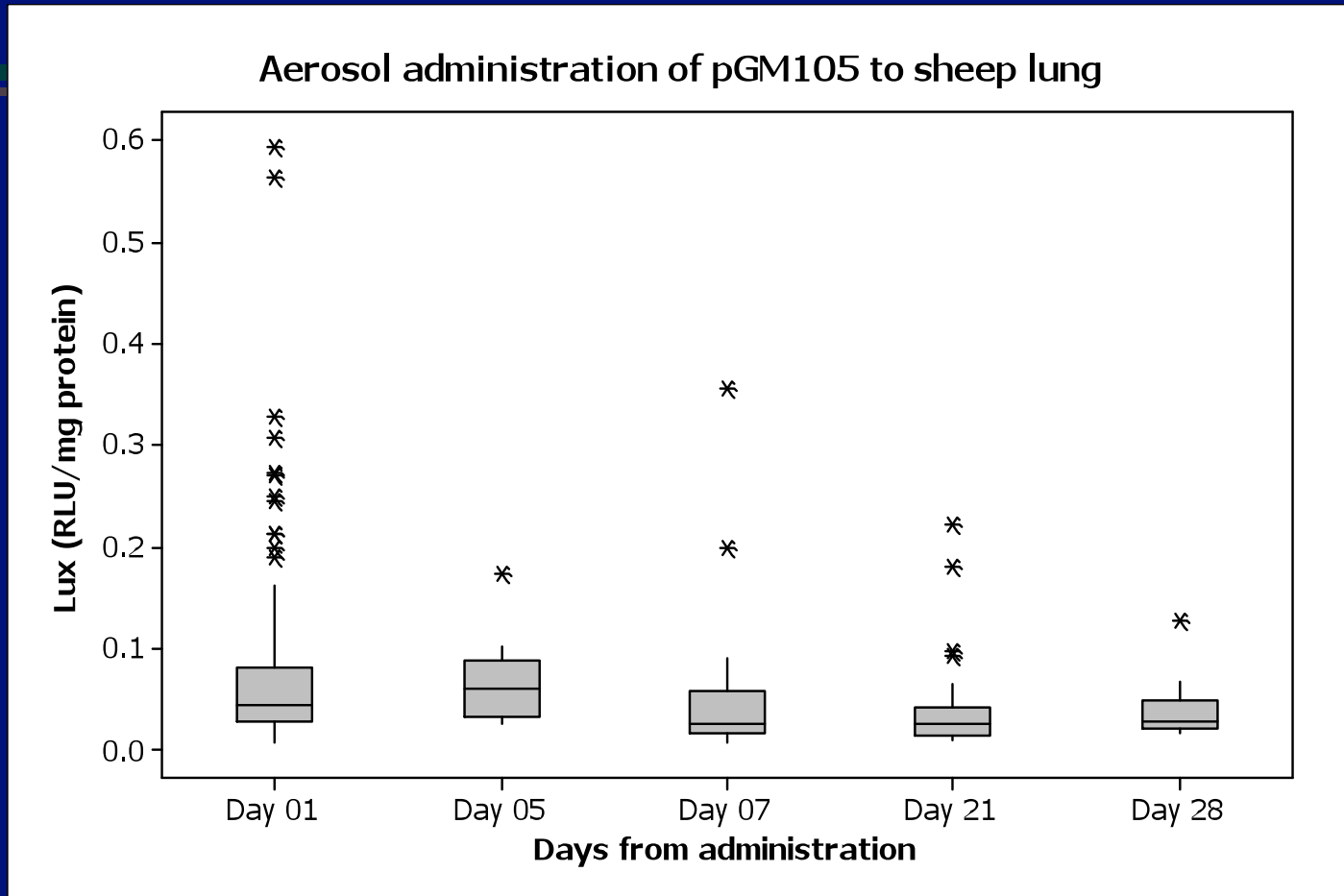
Plasmids driven by polyubiquitin C (pGM105) or human elongation factor 1-alpha (pGM144) promoters direct long lasting expression in mouse lung



Posters
#1008
#1018

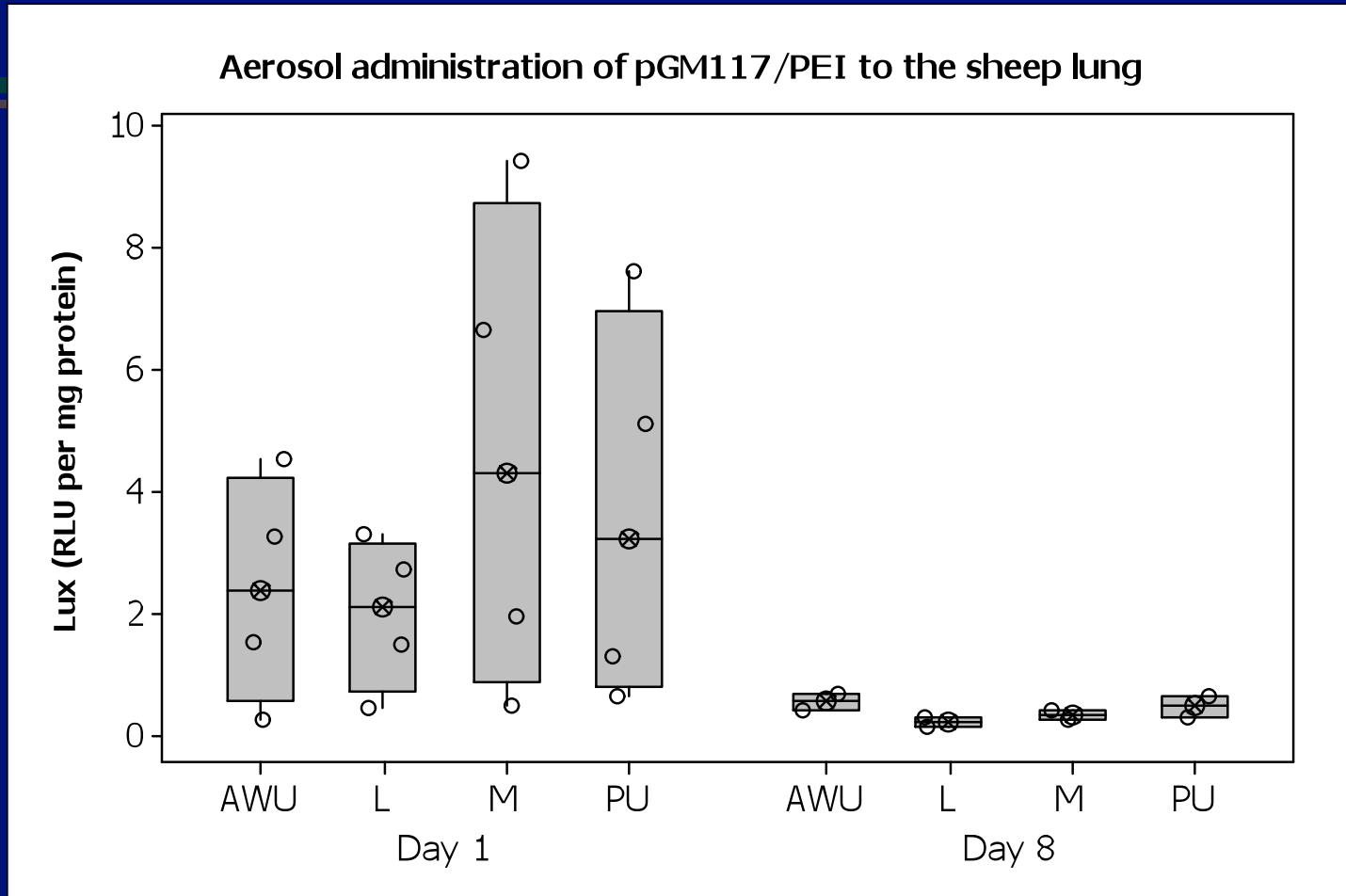
- Aerosol delivery of 10ml pDNA/PEI complexes at a pDNA concentration of 0.2mg/ml using a PARI LC plus nebuliser

UbC promoter plasmid pGM105 directs low and transient expression in sheep lung in vivo



- 13 sheep with pGM105 complexed with PEI. Delivered by PARI LC Plus.
 - 20ml or 80ml volume @ Days 1, 5, 7, 21, 28
 - A single sheep at the highest dose gave positive lux at day 1.

Plasmid pGM117 (UbC promoter/CMV enhancer) directs transient expression in sheep lung in vivo

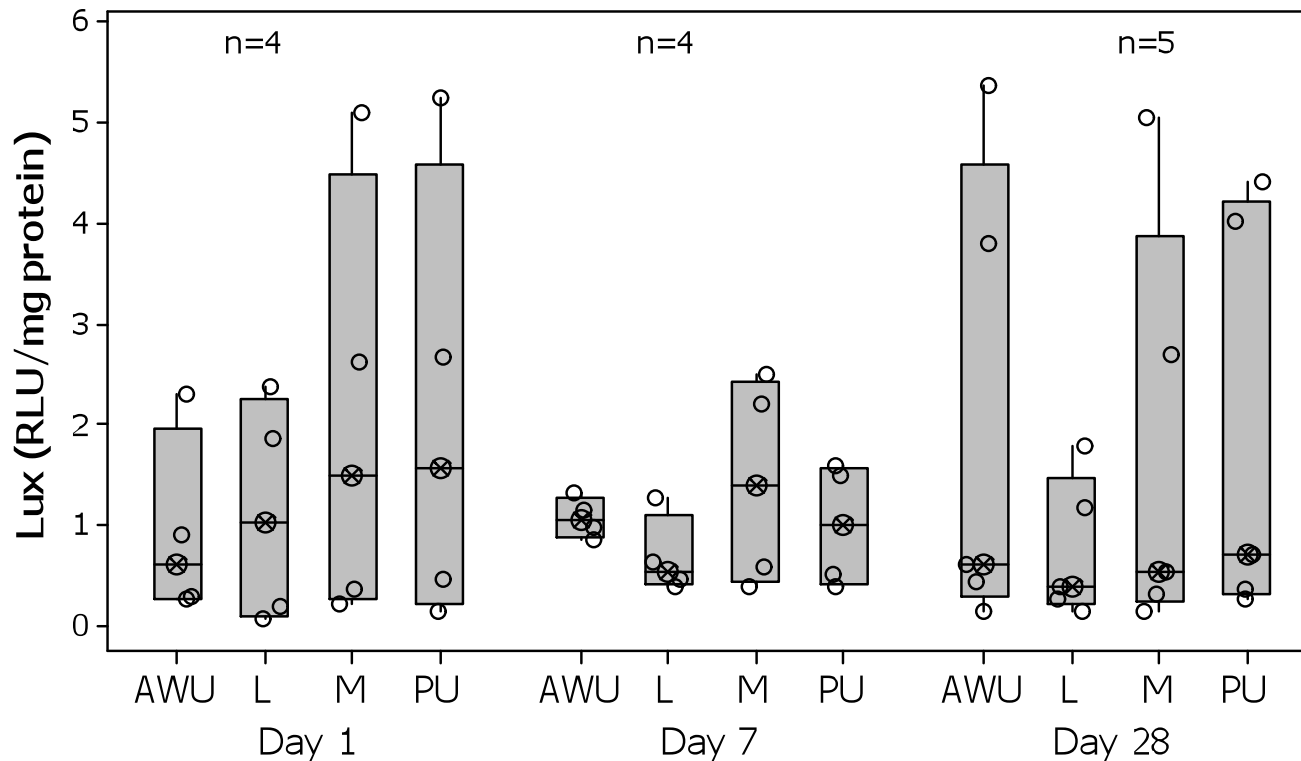


Concentrated pDNA/PEI formulation (1.6mg/ml pDNA) See Poster #1068

Lung segments sliced transversely and divided into AWU- airway upper, PU- parenchyma upper
M-middle and L-lower regions

Plasmid pGM144 (EF1alpha promoter/CMV enhancer) directs persistent expression in sheep lung in vivo

Aerosol administration of pGM144/PEI to the sheep lung.



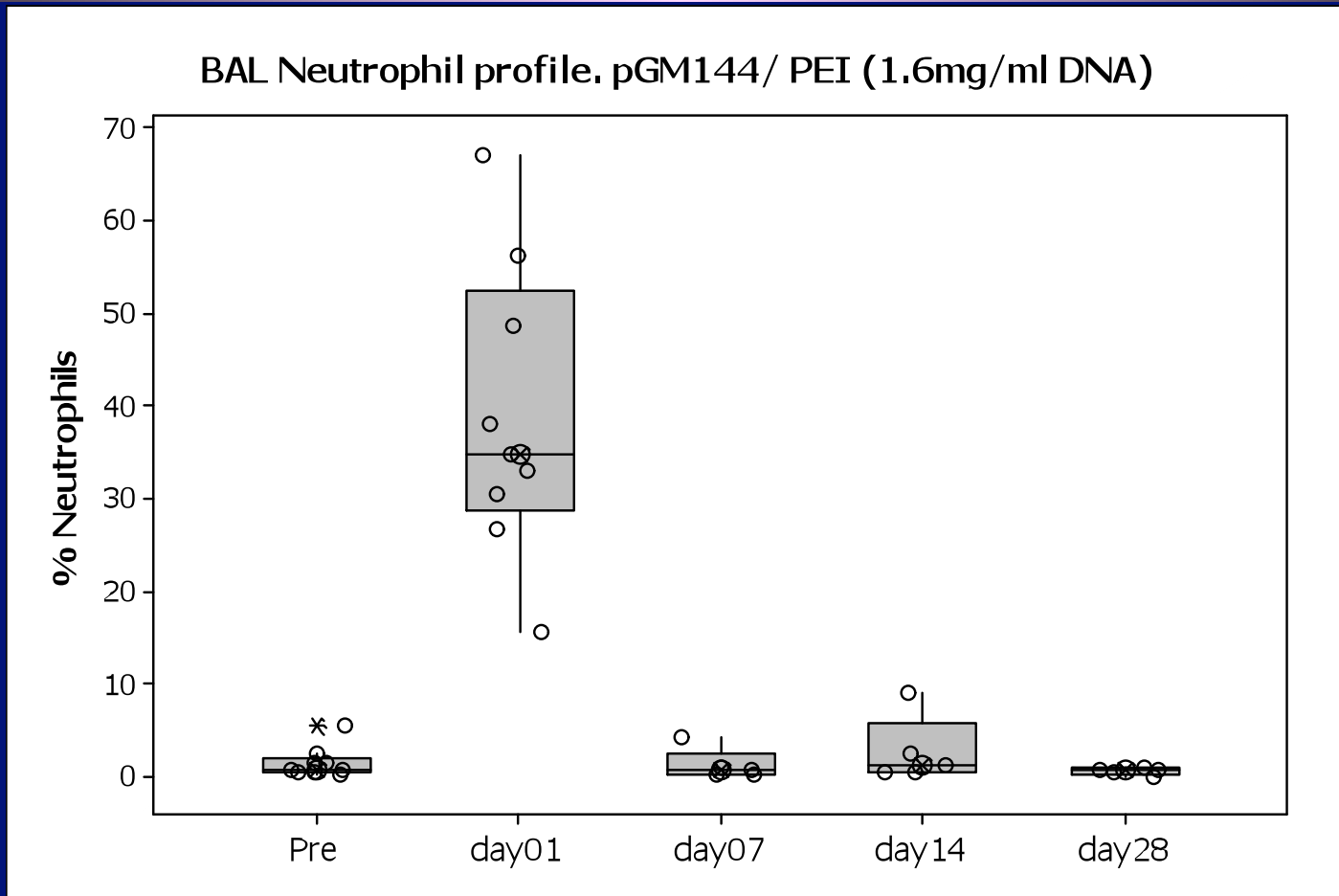
- pGM144. Final conc DNA 1.6mg/ml. 20ml volume delivered by PARI LC Plus.

Removal of CpG motifs from plasmid significantly diminishes inflammatory response following instillation to mouse lung

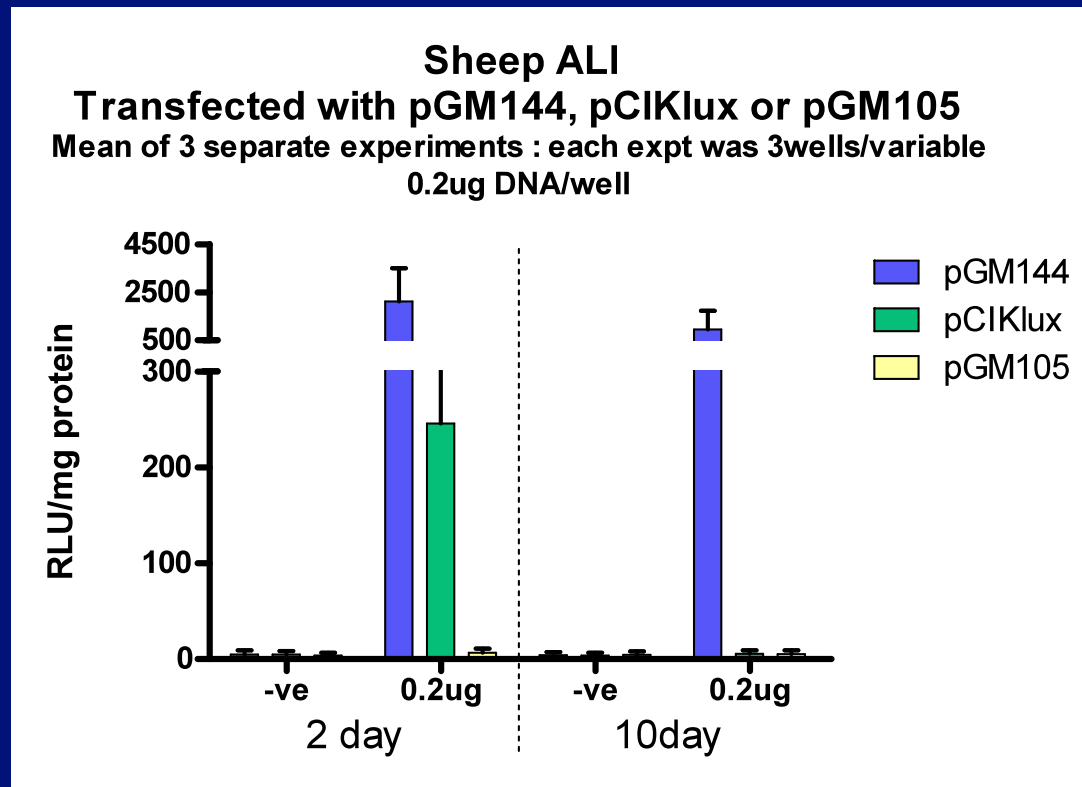
- pDNA/Cationic lipid GL67 instillation
- Mouse BAL Fluid @ 24 hours
 - ↓ TNF alpha
 - ↓ IFN gamma
 - ↓ IL-12
 - ↓ Neutrophil counts

Poster #1015. CpG-dependent inflammatory response after delivery of Lipid/pDNA complexes the mouse lung

Transient increase in bronchoalveolar lavage (BAL) neutrophils remains in absence of CpG motifs



Plasmid expression profile in primary cultures of ovine airway epithelial cells



Cells transfected in suspension then cultured at air-liquid interface (ALI)
Expression profile for each promoter reflects in vivo profile.

Persistent expression of CFTR protein from hEF1alpha promoter in primary airway cells



Cells transfected in suspension then cultured at air-liquid interface (ALI)
Plasmid pGM169. EF1alpha promoter, CMV enhancer CFTR cDNA.

Antibody G449.

Summary

- Sheep studies: Persistent expression from EF1alpha promoter but not UbC
 - Whole lung aerosol in vivo
 - Primary airway cells (ALI culture)
- Product for clinical use
 - Expression beyond 28 days
 - Clinically realistic dosing intervals
- Pilot lung trial for promoter activity in human

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- Deborah Gill
- Lee Davies
- Ian Pringle
- Rebecca Coles

- **LONDON**

- Eric Alton
- Uta Griesenbach
- Jane Davies

UK CFGTC Abstracts

Abs	Title	Authors/Session
1068	Aerosol delivery of concentrated pDNA/PEI formulations to the sheep lung	L. Davies Synthetic Vehicles Sunday 08:30
948	Long-term stability of aqueous pDNA/PEI complexes	G. Nunez-Alonso Poster Session II
1008	Influence of the human and murine CMV enhancer on the duration of expression from CpG-free plasmid vectors in the mouse lung	A-M. Green Poster Session II
1015	CpG-dependent inflammatory response after delivery of Lipid/pDNA complexes the the mouse lung	R. Bazzani Poster Session II
1017	In vivo imaging of Cationic Lipid 67-mediated gene transfer in the mouse lung	U. Griesenbach Poster Session II
1018	Generation of a CpG-free clinical trial plasmid for cystic fibrosis lung gene therapy	I. Pringle Poster Session II