

TOWARDS GENE THERAPY FOR CYSTIC FIBROSIS: BIO-DISTRIBUTION OF GL67A/pGM169 DNA AND mRNA FOLLOWING AEROSOL DELIVERY TO THE MOUSE LUNG



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Overview

The UK CFGTC are undertaking clinical trials of a gene therapy for Cystic Fibrosis (CF) in 2008 and 2009

Clinical studies are planned for aerosol delivery of single and multiple doses of Genzyme Lipid 67 (GL67A) complexed plasmid DNA

Preclinical studies have indicated that this formulation has the following advantages:-

1. CpG-free¹ plasmid reduces the inflammatory responses in-vivo
2. Greatly improved duration of expression
3. Ability to repeat administer formulation
4. Stable aerosol formulation using clinical nebulisers



Aims of this Study

In support of our forthcoming clinical trials we assessed:-

1. The duration of expression from pGM169 in the mouse lung
2. Evidence of aberrant pGM169 in other mouse organs
3. The levels and persistence of pGM169 DNA in the mouse organs

Mouse Aerosol Model

1. 12 Female & 12 Male BALB/c mice (6-8 weeks) placed in aerosol exposure chamber
2. GL67A/pGM169 formulation (60 ml/150 mg pGM169) aerosolised to chamber with a Pari LC+ nebuliser
3. At d1, d14, d28 & d56 post delivery, the internal organs of 3 male and 3 female mice were harvested, taking great care to avoid cross contamination
4. Total DNA and total RNA was extracted from the lungs, liver, gonads and spleen using the Qiagen AllPrep method
5. Levels of pGM169 DNA and mRNA were detected by Taqman RT-PCR

Sample Processing Summary

1. RNA treated with two rounds of DNase treatment during purification
2. RNA concentration determined by RiboGreen assay (Invitrogen) and DNA concentration determined by PicoGreen assay

Mouse Organ	Treated lung	Naive lung	Liver**	Spleen	Testes	Ovaries
% Total DNA Analysed by Taqman	0.004*	2.1	6.5	3.2	3.2	10.8
% Total RNA Analysed by Taqman	4.0	4.0	12	6.0	6.0	20.0

* 1000 fold dilution required before samples were analysed by Taqman for DNA

** Only 100 mg of the tissue processed

Taqman RT-PCR Detection of pGM169 mRNA and DNA



pGM169 mRNA detected by FAM-labelled Taqman Assay (A)

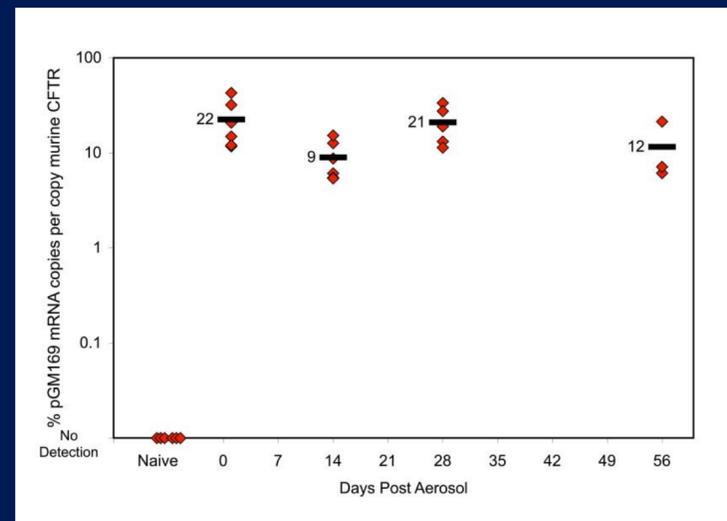
Assay sensitivity around 1-5 copies pGM169 mRNA per reaction

For more details of assay, see poster by Stephanie Sumner-Jones

Copies of pGM169 mRNA normalised against copies of murine CFTR

DNA Assay (B) sensitive to 5 copies of DNA per reaction

Duration of GL67A/pGM169 mRNA Expression in Mouse Lung



High levels of pGM169 mRNA detected in the mouse lung

Mean levels equivalent to 9-22 % endogenous CFTR mRNA levels

No difference between expression at any timepoints (Kruskal Wallis P>0.05)

No difference in expression between male and female mice (not shown)

Bio-Distribution of pGM169 mRNA in Other Mouse Organs

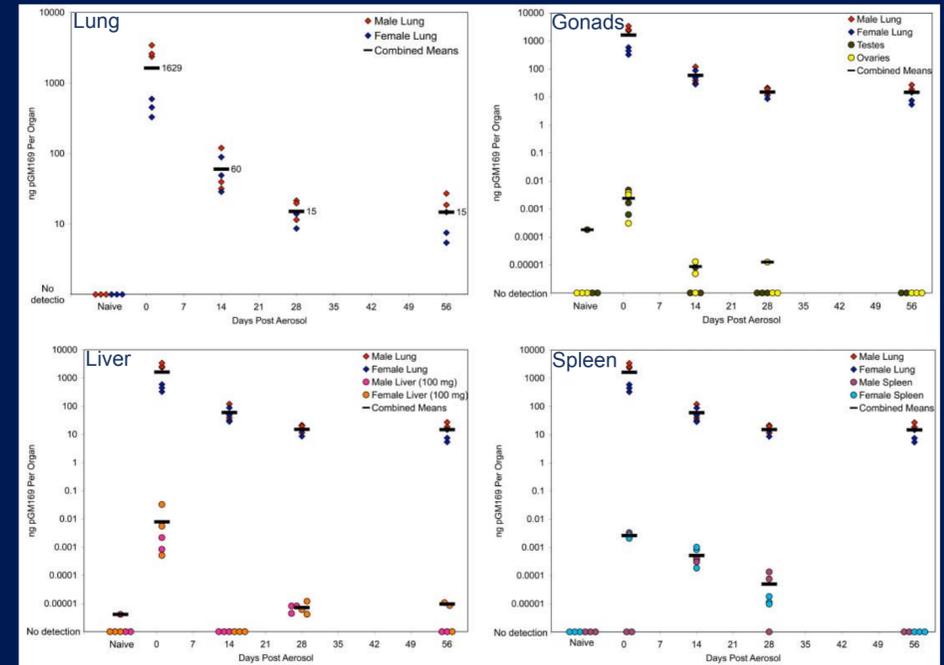
mCFTR expression detected in all other tissues

No detection of pGM169 mRNA in any other tissue at any timepoint

Aerosol delivery restricts GL67A/pGM169 expression to the lung

Bio-Distribution of pGM169 DNA in Mouse Organs

Taqman pGM169 DNA results normalised to ng pGM169 per organ



Lung: High levels of pGM169 DNA detected

Sharp fall between d1 and d28

Gonads: Detection at d1 and d14, six logs lower than lung levels

No detection of pGM169 DNA at d56

Liver: Detection at d1 and d28, 5-7 logs lower than the lung

2 mice positive but at limit of detection at d56

Spleen: Detection d1-d28, 6 logs lower than the lung

Overall, very low levels of pGM169 detected in the non-target organs

Low levels in spleen, liver and gonads may simply be due to contamination from mouse lung or skin during harvesting

No positive detection in the gonads at d56, therefore very low risk of germ line transmission

CONCLUSIONS

1. Long lasting, lung-specific pGM169 gene expression
2. Very high levels of pGM169 DNA in the mouse lung
3. Low levels of pGM169 DNA in other non-target organs
4. No long term persistence of pGM169 DNA in the gonads

1. Utilizing CpG-free plasmid technology (Cayla-InvivoGen, Toulouse, France)

2. pGM169 manufactured by VGX Pharmaceuticals (Houston, TX, USA).