MULTIPLE DOSES OF LIPID-MEDIATED GENE THERAPY NEBULISED TO THE MOUSE LUNG SHOW ROBUST AND SUSTAINED CFTR EXPRESSION

S.C. Hyde[1], S.G. Sumner-Jones[1], I.A. Pringle[1], S.H. Cheng[2], R. Scheule[2], E.W.F.W. Alton[1], J.C. Davies[1],T. Higgins[1], A.C. Boyd[1], J.A. Innes[1], D.J. Porteous[1], D.R. Gill[1] and U. Griesenbach[1]

1. On behalf of The UK Cystic Fibrosis Gene Therapy Consortium; 2. Genzyme Corporation, Cambridge, MA, USA

steve.hyde@ndcls.ox.ac.uk www.genemedresearch.ox.ac.uk www.cfgenetherapy.org.uk Poster download available

INTRODUCTION

- Multidose non-viral gene therapy is being considered for Cystic Fibrosis lung disease
- Such therapy requires CFTR expression that is high-level, sustained and can be repeatedly administered
- CpG-free pGM169 plasmid complexed with cationic liposome GL67A allows CFTR expression lasting several months in the mouse lung after a single nebulised dose
- To progress into multidose studies, a GLP toxicology and biodistribution study in rodents was performed by two CROs
- Biodistribution and duration of CFTR expression in the target organ (lungs) are presented here

METHODS

- Aerosol delivery: after conditioning, mice were exposed to aerosolised pGM169/GL67A at two-weekly intervals by nose-only exposure, for 0.5, 2 or 6 hours (Low, Medium and High dose groups, respectively). Mice exposed to air for 6 hours served as controls. Aerosols were generated with an AeroEclipse II airjet nebuliser which is the intended device for clinical administration.
- Organ harvesting and processing: groups of mice (males & females; n=4-10) were sacrificed after 1, 6 or 12 doses and organs were analysed for plasmid DNA and/or vector-specific CFTR mRNA using quantitative (RT-)PCR. Extraction of DNA and RNA was performed using AllPrep96 (QIAGEN, Crawley, UK) with on-column (QIAGEN) and in-solution (Ambion, Applied Biosystem, Warrington, UK) DNase. DNA from whole blood was extracted using DNeasy® Blood and Tissue Kit.
- Quantitative TaqMan qPCR: methods for (RT-)PCR were essentially as described previously (Rose *et al.*, 2002, Gene Ther., Hyde *et al.*, 2008, Nat. Biotech.) using primer sets specific for transgene-derived CFTR mRNA and endogenous mouse CFTR mRNA, and a separate primer set specific for soCFTR2 cDNA in pGM169 for DNA qPCR. Important changes to the procedures in order to comply with GLP standards included the use of an Internal Positive Control (Applied Biosystems) in each qPCR reaction on pGM169 mRNA and DNA, although this raised the detection limit from 5 copies/qPCR reaction for DNA and 12.5 copies/qPCR reaction for mRNA, to 25 and 62.5, respectively. For non-target organs, up to 750ng total DNA (diluted where necessary) and 0.2-3.6µg total RNA (neat) were analysed per qPCR reaction; for lungs, 10-30pg for day 1 samples and 10pg-1ng for day 56 and 147 DNA samples and 0.25-0.4µg total RNA (neat) were analysed per qPCR reaction.

transgene-derived CFTR mRNA: EFI_soCFTR-13F 5'-TCTCCCTCCTGTGAGTTTGGTT-3'; EFI_soCFTR-51A 5'-6FAM-CTAG-CCACCATGCAGAGAAGCCCTCTG-TAMRA-3'; EFI_soCFTR-98R 5'-GCTCACCACAGAGGCCTTCT-3'; EFI-soCFTR-116R 5'-CCAGCTGAAGAACAGCTTGCT-3' (cDNA synthesis step only)

endogenous murine cftr mRNA: mCFTR-24F 5'-AGCCAGCTTTATCTCCAAACTCTTC-3'; mCFTR-50A 5'-VIC-TCAGCTGGA-GCACAGC-NFQ-3'; mCFTR-91R 5'-GCTGTCTGTACCCTTTCCTCAAA-3' (cDNA synthesis and qPCR steps)

pGM169 DNA: 169_DNA-5095F 5'-GGAACAGCTCCAAGTGCAAGA-3'; 169_DNA-5117A 5'-6FAM-CAAGCCCCAGATTGCTG-CCCTG-TAMRA-3'; 169_DNA-5174R 5'-CCTGGTGTCCTGCACTTCCT-5'

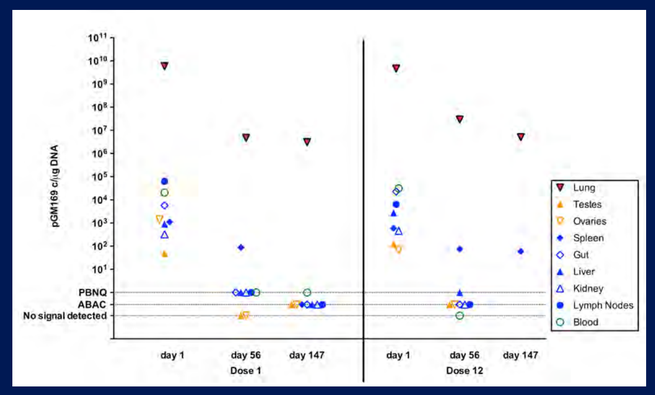
CONCLUSIONS

- These studies further support progression into our planned multidose clinical trial designed to generate clinical benefit in CF patients
- Multiple nebulised doses of pGM169/GL67A result in long-lasting, cumulative CFTR mRNA expression that can increase with successive doses
- This confirms our clinical strategy to deliver multiple doses in order to maximise CFTR expression

RESULTS

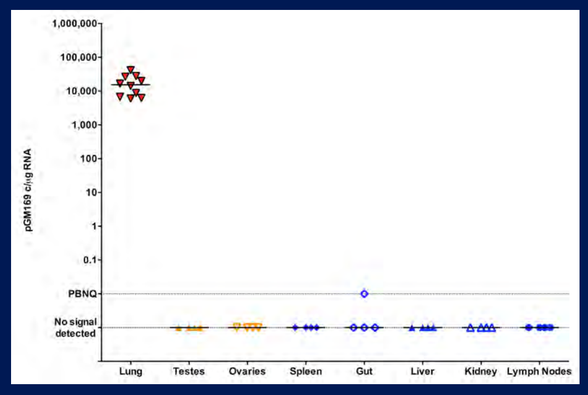
Biodistribution to non-target organs

- Levels of plasmid DNA in non-target organs were several orders of magnitude lower than in the lungs at day 1 and in most cases fell to background levels by day 56 after the first and the twelfth dose. pGM169 DNA remained detectable at very low levels in the spleen of 8 out of 10 mice, at day 147 after the twelfth dose, although it was at background levels at day 147 after a single dose.
- Importantly, when selected RNA samples from organs containing the highest levels of pGM169 DNA (from the Dose 12 day 1 cohort) were analysed for pGM169 mRNA, none had any quantifiable signal.



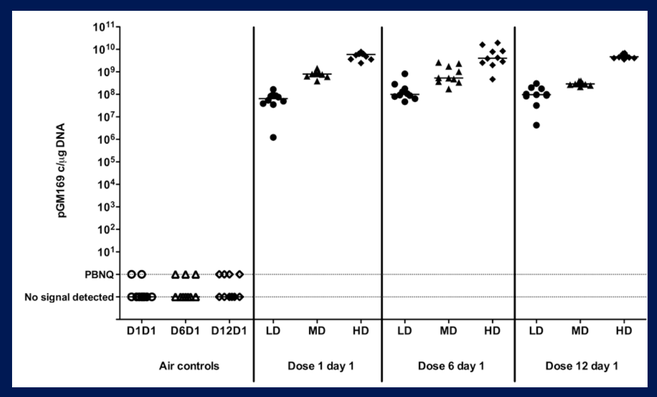
Detection of pGM169 in DNA from organs of mice treated with one dose or twelve doses (6hr each) of pGM169/GL67A, harvested at day 1, 56 or 147. Each symbol represents the group median (n=5/gp for gonads, n=10 for others). PBNQ = positive but not quantifiable pGM169 signal; ABAC = signal at or below air controls.

Detection of pGM169 in RNA from organs of mice treated with twelve doses (6hr each) of pGM169/GL67A, harvested at day 1. Each symbol represents a single mouse. PBNQ = positive but not quantifiable pGM169 signal. The bar indicates the group median.

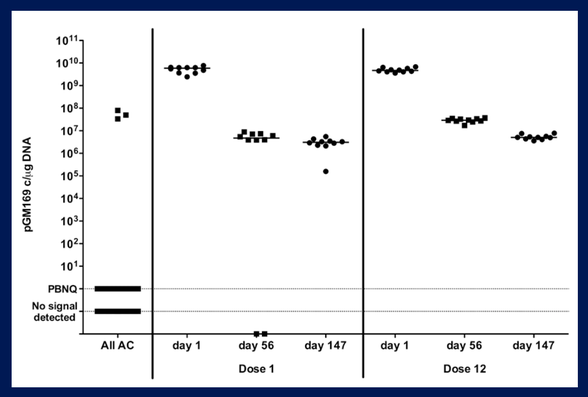


Delivery to target organ

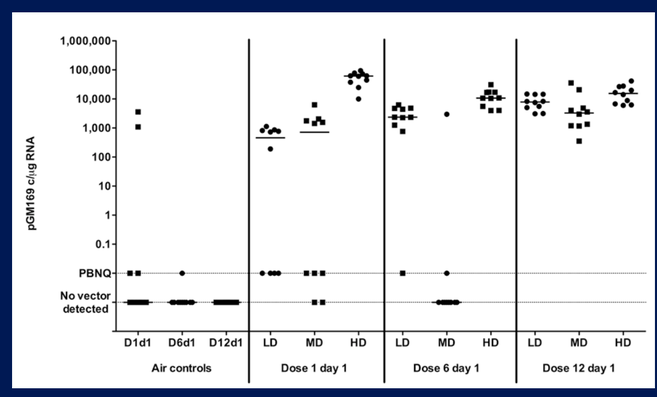
- A significant dose response was observed between duration of inhalation and the quantity of plasmid DNA present in the lungs 1 day after delivery of one, six and twelve doses ($p<0.0001$; Spearman correlation). However, there was no significant accumulation of pGM169 DNA with repeated doses at each level (Low, Medium or High dose).
- Plasmid DNA remained detectable in the lungs of animals for up to 21 weeks after a single dose or after the last of twelve doses.



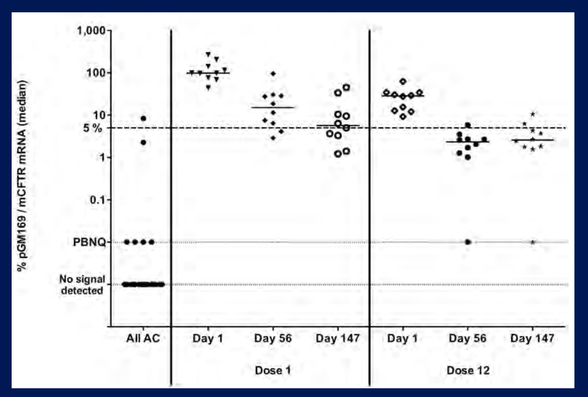
Detection of pGM169 in DNA from lungs of mice treated with one, six or twelve doses of pGM169/GL67A for 0.5 (LD), 2 (MD) or 6 (HD) hours each, and harvested at day 1; and mice treated with one or twelve doses (6hr each) and harvested at day 1, 56 or 147. Each symbol represents a single mouse. PBNQ = positive but not quantifiable pGM169 signal. The bar indicates the group median.



- When CFTR mRNA was measured in the lungs, low levels were detected after a single dose in the Low and Medium groups, with increased signal in the High group ($p<0.001$; equivalent to ≥100% endogenous levels). Importantly, after 12 doses, a cumulative treatment effect was noted such that high mRNA levels were observed for all animals in all treatment groups.
- Robust levels of CFTR mRNA remained in the lung for at least 21 weeks after the final exposure.



Detection of pGM169 in RNA from lungs of mice treated with one, six or twelve doses of pGM169/GL67A for 0.5 (LD), 2 (MD) or 6 (HD) hours each, and harvested at day 1; and mice treated with one or twelve doses (6hr each) and harvested at day 1, 56 or 147. Each symbol represents a single mouse. PBNQ = positive but not quantifiable pGM169 signal. The bar indicates the group median. The dashed line at 5% indicates the target level of correction based on cell mixing experiments.



Produced by Stephanie Sumner-Jones, Gene Medicine Group & The Medical Informatics Unit, NDCLS, University of Oxford, Telephone +44 (0)1865 220545