



THE UNIVERSITY *of* EDINBURGH

## Edinburgh Research Explorer

# A Phase I/IIa Safety and Efficacy Study of Nebulized Liposome-mediated Gene Therapy for Cystic Fibrosis Supports a Multidose Trial

### Citation for published version:

Alton, EFWF, Boyd, AC, Porteous, DJ, Davies, G, Davies, JC, Griesenbach, U, Higgins, TE, Gill, DR, Hyde, SC, Innes, JA & UK Cystic Fibrosis Gene Therapy Consortium \* 2015, 'A Phase I/IIa Safety and Efficacy Study of Nebulized Liposome-mediated Gene Therapy for Cystic Fibrosis Supports a Multidose Trial', *American Journal of Respiratory and Critical Care Medicine*, vol. 192, no. 11, pp. 1389-92. <https://doi.org/10.1164/rccm.201506-1193LE>

### Digital Object Identifier (DOI):

[10.1164/rccm.201506-1193LE](https://doi.org/10.1164/rccm.201506-1193LE)

### Link:

[Link to publication record in Edinburgh Research Explorer](#)

### Document Version:

Peer reviewed version

### Published In:

American Journal of Respiratory and Critical Care Medicine

### General rights

Copyright for the publications made accessible via the Edinburgh Research Explorer is retained by the author(s) and / or other copyright owners and it is a condition of accessing these publications that users recognise and abide by the legal requirements associated with these rights.

### Take down policy

The University of Edinburgh has made every reasonable effort to ensure that Edinburgh Research Explorer content complies with UK legislation. If you believe that the public display of this file breaches copyright please contact [openaccess@ed.ac.uk](mailto:openaccess@ed.ac.uk) providing details, and we will remove access to the work immediately and investigate your claim.



**A Phase I/IIa safety and efficacy study of nebulised liposome-mediated gene therapy for cystic fibrosis supports a multidose trial**

Eric WFW Alton\*, A Christopher Boyd\*, Steve Cunningham\*, Gwyneth Davies\*, Jane C Davies\*, Deborah R Gill\*, Uta Griesenbach\*, Tracy E Higgins\*, Stephen C Hyde\*, J Alastair Innes\*, David J Porteous\*,

On behalf of the UK Cystic Fibrosis Gene Therapy Consortium

\*Senior Authors

**Address correspondence to:** Jane C Davies Department of Gene Therapy, National Heart and Lung Institute, Manresa Rd, London SW3 6LR, UK (Email: [j.davies@imperial.ac.uk](mailto:j.davies@imperial.ac.uk) Tel: 0207-594-7973)

From Royal Brompton and Harefield NHS Foundation Trust, London, UK (AGN, BFK, BL, DMG, DMH, JD, JJ, NJ, SS), Imperial College, London, UK (AB, AS, CJS, CM, EFWFA, FC, FM, GD, GR, JCD, KJB, LH, MC, MCM, MW, NN, NV, NSD, PC, SD, SNS, SS, TB, TEH, UG), Western General Hospital, Edinburgh, UK (HS, PR, AW, NB, MD, APG, JAI), University of Edinburgh, Edinburgh, UK (ACB, JB, HED, AD, JSRG, LH, JP-L, DJP, BJS), Royal Hospital for Sick Children, Edinburgh, UK (SC), Centre for Population Health Sciences, University of Edinburgh, UK (GDM), NDCLS, Radcliffe Department of Medicine, University of Oxford, UK (LAD, DM, DRG, SCH, IAP, SGSJ ), Gene Therapy Program, Department of Pathology and Laboratory Medicine, University of Pennsylvania, Pennsylvania, USA (RC, MPL, JMW), Genzyme, a Sanofi Company, Framingham, USA (SHC,RKS,PW-H), NHS Blood & Transplant, Bristol, UK (PL-E,KS), and The Roslin Institute & R(D)SVS, University of Edinburgh, UK (DDSC, GM)

## **Author contributions**

Conception and design of programme: ACB, APG, DRG, DJP, EFWFA, GD, JAI, JCD, SC, SCH, TEH, UG

Design of formulation: ACB, DRG, DJP, EFWFA, GD, IAP, JAI, JCD, LAD, PW-H, RKS, SC, SCH, SGS-J SHC TEH, UG

Preclinical studies: ACB, DDSC, GM, UG

Recruitment and performance of study related procedures: AS, BFK CJS, FC, FM, GD, GR, JCD, KJB, KS NJ, NV, NSD, PC, PL-E, SD, TB

Sample, imaging, respiratory physiological and electrophysiological test analysis: AB, ACB, AD, AS, AGN, APG, AW, BJS, CJS, CM, DJP, DMH, EFWFA, FC, FM, GD, GR, HED, HS, JAI, JB, JCD, JD, JJ, JMW, JP-L, JSRG, KJB, LH, LH, MC, MCM, MD, MPL, MW, NB, NN, NV, NSD, PC, PR, RC, SD, SGS-J, SNS, SS, SS, TB, TEH, UG

Analysis and interpretation of trial data: DA, EFWFA, ACB, SC, GD, GM, JCD, DG, DMG, UG, SH, DP, TEH, BL, MY-L

Writing of manuscript: All authors

Senior authors: ACB, DRG, DJP, EFWFA, GD, JAI, JCD, SC, SCH, TEH, UG

The research was funded by the UK Cystic Fibrosis Trust and supported by the National Institute for Health Research (NIHR) Respiratory Biomedical Research Unit at the Royal Brompton and Harefield NHS Foundation Trust and Imperial College London and the Biomedical Research Centre based at Imperial College Healthcare NHS Trust and Imperial College London.

**Running Title:** Single dose gene therapy for CF

The vast majority of treatments for cystic fibrosis (CF) target the downstream consequences of the disease and are incompletely effective. The success of the cystic fibrosis transmembrane conductance regulator (CFTR) potentiator, ivacaftor, has illustrated the clinical benefits arising from restoration of CFTR protein function (1). This agent is applicable as a monotherapy for a minority of patients with specific, rare mutations. *CFTR* gene therapy, a mutation-independent alternative, has demonstrated proof-of-principle for gene transfer in animal models and human trials, but only one study (using a viral vector) has unsuccessfully assessed whether clinical outcomes can be improved (2). In preparation for a Phase IIb clinical trial of repeatedly administered, non-viral, liposome-mediated *CFTR* gene transfer assessing clinically-meaningful outcomes (3), the UK CF Gene Therapy Consortium ([www.cfgenetherapy.org.uk](http://www.cfgenetherapy.org.uk)) undertook a single application, safety and dose ranging study (NCT00789867

Some of the results of these studies have been previously reported in the form of abstracts. (4, 5)

The chosen plasmid DNA expresses CFTR under the control of the hCEFI sequence (6), a modified EF1a promoter aiming for extended duration of expression (7) and was rendered CpG-free to minimise a host inflammatory response (6). The cationic lipid, GL67A, was chosen on the basis of extensive preclinical testing (8). Following informed consent, adult CF subjects received a single nebulised +/- nasal dose of pGM169/GL67A. Reconstitution and preparation of pGM169/GL67A was undertaken on the day and doses delivered in sealed negative-pressure cubicles following pre-treatment with inhaled salbutamol (albuterol). Pre-planned adjunctive therapies including ibuprofen, prednisolone or paracetamol were administered to some patients.

The primary outcome of the clinical study was safety; assessment included examination, standard haematology/biochemistry, adverse events, spirometry, lung clearance index, chest CT, gas transfer, bronchial biopsy histology and immune markers. pGM169-specific DNA and mRNA were measured on nasal and lower airway brushings, with potential difference also measured bronchoscopically and nasally. For the latter, 'responders' were defined as demonstrating chloride secretion  $\geq 5$  mV more than their mean pre-dose value and greater than any of their pre-dose responses.

A total of 35 subjects (Table 1) received a nebulised dose (5 ml n=8, 10 ml n=10, 20 ml n=17) via an AeroEclipse II (Trudell) breath-actuated nebuliser (9). Three subjects undertook slow delivery (~ 75 min versus 25 min for each 5 ml). Standard spray devices were used for nasal delivery (2 ml, n=21). Based on pre/post device weighing, 88.7 (2.9)% of expected nebulised dose and 94.5 (15.0)% of expected nasal dose was delivered. There were two serious adverse events (SAEs): one occurred following the pre-dosing bronchoscopy (swelling of the uvula related to intubation) and led to observation overnight in hospital; the other was an episode of pancreatitis occurring around day 10 post dosing (10 ml nebulised cohort). The subject was exocrine pancreatic sufficient and had likely experienced previous similar, but undiagnosed episodes.

Overall, in the trial, 94.3% of subjects experienced at least one adverse event (AE), the majority of which were mild to moderate in severity and resolved spontaneously, or with standard antipyretics. The commonest occurred on the day of dosing and largely resolved within 24-48 hours (Table 1): Typically, within the first few hours post-dosing, a mild, self-limiting flu-like systemic response was seen, most frequently in the 20 ml patients. This was not affected by slow delivery or co-administration of ibuprofen or prednisolone, but was clearly dose-related and reduced by paracetamol. Symptoms of headache and/ or tiredness were reported by 82%, 70% and 13%, and raised serum inflammatory markers recorded in 100%, 60% and 63% of the 20, 10 and 5 ml groups respectively, with dose related trends in maximal values. No patient dosed with 5 ml had a temperature >38°C (Table 1). A relatively asymptomatic, dose-related, restrictive drop in spirometry was also observed, with no change in respiratory rate or oxygen saturation. No patient dosed with 5 ml showed a >20% relative fall in FEV<sub>1</sub> (Table 1). The 20 ml group showed a small, significant (p<0.05) mean (SD) drop in gas transfer (transfer factor for carbon monoxide corrected for alveolar volume and haemoglobin concentration (KCOc)) on day 2 of 4.5(6.0)% which had returned to baseline values by day 14. No changes were seen in the other cohorts. Two of the 20 ml patients had small areas of ground glass opacity reported on their day 2 chest CT scans, which had resolved by day 14. No significant changes were seen in endobronchial histology (20 ml; n=10).

Consistent with the proposed excretion route for lipids, small but significant serum creatinine rises *within* the normal range could be detected 8 hours post dosing in the 20 and

10 ml group but not the 5 ml cohort; there were no other biochemical changes. Bilirubin rose on day 1 in all dosing groups, as with creatinine remaining within the normal range, and normalised by day 2. There was no evidence of immune responses based on double-stranded DNA antibodies or human CFTR-specific T cells.

Lung clearance index (LCI), a sensitive marker of pulmonary dysfunction (10) was included as a safety assay. Fourteen 20 ml patients with paired pre and 28 days post-dosing values, showed a small, but significant increase (ie a deterioration; Fig1a). In contrast, and unexpectedly, on post hoc analysis, 11/14 patients in the lower dosing groups (5 and 10 ml) showed a small but significant improvement (Fig 1a).

With respect to bronchial samples, ten patients (all 20 ml) had paired pre- and post-dosing bronchoscopies. pGM169-specific DNA was detected in all bronchial brushing samples at levels ~x1000-fold higher than in the nasal samples. pGM169-specific mRNA was detected in 2/10 post-dosing samples. Paired bronchoscopic potential difference (PD) measurements were interpretable on 8/10 patients. There was a trend towards an increase in chloride secretion (Fig 1b), but no changes in sodium-related parameters.

With respect to nasal samples, pGM169-specific DNA was detected in all 15 brushing samples taken between day 2 and day 14 post-dosing and in 2/6 samples at day 28. pGM169-specific mRNA was detected in 3/21 post-dosing samples, all positive samples being observed at either day 14 (n=2) or 28 (n=1). In keeping with previous published data, there were no changes in sodium parameters on nasal PD. In contrast, 6/16 subjects (37.5%) demonstrated a 'response' in terms of chloride secretory capacity. Responses were seen most commonly in the zero chloride perfusion phase and at the 14 day time point; they were of sustained duration in one subject. (Fig 1c).

These data were important in informing the design of the Phase IIb trial. Thus, based on these findings, 5 ml was selected as the optimal dose with paracetamol being used as an adjuvant to minimise the risk of unblinding. Although well-tolerated, the side effects of the 20 ml doses were considered prohibitive for use in a repeated administration trial. We consider that the efficient delivery of large volumes of viscous fluid into the airways led acutely to both the flu-like and restrictive responses, analogous to those seen post-bronchoalveolar lavage, and masking the effect of plasmid DNA CpG depletion. At lower

volumes, the latter effect was 'revealed', allowing safe dosing of 5 ml. The unexpected improvement in LCI after only one administration at the lower doses was intriguing; larger numbers and longer follow-up are needed to confirm or refute this finding. The variable responses both in molecular and CFTR functional terms underscore the technical challenges inherent in these assays and the limited sensitivity to low levels of gene expression (11). The clean safety profile and encouraging improvements in a sensitive measure of airway health, lend support to progression to a Phase IIb, multidose trial designed to detect clinical improvements following prolonged administration.



## References

1. Ramsey BW, Davies J, McElvaney NG, Tullis E, Bell SC, Dřevínek P, Griese M, McKone EF, Wainwright CE, Konstan MW, Moss R, Ratjen F, Sermet-Gaudelus I, Rowe SM, Dong Q, Rodriguez S, Yen K, Ordoñez C, Elborn JS; VX08-770-102 Study Group. A CFTR potentiator in patients with cystic fibrosis and the G551D mutation. *N Engl J Med.* 2011;365:1663-72.
2. Griesenbach U, Alton EW; UK Cystic Fibrosis Gene Therapy Consortium. Gene transfer to the lung: lessons learned from more than 2 decades of CF gene therapy. *Adv Drug Deliv Rev.* 2009 27;61:128-39.
3. Alton EW, Armstrong DK, Ashby D, Bayfield KJ, Bilton D, Bloomfield EV, Boyd AC, Brand J, Buchan R, Calcedo R, Carvelli P, Chan M, Cheng SH, Collie DD, Cunningham S, Davidson HE, Davies G, Davies JC, Davies LA, Dewar MH, Doherty A, Donovan J, Dwyer NS, Elgmati HI, Featherstone RF, Gavino J, Gea-Sorli S, Geddes DM, Gibson JS, Gill DR, Greening AP, Griesenbach U, Hansell DM, Harman K, Higgins TE, Hodges SL, Hyde SC, Hyndman L, Innes JA, Jacob J, Jones N, Keogh BF, Limberis MP, Lloyd-Evans P, Maclean AW, Manvell MC, McCormick D, McGovern M, McLachlan G, Meng C, Montero MA, Milligan H, Moyce LJ, Murray GD, Nicholson AG, Osadolor T, Parra-Leiton J, Porteous DJ, Pringle IA, Punch EK, Pytel KM, Quittner AL, Rivellini G, Saunders CJ, Scheule RK, Sheard S, Simmonds NJ, Smith K, Smith SN, Soussi N, Soussi S, Spearing EJ, Stevenson BJ, Sumner-Jones SG, Turkkila M, Ureta RP, Waller MD, Wasowicz MY, Wilson JM, and Wolstenholme-Hogg P. Repeated nebulisation of non-viral CFTR gene therapy in patients with cystic fibrosis: a randomised, double-blind, placebo-controlled, phase 2b trial. *Lancet Respir. Med.* 2015.
4. Davies JC, Gill D, Griesenbach U, Voase N, Davies G, Higgins T, Innes JA, Boyd C, Porteous D, Hyde S, Alton EFW. Evaluation of safety and gene expression with single dose of pGM169/GL67A administered to the nose and lung of individuals with CF: The UL CF Gene Therapy Consortium Pilot Study. *Pediatric Pulmonology Supplement* 32, 2009 (Abstract 268)
5. Davies JC, Gill D, Griesenbach U, Voase N, Davies G, Higgins T, Innes JA, Boyd C, Porteous D, Hyde S, Alton EFW. Evaluation of safety and gene expression with single dose of pGM169/GL67A administered to the nose and lung of individuals with CF: The UK CF Gene Therapy Consortium Pilot Study. *Thorax* 2009;64:S141

6. Hyde SC, Pringle IA, Abdullah S, Lawton AE, Davies LA, Varathalingam A, Nunez-Alonso G, Green AM, Bazzani RP, Sumner-Jones SG, Chan M, Li H, Yew NS, Cheng SH, Boyd AC, Davies JC, Griesenbach U, Porteous DJ, Sheppard DN, Munkonge FM, Alton EW, Gill DR. CpG-free plasmids confer reduced inflammation and sustained pulmonary gene expression. *Nat Biotechnol.* 2008 May;26(5):549-51.
7. Gill DR, Smyth SE, Goddard CA, Pringle IA, Higgins CF, Colledge WH, Hyde SC. Increased persistence of lung gene expression using plasmids containing the ubiquitin C or elongation factor 1 $\alpha$  promoter. *Gene Ther.* 2001;8:1539-46.
8. McLachlan G, Davidson H, Holder E, Davies LA, Pringle IA, Sumner-Jones SG, Baker A, Tennant P, Gordon C, Vrettou C, Blundell R, Hyndman L, Stevenson B, Wilson A, Doherty A, Shaw DJ, Coles RL, Painter H, Cheng SH, Scheule RK, Davies JC, Innes JA, Hyde SC, Griesenbach U, Alton EW, Boyd AC, Porteous DJ, Gill DR, Collie DD. Pre-clinical evaluation of three non-viral gene transfer agents for cystic fibrosis after aerosol delivery to the ovine lung. *Gene Ther.* 2011;18:996-1005.
9. Davies LA, Nunez-Alonso GA, McLachlan G, Hyde SC, Gill DR. Aerosol delivery of DNA/liposomes to the lung for cystic fibrosis gene therapy. *Hum Gene Ther Clin Dev.* 2014;25:97-107.
10. Kent L, Reix P, Innes JA, Zielen S, Le Bourgeois M, Braggion C, Lever S, Arets HG, Brownlee K, Bradley JM, Bayfield K, O'Neill K, Savi D, Bilton D, Lindblad A, Davies JC, Sermet I, De Boeck K; European Cystic Fibrosis Society Clinical Trial Network (ECFS-CTN) Standardisation Committee. Lung clearance index: evidence for use in clinical trials in cystic fibrosis. *J Cyst Fibros.* 2014;13:123-38.
11. Rose AC, Goddard CA, Colledge WH, Cheng SH, Gill DR, Hyde SC. Optimisation of real-time quantitative RT-PCR for the evaluation of non-viral mediated gene transfer to the airways. *Gene Ther.* 2002;9:1312-20.