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EFFICACY AND MECHANISM EVALUATION

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on behalf of the UK Cystic Fibrosis Gene Therapy Consortium

NHS
National Institute for Health Research

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This report

Abstract

A randomised, double-blind, placebo-controlled trial of repeated nebulisation of non-viral cystic fibrosis transmembrane conductance regulator (*CFTR*) gene therapy in patients with cystic fibrosis

Eric WFW Alton,[1][*][†] David K Armstrong,[2] Deborah Ashby,[1] Katie J Bayfield,[1] Diana Bilton,[1,3] Emily V Bloomfield,[1] A Christopher Boyd,[2†] June Brand,[2] Ruaridh Buchan,[4] Roberto Calcedo,[5] Paula Carvelli,[1] Mario Chan,[1] Seng H Cheng,[6] David S Collie,[7] Steve Cunningham,[8†] Heather E Davidson,[2] Gwyneth Davies,[1] Jane C Davies,[1,3†] Lee A Davies,[9] Maria H Dewar,[4] Ann Doherty,[2] Jackie Donovan,[1,3] Natalie S Dwyer,[1] Hala I Elgmati,[2] Rosanna F Featherstone,[1] Jemyr Gavino,[1] Sabrina Gea-Sorli,[1] Duncan M Geddes,[1,3] James SR Gibson,[2] Deborah R Gill,[9†] Andrew P Greening,[4] Uta Griesenbach,[1†] David M Hansell,[1,3] Katharine Harman,[1] Tracy E Higgins,[1†] Samantha L Hodges,[1] Stephen C Hyde,[9†] Laura Hyndman,[2] J Alastair Innes,[4†] Joseph Jacob,[1,3] Nancy Jones,[1,3] Brian F Keogh,[1,3] Maria P Limberis,[5] Paul Lloyd-Evans,[10] Alan W Maclean,[2] Michelle C Manvell,[1] Dominique McCormick,[9] Michael McGovern,[2] Gerry McLachlan,[7] Cuixiang Meng,[1] M Angeles Montero,[1,3] Hazel Milligan,[4] Laura J Moyce,[9] Gordon D Murray,[11†] Andrew G Nicholson,[1,3] Tina Osadolor,[1,3] Javier Parra-Leiton,[2] David J Porteous,[2†] Ian A Pringle,[9] Emma K Punch,[1] Kamila M Pytel,[1] Alexandra L Quittner,[12] Gina Rivellini,[1] Clare J Saunders,[1] Ronald K Scheule,[6] Sarah Sheard,[1,3] Nicholas J Simmonds,[1,3] Keith Smith,[10] Stephen N Smith,[1] Najwa Soussi,[1] Samia Soussi,[1] Emma J Spearing,[1] Barbara J Stevenson,[2] Stephanie G Sumner-Jones,[9] Minna Turkkila,[1] Rosa P Ureta,[1] Michael D Waller,[1] Marguerite Y Wasowicz,[1] James M Wilson[5] and Paul Wolstenholme-Hogg[6] on behalf of the UK Cystic Fibrosis Gene Therapy Consortium

ABSTRACT

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Background: Cystic fibrosis (CF) is a chronic, life-limiting disease caused by mutations in the CF transmembrane conductance regulator (*CFTR*) gene leading to abnormal airway surface ion transport, chronic lung infections, inflammation and eventual respiratory failure. With the exception of the small-molecule potentiator, ivacaftor (Kalydeco®, Vertex Pharmaceuticals, Boston, MA, USA), which is suitable for a small proportion of patients, there are no licensed therapies targeting the basic defect. The UK Cystic Fibrosis Gene Therapy Consortium has taken a cationic lipid-mediated *CFTR* gene therapy formulation through preclinical and clinical development.

Objective: To determine clinical efficacy of the formulation delivered to the airways over a period of 1 year in patients with CF.

Design: This was a randomised, double-blind, placebo-controlled Phase IIb trial of the *CFTR* gene–liposome complex pGM169/GL67A. Randomisation was performed via InForm™ version 4.6 (Phase Forward Incorporated, Oracle, CA, USA) and was 1 : 1, except for patients in the mechanistic subgroups (2 : 1). Allocation was blinded by masking nebuliser chambers.

Settings: Data were collected in the clinical and scientific sites and entered onto a trial-specific InForm, version 4.6 database.

Participants: Patients with CF aged ≥ 12 years with forced expiratory volume in the first second (FEV_1) between 50% and 90% predicted and any combination of *CFTR* mutations. The per-protocol group (≥ 9 doses) consisted of 54 patients receiving placebo (62 randomised) and 62 patients receiving gene therapy (78 randomised).

Interventions: Subjects received 5 ml of nebulised pGM169/G67A (active) or 0.9% saline (placebo) at 28 ($\pm$5)-day intervals over 1 year.

Main outcome measures: The primary end point was the relative change in percentage predicted FEV_1 over the 12-month period. A number of secondary clinical outcomes were assessed alongside safety measures: other spirometric values; lung clearance index (LCI) assessed by multibreath washout; structural disease on computed tomography (CT) scan; the Cystic Fibrosis Questionnaire – Revised (CFQ-R), a validated quality-of-life questionnaire; exercise capacity and monitoring; systemic and sputum inflammatory markers; and adverse events (AEs). A mechanistic study was performed in a subgroup in whom transgene deoxyribonucleic acid (DNA) and messenger ribonucleic acid (mRNA) was measured alongside nasal and lower airway potential difference.

Results: There was a significant ($p = 0.046$) treatment effect (TE) of 3.7% [95% confidence interval (CI) 0.1% to 7.3%] in the primary end point at 12 months and in secondary end points, including forced vital capacity (FVC) ($p = 0.031$) and CT gas trapping ($p = 0.048$). Other outcomes, although not reaching statistical significance, favoured active treatment. Effects were noted by 1 month and were irrespective of sex, age or *CFTR* mutation class. Subjects with a more severe baseline FEV_1 had a FEV_1 TE of 6.4% (95% CI 0.8% to 12.1%) and greater changes in many other secondary outcomes. However, the more mildly affected group also demonstrated benefits, particularly in small airway disease markers such as LCI. The active group showed a significantly ($p = 0.032$) greater bronchial chloride secretory response. No difference in treatment-attributable AEs was seen between the placebo and active groups.

Conclusions: Monthly application of the pGM169/GL67A gene therapy formulation was associated with an improvement in lung function, other clinically relevant parameters and bronchial CFTR function, compared with placebo.

Limitations: Although encouraging, the improvement in FEV_1 was modest and was not accompanied by detectable improvement in patients' quality of life.

Future work: Future work will focus on attempts to increase efficacy by increasing dose or frequency, the coadministration of a CFTR potentiator, or the use of modified viral vectors capable of repeated administration.

Trial registration: ClinicalTrials.gov NCT01621867.

Funding: This project was funded by the Efficacy and Mechanism Evaluation (EME) programme, a Medical Research Council and National Institute for Health Research partnership.

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List of abbreviations

5PL	five-parameter logistic	DMPE-PEG5000	1,2-dimyristoyl-*sn*-glycero-3-phosphoethanolamine-*n*-[methoxy (polyethylene glycol 5000)] (ammonium salt)
AAV	adeno-associated virus		
AE	adverse event		
ANCOVA	analysis of covariance	DNA	deoxyribonucleic acid
ATS	American Thoracic Society	DNase	deoxyribonuclease
BAL	bronchoalveolar lavage	DOPE	1,2-dioleoyl-*sn*-glycero-3-phosphoethanolamine
BMI	body mass index		
BP	blood pressure	dsDNA	double-stranded DNA
BSA	bovine serum albumin	ELISA	enzyme-linked immunosorbent assay
cAMP	cyclic adenosine monophosphate		
cDNA	complementary deoxyribonucleic acid	EME	Efficacy and Mechanism Evaluation
		ERS	European Respiratory Society
CF	cystic fibrosis	FEV_1	forced expiratory volume in the first second
CFF TDN	Cystic Fibrosis Foundation Therapeutic Development Network		
		FPF	fine particle fraction
CFQ-R	Cystic Fibrosis Questionnaire – Revised	FVC	forced vital capacity
		GL67	cholest-5-en-3-ol (3β)-, 3-[(3-aminopropyl)[4-[(3-aminopropyl)amino]butyl]carbamate]
CFTR	cystic fibrosis transmembrane conductance regulator (gene)		
CI	confidence interval	GMP	good manufacturing practice
CMV	cytomegalovirus	GT	gene therapy treated
CON	anaesthetic control	GTAC	Gene Therapy Advisory Committee
CONSORT	Consolidated Standards of Reporting Trials	H&E	haematoxylin and eosin
		ICTU	Imperial College Trials Unit
CpG	cytosine–phosphate–guanidine	IL-8	interleukin 8
CRO	clinical research organisation	IRT	immunoreactive trypsinogen
CRP	C-reactive protein		
CT	computed tomography	ITT	intention to treat
DC-Chol	3beta-[N-(N′,N′-dimethylaminoethane)carbamoyl]cholesterol	KCO	transfer coefficient
		KCOc	corrected transfer coefficient
		LCI	lung clearance index
DMEC	Data Monitoring and Ethics Committee	LOQ	limit of quantification

MBW	multiple breath washout	rhDNase	recombinant human DNase
MEF	mid-expiratory flow	RNA	ribonucleic acid
MET	metabolic equivalent	SAE	serious adverse event
MHRA	Medicines and Healthcare products Regulatory Agency	SAP	statistical analysis plan
		SD	standard deviation
MMAD	median mass aerodynamic diameter	SEM	standard error of the mean
		SF6	sulphur hexafluoride
mRNA	messenger ribonucleic acid	soCFTR2	CpG-free version of the *CFTR* coding sequence
MRSA	meticillin-resistant *Staphylococcus aureus*		
		SOP	standard operating procedure
NIHR	National Institute for Health Research	TE	treatment effect
		TLCO	transfer factor of the lung for carbon monoxide
ORO	Oil Red O dye		
PBMC	peripheral blood mononuclear cell	TLCOc	corrected transfer factor of the lung for carbon monoxide
PBNQ	positive but not quantifiable		
PBS	phosphate-buffered saline	TSC	Trial Steering Committee
PD	potential difference	UK CFGTC	UK Cystic Fibrosis Gene Therapy Consortium
pDNA	plasmid DNA		
PP	per protocol	VA	alveolar volume
qPCR	quantitative polymerase chain reaction	VO_{2max}	maximal oxygen uptake
qRT-PCR	quantitative reverse transcription PCR		

Plain English summary

Cystic fibrosis (CF) is an inherited disease that significantly shortens life because of severe lung disease. Only one treatment aimed at the underlying cause is currently available and this is suitable for only the 4% of patients who have specific types of gene mutations; standard care for all other patients targets the symptoms of the disease rather than the cause.

Gene therapy aims to insert a normal copy of the gene back into the lungs' cells and restore function. In the UK CF Gene Therapy Consortium we have (1) identified the best vector with which to gain cell entry; (2) designed a piece of genetic material capable of safely expressing the missing protein for a prolonged duration; (3) found out how we can deliver this to the lungs using a clinical nebuliser; (4) tested safety in two animal models; and (5) confirmed a safe dose in a single-administration trial.

In this trial, CF patients (aged ≥ 12 years) randomly received gene therapy or placebo every month for 1 year. The primary outcome was a change in lung health, measured by a standard breathing test [forced expiratory volume in the first second (FEV_1)]. In addition, we included a number of secondary and safety outcomes.

One hundred and sixteen patients received at least nine doses and were analysed for efficacy. After 12 months, a statistically significant, but modest, difference was observed between the two groups for FEV_1; this was supported by trends in other outcomes. Effects were independent of gene mutation, age and sex. There were no significant safety concerns.

We suggest the results reported here provide proof of concept that repeated administration of CF transmembrane conductance regulator (*CFTR*) gene therapy can alter clinically relevant outcomes, providing another step along the path of translational CF gene therapy.

Scientific summary

Background

Cystic fibrosis (CF) is a chronic, life-limiting disease caused by mutations in the CF transmembrane conductance regulator (*CFTR*) gene encoding a chloride ion channel active on the apical surfaces of epithelia. Although it is a multisystem disease, the major morbidity and mortality is as a result of lung disease. In the conducting airways, lack of normal CFTR protein function leads to airway surface dehydration and impairment of the body's primary innate defence system, mucociliary clearance. Bacterial infections ensue from early life and the aggressive inflammatory response that results ultimately leads to irreversible airway scarring in the form of bronchiectasis and respiratory failure. Approximately 95% of CF patients die of respiratory failure unless a transplant is performed. The median age of death in the UK currently is 29 years.

With the exception of one new drug, the small-molecule potentiator ivacaftor (Kalydeco®, Vertex Pharmaceuticals, Boston, MA, USA), there are no licensed therapies targeting the basic defect. This drug is currently suitable for only a minority of patients (4–5%) with particular, relatively rare, *CFTR* gene mutations. All the other clinically available treatments target downstream consequences of the disease rather than the cause, and at best delay, rather than prevent, the decline in lung function.

Gene therapy, whereby a normal copy of the *CFTR* gene is introduced into cells of the conducting airways, has been considered for some time to be an attractive option, as, unlike the small-molecule approach, it would be mutation independent. Viral vectors have proved problematic because of immune responses occurring on repeat application, but non-viral approaches do not suffer from the same problem. Proof of principle has been confirmed for non-viral gene therapy, although trials have largely been single application and outcomes have been molecular rather than clinical.

The UK Cystic Fibrosis Gene Therapy Consortium (UK CFGTC) comprises scientists, clinicians and allied health professionals from three sites in the UK: University of Edinburgh, Imperial College London and University of Oxford. The consortium has been working together for more than a decade with the aim of developing clinically applicable gene therapy for patients with CF. In our wave I programme, we have chosen the most optimal non-viral vector; designed a plasmid capable of long-duration expression, with limited proinflammatory potential; identified the optimal nebuliser delivery system; and tested the product in two preclinical animal models and a single-application safety and dose-ranging trial.

Objective

The primary objective of this trial was to determine the clinical efficacy of the formulation delivered to the airways over a period of 1 year in patients with CF.

Design

This was a randomised, double-blind, placebo-controlled Phase IIb trial of the *CFTR* gene–liposome complex, pGM169/GL67A. Randomisation was performed via InForm™ version 4.6 (Phase Forward Incorporated, Oracle, CA, USA) and was 1 : 1, except for patients in the mechanistic subgroups (2 : 1). Allocation was blinded by masking nebuliser chambers.

Setting

Data were collected as the clinical and scientific sites and entered onto a trial-specific InForm, version 4.6 database.

Participants

Eligible CF subjects were at least 12 years old and had mild to moderate lung disease, with forced expiratory volume in the first second (FEV_1) between 50% and 90% predicted. Subjects could have any combination of *CFTR* mutations. Exclusion criteria included infection with organisms related to an increased rate of disease progression or posing a cross-infection risk (meticillin-resistant *Staphylococcus aureus, Mycobacterium abscessus* and the *Burkholderia cepacia* complex).

Intervention

Following a successful screening visit, subjects received 5 ml of pGM169/lipid 67A (GL67A) (active) or 0.9% saline (placebo) at 28 ($\pm$5)-day intervals over 1 year. Based on previous trial data identifying cytosine–phosphate–guanidine (CpG) motifs within the bacterially derived deoxyribonucleic acid (DNA) as the most likely cause of mild flu-like responses, and with an aim to increase the duration of expression, our chosen formulation comprises a plasmid, pGM169, encoding the *CFTR* gene driven by a CpG-free human cytomegalovirus enhancer/elongation factor 1a (*hCEFI*) enhancer/promoter. The cationic lipid is made up of three components to optimise DNA binding, stability and gene transfer: (1) cholest-5-en-3-ol (3β)-, 3-[(3-aminopropyl)[4-[(3-aminopropyl)amino]butyl]carbamate] (GL67); (2) 1,2-dioleoyl-*sn*-glycero-3-phosphoethanolamine (DOPE); and (3) 1,2-dimyristoyl-*sn*-glycero-3-phosphoethanolamine-*n*-[methoxy (polyethylene glycol 5000)] (ammonium salt) (DMPE-PEG5000) formulated at a 1 : 2 : 0.05 molar ratio. Components were mixed following stringent standardised operating procedures by unblinded trial pharmacists on the day of dosing. The formulation was nebulised via a breath-actuated nebuliser, the AeroEclipse® II (Trudell Medical International Europe Ltd, Nottingham, UK), which had been masked and locked to prevent unblinding of patients or trial team. A subgroup of patients also received doses to the nasal epithelium via a standard nasal spray device. Randomisation was 1 : 1 except for the mechanistic subgroups, which were randomised 2 : 1 in favour of active treatment to enrich for this group.

Main outcome measures

The primary end point was the relative change in percentage predicted (based on Stanojevic reference ranges) FEV_1 over the 12-month period. Secondary clinical outcomes included other physiological tests [spirometric values, lung clearance index (LCI) assessed by multibreath washout using sulphur hexafluoride as a tracer gas, exercise testing and activity monitoring], structural changes on a computed tomography (CT) scan and a disease-specific, validated quality-of-life questionnaire. We also assessed inflammatory markers, infection burden and a large number of additional safety measures and collected data on adverse events (AEs). A mechanistic study was performed in two subgroups: one group of patients underwent bronchoscopies pre and post dosing, during which CFTR function was assessed with lower airway potential difference (PD) and samples were obtained for transgene DNA and messenger ribonucleic acid (mRNA) quantification as well as histology; the second group underwent similar measures in the nose after additional nasal dosing.

Results

We recruited 136 patients into the intention-to-treat cohort; the active and placebo groups were well matched at baseline with regard to age, sex, lung function severity and *CFTR* mutation class. The per-protocol cohort was predefined as those patients receiving at least 9 monthly doses of trial formulation; it consisted of 54 patients receiving placebo and 62 receiving gene therapy. The uneven split relates to the 2 : 1 randomisation in the mechanistic subgroup. There was a significant ($p = 0.046$) treatment effect (TE) of 3.7% [95% confidence interval (CI) 0.1% to 7.3%] in the primary end point of relative change in percentage FEV_1 at 12 months. There were also significant TEs in secondary end points, including forced vital capacity (FVC) ($p = 0.031$) and gas trapping on CT scans ($p = 0.048$); supportive, non-statistically significant changes were seen in the majority of other outcomes. Effects were noted by 1 month and were irrespective of sex, age or *CFTR* mutation class. Subjects with a more severe baseline FEV_1 had a FEV_1 TE of 6.4% (95% CI 0.8% to 12.1%) and larger responses in most other outcomes. However, the milder group also demonstrated trends towards a TE in the small airway measure, LCI, confirming that this group of patients may still benefit. The active group showed a significantly ($p = 0.032$) greater bronchial chloride secretory response; overall, there were no significant changes in nasal PD but some actively treated patients demonstrated improved chloride secretion. Plasmid DNA was detectable in the majority of samples from both upper and lower airways, although mRNA was not detectable; this assay is known to lack sensitivity. The formulation was safe with no evidence of immune responses and no differences in treatment-attributable AEs seen between the placebo and active groups.

Conclusions

The UK CFGTC has conducted the first trial of non-viral *CFTR* gene therapy designed specifically to detect clinical benefit. The trial formulation was designed to (1) permit repeated application over a period of time sufficient to determine change in clinically relevant outcomes; (2) minimise inflammatory responses by removing all CpG motifs; and (3) lead to extended duration of expression with a non-viral, humanised promoter. Monthly application of the pGM169/GL67A gene therapy formulation was associated with a significant improvement in the primary outcome, FEV_1. There were also significant improvements in FVC and gas trapping on CT scans, with supportive signals in other outcomes. Signals were larger in patients entering the trial with lower lung function, although they were apparent across the spectrum of disease severity. Evidence for *CFTR* expression was seen in the lower airway with changes in bronchial PD. The formulation did not lead to the generation of host immune responses and was confirmed as safe. The approach is of relevance to CF patients, independent of their underlying *CFTR* gene mutation.

Limitations

Although encouraging, the difference in FEV_1 between groups was modest and was not accompanied by detectable improvement in the quality of life of patients. The molecular assays appear to lack efficacy, and, although supportive changes were observed in bronchial PD, the mechanistic subgroups were underpowered.

Future work

The almost doubled benefit in patients in the more severe half of the group, based on $FEV_1 < 70\%$, is probably, in our opinion, related to increased proximal airway drug deposition. This provides encouraging support for improved outcomes if higher doses could be delivered. We will seek to explore this in a future trial, by increasing each dose, decreasing the dosing interval or, possibly, by maximising transgene-derived CFTR function with the coadministration of a potentiating drug. The consortium is also developing a pseudotyped lentivirus that leads in preclinical testing to high-level gene expression.

Trial registration

This trial is registered as ClinicalTrials.gov NCT01621867.

Funding

Chapter 1 Introduction and background

Cystic fibrosis

Pathophysiology

Cystic fibrosis (CF), a common, genetically inherited disease, is caused by mutations in the CF transmembrane conductance regulator (*CFTR*) gene. Inherited in an autosomal recessive fashion, it has a prevalence of approximately 1 : 2500 and a carrier rate of around 1 : 25 in the Caucasian population. It is estimated that there are approximately 70,000–100,000 people with CF worldwide and over 10,000 on the current UK registry. It is a multisystem disease, primarily affecting the lungs, but also with significant pancreatic, liver and gastrointestinal involvement and infertility in the majority of males. Lung involvement occurs from an early age with intermittent then chronic bacterial infection, inflammation and eventual bronchiectasis, fibrosis and death from respiratory failure.[1] People affected with CF are particularly susceptible to certain bacteria, including *Staphylococcus aureus* Rosenbach 1884, *Pseudomonas aeruginosa* (Schroeter 1872) Migula 1900 and *Burkholderia cepacia* (Palleroni and Holmes 1981) Yabuuchi *et al.* 1993, although more recently the use of molecular detection techniques has revealed much more diverse bacterial populations present in the lower airway than hitherto suspected.[2]

The gene responsible for CF was identified in 1985 and found to be localised to the long arm of chromosome 7, position 7q21–24,[3] with the sequence being fully identified in 1989.[4] Over 2000 mutations in the *CFTR* gene have been described, although not all are disease-causing; some mutations still produce a functional protein and are probably more accurately termed benign polymorphisms. In its normal, wild-type, form, the *CFTR* gene encodes a 1480-amino-acid protein[5] that sits on the cell surface and acts as a cyclic adenosine monophosphate (cAMP)-regulated ion channel. CFTR is a direct conductor of chloride and (probably) bicarbonate ions and interacts closely with the major sodium-absorbing channel, epithelial sodium channel (ENaC), inhibiting its activity. Defects in CFTR lead to reduced chloride secretion and hyperabsorption of sodium and, thus, water, the combined effect of which is dehydration of the cell surface.[6] Mucociliary clearance is impaired and the infective/inflammatory sequelae of this ensues. It is not known if the decreased volume of airway surface liquid alone causes the predisposition to infection, if the immune system is also affected causing a defect in pathogen killing,[7] or whether or not other environmental factors within the CF airway prevent the innate immune system functioning as efficiently as in individuals without CF.

Clinical presentation, diagnosis and disease manifestation

The majority of today's older children and adults, the population included in this trial, will have been diagnosed once they had developed symptoms suggestive of the disease. In many countries, the UK included, this picture has changed dramatically with the introduction of newborn screening; currently, infants are diagnosed around 3–4 weeks of age based on dried-blood-spot screening.[8] The screens differ somewhat globally, but the UK test measures immunoreactive trypsinogen (IRT) and pancreatic enzyme levels, which are increased in CF because of pancreatic duct obstruction. Samples with high levels of IRT are sent for mutation testing. This allows identification and initiation of treatment presymptomatically and before the downstream sequelae have arisen.

The gold standard diagnosis is based on measuring chloride ion levels in sweat samples. Lack of chloride ion reabsorption by CFTR in the sweat gland leads to high levels being lost in the sweat, typically > 100 mmol/l in comparison with values of < 30 mmol/l in healthy individuals.[9] Diagnosis can also be established on *CFTR* mutation testing, although, because of the large number of mutations, a negative test cannot rule out the diagnosis unless the entire *CFTR* gene has been sequenced; this is rarely employed as a first-line diagnostic tool and is reserved for difficult diagnoses. However, whereas previously it may have been sufficient to secure a diagnosis on the basis of a sweat test, with the recent development of mutation-specific small-molecule treatments,[10] it is becoming increasingly necessary for a patient's mutation(s) to be defined.

Disease presentation may be respiratory or nutritional and is often both.[1] Respiratory problems present as cough, wheeze, lower respiratory tract infections, sputum production and upper airway disease including nasal polyps and sinusitis. Lung health deteriorates over time, largely driven by infection and the host inflammatory response. Disease is thought to begin in the small airways, although this may just reflect a greater impact of obstruction at this site. Airway obstruction related to mucus accumulation and airway wall inflammation is measurable on physiological testing, the most commonly employed being forced expiratory manoeuvres with spirometry. Over time, and despite optimal treatment at specialist centres, lung function declines. A major contributor to this decline is episodes of pulmonary exacerbation, periods of increased symptoms and acute drop in lung function that is often not fully regained after treatment.[11]

The most common manifestation of the pancreatic exocrine disease, present in > 95% of patients, is poor weight gain with steatorrhoea. Later, patients may develop pancreatic endocrine dysfunction with impaired glucose metabolism and CF-related diabetes mellitus; this has respiratory consequences that are poorly understood, but is clearly associated with an increased rate of progression of lung disease.

Conventional management of patients with cystic fibrosis

Cystic fibrosis is a multisystem disease and treatment can be optimally conducted only with the help of a full multidisciplinary team (CF physician, specialist nurse, physiotherapist, dietitian, clinical psychologist, pharmacist) and input from ancillary specialists with expert knowledge of CF (ear, nose and throat surgeon, obstetrician, endocrinologist). CF patients should be seen at least every 3 months by the core CF team. A large number of treatment guidelines have been published.[12–14]

The main aims of respiratory care are clearance of mucus, prevention and early eradication of infection and suppression of infection once chronic. Sputum clearance is achieved with regular physiotherapy alongside adjunctive inhaled mucoactive agents: hypertonic saline has been shown to aid clearance,[15] most likely be rehydrating the airway surface and stimulating cough. Recombinant human deoxyribonuclease (DNase) (dornase alfa; Pulmozyme®, Roche Products Ltd) breaks down the high levels of deoxyribonucleic acid (DNA) in the CF airway resulting from degradation of neutrophils and decreases mucus viscosity.[16] Regular microbiological surveillance is essential for the early detection of infecting bacteria; sputum samples or cough swabs are collected at every patient contact. *P. aeruginosa* is an extremely common pathogen, often being acquired first in childhood and becoming chronic in adulthood in around 60–70% of patients. Its presence is associated with a more rapid decline in lung function and, therefore, aggressive eradication is employed when it is first encountered.[17] Once it becomes chronic, long-term nebulised antibiotics such as colistimethate sodium (Colomycin®, Forest Laboratories UK Ltd) or the aminoglycoside, tobramycin for inhaled solution (Tobi®, Novartis Pharmaceuticals UK Ltd), are administered to maintain suppression of bacterial numbers and thereby attempt to limit the host inflammatory response.[18] There are a small number of other organisms that pose particular problems, either because of high transmissibility [e.g. meticillin-resistant *Staphylococcus aureus* (MRSA) and *B. cepacia*], or because they are related to a more rapid rate of clinical decline [e.g. *Mycobacterium abscessus* (Moore and Frerichs 1953) Kusunoki and Ezaki 1992]. These organisms are difficult to treat and, furthermore, such patients often miss out on inclusion in clinical trials of new drugs, either because of infection control issues or concerns over baseline stability. Periods of pulmonary exacerbation are treated with increased attention to airway clearance and antibiotics, often given intravenously and requiring hospital admission.

Therefore, CF management not only poses a huge burden on the patients and their families, but also carries with it substantial health-care system costs.[19] Ultimately, once respiratory failure has ensued, lung transplantation is the only option. There is a shortage of organs in most developed countries and many patients die while on the waiting list. Even after receiving a transplant, complications frequently occur, most importantly the development of bronchiolitis obliterans, although some patients may do well for many years.[20] Currently in the UK, median age at death is around 29 years of age.[21] Thus, despite expensive and burdensome treatments, life expectancy is significantly reduced for CF patients. Progress is being made in several areas of drug development, for example with newer antibiotics, but many feel that a significant improvement in health is likely to be achievable only with novel strategies to tackle the basic

defect in the *CFTR* gene rather than these downstream consequences. The next sections describe the progress that has been made in small-molecule and gene therapy fields to this end.

Novel small-molecule cystic fibrosis transmembrane conductance regulator modulators

An understanding of the various mechanisms by which different mutations lead to CFTR dysfunction has led to the grouping of mutations into classes.[22]

Class I mutations lead to a premature 'stop' in the messenger ribonucleic acid (mRNA) and a short, non-functioning protein. Mutations of class II encode a structurally abnormal, misfolded protein that does not traffic to its site of action on the apical cell surface but is removed by the endoplasmic reticulum and degraded. Class III–VI mutations reach the cell surface but fail to function appropriately: class III mutations have decreased activation of the channel and remain closed; and class IV mutations cause decreased conductance of ions across the channel. Class V mutations encode proteins with splice-site mutations and result in reduced amounts of CFTR at the cell surface, so some function occurs but at a reduced level. Class VI mutations lead to a shortened half-life because of protein instability and may also impair CFTR's regulation of neighbouring channels. Many mutations lead to defects in more than one of these classes; for example, F508del (previously ΔF508), the most common mutation worldwide, is clearly a class II misfolding defect, but also opens infrequently and has a short half-life, so also possesses class III and VI features.

Treatments for class I mutations

Class I, or nonsense, mutations account for approximately 5–10% of the worldwide CF population, although the incidence is increased to around 60% among Israeli Jewish CF subjects.[23,24] Based on the initial observation that premature truncation codons could be over-read in the presence of gentamicin (Garamycin®, Schering-Plough)[24–27] one synthetic agent, ataluren (previously known as PTC124; Translarna™, PTC Therapeutics), has progressed to clinical trials.[28] This drug was administered orally to CF patients aged ≥ 6 years with at least one class I mutation in a parallel-design randomised controlled trial. Despite encouraging Phase II data, neither the primary end point (change in forced expiratory volume in the first second; FEV_1) nor multiple secondary end points were met. A post hoc subgroup analysis demonstrated a treatment effect (TE) in the subgroup who were not receiving nebulised aminoglycoside antibiotics, which may have been interfering with the mechanism of action. A further trial is planned, but to date these patients do not have any other mutation-specific drug approaches in the pipeline.

Treatments for class III mutations

Ivacaftor (Kalydeco®, Vertex Pharmaceuticals, Boston, MA, USA) is the first mutation-specific drug to be approved in the USA, Europe and Australasia. It is a CFTR potentiator, which increases the channel's open probability. Clinical trial results prove, for the first time, that improving CFTR function can lead to significant benefits.

Class III mutations lead to a protein that remains closed for almost 100% of the time, a so-called 'gating' defect. The most common class III mutation is G551D (new nomenclature Gly551Asp). Other class III mutations are rare and together probably account for only a further 1% of patients.

Ivacaftor was identified via high-throughput screening technology,[29–31] and progressed rapidly through preclinical testing into CF clinical trials, based on significant financial support from the CF Foundation in the USA. Twice-daily oral administration in patients with a G551D mutation led to a 10% absolute improvement in FEV_1, a reduction in pulmonary exacerbations and improved quality-of-life scores; additionally, it seemed to have benefits outside the lungs, with improvement in weight and body mass index (BMI) thought to be related to gut bicarbonate secretion. It was initially licensed (and is now available in the UK) for patients with the G551D mutation, but a subsequent trial demonstrated similar benefit for the small group of patients with other class III mutations,[10] for which the drug has now also been licensed and is awaiting funding approval. Furthermore, a trial in patients with the class IV mutation R117H (NCT01614457), which demonstrated smaller benefits, has led, to date, to the US Food and Drug Administration approval and is being considered by the European Medicines Agency.

Treatments for class II mutations

Class II defects are present in the majority of patients with CF worldwide. F508del is carried by approximately 70–80% of the CF population, with 50% of the total CF population being homozygous.[32] As described above, the vast majority of class II CFTR is degraded by intracellular processes and fails to reach the cell surface. The absolute amount that escapes this degradation differs between individuals and, may account, in part, for the phenotypic variation observed. The concept of CFTR 'correctors' has been around for many years, although the term has only recently been coined. In the 1980s, an observation was made that low temperatures and the compound glycerol, stabilised misfolded CFTR protein and facilitated trafficking; the term 'molecular chaperone' was coined for a drug with these capabilities.[32,33] High-throughput screening was conducted by several commercial and academically funded groups and one of these, Vertex Pharmaceuticals, identified a promising small molecule, named VX-809 (lumacaftor, Vertex Pharmaceuticals). In vitro chloride transport could be achieved at 14% of wild-type CF levels.[34] The results from the first Phase II trial in 89 patients were somewhat disappointing. As the drug is given systemically, sweat chloride is a convenient biomarker and had shown utility in trials of potentiator agents. In this trial, there was a statistically significant reduction in sweat chloride, confirming proof of concept, but the magnitude was small at around 7 mmol/l. In addition, no change was seen in nasal potential difference (PD), a localised bioelectrical read-out of CFTR ion transport, nor clinical parameters within the 4-week treatment period.[35] As well as a trafficking defect, F508del CFTR protein is abnormally gated. It was, therefore, hypothesised that VX-809 could improve the localisation of CFTR protein to the cell surface, but once there it would need additional help to function; this led to the notion of combination trials with potentiators.

Ivacaftor, which had been shown to have dramatic improvement in the movement of ions across the cell membrane in class III mutations,[29–31] was considered unlikely to benefit patients homozygous for F508del, and this was borne out in a Phase II study of 140 patients.[35] However, in vitro, combining VX-809 with ivacaftor doubled the chloride transport compared with that of VX-809 alone. A large Phase III global clinical trial programme has recently been reported to demonstrate a statistically significant but relatively modest 2–4% absolute improvement in percentage predicted FEV_1 and to reduce the frequency of pulmonary exacerbations.[36] It did not show clinical benefit in patients with only one copy of F508del. Vertex is also developing an alternative corrector, VX-661. It possesses a possible advantage over lumacaftor in that its metabolic pathway does not interact with that of VX-770 and achieving therapeutic levels may be easier. Phase II clinical trials of VX-661 alone, and in combination with ivacaftor, in CF patients homozygous for F508del have been encouraging and a Phase III programme is planned.

Gene therapy

In contrast to the mutation-specific approach described above, gene therapy, described as 'the introduction or alteration of genetic material within a cell or organism with the intentions of curing or treating a disease',[37] has potential for CF patients with mutations in any class. The first studies of human administration of healthy copies of the *CFTR* gene in CF patients were developed very soon after the gene was cloned in 1989.[38]

Two components are required in a gene therapy product: (1) a normal copy of the *CFTR* gene (along with required regulatory constructs); and (2) a gene transfer agent, either a viral or non-viral vector. The choice and suitability of such a vector depends on a number of factors, such as the size of the gene to be carried, the requirement for, and tolerability of, repeated administration and the target cells for treatment.

Choice of vector and clinical trials

Clinical trials of *CFTR* gene transfer to CF subjects have been undertaken using a variety of viral vector systems, including adenovirus and adeno-associated virus (AAV). Encouragingly, adenovirus-based gene transfer led to the correction of the cAMP-stimulated CFTR chloride channel defect in the nose of treated CF subjects.[39,40] However, the efficiency of adenovirus-based gene transfer to human tissues appears to be

low,[41,42] and is associated with lung inflammation[43,44] and the development of neutralising immune responses that, to date, has precluded repeated administration.[45] AAV-based *CFTR* gene transfer appears less proinflammatory,[46–48] although repeated administration is also inhibited by immunological reactivity.[49]

Non-viral vectors have also been used in clinical trials of *CFTR* gene transfer to CF subjects. Ten clinical trials have been performed worldwide to date,[50–58] six of which have been in the UK [Gene Therapy Advisory Committee (GTAC) numbers: 002, 007, 008, 009, 015 and 140] and conducted by current groups within the UK CF Gene Therapy Consortium (UK CFGTC). The first of these non-viral-formulation clinical trials assessed the safety and efficacy of a single nasal delivery of plasmid DNA (pDNA) containing the *CFTR* complementary DNA (cDNA) under the transcriptional control of the simian virus 40 (SV40) promoter complexed with 3beta-[N-(N',N'-dimethylaminoethane)carbamoyl]cholesterol (DC-Chol)–dioleoyl-*sn*-glycero-3-phosphoethanolamine (DOPE) liposomes (GTAC number: 002).[50] Evidence for pDNA transfer, vector-derived *CFTR* mRNA expression and partial correction of the CFTR chloride ion channel defect was obtained in a subset of subjects. Similar modest, but positive evidence of *CFTR* gene transfer and function were obtained after nasal delivery of a pDNA containing the *CFTR* cDNA under the transcriptional control of the respiratory syncytial virus 3' long terminal repeat (RSV 3'LTR) promoter complexed with DC-Chol–DOPE liposomes (GTAC number: 007).[51] Alternative formulations in which pDNAs containing the *CFTR* cDNA under the transcriptional control of the human cytomegalovirus (CMV) immediate early enhancer/promoter were complexed with 1,2-dioleoyl-3-trimethylammoniumpropane liposomes (GTAC number: 008),[52] *p*-ethyl-dimyristoylphosphaditylcholine–cholesterol liposomes,[53] or a polycationic peptide consisting of a N-terminal cysteine followed by 30 lysine residues[54] were broadly shown to be similarly effective. Importantly, in an additional clinical trial utilising the RSV 3'LTR promoter plasmid complexed with DC-Chol–DOPE liposomes, repeated nasal delivery of a non-viral formulation was shown to be equivalently effective after each of three successive administrations (GTAC number: 015).[55] Crucially, no important safety considerations were raised after nasal application of any of these formulations. Collectively, these clinical data provided proof of principle for CF non-viral gene therapy, but highlighted the need for formulations with enhanced efficacy. Extensive chemical optimisation of the promising DC-Chol formulation by Genzyme Inc. (Cambridge, MA, USA) generated the cationic lipid cholest-5-en-3-ol (3β)-,3-[(3-aminopropyl)[4-[(3-aminopropyl)amino]butyl]carbamate] (GL67) with improved gene transfer potency,[59] well-characterised safety parameters[60] and desirable stability during aerosolisation.[61] Clinical trials with GL67-based non-viral formulations have been encouraging. A single nasal administration of a pDNA containing the *CFTR* cDNA under the transcriptional control of the CMV promoter (plasmid pCF1-CFTR) complexed with GL67–DOPE was shown to be safe, to direct vector-derived mRNA expression and to produce an overall ≈ 20% correction of the CFTR chloride ion channel defect.[56] For efficient jet nebuliser-directed aerosol delivery, 1,2-dimyristoyl-*sn*-glycero-3-phosphoethanolamine-*n*-[methoxy (polyethylene glycol 5000)] (ammonium salt) (DMPE-PEG5000) was added to the GL67–DOPE to generate a formulation termed GL67A that consisted of a mixture of GL67–DOPE–DMPE-PEG5000 at a 1 : 2 : 0.05 molar ratio[61] (for further details see *GL67A cationic lipid*). The GL67A formulation lacking pDNA was shown to be safe and well tolerated after a single lung administration in healthy volunteers.[62] Delivery of pCF1-CFTR complexed with GL67A to the lungs[57] or nose and lungs (GTAC number: 009)[58] of CF subjects led to an overall ≈ 25% correction of the CFTR chloride ion channel defect. However, CFTR expression in these subjects, and those in all other clinical trials described above, diminished quickly, such that it was essentially undetectable within 1 week of administration. Furthermore, pCF1-CFTR/GL67A lung administration was associated with a mild flu-like syndrome that resolved within 36 hours.[57,58]

Further in vivo preclinical studies have identified cytosine–phosphate–guanidine (CpG) dinucleotides in the pCF1-CFTR pDNA as the likely cause of the mild flu-like symptoms following dosing and may also impact on the duration of pDNA-mediated transgene expression. The CpG motifs delivered by the pCF1-CFTR/GL67A formulation appear to be sensed by the innate immune system via the toll-like receptor 9 (TLR9) pathway.[63] Stimulation of the TLR9 pathway led to a host inflammatory reaction typified by the elevation of inflammatory cytokines within the lung milieu and recruitment of lung neutrophils.[64]

Thus, proof of principle for non-viral *CFTR* gene therapy had been demonstrated, but improvements were needed specifically with regard to duration of expression and pro-inflammatory responses.

UK Cystic Fibrosis Gene Therapy Consortium

At this stage, in 2001, and in response to an initiative launched by the UK CF Trust, the three UK sites active in CF gene therapy (University of Edinburgh, Imperial College London/Royal Brompton Hospital and University of Oxford) came together to form the UK CF Gene Therapy Consortium (www.cfgenetherapy.org.uk/). The aim of the consortium is to develop clinically relevant gene therapy for patients with CF. The wave 1 programme was based on the recognition that repeated application would necessitate a non-viral vector and aimed to identify the best, currently available non-viral formulation coupled with a long-lasting plasmid with little or no proinflammatory potential and to take this into a clinical trial designed and powered, for the first time, to detect *clinical* benefit. The repeated-dose trial is the focus of this report. Work packages in the wave 1 programme leading up to this point have included a study of outcome measures on patients undergoing a pulmonary exacerbation;[65] a large, longitudinal study of outcome measures in stable patients (from which power calculations were made for this trial); and a single-application safety and dose-ranging pilot study (see *Choice of dose and adjunctive treatments: single-dose pilot study*). The consortium is also working on a wave 2 programme developing and assessing a pseudotyped lentiviral vector, which seems to be repeatedly administrable. Although gene transfer efficiency seems greater with this approach than with our non-viral equivalents, the requirement for preclinical development and toxicity testing placed this further in the future and led to the initial focus on wave 1.

Section *pGM1/GL67A for clinical trial use* describes our chosen clinical trial formulation in detail and the rationale for its development.

pGM1/GL67A for clinical trial use

Composition

We found GL67A (further details in *GL67A cationic lipid*) to be superior to others in both small and large animal models, having undertaken an extensive preclinical assessment of available non-viral formulations. GL67A has previously been administered to both healthy volunteers and CF subjects, including in a single-dose nebuliser study[66] and our recently completed safety study *Evaluation of safety and gene expression with a single dose of pGM169/GL67A administered to the nose and lung of individuals with cystic fibrosis* (sponsor's protocol number: cro851; EudraCT reference: 2007-004050-85).[62] For both the single-dose safety study and the trial described in this report, we made substantial changes to the *CFTR* plasmid, which was specifically designed with the following features:

- The promoters most commonly used are of viral origin; we had previously incorporated a CMV promoter which, although capable of high-level gene expression, is usually of short duration. The current formulation contains a human elongation factor 1 alpha (*EEF1A1*) gene promoter. Our preclinical work demonstrated more sustained levels of expression (up to 2 months) with this new promoter; this could translate into a less frequent dosing regimen. The gene expression profile observed in the recent single-dose study supported this hypothesis, with relatively few changes observed at early time points and several subjects demonstrating functional changes in nasal PD several months after dosing.
- Unmethylated CpG dinucleotides are present in high numbers in pDNA used in gene therapy trials. These bacterially derived components are recognised by humans as foreign and likely responsible for the flu-like responses, reported in our previous trial in the group receiving DNA/lipid but not lipid alone. Although these responses were mild and self-limiting, requiring only treatments with antipyretics, we considered them undesirable in a repeated-dose trial. In an attempt to reduce such an inflammatory response, the new plasmid has been rendered completely CpG free.

Plasmid

pGM169 is a covalently closed, circular, double-stranded pDNA molecule of 6549 base pairs purified from bacteria. It is based on a novel CpG-free plasmid backbone described in the international patent application PCT/GB2007/00110433. A diagrammatic representation of pGM169 is presented in *Figure 1*. pGM169 contains a CpG-free version of the *CFTR* coding sequence termed soCFTR2 under the transcriptional control of a novel CpG-free human CMV enhancer/elongation factor 1 alpha (hCEFI) enhancer/promoter. Other plasmid elements include a CpG-free version of the bovine growth hormone polyadenylation sequence, a CpG-free version of the R6K bacterial plasmid origin of replication and a CpG-free version of the kanamycin resistance gene under the transcriptional control of the CpG-free synthetic bacterial promoter sequence termed EM7.

Good manufacturing practice (GMP) manufacture of pGM169 was conducted by VGXi Inc. (The Woodlands, TX, USA). Bacteria containing the plasmid were fermented to a high density and harvested. The bacteria were then lysed to release their contents, including the plasmid, into solution. The lysate was subjected to three significant purification steps: (1) solid/liquid separation, (2) ion-exchange chromatography and (3) hydrophobic interaction chromatography. Subsequently, the purified plasmid was concentrated and desalted by ultrafiltration/diafiltration into a sterile 8 mM sodium chloride (NaCl) solution and finally subjected to aseptic
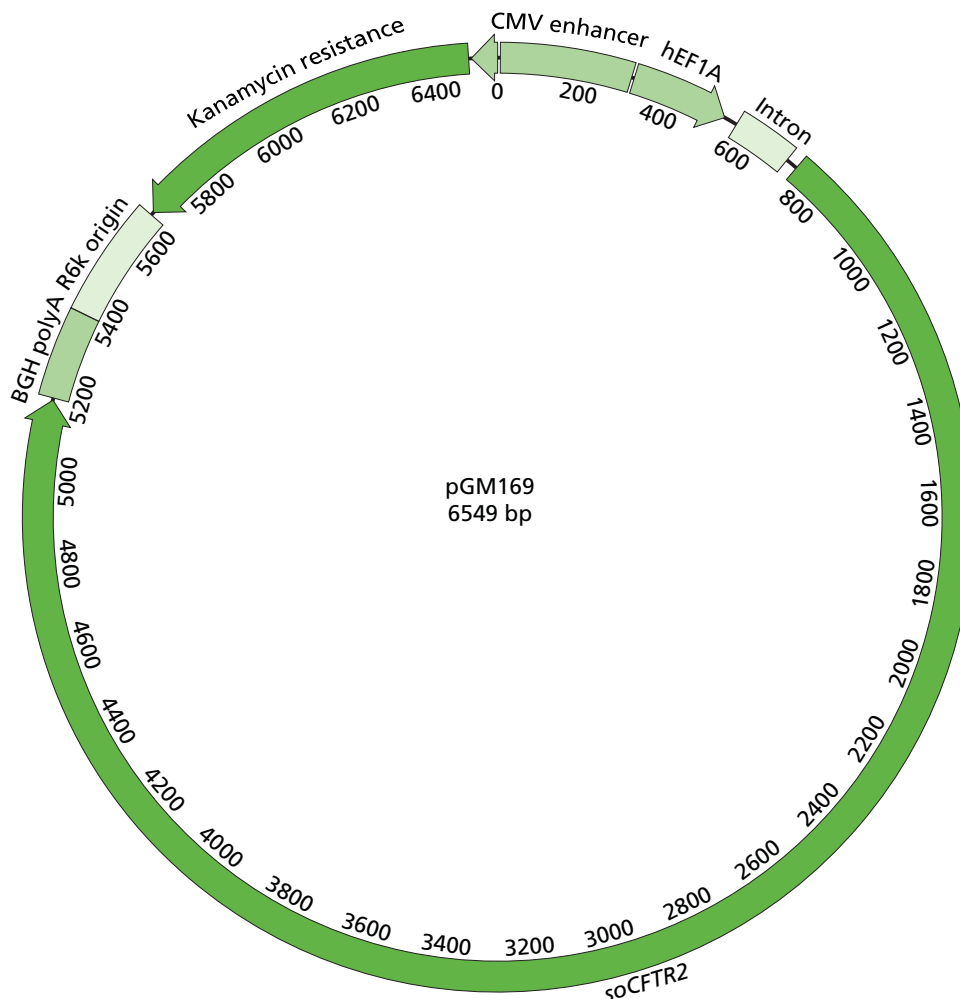
FIGURE 1 Plasmid pGM169. The basic features of pGM169 (proceeding clockwise from 0 base pairs) are the CpG-free human cytomegalovirus enhancer/elongation factor 1 alpha enhancer/promoter; a CpG-free synthetic intron sequence to enhance mRNA splicing; a CpG-free version of the *CFTR* coding sequence termed *soCFTR2*; a CpG-free version of the bovine growth hormone polyadenylation sequence; a CpG-free version of the R6K bacterial plasmid origin of replication; a CpG-free version of the kanamycin resistance gene; and a CpG-free synthetic bacterial promoter sequence termed EM7. bp, base pair; BGH, bovine growth hormone.

filtration to provide the bulk drug substance. This bulk was aseptically filled into single-unit vials and stored at ≤ −70 °C. To prepare the final drug substance, single or multiple pooled lots of bulk drug substance were, if necessary, diluted to 5.3 ± 0.3 mg/ml with sterile 8 mM NaCl and then filled into 10-ml clear glass vials at a fill level of 5.2 ± 0.2 ml. Vials were stored at –80 °C. The material is stable for at least 3 years.

GL67A cationic lipid

The cationic lipid mixture GL67A is an excipient, consisting of a mixture of three components (the structure of which is shown in *Figures 2–4*; GL67, DOPE, and DMPE-PEG5000) formulated at a 1 : 2 : 0.05 molar ratio.

The final formulation containing GL67, DOPE and DMPE-PEG5000 at a 1 : 2 : 0.05 molar ratio and a GL67-to-DNA ratio of 0.75 : 1 was termed GL67A.

Good manufacturing practice-grade GL67 was manufactured by Sanofi-Genzyme (Haverhill, UK). GMP-grade DOPE and DMPE-PEG5000 were purchased from Avanti Polar Lipids (Alabaster, AL, USA). GL67A was formulated from GMP-grade GL67, DOPE and DMPE-PEG5000 by OctoPlus N.V. (Leiden, Netherlands). Briefly, the individual constituents were first dissolved in 2-methylpropan-2-ol (9 : 1 weight-to-weight ratio of 2-methylpropan-2-ol to water) and then mixed in appropriate quantities to obtain a GL67–DOPE–DMPE-PEG5000 molar ratio of 1 : 2 : 0.05. After sterile filtration the lipid mixture was
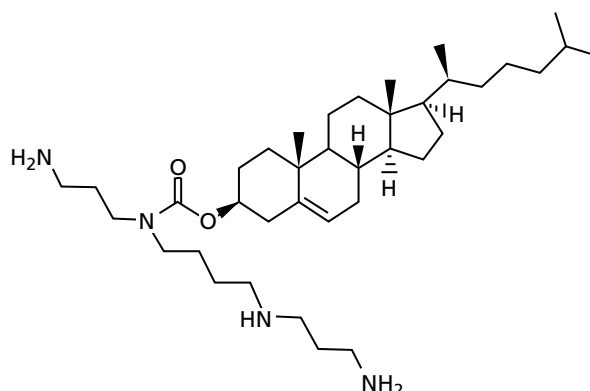
FIGURE 2 Structure of GL67. GL67 is included in the GL67A cationic lipid mixture for its DNA-binding properties (i.e. binding of pGM169).
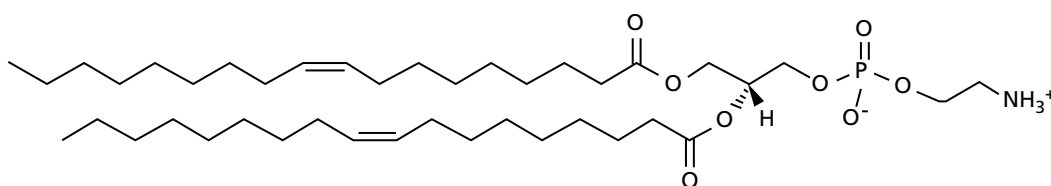
FIGURE 3 Structure of DOPE. DOPE is included in the GL67A cationic lipid mixture for its endocytosis-inducing properties.
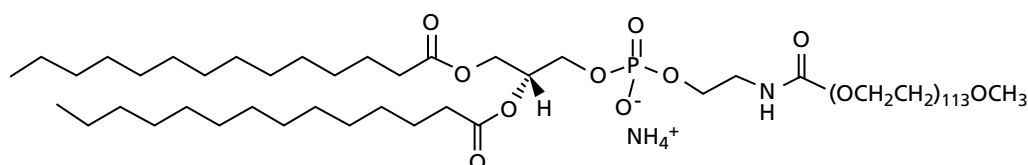
FIGURE 4 Structure of DMPE-PEG5000. DMPE-PEG5000 is included in the GL67A cationic lipid mixture for its charge-shielding properties that facilitate final preparation of the pGM169/GL67A drug product and allow efficient nebulisation.

dispensed into individual 10-ml glass lyophilisation vials such that each vial contained 38.8 mg of GL67, 93.7 mg of DOPE and 17.8 g of DMPE-PEG5000. The vials were freeze-dried under nitrogen gas for approximately 94 hours at temperatures ranging from –50 °C to +10 °C. The vials were capped with aluminium crimp caps, coded and stored at –80 °C. The material is stable for at least 3 years.

Preclinical toxicology of the selected formulation: murine

To comply with the Medicines and Healthcare products Regulatory Agency (MHRA) regulations, a multidose toxicology study in rodents (mice) was performed under good laboratory practice and the study was outsourced to a clinical research organisation (CRO).

Protocol development and selection of clinical research organisations

The protocol was developed with the help of a toxicology consultant (Professor Anthony Dayan, Professor of Toxicology at Barts Hospital, London, UK). In addition to standard toxicology, assessment of DNA and mRNA biodistribution into organs other than the lung was assessed using molecular assays. The study was tendered to six national and international CROs, and selection was based on expertise in conducting inhalation toxicology studies and molecular analysis, costs and flexibility. The inhalation study and toxicology assessment were placed with Charles River (Edinburgh, UK) and the molecular analysis at BioReliance (Glasgow, UK). The studies were performed in several stages, detailed in *Inhalation study and toxicology assessment at Charles River* and *Molecular analysis at BioReliance*.

Inhalation study and toxicology assessment at Charles River

This was performed in three phases: (1) analytical development; (2) a feasibility study; and (3) the main toxicology study.

Analytical development

This phase was used to develop methodologies, validation and specifications for the analysis of pDNA and liposome in inhalation toxicology studies. An important aspect was to show that the AeroEclipse® II (Trudell Medical International Europe Ltd, Nottingham, UK) nebuliser to be used in the trial was able to generate droplet sizes suitable for mouse inhalation studies.

Feasibility study

This showed that mice tolerated the prolonged (6 hour = maximum feasible dose) exposure in the nebulisation chamber and that gene transfer and gene expression could be achieved using the inhalation chambers used at Charles River.

Main toxicology study

This consisted of four dosing cohorts (low, medium, high and air control), corresponding to approximately 5-, 20- and 60-fold the anticipated human dose (mg/kg). Mice were exposed on 12 occasions over a 6-month period to mimic the human trial protocol (consistent with relevant guidelines). Controls were exposed to air for 6 hours. The study involved approximately 700 mice (including animals dosed for assessment of gene expression and biodistribution; see *Molecular analysis at BioReliance*). Standard non-invasive assessments were made at regular intervals and post-mortem toxicology undertaken 2 weeks or 3 months after the last dose. Additionally, molecular analyses to assess gene expression and biodistribution were performed. The treatment was well tolerated by all mice. In the high-dose group, small increases in lung weight and circulating neutrophils were seen 2 days after the last dose (dose 12); this was not observed in the cohort sacrificed 14 days after the last dose. Two weeks after administration of the last dose scattered alveolar macrophages were seen on histology in the low- and medium-dose groups. Multifocal alveolar foamy macrophage accumulation and occasional inflammatory changes were recorded in the high-dose group. Fat-laden macrophages were observed by Oil Red O (ORO; VWR International Ltd, Lutterworth, UK) staining. There was no evidence of structural remodelling. All other organs were unremarkable. Three months after administration, findings were no different from baseline in the low-dose group, but were still observed in the medium- and high-dose animals.

Molecular analysis at BioReliance

The study was again performed in three phases: (1) assay transfer; (2) validation of extraction methods; and (3) the main biodistribution study.

Assay transfer

Molecular assays [quantitative polymerase chain reaction (qPCR) and quantitative reverse transcription PCR reaction (qRT-PCR) for the detection of pGM169 were developed by the UK CFGTC and the know-how and experimental details were transferred to BioReliance where the protocols were validated to ensure that sensitivity and reproducibility of the assays were compliant with regulatory requirements.

Validation of extraction

The validation of extraction study demonstrated that the clinical trial plasmid could be successfully retrieved from all tissue selected for the biodistribution and gene expression studies (lung, gonad, gut, spleen, kidney, liver, lymph nodes and blood), as well as determining the detection limits of the assays.

Main biodistribution study

The main biodistribution study was designed to assess the level and persistence of DNA and pGM169-specific mRNA in the above organs. Male and female mice ($n = 10$ per treated group) were sacrificed after one, six and 12 doses and organs analysed using qPCR. A dose–response relationship was observed between duration of inhalation and the quantity of pDNA present in the lungs 1 day after delivery of 1, 6 and 12 doses ($p < 0.0001$; Spearman's rank correlation coefficient). pDNA remained detectable in the lungs of animals for up to 21 weeks after the final (high) dose. Levels of pDNA in non-target organs were several orders of magnitude lower than the lungs at day 1 and generally did not persist for > 2 months. pGM169-specific mRNA was not detectable in any organs other than the lung. When CFTR mRNA was measured in the lungs, low levels were detected after a single dose in the low- and medium-dose groups, with increased signal in the high-dose group ($p < 0.001$; equivalent to $\geq 100\%$ endogenous levels). Robust levels of *CFTR* mRNA remained in the lung for at least 21 weeks after the final exposure.

In conclusion, all animals tolerated the treatment well. The transient and dose-related systemic inflammatory responses and drop in lung function observed in the single-dose pilot study (see *Choice of dose and adjunctive treatments: single-dose pilot study*) were not replicated in non-CF mice. Mice only developed mild systemic inflammation at the highest dose (≈ 50-fold of the human dose) possibly highlighting species differences or an increased response to the lipid–DNA complexes in the inflamed human CF lung. The mouse multidose toxicology study supports progression into a multidose CF gene therapy trial. Key data are summarised in Alton *et al.*[67]

Preclinical toxicology of the selected formulation: ovine

Following MHRA discussions, the UK CFGTC also used its in-house sheep core facility to undertake a study of nine monthly administrations of 10 ml of the therapeutic product administered via the inhalation route. Repeated gene delivery was very well tolerated by the animals, with no obvious clinical signs throughout the study. The majority of changes observed were mild and were observed both in anaesthetic controls (CONs) and gene therapy-treated (GT) animals. Some mild changes in animal body weight and haematological parameters were observed at day 1 in both groups, and are likely to be a consequence of the anaesthetic procedure; for example, food was withheld from animals the night before an anaesthetic procedure to avoid bloating as a result of rumen gas accumulation. Specifically, the absence of a gene therapy-related increase in the acute-phase protein haptoglobin contrasts with previous studies that demonstrated a significant rise following a 20-ml dose of the same pGM169/GL67A complex. Although an upwards trend in the serum creatinine levels was observed in the GT group, the increase was small, all values were within the normal reference range and final values after the 4-week recovery period were lower than baseline levels.

A gene therapy-specific effect was seen in bronchoalveolar lavage (BAL), which was assessed after doses 1, 5 and 9. This was manifested by increases in total BAL cell number, neutrophils, macrophages and lymphocytes. These changes are consistent with the previously observed changes in the sheep lung at day 1 after a 20-ml dose. However, encouragingly, rather than being exacerbated by repeated delivery, the response was less pronounced after dose 9 and the observed changes had always fully resolved by day 15 after delivery. The increase in BAL macrophage and lymphocyte numbers was only observed after dose 1. There were no significant changes in any aspect of lung function attributable to the gene therapy administration.

In the lungs, common to all three groups (GT, CON and untreated animals), was minimal/mild lymphoplasmacytic, and sometimes eosinophilic, inflammation around bronchioles and some blood vessels. This was present in most samples, and is consistent with background immunosurveillance and/or indicative of previous parasite exposure. Alveolar histiocytosis was also common to all three groups, and is not unusual in the ovine lung, especially if sheep are not specific pathogen free or in climate- or pathogen-controlled housing. There were no notable changes that suggest accumulation of lipid related to gene therapy treatment. No adverse effect of the test article was observed on lung morphometry, indicating no changes in alveolar septal thickness or alveolar size after nine doses. In the additional organs assessed, there was nothing to point to an adverse effect of treatment. Lesions present were consistent with background inflammation or immunosurveillance, were incidental and present in all three groups.

Choice of nebuliser for clinical trial programme

In total, five commercially available, Conformité Européenne marked jet nebuliser devices were compared (four PARI Medical Ltd breath-enhanced design nebulisers and one inherently breath-actuated nebuliser: the AeroEclipse® II, Trudell Medical International Europe Ltd, Nottingham, UK).

The different investigations focused on:

1. Median mass aerodynamic diameter (MMAD) aerosol droplet size and fine particle fraction (FPF; that proportion of the aerosol droplets $\leq 5\,\mu m$, which are those thought likely to be deposited within the airways) with two commercially available nebuliser compressor devices, or with continuous gas supplies operating at either 29 psi or 50 psi.
2. Aerosol delivery rate, and inhalation efficiency (the proportion of the produced aerosol that can be retained within the lung rather than immediately exhaled) in breath actuated and non-breath actuated modes at an operating gas pressure of 50 psi.
3. pDNA stability during the aerosolisation process. Jet nebulisation results in a modest degree of non-specific pDNA damage.[68] The damage is associated with loss of supercoiled pDNA forms, an increase in linear forms and the appearance of degraded DNA after agarose gel electrophoresis.

All test nebuliser device and operating gas supply combinations resulted in the generation of respirable pCIKLux/GL67A aerosol droplets. A reduction in aerosol MMAD and an increase in aerosol FPF were generally observed with increasing operating pressures. Operating the test nebuliser devices under breath actuation conditions reduced the rate of aerosol delivery, but increased the inhalation efficiency. Both Pari LC+ (PARI Medical Ltd, Surrey, UK) and AeroEclipse® II devices impart a modest degree of pDNA damage during aerosolisation. Of the five nebuliser devices evaluated, only the AeroEclipse® II device is capable of operating in a breath-actuated mode. Given the considerable enhancement in inhalation efficiency of pCIKLux/GL67A aerosol observed and the lack of inferiority in the other parameters measured, the AeroEclipse II was selected as the preferred nebuliser device for clinical studies of pGM169/GL67A. The measured MMAD of pGM169/GL67A aerosols using the AeroEclipse® II was $3.4 \pm 0.1\,\mu m$, with a FPF of $71.4\% \pm 1.5\%$.[69] To confirm that nebulisation does not alter the ability of pGM169/GL67A to generate

functional chloride channels, HEK-293T cells [American Type Culture Collection (ATCC), Teddington, UK] were transfected with pGM169/GL67A. The cells were collected prior to nebulisation and at the end of nebulisation (residual volumes in the nebuliser) and an iodide efflux assay, commonly used to assess CFTR function, was performed as previously described.[70] Levels of cAMP-mediated iodide efflux were similar when using pGM169/GL67A before and after nebulisation (data not shown).

Choice of dose and adjunctive treatments: single-dose pilot study

Dose ranging was undertaken in the single-dose trial (manuscript in preparation). In brief, we observed that, despite depletion of pro-inflammatory CpG motifs, 20-ml nebulised doses led to systemic inflammatory responses and an acute reduction in pulmonary function; although these were largely well tolerated, we considered them unacceptable for repeated application. The side effects were clearly dose related. The group receiving 5-ml doses had no, or very low-level, fever, minimal rises in systemic inflammatory markers and small, self-limiting reductions in lung function after dosing; 10 ml was somewhere in between and, although probably acceptable, we had concerns that blinding, of either patients or staff, could be compromised. A 5-ml dose was therefore chosen for the current trial. In addition, standard doses of the antipyretic agent paracetamol post dosing appeared to further reduce both symptoms and inflammatory markers and was administered within 2 hours of dosing and again 6 hours later, with a specific aim to ensure patient blinding.

Summary

Cystic fibrosis is a life-limiting disease; currently, there is no treatment targeted at the basic defect in the majority of patients. The UK CFGTC considers that gene therapy has the potential for broad, mutation-independent applicability and has set out to design a clinical trial of the optimal non-viral formulation powered to detect clinical benefit.

In *Chapter 2,* the specific design of the trial, along with background and rationale for the choice of outcome measures, is discussed.

Chapter 2 Methods: clinical trial design and outcome measures

This trial was a placebo-controlled, parallel trial of 12 monthly doses of the gene therapy product or placebo. Given the chronic nature of the disease, this time period was judged to be an adequate one over which to observe potential improvements in clinical outcomes, while not overburdening patients. It is a time period chosen in similarly designed trials of other agents.

Trial formulation

- Active: GL67A nebulised and applied via nasal spray.
- Placebo: 0.9% saline.

Trial objectives

1. To assess the clinical benefit of the gene therapy formulation when given on a monthly basis over a period of 1 year.
2. To assess the safety and tolerability of the formulation over the same period.
3. To assess gene expression of the formulation over the same period.

Additional research questions

1. Will markers of molecular efficacy (mRNA and PD) measured in the lung correlate with changes in clinical end points?
2. Will markers of molecular efficacy measured in the nose correlate with these markers measured in the lungs in the same patients?
3. Will molecular efficacy increase with repeated administration when measured serially in the same patient?
4. Will it be possible to identify the phenotype of responders or non-responders to allow stratification of patients for future gene therapy trials?
5. Will it be possible to identify stratifiable biomarkers from the secondary outcomes, based on disease severity?
6. Will one (or more) of the secondary outcome measures provide different or better information than FEV_1, allowing it to be proposed as a novel registrable biomarker for future trials?
7. Will the extensive preclinical molecular efficacy data produced in support of this study correlate with similar data in humans?
8. Will the extensive two species toxicology package produced in support of this study correlate with the findings in humans?

Trial sponsorship, oversight and approvals

The protocol was developed by the UK CFGTC Strategy Group members and trial statistician (GM) and sponsored by Imperial College London, London, UK.

The protocol was submitted to the GTAC on 7 October 2011 and received approval on 8 March 2012 (GTAC number 184). Clinical trials authorisation was received from the MHRA on 2 February 2012 (EudraCT number 2011-004761-33) and approvals from research and development at all clinical sites was obtained prior to commencing. The trial is registered on clinicaltrials.gov (NCT01621867) and ISRCTN (Current Controlled Trials ISRCTN71164341).

Trial outcome

For a detailed description see *Detailed description of outcome measures and assays*.

Relative change from baseline in percentage predicted FEV_1.

Major secondary outcomes
These include:

- change in other spirometric values [forced vital capacity (FVC), mid-expiratory flow 25–75% ($MEF_{25-75\%}$)]
- lung clearance index (LCI)
- structural parameters on computed tomography (CT) scans of the chest
- a validated quality-of-life score (as measured by the Cystic Fibrosis Questionnaire – Revised; CFQ-R).[71]

Other secondary outcomes
Other measures of lung physiology including:

- exercise capacity [maximal oxygen uptake (VO_{2max})] and activity monitoring
- gas transfer
- Inflammatory markers:

  - blood – white blood cell count and differential count, C-reactive protein (CRP), serum calprotectin
  - sputum – soluble inflammatory markers, cellular inflammation, 24-hour weight, solid content, DNA content, microbiology.

Adverse events (AEs) and other safety measures including:

- blood biochemistry
- haematology
- liver and renal function
- urinary markers
- histology
- fat-laden airway cells.

Mechanistic outcomes

- Transgene-specific DNA and mRNA on bronchial and nasal brushings.
- Nasal and lower airway PD measurements (CFTR protein function).

Statistical considerations in design and analysis

Proposed sample and effect sizes

The sample size derivation was based on outcomes of previous clinical trials in CF and available data on what is widely considered the minimum clinically meaningful difference. Over the last two decades, a relatively small number of new drugs have been licensed for the treatment of patients with CF and adopted internationally as standards of care. Those of particular note include the mucolytic agent, recombinant human DNase [rhDNase; dornase alpha (Pulmozyme®, Roche)]; tobramycin for the inhaled solution; and the macrolide, azithromycin.

(a) Recombinant DNase acts by breaking down neutrophil-derived DNA, which contributes significantly to mucus viscosity. In the most widely cited double-blind, placebo-controlled trial, the drug led to a relative improvement in FEV_1 of 5.8%; this was accompanied by a significant reduction in the frequency of infective exacerbations.[72] This agent is now a licensed product in routine clinical use worldwide.

(b) Tobramycin for inhalation solution (TOBI) was designed as an antipseudomonal antibiotic to be administered on alternate months, in contrast to other agents that are administered continuously. At the end of a 6-month trial period, the relative improvement in FEV_1 was around 7%.[73] This agent is now considered the gold standard by the regulatory agencies both in Europe and in the USA, to the extent that all other inhaled antibiotics are required to undergo head-to-head testing with it.

(c) Azithromycin is an orally bioavailable macrolide that was first considered as a useful therapy in CF after significant success in Japanese panbronchiolitis sufferers. Its mechanism of action is unknown, but is thought likely to relate to its known anti-inflammatory actions. Several trials have been conducted in CF, the largest of which reported a 6.2% TE on FEV_1 and a significant reduction in the frequency of exacerbations.[74] Azithromycin is now in widespread clinical use for CF.

Our decision to power for a relative change of 6% in FEV_1 was made, in part based on these data, and in part on pragmatic considerations of patient recruitment feasibility and cost of materials. We suggest that the current consensus supports this change as being a clinically meaningful improvement. Based on run-in study data [longitudinal assessment of clinical measurements in patients with cystic fibrosis in preparation for a clinical trial of CFTR gene therapy (unpublished data, Gene Therapy Consortium)], and using the mean of two measurements at baseline and the mean of two measurements at 12 months, we estimate the standard deviation (SD) of the percentage change in percentage predicted FEV_1 over 12 months to be 10.0%. The use of duplicate measurements reduces variability substantially, with resultant increases in power. The corresponding SD using only single measurements is 12.2%. We also demonstrated, using data from the run-in study, that analysis of percentage predicted FEV_1 is more sensitive than analysis of absolute FEV_1; the corresponding SD for percentage change from baseline based on duplicate measurements was 11.6% for absolute FEV_1. With the SD of 10.0%, a total sample of 120 evaluable patients would provide 90% power at the 5% significance level (2-sided) to detect a difference of 6% between the randomised groups in the mean change from baseline. With a plan to recruit 130 patients, we would allow a safety margin for subjects leaving the study prior to completion. This number also allows us to be powered at $\geq 80\%$ to detect changes in the secondary outcomes LCI and CT scan parameters, which were smaller than we had previously observed in a study of intravenous antibiotics.[65] The above power calculation was conservative in that covariate adjustment can be anticipated to increase the statistical power.

Statistical analysis

We reviewed the approach taken to the analysis of FEV_1 in over 40 published randomised trials of interventions in CF. There is no consistent approach, with roughly half of the trials using absolute changes from baseline (for either absolute FEV_1, measured in litres, or percentage predicted FEV_1) and the other half using relative changes (i.e. percentage change from baseline in terms of either absolute FEV_1 or percentage predicted FEV_1). Analysis of absolute changes is typically based on an analysis of covariance (ANCOVA) with baseline FEV_1 as a covariate, and in some instances relative changes were also analysed with an ANCOVA to adjust for baseline FEV_1.[75] The clinical investigators were of the opinion that in the context of this trial and, in particular, given the age range of the recruits, the clinically relevant measure of TE is the

relative (percentage) change from baseline in the percentage predicted FEV_1 and not the absolute change. The primary analysis was designed to compare the two randomised groups in terms of the mean percentage change in percentage predicted FEV_1 from baseline to end of treatment.

An ANCOVA model was designed to include baseline percentage predicted FEV_1, together with the other variables used in the randomisation algorithm, as covariates. 'Baseline' will be defined as the mean of the FEV_1 values from the two pretreatment assessments. 'End of treatment' will be taken as the mean of the values obtained at 14 days and 28 days following the final treatment. The TE will be presented as an adjusted difference in mean percentage change along with its corresponding 95% confidence interval (CI). No interim efficacy analyses were planned. As a sensitivity analysis the above analysis would be repeated, but with the logarithm of the end of treatment percentage predicted FEV_1 taken as the response variable, and the logarithm of the baseline percentage predicted FEV_1 included as a covariate in place of its raw value. An exploratory analysis compared the two randomised groups in terms of the evolution of FEV_1 over the 12 months of treatment. The study was not adequately powered to explore subgroup effects for the primary outcome measure, although we did plan to look at the stability of the TE over subgroups defined by the covariates included in the ANCOVA model. The formal analyses were performed by including interaction terms in the model. A similar approach could be used with certain secondary outcome measures that are closer to the direct mechanism of action of the study intervention, as there is likely to be more statistical power with such variables to explore subgroup effects, which could support a 'stratified medicine' approach to the use of gene therapy.

The intention-to-treat (ITT) group was defined as patients randomised and having any subsequent follow-up data. The per-protocol (PP) population was defined as patients receiving ≥ 9 doses. Demographic and safety data are presented for both populations. All efficacy data are presented for the PP population.

Subject identification, recruitment and randomisation

Inclusion criteria

1. Cystic fibrosis confirmed by sweat testing or genetic analysis.
2. Males and females aged ≥ 12 years.
3. FEV_1 between 50% and 90% predicted inclusive (Global Lung Function Initiative reference ranges; www.ers-education.org/guidelines/global-lungfunction-initiative.aspx).
4. Clinical stability at screening defined by:

   i. not on any additional antibiotics (excluding routine, long-term treatments) for the previous 2 weeks
   ii. no increase in symptoms such as change in sputum production/colour, increased wheeze or breathlessness over the previous 2 weeks
   iii. no change in regular respiratory treatments over the previous 4 weeks
   iv. if any of these apply, entry into the study can be deferred.

5. Prepared to take effective contraceptive precautions for the duration of their participation in the study and for 3 months thereafter (as stated in GTAC guidelines)
6. If taking regular rhDNase and is willing, and considered able by independent medical carers, to withhold treatment for 24 hours before and 24 hours after the gene therapy dose
7. Written informed consent obtained.
8. Permission to inform general practitioner of participation in study.

Exclusion criteria

1. Infection with *Burkholderia cepacia* complex organisms, MRSA or *M. abscessus.*
2. Significant nasal pathology including polyps, clinically significant rhinosinusitis, or recurrent severe epistaxis (nose bleeds; nasal cohort only).
3. Acute upper respiratory tract infection within the last 2 weeks (entry can be deferred).

4. Previous spontaneous pneumothorax without pleurodesis (bronchoscopic subgroup only).
5. Recurrent severe haemoptysis (bronchoscopic subgroup only).
6. Current smoker.
7. Significant comorbidity including:

    i. moderate/severe CF liver disease (varices or significant, sustained elevation of transaminases: alanine transaminase/aspartate transaminase levels of > 100 IU/l)
    ii. significant renal impairment (serum creatinine levels of > 150 μmol/l)
    iii. significant coagulopathy (bronchoscopic group only)

8. Receiving second-line immunosuppressant drugs, such as methotrexate, ciclosporin and intravenous immunoglobulin preparations.
9. Pregnant or breastfeeding.

Patient recruitment

We had originally anticipated that the majority of trial participants would be recruited from an earlier study (the Gene Therapy Run-in) assessing outcome measures at periods of stability (unpublished data, Gene Therapy Consortuim). However, delays because of funding issues and the resultant drop-off in patient interest meant that recruitment had to be spread more widely. Initial patients were recruited by study staff from the adult and paediatric clinics at the Royal Brompton Hospital and from across Scottish CF centres. Once it was apparent that the pace of recruitment was slower than required, we activated participant identification centres. Local CF centres were activated in November 2012 and nationwide CF centres followed in January 2013; in total, 16 outside centres referred patients. These patients attended one of the three sites for trial-related visits, but continued their clinical care at their referring hospital. All patients had read a detailed, age-specific information sheet and underwent a thorough briefing before signing consent. Parents provided consent for participation of paediatric subjects, who were also asked for their assent to take part. Patient information sheets are available in *Appendix 1*.

Randomisation

Randomisation was performed by trial staff following a successful screening visit using the electronic InForm™, version 4.6 (Phase Forward Incorporated, Oracle, CA, USA) system. Patients consenting to participate in one or more of the mechanistic subgroups were randomised 2 : 1 to enrich for subjects receiving active treatment; all others were randomised on a 1 : 1 basis. The data required for randomisation and stratification [centre (London or Edinburgh), age (< 18/≥ 18 years), FEV_1 (< 70/≥ 70%) and inclusion in the mechanistic subgroups] were entered by a member of the trial team, which led to the generation of a unique patient number corresponding to a blinded randomised arm of the trial. In the event of computer system failure, a prearranged manual randomisation method was available from the InForm team. The unique patient number was entered onto the subject's prescription sheet and submitted to the trial pharmacists who had access to the unblinding code and prepared the active or placebo product as appropriate.

Preparation of dose and blinding

Assembly of pGM169/GL67A complexes

The pGM169 drug substance and GL67A cationic lipid mixture are imported into the UK by The Clinical Biotechnology Centre of the University of Bristol, Bristol, UK, by a qualified person acting on behalf of the UK National Blood Service, a division of the NHS Blood and Transplant Special Health Authority. The dose preparation is described in full in *Appendix 2*. Briefly, immediately prior to use, pGM169 and GL67A are thawed to room temperature and GL67A is hydrated in water for injection. pGM169/GL67A complexes are prepared by rapidly mixing 5 ml of pGM169 and 5 ml of GL67A using a disposable syringe-based static mixer device [Plas Pak Industries, Norwich, CT, USA (*Figure 5*)]. The final dosage form of pGM169/GL67A contains nominally 2.6 mg/ml of pGM169, 3.7 mg/ml of GL67, 8.9 mg/ml of DOPE and 1.7 mg/ml of DMPE-PEG5000. The shelf life of the final formulation was 6 hours.
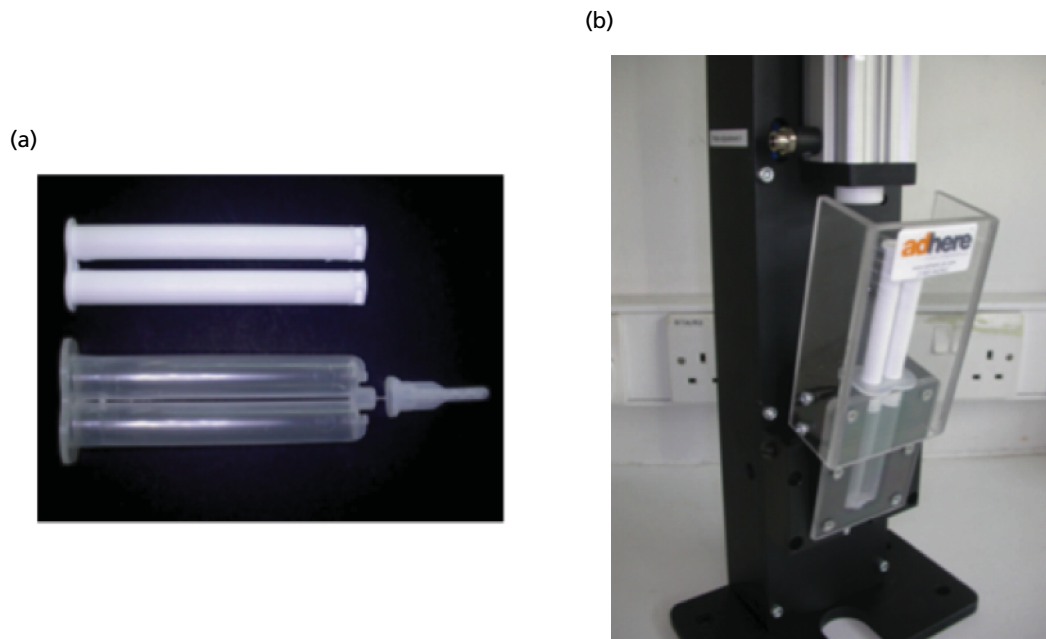
FIGURE 5 Mixing of the trial formulation. The pGM169 and GL67A were mixed in a dual-barrel mixing device by loading the components into the syringe (a) and using a fixed-rate plunge mechanism (b) to deliver this into the receiving container.

Blinding

pGM169/GL67A or placebo (0.9% saline) were then filled into AeroEclipse II breath-actuated nebulisers by unblinded trial pharmacists. To avoid any inadvertent unblinding by clinical staff, nebulisers were taped and a tamper-proof seal was attached (*Figure 6* and see *Appendix 3* for details). Clinical trial staff, participants and trial analysts were blind to allocation until database lock. The 10-ml volumes were placed in opaque nasal spray devices (GlaxoSmithKline parts number AR5989 30 ml bottle/AR9488 30 ml actuator) and the device was primed.
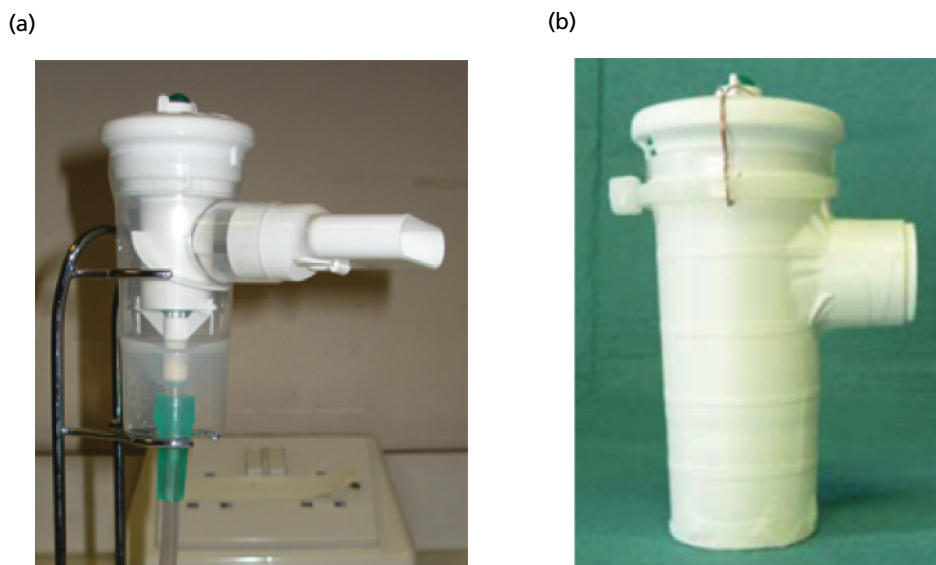
FIGURE 6 Masking of the nebuliser pots to ensure blinding. AeroEclipse II nebuliser pots are transparent (a) as the gene therapy formulation has a milky appearance that contrasts clearly with that of the 0.9% saline (clear, colourless liquid), it was necessary to mask the pots, which was achieved with tape and (b) a tamper-proof seal was applied to the lid, which also prevented any unintentional spillages.

Administration of doses to trial participants

Patients were pretreated with 200–400 μg of salbutamol via a metered-dose inhaler and spacer device approximately 20 minutes prior to receiving the trial treatment to prevent bronchospasm in response to the hypotonic nature of the trial preparation. Nebulisation took place in sealed negative-pressure cubicles, with external venting to limit spread within the hospital setting; patients were observed and communicated with through a glass window by the trial team. The 5-ml aliquot was nebulised during cycles of tidal breathing while patients were wearing a nose clip (*Figure 7*) for 3 minutes, following which the nebuliser air supply (8 l/minute) was turned off for a 2-minute rest period. Nebulisation continued for 40 minutes, which was predetermined as a period after which delivery was complete. Patients in the nasal subgroup administered one actuation of the nasal spray device to each nostril during the 2-minute 'off-nebuliser' period, which resulted in an approximate 1-ml dose to each nostril (see *Figure 7*). After dosing, patients either remained in the cubicle for 40 minutes, or were able to leave wearing a mask to limit spread of exhaled trial product in the unit. They were observed for 1–2 hours, but no spirometry or temperature measurements were made, to limit the potential for unblinding related to transient dose-related fever or drop in lung function. Patients were administered paracetamol (dose adjusted for body weight if < 40 kg) within 2 hours of dosing and asked to take another dose 6 hours later, once at home. This was again to prevent unblinding of the active group.

(a) (b)
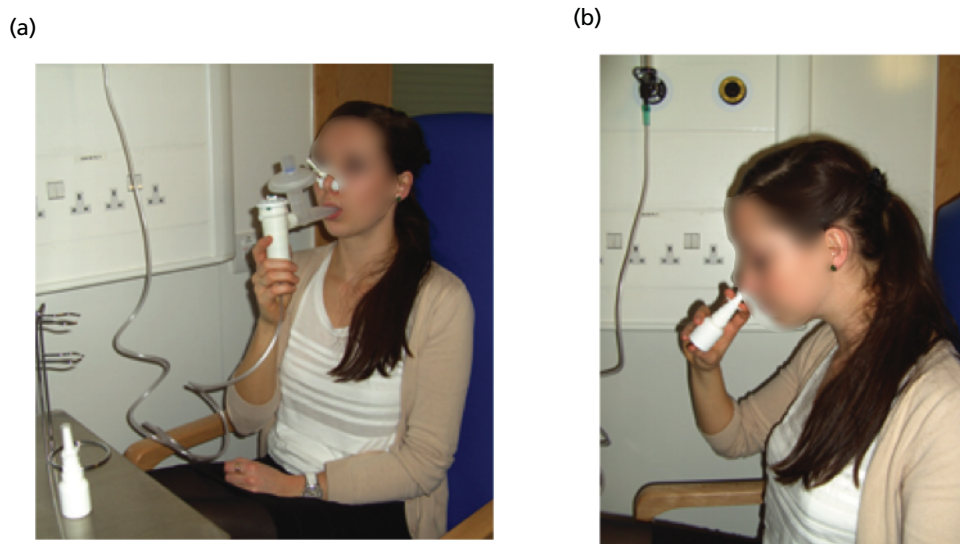
FIGURE 7 Dose administration. Nebulisation of the trial product in a sealed, negative-pressure cubicle while subject (a) wears a nose clip and; (b) receives delivery of nasal dose. A member of the study team illustrates the technique.

Study visits and interventions

Early patient safety cohort adaptive design

An adaptive early recruitment phase was designed to permit the early identification of any cumulative side effects: 20 subjects (10 active treatment and 10 placebo) would receive three doses at 4-weekly intervals before any further subjects were dosed. In addition to the visits described below undertaken by the entire cohort, they were also seen on day 2 post each dose. Clinical examination findings, lung function, gas transfer and systemic inflammatory markers would then be reviewed in an unblinded fashion by the Data Monitoring and Ethics Committee (DMEC).

- Should data prove satisfactory, these subjects would continue with subsequent visits and the remainder of the cohort will begin dosing.
- Should the data be of sufficient concern to the DMEC that progression was unacceptable, a second cohort of 20 patients would receive three doses of the formulation at a 2.5-ml dose, followed by DMEC review in a similar fashion.

  - If these data were acceptable, the trial would continue with all subjects receiving a 2.5-ml dose of either gene therapy or placebo. In this instance, the initial cohort would be discontinued and an additional 20 'naive' subjects recruited.
  - Should the DMEC have considered these data unacceptable, the trial would have been halted.

The adaptive design was not in fact required as there were no concerning safety signals. Safety data from this cohort are presented in *Chapter 5*.

Schedule of trial visits

Scheduled study visits and interventions are shown in *Table 1*.

TABLE 1 Scheduled study visits and interventions

Summary of study assessments

Week / Visit name	E&C	Intro	Screen	nPD1[a]	nPD2[a]	nPD3[a]	Bronch[a]	0 / Dose 1	D2a	4 / Dose 2	D2b	8 / Dose 3	D2c	12 / Dose 4	16 / Dose 5	20 / Dose 6	24 / Dose 7	28 / Dose 8	32 / Dose 9	36 / Dose 10	40 / Dose 11	44 / Dose 12	46 / F/U1	48 / F/U2	49 / Bronch
Consent/history/examination																									
Informed consent	x[b]	x[b]	x[b]				x[c]																		
Medical history	x	x	x				x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Physical examination	x	x	x				x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Quality-of-life questionnaire (CFQ-R)	x	x	x					x						x			x			x				x	
Vital signs	x	x	x				x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Pulse oximetry	x	x	x				x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Prior medications	x[b]	x[b]	x[b]																						
Concomitant medications			x				x	x		x		x		x	x	x	x	x	x	x	x	x	x	x	x
Check diary comments and reissue diary								x		x		x		x	x	x	x	x	x	x	x	x	x	x	
AEs	x	x	x				x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Download PiKo-6 device (nSpire Health Ltd, Hertford, UK)			x[d]																						
Sweat test[e]	x[e]	x[e]	x[e]																						
Samples[f]																									
Blood sampling	x	x	x					x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	
Urine sampling	x	x	x					x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	
Sputum[g]	x[h,i]	x[h,i]	x[h,i]					x[h]	x	x	x	x	x	x		x			x	x			x[j]	x[j]	
Sputum for NTM	x[k]	x[k]	x[k]					x[k]		x													x[k]	x[k]	
24-hour sputum weight	x	x	x					x	x	x	x	x	x	x				x						x	

TABLE 1 Scheduled study visits and interventions (*continued*)

Summary of study assessments

Visit name (Week)	E&C	Intro	Screen	nPD1[a]	nPD2[a]	nPD3[a]	Bronch[a]	Dose 1 (0)	D2a	Dose 2 (4)	D2b	Dose 3 (8)	D2c	Dose 4 (12)	Dose 5 (16)	Dose 6 (20)	Dose 7 (24)	Dose 8 (28)	Dose 9 (32)	Dose 10 (36)	Dose 11 (40)	Dose 12 (44)	F/U1 (46)	F/U2 (48)	Bronch (49)
Investigations[f]																									
LCI		x[h]	x[h]					x[h]						x			x			x			x[h]	x	
Exercise bike test			x[k]					x[k]							x[k]					x				x	
Activity monitor			x													x							x		
CT scan			x											x										x	
Gas transfer			x				x		x		x		x	x			x							x	x
Bronchial blood flow measurement[m]			x											x										x	
Spirometry	x		x				x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Dosing																									
Randomisation[n]								x																	
Study dose administration								x		x		x		x	x	x	x	x	x	x	x	x			
Nasal/bronchoscopy subgroups only																									
Nasal PD	x[o]	x[o]	x[o]	x[o]	x[o]	x[o]						x[p]		x[m]		x[p]	x		x[p]	x[m]			x[h]	x[h]	x[h]
Nasal brushing			x[q]			x[q]																	x[k]	x[k]	x[k]
Bronchoscopy							x																		x

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Summary of study assessments

Week								0		4		8		12	16	20	24	28	32	36	40	44	46	48	49
Visit name	E&C	Intro	Screen	nPD1[a]	nPD2[a]	nPD3[a]	Bronch[a]	Dose 1	D2a	Dose 2	D2b	Dose 3	D2c	Dose 4	Dose 5	Dose 6	Dose 7	Dose 8	Dose 9	Dose 10	Dose 11	Dose 12	F/U1	F/U2	Bronch
Predischarge administration																									
Instructions regarding home spirometry (PiKo-6)	x		x					x		x		x		x	x	x	x	x	x	x	x	x	x		
Instructions regarding use of symptom diary	x		x					x		x		x		x	x	x	x	x	x	x	x	x	x		
Schedule next study appointment	x		x					x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x		

Bronch, bronchoscopy; D2a, Day 2a; D2b, Day 2b; D2c, Day 2c; E&C, eligibility and consent; FU1, follow-up 1; FU2, follow-up 2; intro, introduction; nPD1, nasal potential difference on occasion 1; nPD2, nasal potential difference on occasion 2; nPD3, nasal potential difference on occasion 3; NTM, non-tuberculous mycobacteria.

a May occur before screening.
b First point of contact only.
c Bronchoscopy-specific consent only.
d Patients who have undergone introductory visit only.
e One time point pre-dosing if required.
f On dosing visits, must be pre-dose.
g If no sputum available, cough swab to be performed.
h Two time points only.
i Can induce at these time points; required once pre and once post dosing.
j For intensively monitored cohort only.
k One time point only.
l Limited cut.
m If available; timing may vary.
n To be conducted within week prior to visit.
o Three occasions only.
p Optional at day 14 post dosing.
q One occasion only with final nasal PD.
Subgroups in green areas: intensive cohort, new subjects, nasal/bronchial cohorts.

Detailed description of outcome measures and assays

Clinical examination

Clinical examination included recording pulse rate, blood pressure (BP), respiratory rate, temperature, pulse oximetry, height, weight and lung auscultation. Patients were assessed by a team comprising medical, nursing and respiratory physiology personnel.

Spirometry

Forced expiratory manoeuvres from which the FEV_1 and the total volume exhaled (FVC) are conventional measures of airway patency and lung health, and are widely used both clinically and in trial settings for CF. The MEF (flow at varying percentages of vital capacity) are thought to more closely reflect small, distal airway disease, but are recognised as being more inherently variable. In CF, FEV_1 is closely linked to survival and is the most widely used primary outcome in clinical trials of respiratory interventions. It is recognised by regulatory agencies as a surrogate outcome and for this reason, alongside our longitudinal data demonstrating good power, it was chosen as the primary outcome for this trial. Spirometry was performed on the EasyOne spirometer (New Diagnostic Design Technologies, Zurich, Switzerland) with disposable mouthpiece and filter according to the American Thoracic Society (ATS)/European Respiratory Society (ERS) criteria.[76] A minimum of three measurements and the best FEV_1, FVC and $MEF_{25-75\%}$ were recorded in absolute values. These values were converted to percentage predicted values based on the Stanojevic references.[77]

Lung clearance index

The LCI is a measure derived from a multiple breath washout (MBW), which provides a global measurement of ventilation inhomogeneity. It can be performed either with inhalation of an inert tracer gas, such as sulphur hexafluoride (SF6), or by using 100% oxygen to wash out resident nitrogen. In the case of an exogenous tracer, the gas is inspired until equilibrium is reached between the inhaled and exhaled air at which point the gas source is removed. The number of lung volume turnovers required until the expired tracer gas concentration falls to an arbitrary 1/40th of its starting value is used to calculate the LCI. Individuals with lung disease and greater ventilation inhomogeneity require longer to clear the tracer gas and, therefore, will have a higher (more abnormal) LCI.

Many different systems have been used to measure MBW in clinical trials in CF and these have been summarised in a recent ATS/ERS consensus document.[78] Although the mass spectrometer is considered the gold standard gas analyser, it is very expensive, custom built for MBW and, therefore, not suitable for widespread use.[78] The method adopted in this trial used the tracer gas, SF6, detected by the Innocor™ photoacoustic gas analyser (Innovision, Glamsbjerg, Denmark). Methodology was as described in a recent trial of the small molecule, ivacaftor.[79] In brief, the procedure is performed with a mouthpiece during tidal breathing while patients wear a nose clip (*Figure 8*). Triplicate measures are undertaken, with each test taking approximately 15 minutes. Data are subsequently analysed off-line according to strict standard operating procedures (SOPs) for quality control using software developed within the consortium. Every seventh trace was analysed in duplicate by a corresponding team member from the other site.

Cycle ergometry

This test of exercise capacity was performed on a stationary exercise bike with breath-by-breath analysis on the Innocor photoacoustic gas analyser and was used to calculate VO_{2max}. Subjects were asked to pedal at a set speed of 70 rpm and maintain this speed throughout the test while wearing a nose clip and breathing via a mouthpiece attached to a gas analyser. The resistance increased automatically each minute for as long as pedalling could be sustained. The starting workload and increase in workload was dependent on the patient's height, and calculated using the Godfrey protocol.

- Patients < 120 cm use 10 W starting resistance and 10-W increments.
- Patients 120–150 cm use 15 W starting resistance and 15-W increments.
- Patients > 150 cm use 20 W starting resistance and 25-W increments.

The subject's heart rate, oxygen saturations and Borg scale were measured at rest and at each minute during exercise. The test was stopped by the operator if the patient's oxygen saturation fell below 80%, if they were unable to maintain the required pedalling rate or if there was any concern about the patient's condition. Once the test was complete, patients had a 2-minute cool-down period and were monitored until their oxygen saturation returned to baseline. The Innocor system was validated against standard calibrated VO_2 equipment using paired exercise tests in 12 CF patients. For VO_{2max}, the mean difference (reference equipment – Innocor) was –0.026 l/minute and the 95% CI was –0.27 to 0.22 l/minute.

(a)

FIGURE 8 Lung clearance index. (a) A child performs a LCI on the Innocor gas analyser; (b) wash-in of the tracer gas, SF6 (in green) is undertaken until equilibrium; and (c) the gas supply is disconnected and concentration falls with each subsequent breath as washout occurs (bottom). The black trace denotes flow. (*continued*)

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Body-worn activity monitoring

Patients were asked to wear the SenseWear® body monitoring system™ (BodyMedia, Pittsburgh, PA, USA) for at least 7 whole days at time points throughout the trial. The device uses accelerometry, heat flux, skin temperature and galvanic skin response sensors to gather physiological data on movement and daily physical activity patterns. These armbands have been validated in normal subjects and a number of patient groups.[80] Data from activity monitors were downloaded and analysed using proprietary software. Final data were analysed when there were at least 4 days of data, at least 1 day was on a weekend and there were at least 10 hours of data for each day.

This analysis yields data for the proportion of time that wearers spend at various levels of energy expenditure. For the purposes of summarising these data in the present trial, a comparison was made (between active and placebo groups) of the percentage of time spent in vigorous exercise (defined as > 3 metabolic equivalents; METs) compared with the percentage of time spent at ≤ 3 METs of activity, as well as the mean number of steps taken per day.

Gas transfer

The transfer factor of the lung for carbon monoxide (TLCO) was measured using a single-breath technique (London site: Jaeger Masterscreen, CareFusion, Germany; Edinburgh site: CPL, nSpire Health, England). Subjects, who were seated and wearing a nose clip, were asked to exhale to residual volume, then take a maximal inspiration (required to be > 90% vital capacity) of the test gas (medical air with 0.28% carbon monoxide and 9% helium) prior to a breath hold of 10 seconds' duration and a smooth exhalation. This procedure was repeated at least twice on each test day (minimal interval between tests was 4 minutes). Duplicate estimates of TLCO were required to be within 10% or 1.0 mmol/minute/kPa (whichever was greater) to meet requirements for inclusion. At least two technically acceptable tests from up to 10 attempts were obtained and the mean values were used for analysis. The following values were recorded for each test: single-breath TLCO, alveolar volume (VA), transfer coefficient (KCO; derived from TLCO/VA) and inspiratory vital capacity. TLCO and KCO were corrected for most recent haemoglobin (usually obtained on the same day) and expressed as corrected TLCO (TLCOc) and corrected KCO (KCOc) using standard formulae.

Quality-of-life questionnaire

The validated CFQ-R for either adults or children was completed by subjects at time points throughout the trial, always at the start of a visit before any other study investigations. The questionnaire is available in *Appendix 4*.

Lung computed tomography scanning

High-resolution CT scans were obtained on 64-channel multidetector scanners (SOMATOM Sensation 16 or 64; Siemens Medical Solutions, Erlangen, Germany). On two occasions (pre-dosing and at 28 ± 5 days after dose 12) patients underwent a full scan comprising contiguous thin sections (1.25 mm) through the entire volume of the lungs obtained during inspiration and also interspaced (1-mm sections at 10-mm increments) at end-expiration. A high-spatial-resolution algorithm was used for image reconstruction. All CT scans were scored independently by the same two radiologists specialising in thoracic imaging; scans had been anonymised and scores were assigned with the radiologists blinded to both subject identification and whether pre- or post-dosing. All scoring was performed directly from workstations with access to image manipulation including window settings (default: width 1500 Hounsfield units, centre 500 Hounsfield units). The presence and severity of specific CT scan features were recorded for each lobe as previously described.[81] The features studied were extent of bronchiectasis, severity of bronchiectasis, bronchial wall thickness, small and large airway mucus plugs (all scored per lobe on a range of 0–4 and the results meaned) and gas trapping (scored on a percentage basis) (features illustrated on examples in *Figure 9*). In addition, as a safety outcome, all subjects underwent a limited-cut inspiratory CT scan pre-dosing at the dose 4 visit; these scans were not formally scored, but were reviewed in real time by the clinical radiology team for any acute changes and the reports were provided to the DMEC.

(a)
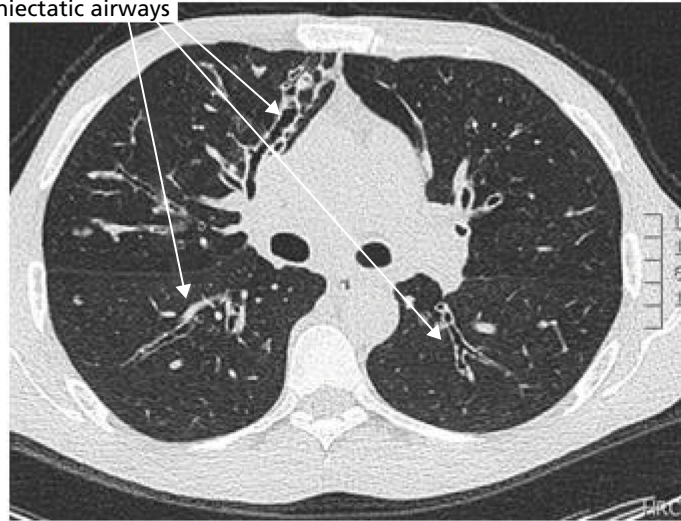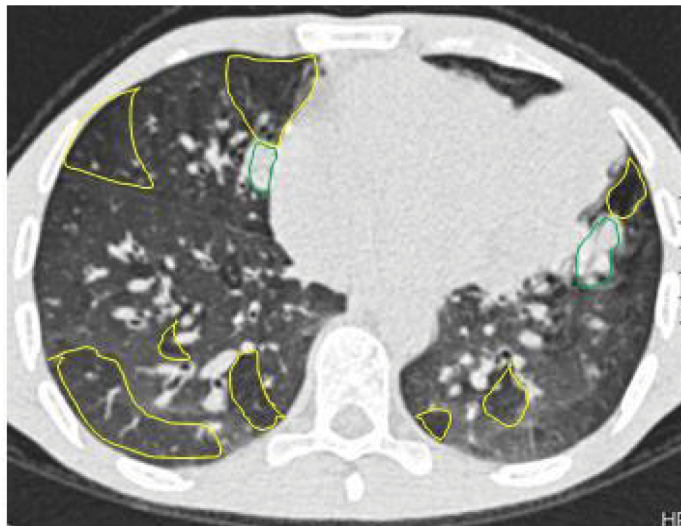
(b)

FIGURE 9 Computed tomography parameters. (a) Examples of bronchiectasis; (b) gas trapping (areas of mosaic attenuation highlighted in yellow where gas has failed to empty from areas of lung on expiration); and (c) an area of dense small mucus plugs in the left-lower lobe highlighted in yellow. (*continued*)

(c)

FIGURE 9 Computed tomography parameters. (a) Examples of bronchiectasis; (b) gas trapping (areas of mosaic attenuation highlighted in yellow where gas has failed to empty from areas of lung on expiration); and (c) an area of dense small mucus plugs in the left-lower lobe highlighted in yellow.

Venepuncture for blood sampling

This was performed by an experienced practitioner after the application of topical anaesthetic cream, if desired by the patient, via a peripheral vein. The use of central indwelling intravenous catheters was discouraged but not prohibited.

Urine sampling

Urine was collected into a sterile container and tested immediately by dipstick.

Sputum sampling

Sputum was collected after spontaneous expectoration whenever possible. In patients unable to expectorate, induction was considered at screening and follow-up visits only; all other visits included administration of study drug and it was considered undesirable to expose the patients to a procedure known to induce a degree of bronchospasm prior to dosing. The induction protocol followed clinical guidelines and included pretreatment with an inhaled bronchodilator (200 µg of salbutamol or equivalent, 15 minutes before test). Hypertonic saline (7%) was administered via a deVilbiss 2000 (DeVilbiss Healthcare Ltd, Tipton, UK) or equivalent ultrasonic nebuliser. Attempts to induce sputum were made during and after nebulisation and FEV_1 was monitored carefully throughout. Prior to dose 1, sputum was expectorated in 72 out of 116 (62%) of patients [active group, 37/62 (60%); placebo group, 35/54 (65%)]. After the last dose, sputum was expectorated in 57 out 116 (49%) of patients [active group: 27/62 (44%); placebo group: 30/54 (56%)].

At certain visits, patients were asked to collect all sputum expectorated over the previous 24 hours into 50-ml Falcon tubes (VWR International Ltd, Leicestershire, UK) and to bring these with them to the study visit.

Nasal potential difference

The characteristic CF ion transport defects, involving the charged ions chloride and sodium, result in an altered PD across the respiratory epithelium, measurable in millivolts. Techniques to measure this for both clinical diagnostic purposes and in the context of trials have been developed.[82,83] When compared with healthy cells expressing *CFTR*, in CF the absent chloride secretion and sodium hyperabsorption result in an apical cell surface that is electrically more negative under basal or resting (non-stimulated) conditions. Amiloride, as a sodium channel blocker, results in a greater reduction (depolarisation) in nasal PD for CF than non-CF subjects and subsequent ionic and pharmacological challenges to stimulate chloride secretion

(via chemical gradients and cAMP stimulation) are largely ineffective in CF but lead to pronounced hyperpolarisation in the presence of functional CFTR (*Figure 10*).

There have been over 20 previous clinical trials of CF gene therapy, which have involved dosing to the nose, lung or both.[84] In trials involving nasal dosing, nasal PD has been used as a main outcome for efficacy.[39,42,50–52,55,58,84–86] However, these tests are inherently variable and gene therapy-related results have been similarly so; correlation of any change in nasal PD with molecular evidence of *CFTR* has also often been lacking.[50,52,58] Where changes in PD have been reported following gene therapy, these have almost uniquely been restricted to the chloride secretion phase rather than correcting sodium hyperabsorption phases. This likely reflects the higher proportion of cells required to be corrected before changes in sodium hyperabsorption can be corrected (> 90% vs. < 10% for restoration of chloride transport).[87–89] Use is not limited to gene therapy evaluation, and recent studies have included trials of the small-molecule drugs ataluren and ivacaftor. Interestingly, while the latter small-molecule potentiator demonstrated significant and large-magnitude clinical changes, changes in nasal PD were much more modest.

For reasons of variability, patients electing to participate in the nasal dosing substudy underwent up to three nasal PDs on different days prior to dosing and two at follow-up appointments. A minority of subjects also underwent on-treatment recordings at other time points, but these are not included in the analysis owing to the small number.
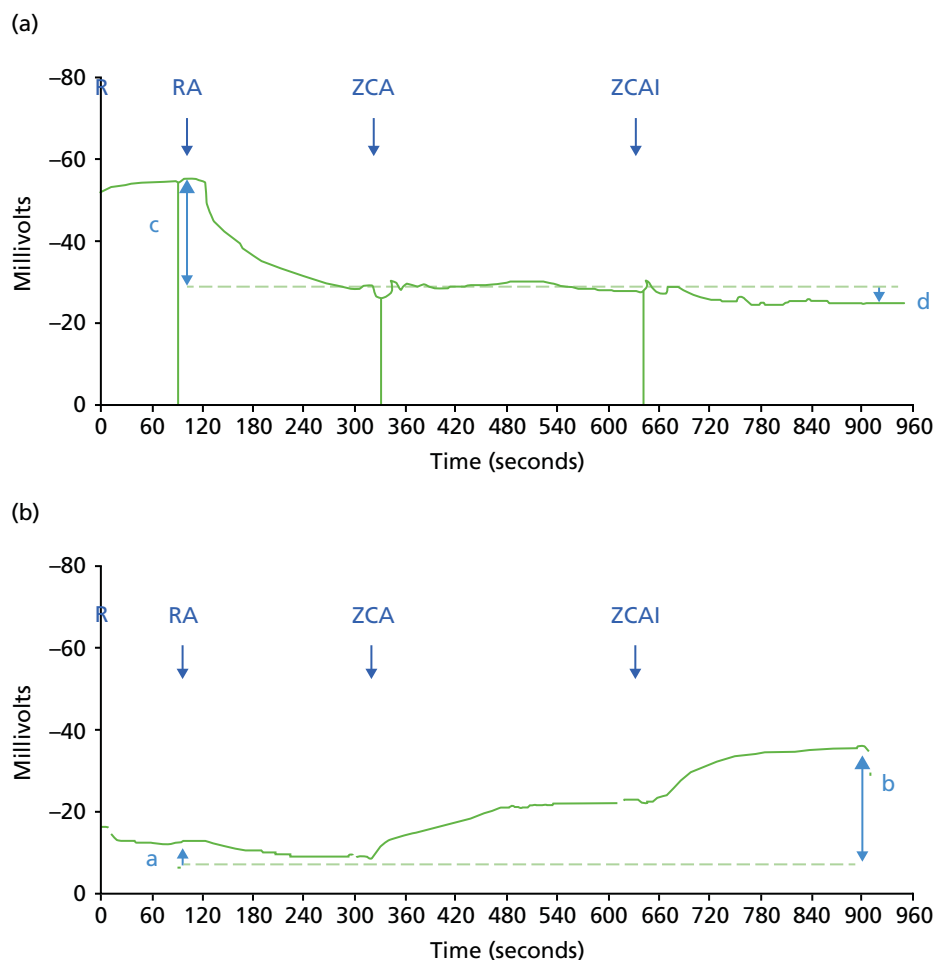
FIGURE 10 Representative nasal PD traces from (a) a CF patient; and (b) a healthy volunteer. The CF basal (Ringer's, R) measures are higher (more negative) than non-CF largely related to sodium hyperabsorption. For the same reason, there is a larger drop (depolarisation) to the sodium channel blocker, amiloride (RA). There is little chloride secretion in response to either a zero chloride solution (ZC, passive gradient) or the cAMP stimulator, isoproterenol (ZCAI), in contrast to the hyperpolarisation observed in the healthy volunteer.

At the time of the first measurement in any subject, a single-use, disposable, dual-lumen catheter (Marquat, France) was fixed in place at the site of maximal unstimulated PD in one nostril; for all subsequent measurements, the same nostril and the same distance into the nasal cavity was used. Solutions comprised chemicals listed in the Cystic Fibrosis Foundation Therapeutic Development Network (CFF TDN)'s SOP[90] and were perfused at room temperature at a rate of 4 ml/minute in the following sequence: Ringer's solution, Ringer's plus amiloride (0.1 mM), Ringer's plus amiloride plus zero chloride and Ringer's plus amiloride plus zero chloride containing isoproterenol (0.01 mM) (*Figure 11*). Post-dosing measurements were obtained after 14 ± 2 days and 28 ± 5 days.

Traces were obtained using a high-impedance, low-resistance voltmeter (LR-4; Logan Research UK Ltd, Rochester, UK) onto a laptop computer and were scored by four investigators experienced in the technique (EWFWA, JCD, SNS and MDW). The four components (baseline, amiloride, zero chloride and isoproterenol responses) were scored individually using standard criteria.

Nasal brushings

Nasal brushings were performed in subjects in the nasal subgroup both pre-dosing and at 28 ± 5 days post final dose from under the middle or inferior turbinate of both nostrils using interdental brushes (Dent-o-care, London, UK). Cells were suspended in 800 μl of phosphate-buffered saline (PBS) and processed for transgene DNA/mRNA analysis (see *Nasal and bronchial brushings*).

Bronchoscopy and associated procedures

Bronchoscopy was undertaken in a subgroup of patients for both efficacy (gene transfer assessed by mRNA on brushings and PD) and safety assays (remodelling or lipid deposition in endobronchial biopsies).

Potential difference

Cystic fibrosis transmembrane conductance regulator-mediated ion transport is clearly of more relevance in the lower airway than the nose, but is more complex and invasive to measure. No standardised technique has been developed and only a few research groups perform this procedure. General anaesthesia is required to overcome the challenges of interference from patient movement or coughing, and to avoid local anaesthetic agents that affect ion channel function. The single operating channel within most fibreoptic bronchoscopes limits the size of the PD catheters used. Although some systems allow for a double-lumen PD catheter to pass through this port, our group has designed a single-lumen technique.[91]

FIGURE 11 Nasal PD. The subject has a double-lumen catheter inserted in one nostril. Solutions are pumped through one lumen while the other is connected to an electrode via conducting cream. There is a reference electrode placed subcutaneously on the forearm.

The technique permits basal and stimulated measurements, although pooling of solutions which then dilute subsequent perfusate needs to be carefully avoided; for this reason, perfusion rate is slower than for nasal measurements and an amiloride phase is omitted. We have previously shown clear separation between CF and non-CF basal PD in the proximal airways, although in both groups PD falls with distal progression and a disease-specific difference is not seen in the most peripheral sites measured. As expected, chloride secretory responses are significantly different between CF and non-CF (*Figure 12*). This technique was used in our previous trial of GL67-mediated *CFTR* (with a previous version of the *CFTR* plasmid incorporating a CMV promoter) and showed significant improvement in chloride secretion on day 2 post dosing.[58]

In the current trial, all bronchoscopies and associated procedures were performed by a single operator (JCD), with the same senior anaesthetist (BK). Solutions (Ringer's and a zero-chloride solution) were manufactured by the pharmacy at Eastbourne Hospital, UK and constituted the chemicals in the CFF TDN SOP,[90] with the exception that amiloride was omitted from the latter. They were warmed prior to use to reduce the formation of microbubbles, but were used at room temperature and perfused at 100 ml/hour (*Figure 13*). Basal (non-stimulated) PD was measured on each wall of the distal trachea with sterile Ringer's solution. Where possible, a stable period (< 1 mV change over 30 seconds) was recorded. Subsequently, at three sites more distally (approximately fifth-generation airways; all in one lung), following a second period with Ringer's solution, the solution was switched to a zero-chloride equivalent containing isoproterenol (10 µM) for 5 minutes. The catheter was removed and reprimed between measurements. Hardware and software were as previously described.[91] Measurements were completed before the samples described below were obtained.

For analysis, recordings (1–3) from the same patient at the same time point were pre-grouped and scored blinded both for acceptability and response. Recordings were only accepted for scoring if (a) the time of any inflexion owing to the zero chloride/isoproterenol solution was approximately 50–80 seconds after onset of perfusion, this duration having previously been shown to represent clearing of the dead space within the catheter (≈50 seconds) and allowing ≈30 seconds for the initiation of any isoproterenol response; and (b) the response fell into one of three categories namely no response, continuous upwards (hyperpolarisation) or continuous downwards (depolarisation) deflection. Recordings were excluded if
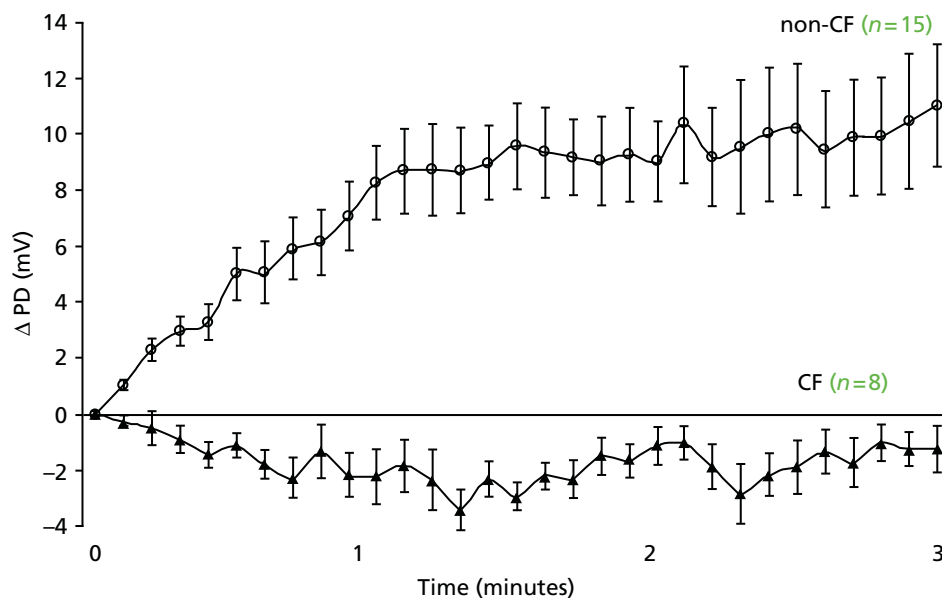
FIGURE 12 Chloride secretory responses on lower airway PD. Summary data demonstrating the increase in millivolts in lower airway PD in non-CF patients following perfusion with a zero-chloride solution containing isoprenaline (indicating chloride secretion). The effect is absent in patients with CF. The pre–post design of measurements in this trial is in an attempt to detect a signal over and above pre-dosing traces in the actively treated group.[91]

Single lumen perfusion and recording catheter on surface epithelium of ≈ fourth generation airway

Laptop

Millivoltmeter and electrodes

Infusion pumps
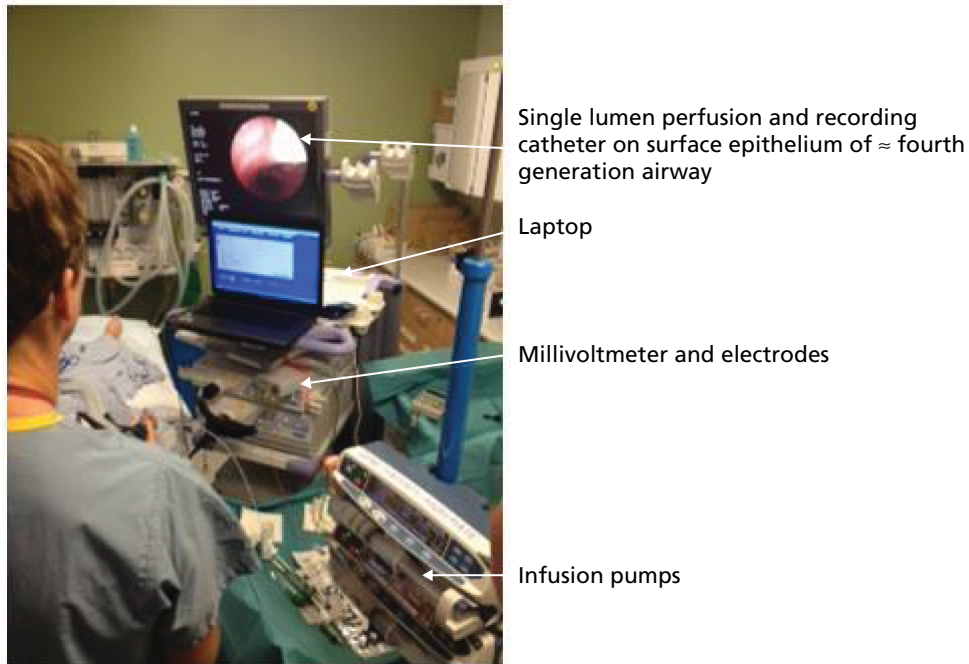
FIGURE 13 Bronchial PD measurement. Under video control, the PD catheter is advanced via the bronchoscope onto the lower airway epithelial surface and can be observed on the screen towards the top of the picture. Solutions are perfused via pumps to the right and recordings made via a millivoltmeter connected to a laptop computer.

(a) the response at the 50- to 80-second time point was unstable (rising or falling); or (b) the deflection was characterised by both a depolarisation and a hyperpolarisation. Traces were scored by four investigators with experience in the technique (EWFWA, JCD, KH and SNS).

Bronchial brushing

Ten bronchial brushing samples were obtained from throughout the airways of the same lung using disposable cytology brushes (BC-202D-5010; Olympus UK, Southend-on-Sea, UK); to prevent cell loss that occurs while resheathing the brush, the entire bronchoscope was removed each time and the brush cut while still exposed at the distal end of the scope. Brushed cells were placed into two aliquots of PBS (five brushings in each) and processed for DNA/mRNA analysis (see *Nasal and bronchial brushings*).

Endobronchial biopsy

Two endobronchial biopsies were obtained from approximately fifth-generation subcarinae with disposable forceps (BC-202D-5010, Olympus UK, Southend-on-Sea, UK; *Figure 14*) and processed as described in *Endobronchial biopsies*. Bronchoalveolar lavage was not undertaken, but, where possible, samples of secretions were sent for microbiological culture.

Post procedure, patients were observed for 4–6 hours prior to discharge.

(a) (b)
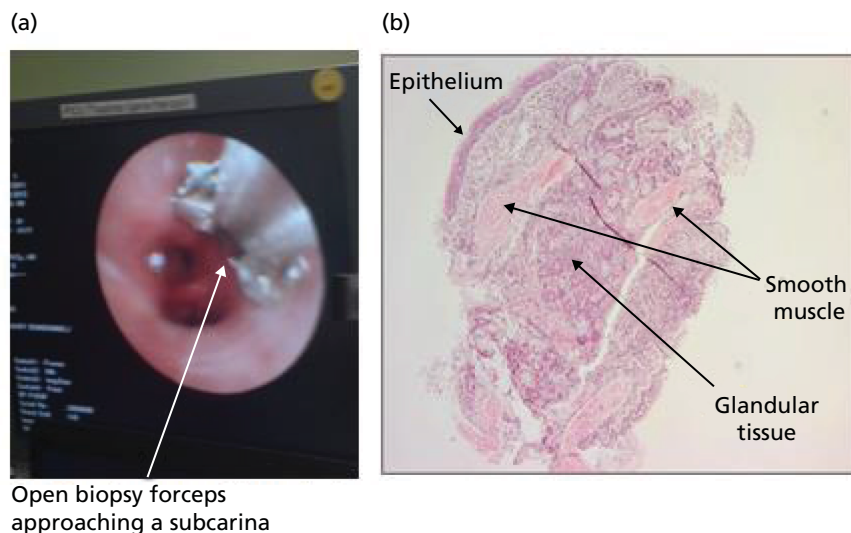
FIGURE 14 Endobronchial biopsy. (a) The biopsy forceps were advanced under direct vision to a subcarina of an approximate third- to fifth-generation airway where two biopsies were obtained; and (b) representative morphology on a haematoxylin and eosin (H&E)-stained section of an endobronchial biopsy demonstrating pseudostratified columnar epithelium and a submucosa containing smooth muscle, glandular tissue and an inflammatory cell infiltrate.

Sample handling, preparation and assays

Blood

Routine clinical
Blood samples for electrolytes, renal function, CRP, full blood count and coagulation screen were processed and reported by the clinical laboratories in accordance with usual routine hospital practice.

Inflammatory markers
Serum was frozen and stored for subsequent cytokine analysis. Serum interleukin 6 was measured on the Beckman Access 2 immunoassay analyser (Beckman Coulter, High Wycombe, Buckinghamshire, UK). An in-house calprotectin enzyme-linked immunosorbent assay (ELISA) was used (intra-assay coefficient of variation = 5.6%; unpublished observations). Calprotectin antibodies were kind gifts of Erling Sundrehagen, Oslo, Norway. Incubations were carried out in a damp box to prevent potential evaporation during the ELISA protocol. Microtitre plates (Immulux HB, Dynex Technologies, Chantilly, VA, USA) were coated overnight at 4 °C with 100 µl of anticalprotectin (mouse antihuman) (Axis-Shield Diagnostics Ltd, Dundee, UK; Diatec mAb Clone Cp5 B1252, Product number 9790) monoclonal antibody at 2.5 µg/ml in coating buffer (KPL/Insight Biotechnology). Plates were blocked with 1% bovine serum albumin (BSA; Sigma-Aldrich Company Ltd, Gillingham, UK) for 1 hour at 37 °C and washed four times with 0.05% Tween-20 (Sigma-Aldrich Company Ltd, Gillingham, UK). Duplicate samples of 100 µl were added to the plate in 1 : 60,000 dilutions for sputum in PBS and 1 : 500 for serum [50% fetal calf serum (Sigma-Aldrich Company Ltd, Gillingham, UK) in PBS]. Positive controls of calprotectin (Immundiagnostik, Oxford Biosystems, Oxford, UK) were included. Human recombinant calprotectin (Cambridge Biosystems, Cambridge, UK) was used to produce 1.56–100 ng/ml standard curves. The samples: anti-calprotectin [chicken antihuman (Axis-Shield Diagnostics, Ltd, Dundee, UK; lot number: 4095804)] polyclonal antibody at 1 : 5000 dilution; 100 µl of alkaline phosphatase-conjugated donkey anti-chicken immunoglobulin G (Jackson Product Code 703-055-155, Jackson ImmunoResearch, Suffolk, UK) at 1 : 5000 dilution were added to wells in three cycles of incubation at room temperature for 1 hour on a platform vibrator (450 rpm) followed by washings. Finally, 100 µl of BluePhos® Microwell Phosphatase Substrate System (Kirkegaard & Perry Laboratories, Inc., Gaithersburg, MD, USA) was added to each well. Plates were incubated in the dark at 600 rpm until the blue colour developed, and reached an absorption of 620 nm on a BioTek plate reader (BioTek UK, Swindon, UK). Sample concentrations were calculated using the five-parameter logistic (5PL) non-linear regression curve-fitting model.[92]

Antibodies against double-stranded DNA (dsDNA) (immunoglobulin G) were processed by the clinical laboratories using a commercial dsDNA assay was performed on the Immunocap 250 analyser (ThermoScientific, ThermoFisher Diagnostics, Milton Keynes, UK) in accordance with routine hospital practice. Out of 232 samples, 218 (94%) were available for analysis. Peripheral blood mononuclear cells (PBMCs) were extracted and human interferon-γ ELISA for the analysis of *CFTR*-specific T cells was performed as previously described.[93] Out of 232 samples, 170 (73%) were suitable for analysis. In the remaining 27% of samples, cell viability was too low to perform the assay. Cell viability was likely affected by storage of PBMCs in liquid nitrogen and shipment of samples from the UK to the USA, where the assays were performed.

Sputum

Samples collected into Falcon tubes for 24 hours prior to a visit were weighed. Freshly expectorated sputum obtained during the trial visit was stored on ice for a maximum of 2 hours and processed as previously described.[65] If patients did not produce sufficient sputum to perform all sputum assays, the following priority for assays was assigned: (1) clinical microbiology (generated from cough swabs if patients were completely non-productive); (2) soluble sputum that generated samples for quantification of interleukin 8 (IL-8), calprotectin and extracellular DNA as well as sputum cells; and (3) sputum solid content. Samples to prepare soluble sputum were available from 130 out of 232 samples (56%).

Microbiology

Samples were processed according to CF-specific protocols in the microbiology laboratories at the clinical centres. Semiquantitative reports of cultured bacteria and fungi (and non-tuberculous mycobacteria pre-dose 1 and at follow-up) were provided. Completely non-productive patients underwent cough swabs for microbiological surveillance following clinical protocols.

Soluble inflammatory mediators

Sputum calprotectin was measured as described above for serum. Sputum IL-8 assays were performed using a commercial kit (Human IL-8 Ultra Sensitive Kit, kit number KAC1301; Biosource/Invitrogen, CA, USA), following the manufacturer's instructions. Plates were read using a Biotek plate reader, and standard curves produced using the 5PL non-linear regression curve-fitting model. Extracellular DNA was quantified in the soluble sputum fraction using the Quant-iT™ PicoGreen® dsDNA Assay Kit (Invitrogen, Paisley, UK) according to the manufacturer's recommendations. Sputum solid content was determined as previously described.[65] Out of the expected 232 samples, 169 (63%) were not available because patients did not expectorate sufficient sputum.

Cell counts

Cells were isolated from solubilised sputum as described (Tracking[65]) and approximately 10^5 sputum cells were cytospun (5 minutes at 500 rpm) onto cytoslides (Thermo Shandon Ltd, Cheshire, UK) (six slides per subject). Slides were air dried and fixed for 10 minutes in 4% formalin (Sigma-Aldrich, St Louis, M0, USA) and subsequently stored at –20 °C until further use. For total and differential cell counts, cells were fixed for 10 minutes in cold methanol (Fisher Scientific, Loughborough, UK), dried and stained with May–Grünwald–Giemsa (MGG quick stain; Bio-Optica, Milan, Italy) using routine histological procedures and neutrophils, lymphocytes, macrophages and eosinophils quantified. Cells were counted on random fields until 300 neutrophils were counted. Total and differential cell counting could be performed on 129 out of 232 (56%) and 111 out of 232 (48%) samples, respectively.

Quantification of intracellular lipid droplets could be performed on 121 out of 232 (52%) of samples and was performed on slides stained with ORO using a protocol adapted from Lian *et al.*[94] Briefly, defrosted slides were washed in distilled water, dried and placed for 3 minutes in 100% propylene glycol (Sigma-Aldrich, St Louis, M0, USA). Slides were then transferred into pre-warmed (60 °C) ORO solution (0.5% weight to volume in propylene glycol) and stained for 10 minutes in a 60 °C oven. Subsequently slides were placed into 85% (v/v) propylene glycol for 3 minutes at room temperature and rinsed three times in distilled water, counterstained with Harris's haematoxylin (VWR International Ltd, Lutterworth, UK)

and mounted in Aquatex mounting medium (VWR International Ltd, Lutterworth, UK). Quantification of lipid staining was performed blinded on each slide. On each slide 300 neutrophils, 100 macrophages and 100 squamous cells were evaluated for the presence of cytoplasmic lipid droplets. The data are presented as a percentage of lipid-containing cells.

Urine

Samples were dipsticked routinely for glucose, protein and blood. At the start of the trial and on visits including CT scans, post-menarche female patients underwent urinary pregnancy testing.

Nasal and bronchial brushings

Deoxyribonucleic acid and ribonucleic acid (RNA) were simultaneously isolated from nasal and bronchial brushing samples using the AllPrep DNA/RNA Mini Kit (QIAGEN, Manchester, UK). Levels of pGM169-specific and endogenous *CFTR* DNA and mRNA were quantified using TaqMan® (Life Technologies, Paisley, UK) qRT-PCR instruments and supplies, following reverse transcription, when appropriate, as described by Rose *et al.*[95] Absolute DNA and in vitro transcribed RNA calibration standards allowed precise copy number quantification. pGM169 DNA levels were determined using a qPCR primer set specific for the soCFTR2 cDNA:

- 50 nM forward primer: 5′-GGAACAGCTCCAAGTGCAAGA-3′
- 900 nM reverse primer: 5′-CCTGGTGTCCTGCACTTCCT-3′
- 100 nM probe: 5′-FAM-CAAGCCCCAGATTGCTGCCCTG-TAMRA-3′.

Levels were normalised to total genomic DNA, as determined using a qPCR primer set specific for the endogenous *CFTR* gene:

- 300 nM forward primer: 5′-CTTCCCCCATCTTGGTTGTTC-3′
- 300 nM reverse primer: 5′-TGACAGTTGACAATGAAGATAAAGATGA-3′
- 100 nM probe: 5′-VIC-TGTCCCCATTCCAGCCATTTGTATCCT-TAMRA-3′.

pGM169-derived mRNA was determined using qRT-PCR. Reverse transcription reactions (20 µl) contained ≤ 5 µl total RNA, 1.25 units of MultiScribe™ Reverse Transcriptase (Life Technologies) and 0.4 units RNAse inhibitor (Life Technologies) and 400 nM of the appropriate primer:

- soCFTR2 mRNA: 5′-CCAGCTGAAGAACAGCTTGCT-3′
- human *CFTR* (hCFTR) mRNA: 5′-CCAGGCGCTGTCTGTATCCT-3′.

pGM169-derived cDNA levels were determined using a qPCR primer set specific for the correctly spliced exon 1–2 soCFTR2 mRNA:

- 300 nM forward primer: 5′-TCTCCCTCCTGTGAGTTTGGTT-3′
- 300 nM reverse primer: 5′-GCTCACCACAGAGGCCTTCT-3′
- 100 nM probe: 5′-FAM-CTAGCCACCATGCAGAGAAGCCCTCTG-TAMRA-3′.

Levels were normalised to endogenous hCFTR mRNA as determined using a qPCR primer set specific for the correctly spliced exon 1–2 endogenous *CFTR* mRNA:

- 300 nM forward primer: 5′-GGAAAAGGCCAGCGTTGTC-3′
- 300 nM reverse primer: 5′-CCAGGCGCTGTCTGTATCCT-3′
- 100 nM probe: 5′-VIC-CCAAACTTTTTTTCAGCTGGACCAGACCAA-TAMRA-3′.

The qPCR reactions (10 µl) contained 0.6–6 ng of total DNA or 2 µl of cDNA and 1 × TaqMan® Universal MasterMix (Life Technologies Manchester, UK). Thermocycling was performed in 384-well plates, on a 7900HT cycler (Applied Biosystems, Thermofisher, Foster City, CA, USA), with SDS 2.2 software, and the following cycling conditions: 50 °C for 2 minutes; 95 °C for 10 minutes; then 40 cycles of 95 °C for

15 seconds and 60 °C for 1 minute. Data were calculated as the percentage of pGM169 specific to endogenous *CFTR* copy number. When samples were positive for pGM169-specific signal, but either quantification fell below the linear range of the standards (limit of quantification; LOQ) or quantification of endogenous *CFTR* signal was negative, they were scored as positive but not quantifiable (PBNQ). Remaining samples that were negative for endogenous *CFTR* signal were scored as not determined. Samples negative for the pGM169-specific signal but positive for endogenous *CFTR* signal were scored as zero. When data are presented as post–pretreatment difference, a conservative approach was adopted for samples scored with the nominal value of PBNQ by substituting a value of either zero or the LOQ as appropriate to maximise the post–pre difference for placebo samples and minimise the post–pre difference for active samples. For bronchial brushings, two nominally identical samples were typically available (each pools of five independent bronchial brushings) and the maximum score was reported.

Endobronchial biopsies

Two biopsies were collected from each patient. One was frozen in 2-methylbutane-cooled liquid nitrogen at –196 °C and the other was immediately fixed in 10% formal saline. The formalin-fixed samples were processed using a Tissue Tek Sakura VIP processor (Sakura Finetek UK Ltd, Thatcham, UK) and embedded in paraffin wax blocks. Three micron sections were then cut from these blocks for haematoxylin and eosin (H&E) staining. For frozen samples, 6- to 8-µm sections were cut for H&E and ORO staining. For H&E staining, slides were thawed at room temperature for 30 minutes, and then placed in Harris's haematoxylin for 1 minute. Subsequently, they were differentiated in acid alcohol for 5 seconds and left to blue in tap water for 5 minutes. They were then counterstained with eosin for 30 seconds and cover-slipped using an aqueous mountant.

For ORO staining, frozen sections were thawed and air dried at room temperature for 30 minutes. Slides were rinsed in 60% 2-propanol then placed in filtered ORO working solution for 15 minutes. Subsequently, the slides were taken out of the ORO solution and excess dye was washed off with 60% 2-propanol, before rinsing in three changes of distilled water. The sections were counterstained using Harris's haematoxylin for 30 seconds in order to visualise the nuclei. The sections were left to blue in running tap water for 5 minutes and mounted with a coverslip using Aquatex (an aqueous mountant). H&E slides were scored independently by two blinded pathologists using a semiquantitative scoring system for goblet cell hyperplasia, basement membrane thickening, presence of chronic inflammatory cells (lymphocytes and plasma cells), neutrophils, eosinophils and seromucinous gland hyperplasia. ORO slides were scored for the presence of lipid-laden macrophages using a semiquantitative scoring system.

Data collection and analysis

Trial database, monitoring and audit

The Imperial College Trials Unit (ICTU) built an InForm database specifically for the trial and the staff working on the trial entered the data from source. The InForm database was monitored by both ICTU on a regular basis and the Imperial College Research Office undertook on-site source data verification. Two patients had 100% source data verification and the remainder had 10%. Any queries were flagged on the database as well as in the case report forms and dealt with by the study team.

Data analysis

A detailed statistical analysis plan (SAP) was drafted by the trial statistician (GDM) and was further refined with input from the Trial Management Group (see *Appendix 5*). The SAP was approved by the Trial Steering Committee (TSC) and finalised ahead of the database being locked and the trial unblinded. Data management was undertaken using Microsoft Excel® (version 14.4.6 for Mac OSX; Microsoft Corporation, Redmond, WA, USA). Descriptive statistics and standard analyses were performed using Prism (Version 5.0c for Mac OSX, Graph Pad Software Inc., San Diego, CA, USA) and or IBM SPSS (version 22.0, IBM Corporation, Armonk, NY, USA). There was no exploration of the impact of missing values in the primary analysis as data were available for 114 out of the 116 PP patients.

Patient and public involvement

Since 2000, when the UK CFGTC started working together, we have had a close relationship with patients and their families, the ethos of the consortium being to take the patients with us along the gene therapy pathway. As well as having a formal patient representative on the TSC and input from the National Institute for Health Research (NIHR) Respiratory Biomedical Research Unit patient group, the UK CFGTC has attended numerous patient and family meetings to discuss the trial programme as well as future research projects. Patient input enabled us to refine the trial design in a number of ways, including the length of time for which a nebuliser may be tolerated and which side effects in an eventual clinical product would be tolerated.

Owing to the length of the study, one of the biggest challenges we had during the trial was patient recruitment. Being so involved with the patient groups enabled us to take advantage of social media and local networks across the UK ensuring that when we opened the patient identification centres patients were already aware of the study and wanted to participate. Importantly, having patients involved at every stage of the research process enabled us to design a product that would be tolerated by the patients if it becomes a licensable product.

In 2013 the patient and public involvement for this project was written up as an INVOLVE case study.[96]

Role of the funder

Chapter 3 Results of clinical efficacy outcomes

Results are reported according to Consolidated Standards of Reporting Trials (CONSORT) guidelines.

Overall timelines

The first patient was screened on 6 June 2012 and the first dose was administered on 13 June 2012. The last patient was screened on 24 June 2013, the last dose was administered on 1 May 2014 and the last follow-up visit was performed on 30 May 2014.

Screening and recruitment

Following prescreening of clinic databases, 191 patients were considered likely to be suitable and agreed to a screening visit. Forty of these were not enrolled, as they failed to fulfil inclusion criteria (see *Figure 15*). Of the 151 who passed screening, 11 patients subsequently withdrew, either because of a change of mind or because of the development of an exclusion criterion. One hundred and forty patients were randomised: 62 (44%) to placebo and 78 (56%) to active treatment. Two patients in each group withdrew post randomisation and were not able to reattend for any further visits; therefore, this left 136 in the ITT cohort, predefined as being randomised and having any follow-up data available. The PP population was predefined as those patients receiving ≥ 9 doses and comprised 116 patients (placebo, $n = 54$; active treatment, $n = 62$). The reasons for 20 patients discontinuing are shown in *Figure 15*. Of note, the first small-molecule CFTR modulator, ivacaftor, was licensed and approved in UK countries during the trial for patients with the G551D mutation. Three of our subjects elected to leave the trial to enable them to receive this treatment; others were prepared to wait until the end of the trial and continued.
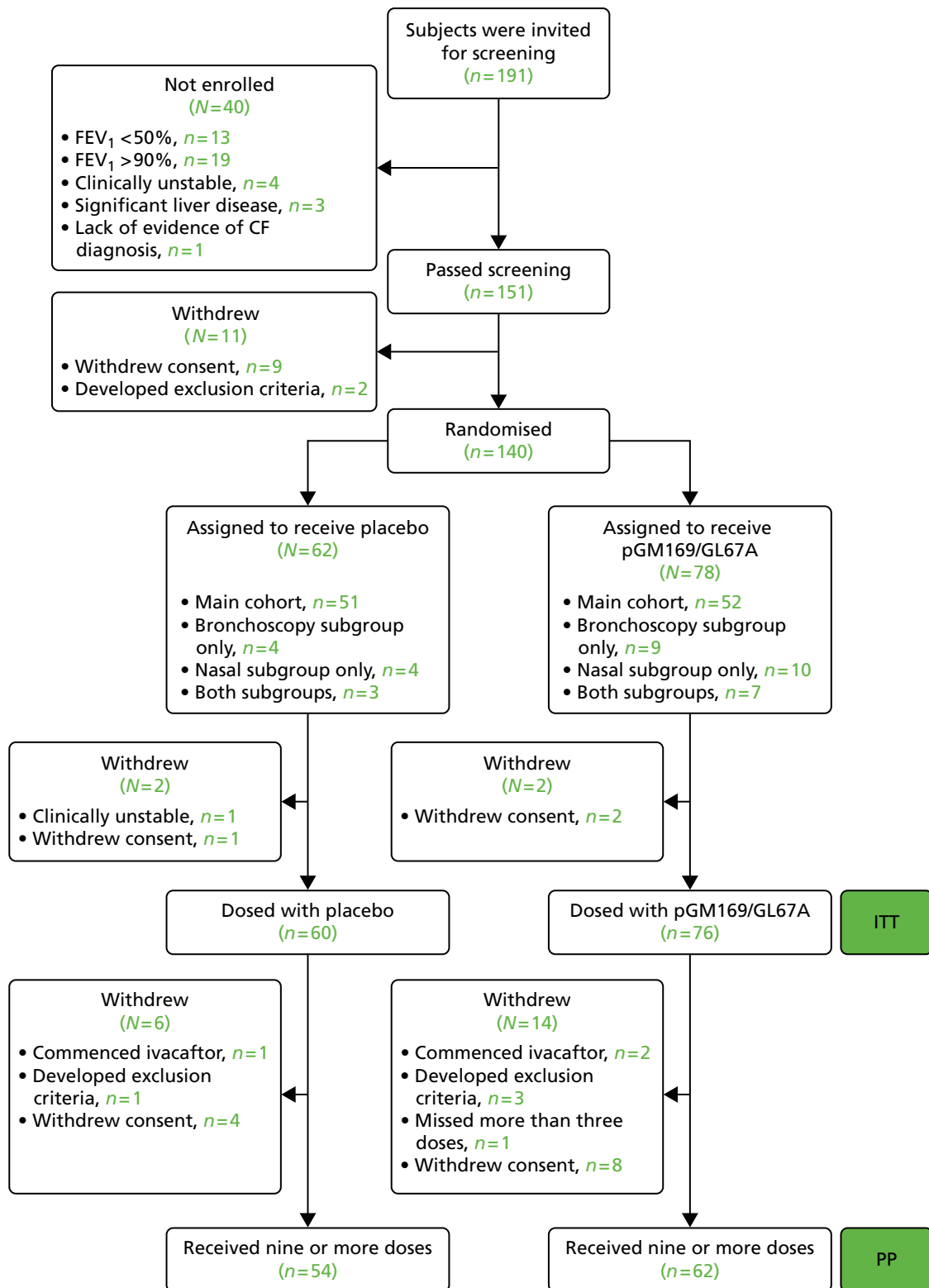
FIGURE 15 The CONSORT diagram showing screening, randomisation and patients dosed. The ITT and PP groups are identified.

Subject demographics

Subjects randomised to active-treatment and placebo groups were well matched at baseline for age (and proportion of paediatric patients aged < 18 years), sex, centre and *CFTR* gene mutation class (F508del/F508del vs. other) (*Table 2*). The two groups were also similar with regard to clinical characteristics including lung function (FEV$_1$%) and BMI, used here as a general measure of nutritional status.

TABLE 2 Baseline demographic data of subjects randomised to the active-treatment and placebo groups. Data are presented for both ITT and PP populations. Groups were well matched

Characteristic	ITT placebo (*n* = 60)	ITT active treatment (*n* = 76)	ITT total (*n* = 136)	PP placebo (*n* = 54)	PP active treatment (*n* = 62)	PP total (*n* = 116)
Age (years)						
Mean	25.9	25.0	25.4	26.0	23.6	24.7
Range	12–64	12–57	12–64	12–64	12–57	12–64
Age distribution, n (%)						
< 18 years	19 (32)	25 (33)	44 (32)	17 (31)	23 (37)	40 (34)
≥ 18 years	41 (68)	51 (67)	92 (68)	37 (69)	39 (63)	76 (66)
Sex, n (%)						
Female	29 (48)	37 (49)	66 (49)	25 (46)	31 (50)	56 (48)
Male	31 (52)	39 (51)	70 (51)	29 (54)	31 (50)	60 (52)
Centre distribution, n (%)						
Edinburgh	28 (47)	27 (36)	55 (40)	24 (44)	22 (36)	46 (40)
London	32 (53)	49 (64)	81 (60)	30 (56)	40 (64)	70 (60)
Height (cm)						
Mean	164.5	164.3	164.4	165.0	163.6	164.3
Range	146.6–191.3	133.8–186.2	133.8–191.3	147.4–191.3	133.8–186.2	133.8–191.3
Weight (kg)						
Mean	60.9	61.1	61.0	61.6	61.0	61.3
Range	37.0–120.7	33.6–105.4	33.6–120.7	37–120.7	33.6–105.4	33.6–120.7
Percentage predicted FEV$_1$						
Mean	67.9	69.2	68.6	69.0	69.9	69.5
Range	48.4–89.9	50.3–89.6	48.4–89.9	49.6–89.9	50.7–89.6	49.6–89.9
BMI						
Mean	22.3	22.3	22.3	22.4	22.4	22.4
Range	16.2–41.0	16.7–40.7	16.2–41.0	16.2–41.0	16.7–40.7	16.2–41.0
Mutation class						
F508del/F508del	29	37	66	26	31	57
F508del/class 1–6	23	28	51	22	23	45
Not F508del/class 1	2	5	7	1	3	4
Heterozygous/ homozygous class 3–6	3	2	5	2	2	4
F508del/unknown class	3	4	7	3	3	6

Primary outcome: relative change in FEV₁ percentage

Out of 116 PP subjects, 114 (placebo, $n = 54$; active treatment, $n = 60$) had paired pre–post-treatment measurements of percentage predicted FEV$_1$. Two patients, despite fulfilling the PP definition of receiving ≥ 9 doses, were excluded from this analysis as they did not have spirometry performed at follow-up visits; in one case, this test was contraindicated owing to a recent surgically induced pneumothorax. The other patient had recently withdrawn from the trial because of time commitments and was unable to return for follow-up measurements. There was a significant ($p = 0.046$; *Figure 16*) TE in the primary outcome, percentage predicted FEV$_1$, with the active-treatment group having an ANCOVA-adjusted 3.7% (95% CI 0.1% to 7.3%) greater change in FEV$_1$ than the placebo group at 12 months' follow-up. The effect was seen from month 1 onwards, with a sustained divergence of the two groups (see *Figure 16*).

Changes in FEV$_1$ over the course of the trial were variable between subjects. *Figure 17* illustrates individual relative improvements from pretreatment to follow-up in a waterfall plot in which improvement is indicated by a positive value. Post hoc analysis showed that 21 subjects [placebo, $n = 6$ (11%); active treatment, $n = 15$ (25%)] demonstrated a change in percentage predicted FEV$_1$ of ≥ 5%.

The TE in the ITT population with spirometry measurements both pre-dosing and within the protocol-defined window after their final dose (placebo, $n = 56$; active treatment, $n = 65$) was 3.6% (95% CI 0.2% to 7.0%; $p = 0.039$). The SAP prespecified an additional sensitivity analysis based on the area under the curve for the percentage predicted FEV$_1$. With this analysis, using the PP population, the estimated TE (in units of percentage predicted FEV$_1$, rather than the relative change) was 1.32% (95% CI –0.48% to 3.12%; $p = 0.15$), consistent with the relative TE observed in the primary analysis.
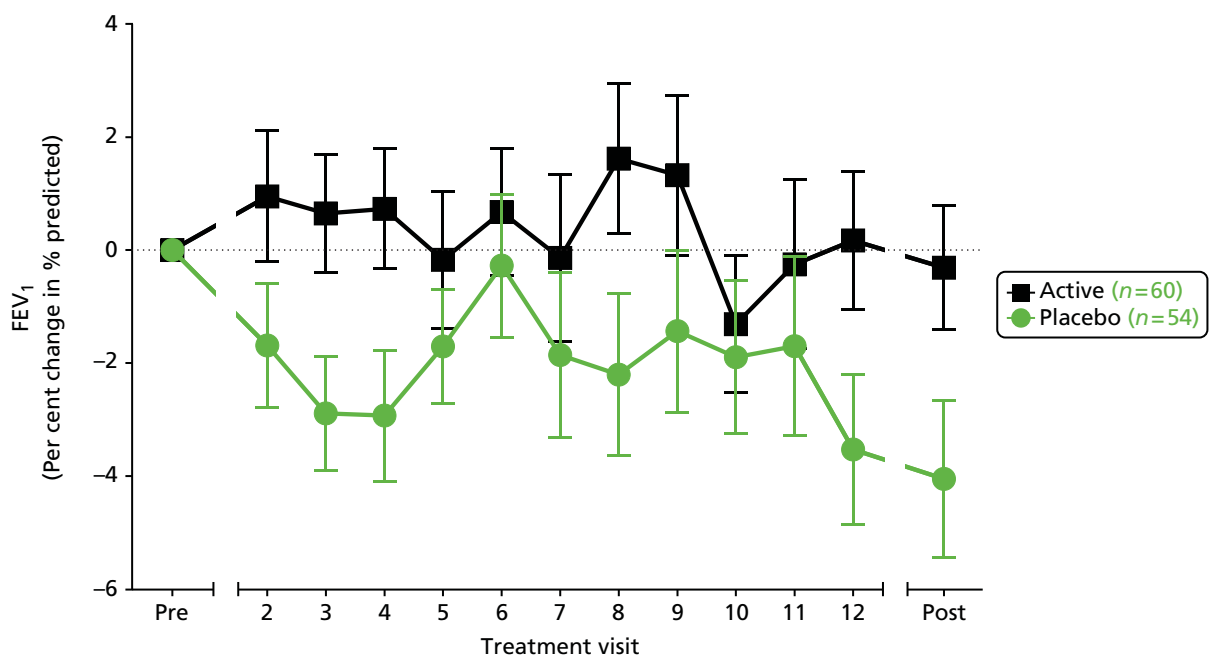
FIGURE 16 Primary end point (relative change in percentage predicted FEV$_1$). Time course of the response of the primary outcome to either placebo (green) or active treatment (black). 'Pre' and 'post' indicate the mean of two measurements at the respective time points. Error bars indicate SEM. There was a significant ($p = 0.046$) TE, with the active-treatment group having an ANCOVA-adjusted improvement of 3.7% (95% CI 0.1% to 7.3%) than placebo at 12 months' follow-up. The effect was seen from month 1 onwards, with a sustained divergence of the two groups. SEM, standard error of the mean.
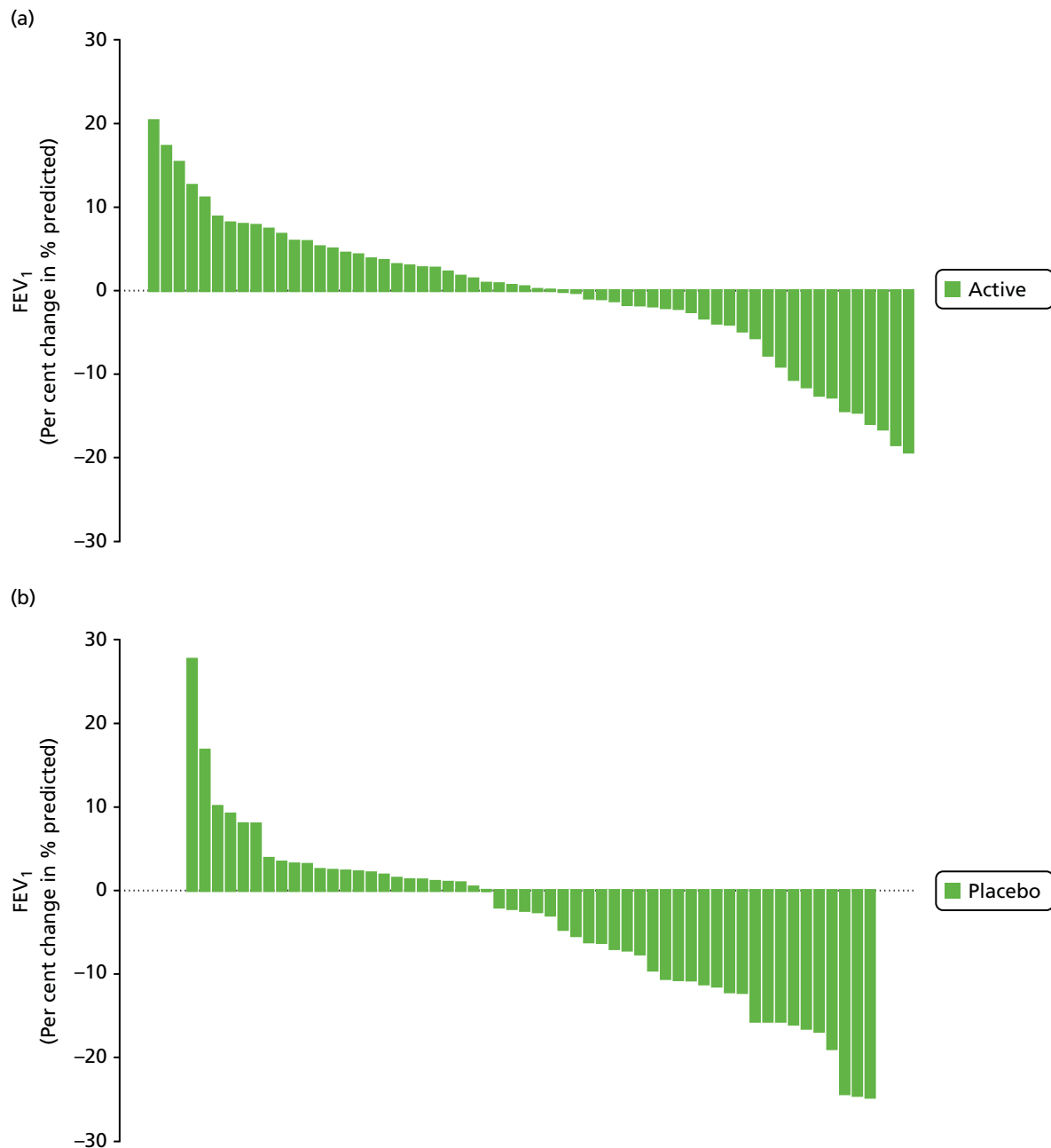
FIGURE 17 The distribution of FEV$_1$ changes in individual patients, shown separately for the two groups. (a) Active treatment; and (b) placebo. Positive values indicate an improvement.

Major secondary outcomes

Physiology

There was also a significant TE in FVC ($p = 0.031$; *Figure 18*; see also *Figures 25* and *26*). No difference was observed in the LCI at the follow-up period, although the serial time point graph (*Figure 19*) demonstrates that the active-treatment group appear to have a better-preserved LCI in the earlier stages of the trial.
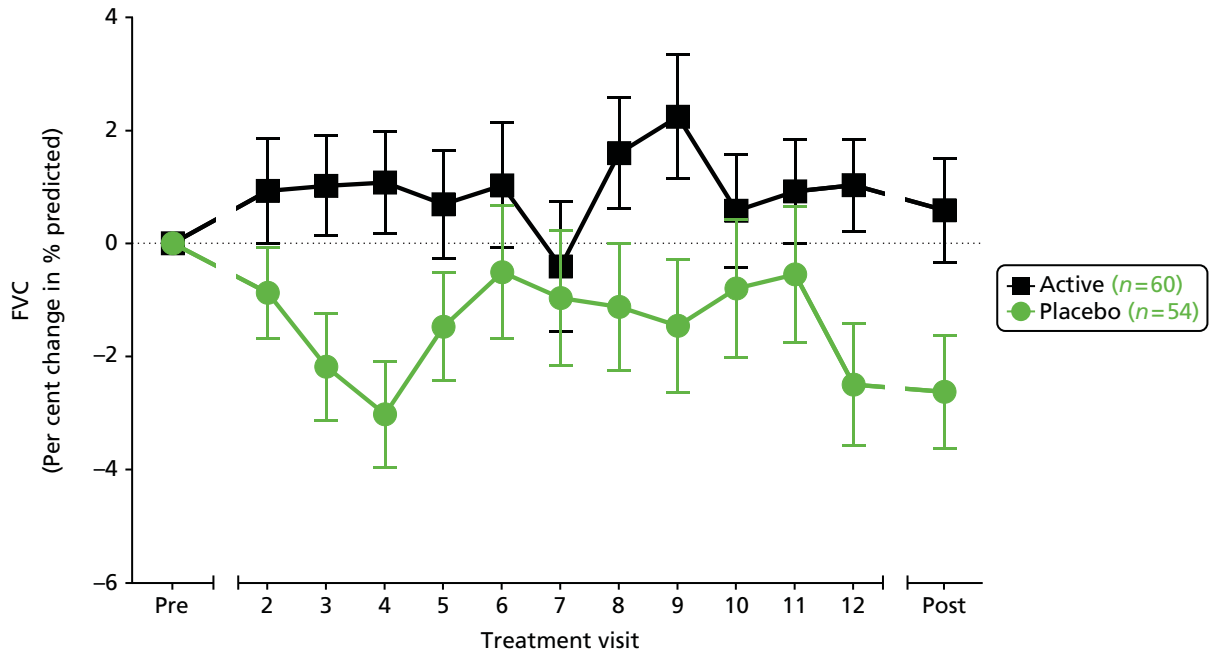
FIGURE 18 Forced vital capacity. Time course of the response of FVC to either placebo or active treatment. 'Pre' and 'post' indicate the mean of two measurements at the respective time points. Error bars indicate SEM. There was a significant ($p = 0.031$) TE, with the active group having an ANCOVA-adjusted improvement of 3.0% (95% CI 0.3% to 5.8%) compared with placebo at 12 months' follow-up. SEM, standard error of mean.
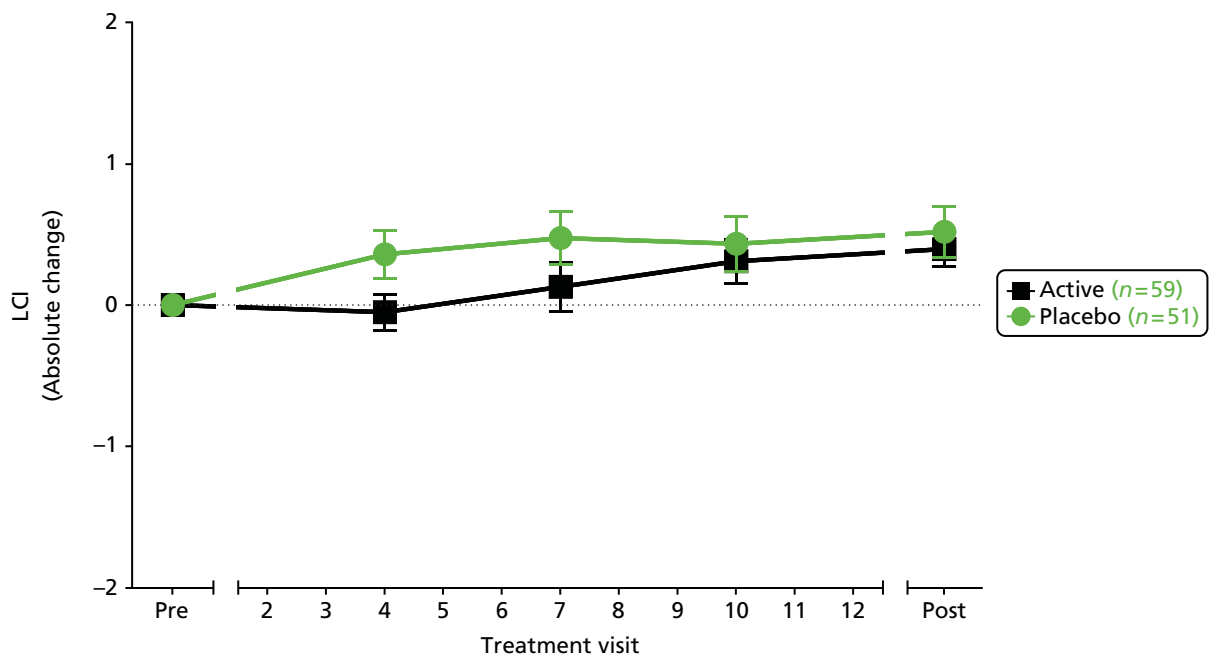
FIGURE 19 Change in LCI. There was no significant TE in LCI, both groups increasing (worsening) slightly over the year of the trial.

Computed tomography scans

Paired pre and post follow-up high-resolution CT scans were available for 115 patients; the one PP patient missing is the same patient also missing from the analysis of the primary end point as she had been lost to follow-up. The other patient missing from the primary outcome is represented here, as CT scanning did not pose the same risks following her pneumothorax as a forced expiratory manoeuvre would have done.

Bronchiectasis is defined as dilatation and thickening of the airways. It is considered irreversible. An intervention applied for this period of time would not therefore be expected to have any impact on the extent of bronchiectasis and this was the case in this cohort (*Figure 20*). Although it was not statistically significant, it was interesting to note a trend for less worsening in the severity of bronchiectasis in the active-treatment group than in the placebo.

Gas trapping is a feature visible on expiratory films when areas of lung fail to empty properly because of airway obstruction. There were increases in the percentage of gas trapping in the placebo group that were not demonstrated in the active-treatment group and which led to a significant TE ($p = 0.048$; *Figure 21*).

Scores of mucus plugging (large or small; *Figure 22*) and airway wall thickness (*Figure 23*) did not differ significantly between the two groups, although, for every parameter, the change was smaller in and, therefore, favoured the active-treatment group.
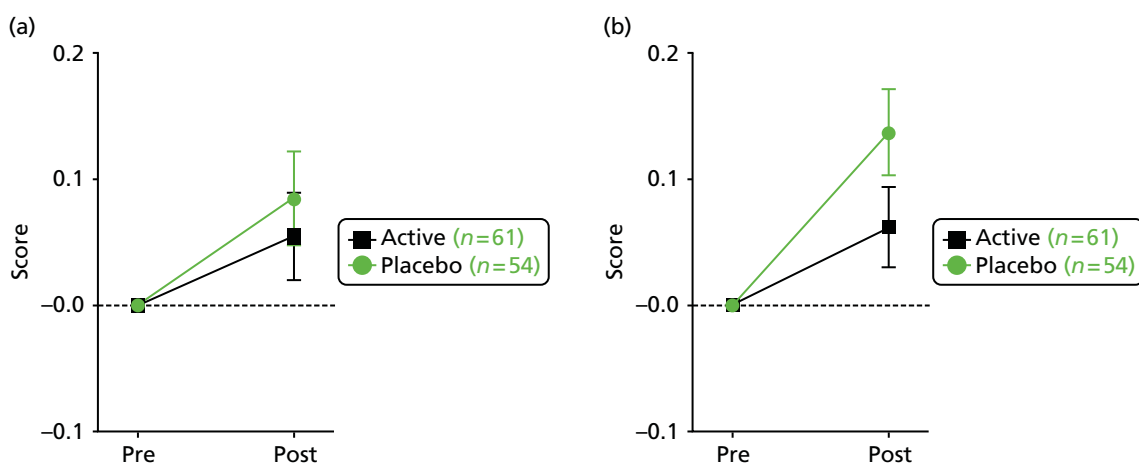
FIGURE 20 Change in CT scores for bronchiectasis (a) extent; and (b) severity.
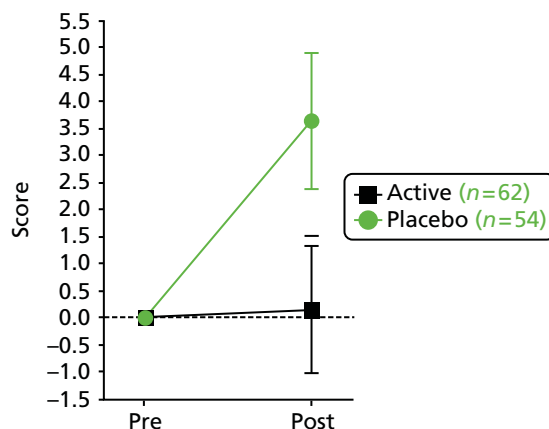
FIGURE 21 Gas trapping on CT scan. Over the course of the study, gas trapping indicative of small airways disease, increased in the placebo group (worsened) but did not in the active-treatment group leading to a statistically significant TE ($p = 0.048$).
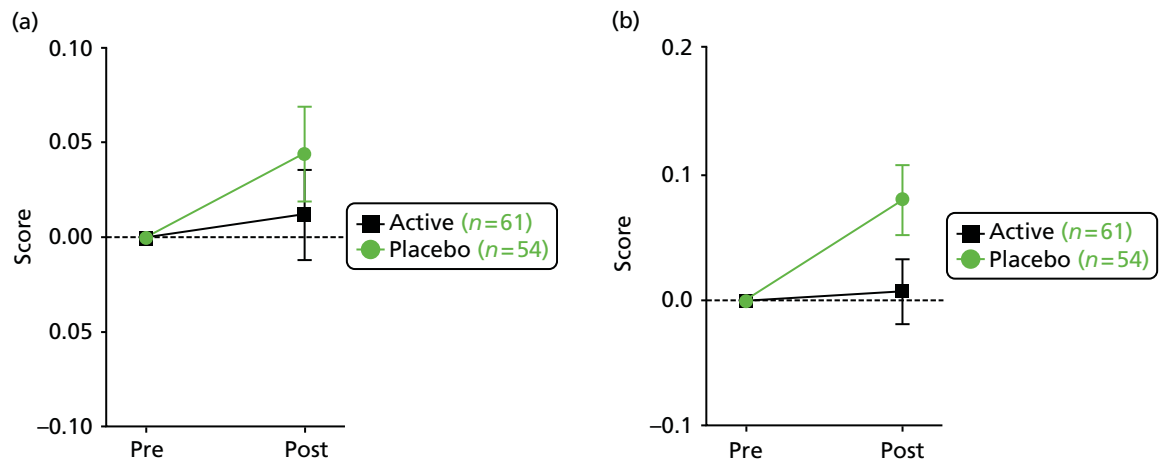
FIGURE 22 Mucus plugging on CT scan. (a) Large mucus plugging; and (b) small mucus plugging (sometimes termed 'tree in bud').
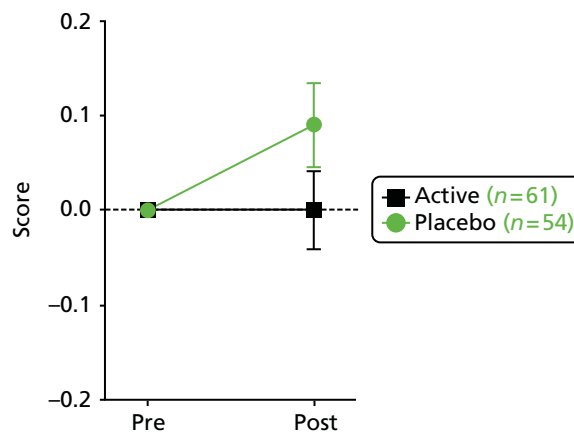
FIGURE 23 Airway wall thickness on CT scan.

Quality-of-life scores

Baseline quality-of-life scores for the domains of major interest in a trial of respiratory treatment (respiratory and physical) were high for the group as a whole (median 87.5%). There were no statistically significant TEs at the end of the trial, although, again, the TE appeared to favour the active-treatment group (*Figure 24*).

Other secondary outcomes

Figures 25 and *26* show that for all the assays, even those which did not reach significance, the standardised TE favoured the active-treatment group.
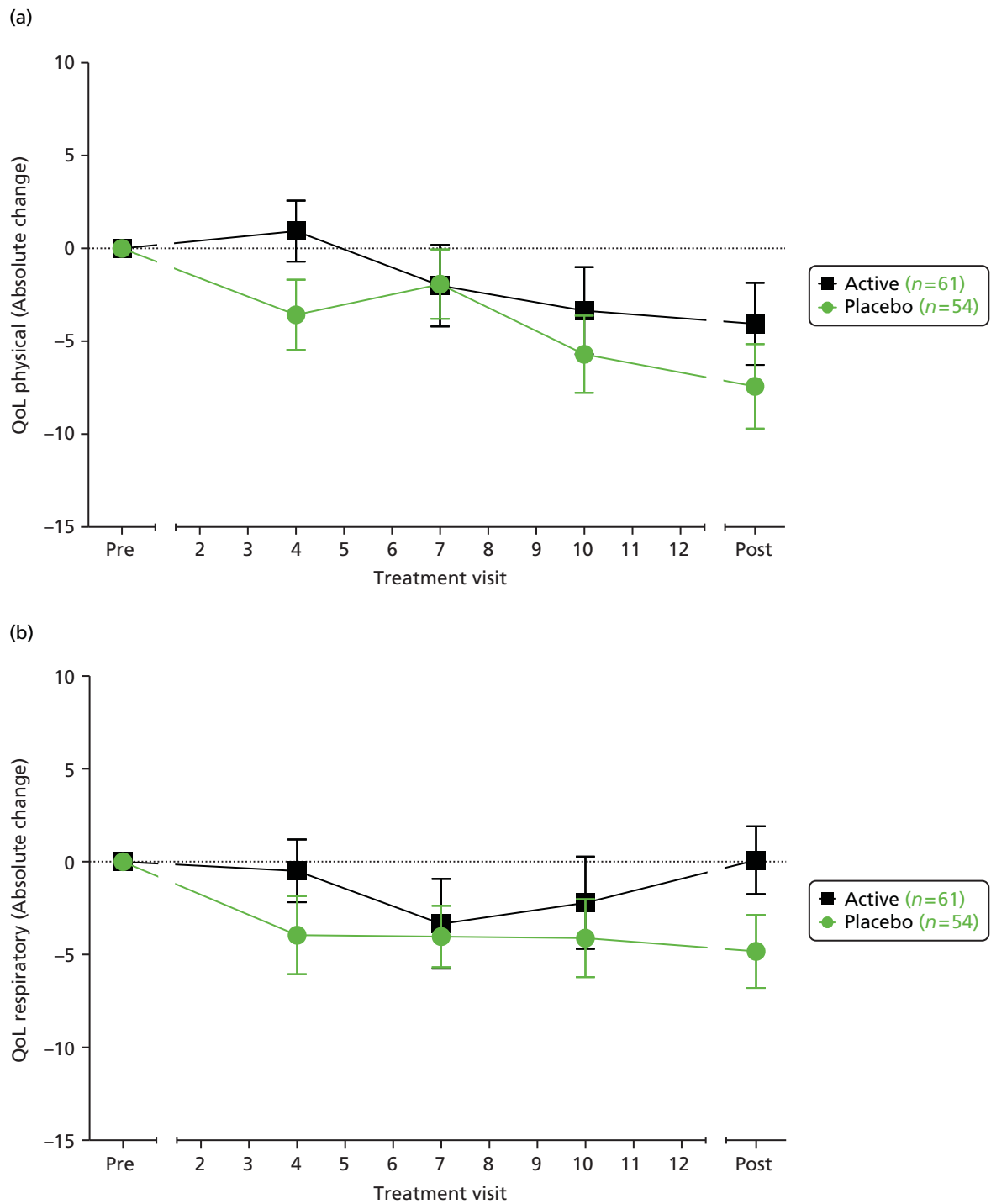
FIGURE 24 Quality-of-life (QoL) scores. Change in the (a) respiratory and (b) physical domains on the validated CF quality-of-life questionnaire CFQ-R.

	n Placebo/active	Pre treatment Mean (SD) [median]		Absolute treatment effect Mean (95% CI)	Standardised treatment effect Mean (95% CI)	Adjusted p-value
Primary end point						
FEV₁	54/60	69.48 (10.50) [69.42]		3.66 (0.07 to 7.25)	0.39 (0.01 to 0.77)	0.046*
Lung function						
FVC	54/60	83.94 (11.54) [82.09]		3.03 (0.29 to 5.78)	0.42 (0.04 to 0.80)	0.031*
MEF₂₅₋₇₅%	54/60	1.63 (0.71) [1.47]		0.07 (−0.08 to 0.22)	0.18 (−0.21 to 0.56)	0.362
LCI	51/59	10.78 (2.45) [10.47]		−0.28 (−0.71 to 0.14)	0.26 (−0.13 to 0.66)	0.187
CT						
Bronchiectasis extent	54/61	1.92 (0.74) [2.17]		−0.03 (−0.13 to 0.07)	0.11 (−0.27 to 0.50)	0.564
Bronchiectasis severity	54/61	1.80 (0.74) [1.92]		−0.08 (−0.17 to 0.02)	0.31 (−0.08 to 0.69)	0.116
Wall thickness	54/61	1.84 (0.60) [1.92]		−0.09 (−0.21 to 0.03)	0.28 (−0.1 to 0.66)	0.146
Large airway mucus plugs	54/61	0.81 (0.38) [0.92]		−0.03 (−0.1 to 0.04)	0.18 (−0.21 to 0.56)	0.369
Small airway mucus plugs	54/61	0.85 (0.33) [1.00]		−0.07 (−0.15 to 0.00)	0.37 (−0.02 to 0.75)	0.061
Gas trapping	54/61	45.6 (12.85) [46.67]		−3.49 (−6.96 to −0.03)	0.39 (0.00 to 0.77)	0.048*
Quality of life (QoL)						
QoL physical	54/61	82.87 (18.9) [87.50]		1.82 (−4.75 to 8.39)	0.11 (−0.28 to 0.50)	0.584
QoL respiratory	54/61	78.78 (14.25) [77.80]		2.08 (−3.06 to 7.22)	0.16 (−0.23 to 0.54)	0.425
Safety						
CRP	48/55	7.32 (9.67) [4.00]		−4.82 (−9.8 to 0.15)	0.39 (−0.01 to 0.80)	0.057
ESR	54/60	12.88 (11.01) [10.50]		−1.86 (−5.21 to 1.48)	0.21 (−0.17 to 0.59)	0.272
WBC count	54/61	8.58 (2.79) [7.90]		−0.52 (−1.28 to 0.24)	0.26 (−0.12 to 0.64)	0.176
KCOc	51/61	1.91 (0.22) [1.91]		0.03 (−0.02 to 0.08)	0.23 (−0.17 to 0.63)	0.257
TLCOc	51/61	8.02 (2.10) [7.81]		0.18 (−0.16 to 0.51)	0.23 (−0.19 to 0.60)	0.302
VA	52/61	4.23 (1.12) [3.99]		0.07 (−0.07 to 0.21)	0.19 (−0.20 to 0.57)	0.331
Sputum 24-hour weight	27/22	10.96 (11.73) [5.92]		−2.96 (−7.97 to 2.05)	0.36 (−0.25 to 0.98)	0.239

Standardised treatment effect
−1.0 −0.5 0.0 0.5 1.0
Favours placebo   Favours active

FIGURE 25 Secondary outcome measures. Forest plot showing the responses of secondary outcome measures to placebo or active treatment. To allow results from different end points to be plotted on a common scale, the estimated TEs were standardised to be presented as multiples of the underlying SD (standardised TE). The size of the circle is proportional to the number of subjects represented and the bars indicate 95% CI. *, statistically significant; ESR, erythrocyte sedimentation rate; WBC, white blood cell.
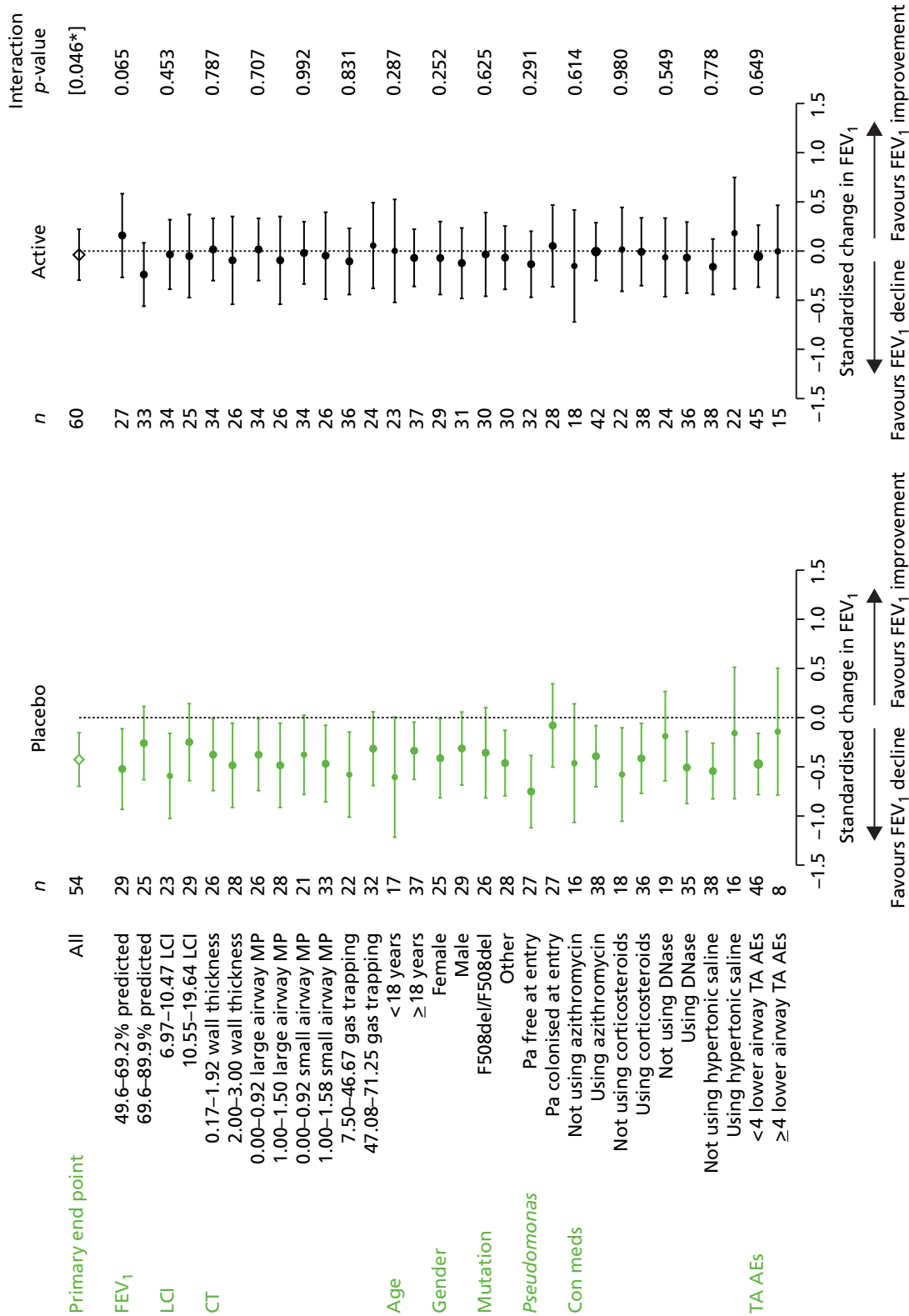
FIGURE 26 Responses of active and placebo groups separately for secondary outcomes. Forest plot showing the responses of the placebo or active-treatment arms when assessed by the predefined subgroup values shown. To allow results from different endpoints to be plotted on a common scale, the estimated TEs were standardised to be presented as multiples of the underlying SD. The size of the circle is proportional to the number of subjects represented and the bars indicate 95% CI. *, statistically significant; Con meds, concurrent medication; MP, mucus plugs; Pa, *P. aeruginosa*; TA AE, treatment-associated AE.

Influence of baseline parameters on primary outcome

We had predefined that the influence of baseline parameters on TE of the primary outcome would be explored in an attempt to define a 'responder' group. We reasoned that differences between responders and non-responders could be based on any of the following:

- Severity of underlying lung disease: patients with milder disease could respond more because the inhaled medication reached further into the airways, or less because they were already so well. We examined this using FEV_1, LCI and CT parameters.
- Age.
- Sex.
- CFTR mutation class: although we expect gene transfer to be mutation independent, this seemed necessary to confirm.
- The presence or absence of *P. aeruginosa*: gene transfer could be affected by the presence of this organism either because of the greater levels of inflammation likely associated with this chronic infection or because of an interaction with the wide array of exoproducts it produces. We did not consider that we had sufficient power to break this down further on the basis of other infections.
- Concomitant medications. Those considered to be particularly important to examine were:

  - the anti-inflammatory agents, azithromycin and corticosteroids, either of which could affect the host response to the medication
  - DNase, which could degrade the DNA in the trial product (of note, patients receiving this drug were asked to withhold it for 24 hours before and after each dose)
  - hypertonic saline, which by influencing mucus clearance could potentially reduce contact time.

- Side effects caused by the trial medication: we considered the hypotheses that those patients who developed either lower respiratory or systemic side effects acutely after dosing could have either a higher (side effects resulting from successful delivery) or smaller (host response limited success of transfection) chance of being a responder. To examine this, we classified patients on the basis of having (1) lower respiratory or (2) flu-like reactions within 48 hours of at least four doses. There were no subjects in the latter group and, therefore, only the lower airway symptoms were examined in this analysis.

For each of the continuous variables, the whole PP group was divided based on median values for that measure. The other variables were divided into two dichotomous groups.

Figure 27 illustrates that TE was independent of age, sex, mutation class, concomitant treatments and response to dosing. However, stratification by baseline disease severity on the basis of percentage predicted FEV_1 suggested a difference in TE between the lower half (49.6–69.2% predicted FEV_1) who had a TE of 6.4% (95% CI 0.8% to 12.1%) and the upper half (69.6–89.9% predicted) of TE 0.2% (95% CI –4.6% to 4.9%) (interaction analysis $p = 0.065$).

This is further illustrated in *Figure 28*, which shows the evolution of FEV_1 over time during the trial in (a) the half of patients with more severe disease and (b) the half with milder disease. The greater TE was not because of the use of 'relative change' magnifying an effect, as this was also apparent when absolute change in FEV_1 percentage was examined (data not shown).

Primary end point		n Placebo/active		Pre treatment Mean (SD) [median]	Absolute treatment effect Mean (95% CI)	Standardised treatment effect Mean (95% CI)	Interaction p-value*
	All	54/60		69.48 (10.51) [69.42]	3.66 (0.07 to 7.25)	0.39 (0.01 to 0.77)	0.046*
FEV₁	49.6–69.2% predicted	29/27		60.58 (5.27) [60.66]	6.42 (0.76 to 12.08)	0.69 (0.09 to 1.29)	0.065
	69.6–89.9% predicted	25/33		78.38 (5.81) [77.89]	0.19 (–4.56 to 4.93)	0.02 (–0.49 to 0.53)	
LCI	6.97–10.47 LCI	23/34		74.36 (8.69) [75.18]	5.26 (–0.10 to 10.61)	0.56 (–0.01 to 1.13)	0.453
	10.55–19.64 LCI	29/25		64.33 (9.58) [63.01]	1.87 (–3.65 to 7.38)	0.20 (–0.39 to 0.79)	
CT	0.17–1.92 wall thickness	26/34		72.97 (10.43) [73.97]	3.71 (–0.96 to 8.37)	0.40 (–0.11 to 0.89)	0.787
	2.00–3.00 wall thickness	28/26		65.98 (9.24) [66.72]	3.68 (–2.24 to 9.59)	0.39 (–0.24 to 1.02)	
	0.00–0.92 large airway MP	26/32		73.75 (10.26) [74.70]	3.18 (–2.31 to 8.66)	0.34 (–0.25 to 0.92)	0.707
	1.00–1.50 large airway MP	28/28		65.43 (8.91) [66.37]	4.68 (0.02 to 9.33)	0.50 (0.01 to 0.99)	
	0.00–0.92 small airway MP	21/34		74.42 (9.97) [75.18]	3.38 (–1.50 to 8.25)	0.36 (–0.16 to 0.88)	0.992
	1.00–1.58 small airway MP	33/26		65.23 (8.87) [65.16]	3.98 (–1.71 to 9.65)	0.43 (–0.19 to 1.03)	
	7.50–46.67 gas trapping	22/36		74.19 (9.51) [75.10]	4.47 (–0.76 to 9.69)	0.48 (–0.09 to 1.03)	0.831
	47.08–71.25 gas trapping	32/24		64.98 (9.29) [64.70]	3.51 (–1.99 to 9.01)	0.38 (–0.22 to 0.96)	
Age	<18 years	17/23		72.38 (9.76) [72.02]	5.73 (–1.97 to 13.42)	0.61 (–0.21 to 1.43)	0.287
	≥18 years	37/37		67.96 (10.63) [67.58]	2.51 (–1.41 to 6.43)	0.27 (–0.15 to 0.69)	
Gender	Female	25/29		69.21 (9.59) [69.42]	3.22 (–2.24 to 8.67)	0.35 (–0.24 to 0.92)	0.252
	Male	29/31		69.74 (11.38) [68.61]	1.80 (–3.09 to 6.67)	0.20 (–0.33 to 0.71)	
Mutation	F508del/F508del	26/30		68.28 (10.10) [66.81]	3.05 (–2.97 to 9.07)	0.33 (–0.32 to 0.97)	0.625
	Other	28/30		70.64 (10.85) [71.27]	3.73 (–0.72 to 8.16)	0.40 (–0.08 to 0.87)	
Pseudomonas	Pa free at entry	27/32		72.68 (9.33) [72.02]	5.83 (1.01 to 10.66)	0.62 (0.11 to 1.13)	0.291
	Pa colonised at entry	27/28		66.06 (10.7) [66.36]	1.24 (–4.51 to 6.98)	0.14 (–0.48 to 0.74)	
Con meds	Not using azithromycin	16/18		72.15 (10.74) [73.52]	2.94 (–4.95 to 10.81)	0.32 (–0.53 to 1.15)	0.614
	Using azithromycin	38/42		68.33 (10.26) [66.89]	3.65 (–0.46 to 7.74)	0.39 (–0.05 to 0.83)	
	Not using corticosteroids	18/22		71.92 (11.04) [73.52]	5.62 (–0.67 to 11.9)	0.60 (–0.08 to 1.27)	0.980
	Using corticosteroids	36/38		68.15 (10.03) [66.91]	3.84 (–1.03 to 8.70)	0.41 (–0.11 to 0.93)	
	Not using DNase	19/24		71.87 (11.32) [73.52]	1.17 (–4.82 to 7.16)	0.13 (–0.52 to 0.76)	0.549
	Using DNase	35/36		68.07 (9.81) [66.89]	4.15 (–0.81 to 9.10)	0.44 (–0.09 to 0.97)	
	Not using hypertonic saline	38/38		70.94 (10.50) [71.66]	3.62 (–0.21 to 7.45)	0.39 (–0.03 to 0.79)	0.778
	Using hypertonic saline	16/22		66.61 (10.35) [66.54]	3.19 (–5.22 to 11.6)	0.34 (–0.56 to 1.23)	
TA AEs	<4 lower airway TA AEs	46/45		69.63 (10.35) [69.64]	3.95 (–0.29 to 8.19)	0.42 (–0.04 to 0.87)	0.649
	≥4 lower airway TA AEs	8/15		68.89 (11.34) [68.48]	1.30 (–6.29 to 8.89)	0.14 (–0.67 to 0.95)	

Standardised treatment effect
–1.5 –1.0 –0.5 0.0 0.5 1.0 1.5
Favours placebo ← → Favours active

FIGURE 27 Stratification of primary outcome measure. Forest plot showing stratification of the primary outcome response by prespecified variables. To allow results from different end points to be plotted on a common scale, the estimated TEs were standardised to be presented as multiples of the underlying SD (standardised TE). The size of the circle is proportional to the number of subjects represented and the bars indicate 95% CI. For all CT values and LCI, lower numbers indicate less severe disease. Con Meds, Concurrent Medication; MP, mucus plugs; Pa, *P. aeruginosa*; TA AE, treatment-associated AE.

(a)

(b)
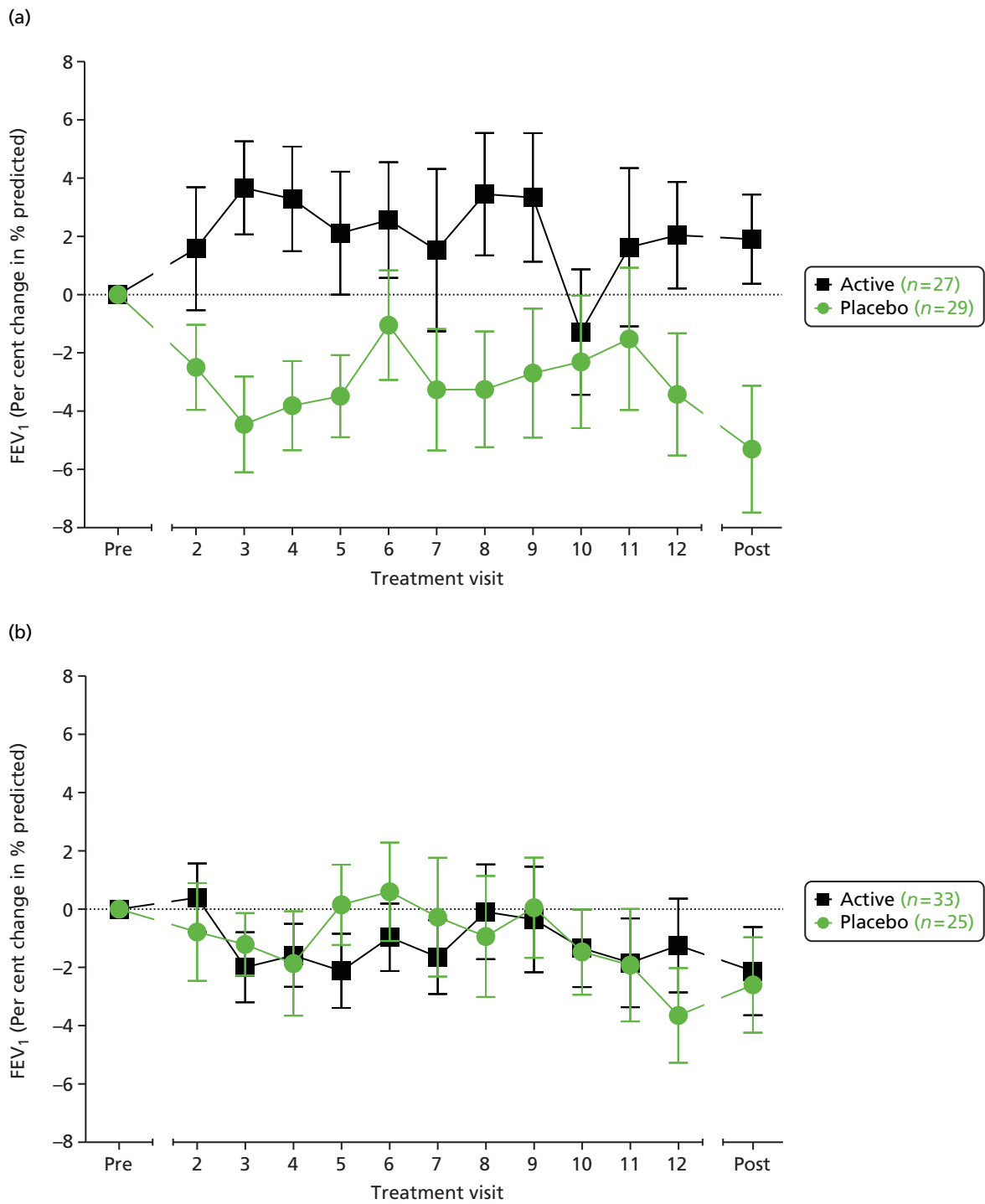
FIGURE 28 Primary outcome stratified by baseline FEV_1. Time course of the primary outcome response stratified by baseline FEV_1 at trial entry. (a) Severe group, baseline FEV_1 49.6–69.2% predicted; and (b) milder group, baseline FEV_1 69.6–89.9% predicted. Error bars indicate SEM. SEM, standard error of the mean.

Influence of baseline FEV$_1$ on other outcomes

Having observed that baseline FEV$_1$ appeared to have an important influence in the magnitude of the TE based on the primary outcome, we next examined other outcome measures for this effect. Subjects were divided into upper and lower halves based on the median value for the entire group, as previously, and TEs were compared. *Figure 29* shows that many of the assays mirrored the effect which had been seen for the primary outcome, namely the more severe group of patients showing an approximate doubling of the TE compared with the values in the whole, unstratified group. This resulted in an absolute improvement (vs. stabilisation) in many of the assays. Of note, in those with less severe FEV$_1$ at trial entry, biomarkers associated with smaller airways, in particular LCI, still showed a TE favouring the active-treatment group.

We confirmed that these benefits seen in the more severe subgroup were not related to an increased number of antibiotic courses during the trial. Both active-treatment and placebo groups received a median of three courses of oral or intravenous antibiotics. In the half with more severe disease, stratified by FEV$_1$, both placebo and active-treatment groups received three courses, while among those with less severe disease, the placebo group received three courses and the active-treatment group received two. Thus, the observed TEs were independent of concurrent antibiotic courses.

Summary of efficacy outcomes

The trial achieved its primary outcome confirming a statistically significant TE favouring the active-treatment group on relative change in FEV$_1$. This was underscored by statistically significant TEs in the secondary outcomes, FVC and gas trapping on CT. Furthermore, for all other secondary outcomes, although not statistically significant, the standardised TE analysis favoured active treatment. In general, these improvements were seen across the group and were independent of sex, age and mutation class. In contrast, the magnitude of TE was influenced by the severity of baseline lung disease assessed by FEV$_1$: patients in the more severe half at baseline experienced larger improvements not only in FEV$_1$ but also in several other outcomes. Changes in the small airway measure, LCI, were still seen in patients with less severe baseline FEV$_1$, suggesting that these patients can still benefit.
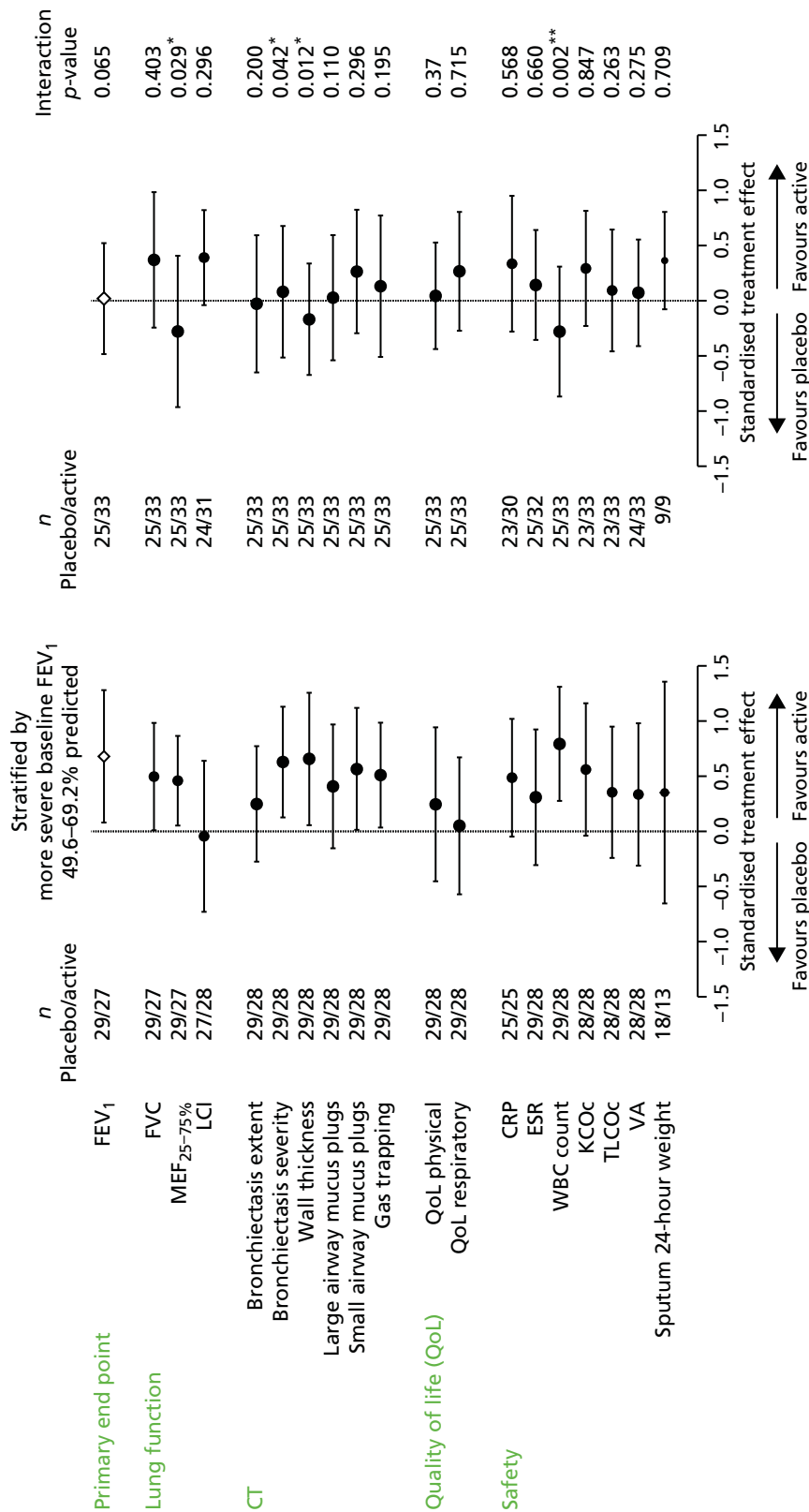
FIGURE 29 Stratification of secondary outcomes. Forest plot showing stratification of secondary outcomes by the severity of baseline FEV_1 at trial entry. To allow results from different end points to be plotted on a common scale, the estimated TEs were standardised to be presented as multiples of the underlying SD (standardised TE). The size of the circle is proportional to the number of subjects represented and the bars indicate 95% CI. ESR, erythrocyte sedimentation rate; WBC, white blood cell.

Chapter 4 Results of mechanistic substudy

Transgene-specific DNA and mRNA

Nasal

In the nasal arm of the substudy, the assay could quantify a dosing-dependent increase in vector-specific DNA in 15 out of 15 active-dose subjects post treatment, although in four of these modest levels of DNA were detected prior to dosing. In the placebo patients, DNA could also be detected in samples from three out of four subjects post dosing (none prior to dosing); no vector-specific mRNA was quantifiable in either group.

Lower airway

In the bronchial arm of the substudy, the assay quantified vector-specific DNA in 10 out of 10 active-treatment patients and zero out of seven placebo subjects post dosing (*Figure 30*); no vector-specific mRNA was quantifiable in either group.
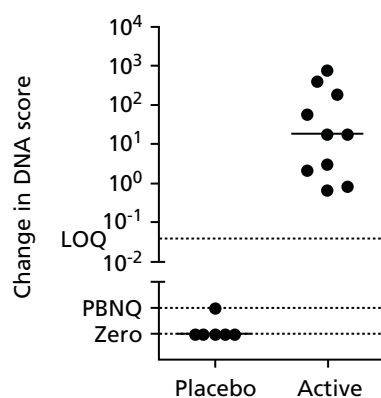
FIGURE 30 Assessment of DNA from bronchial brushings in the placebo ($n = 7$) and active-treatment ($n = 10$) subgroups. Each circle represents an individual patient.

Potential difference measurements

Nasal

The nasal arm comprised 24 patients in the ITT group, 20 of whom were in the PP. One patient had a mean total chloride secretory response ≥ 5 mV pre-dosing and was, therefore, excluded as prespecified in the protocol. Day 28 post-treatment recordings for two active-treatment patients had to be delayed beyond a window of > 7 days of the prespecified interval and were therefore excluded; both patients had usable values at the day 14 post treatment time point. Overall, 75 out of 106 (70.8%) of zero chloride, and 70 out of 106 (66.0%) of isoproterenol recordings were interpretable.

There were no significant changes in baseline values or amiloride responses in either the placebo or active-treatment groups. No significant changes in either the zero chloride or isoproterenol responses were seen. For the zero chloride component 10 out of 14 active-treatment patients and three out of six placebo patients showed net secretion (i.e. a more negative value post treatment than pretreatment); 4 out of 14 active-treatment patients showed mean pre–post-treatment responses (ranging from –3.4 mV to –7.0 mV), which were more negative than the largest placebo response. For the isoproterenol component, 11 out of 12 active-treatment patients and three out of six placebo patients showed net secretion (*Figure 31*).
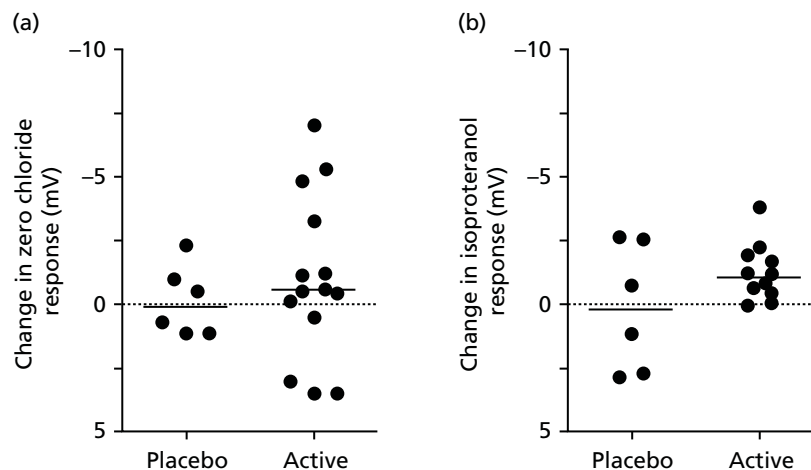
FIGURE 31 The response of the nasal epithelium to perfusion with (a) a zero chloride solution; and (b) a zero chloride solution containing isoproterenol (10 μM). Each symbol indicates the change in this response from trial start to finish for the relevant treatment in an individual patient. The plotted value is the most negative value obtained from all interpretable recordings (range 1–3) at each time point for that patient. A more negative value is in the non-CF direction. The placebo group had a median pre–post trial change of + 0.1 mV (range +1.1 to –2.3 mV) and the active-treatment group –0.6 mV (range +3.5 to –7.0 mV) ($p = 0.509$). The response of the nasal epithelium to perfusion with a zero chloride solution containing isoproterenol (10 μM). The placebo group showed a median pre–post trial change of +0.3 mV (range +2.9 to –2.6 mV) and the active-treatment group –1.0 mV (range +0.1 to –3.8 mV) ($p = 0.424$).

Bronchial

In the bronchial subgroup, one active-treatment patient withdrew after six doses, but consented to post-dosing bronchoscopy within the prespecified timing (28 ± 5 days) after the last dose; values for this patient are included in the analysis. Post-dosing bronchoscopy was performed on two placebo patients 58 and 62 days after their last doses, and on two active-treatment patients 49 and 111 days after their last dose. The data for the two placebo patients are included in the final analysis, on the basis that the natural history of bronchial electrophysiology is unlikely to be influenced by a dose of saline approximately 60 days previously. Values for the two active-treatment patients were omitted from the final analysis given the monthly dosing schedule in the trial based on previously generated gene expression data, although their samples were included in the safety analysis. Overall, 66 out of 102 (64.7%) recordings fulfilled the acceptability criteria.

There were no significant changes in basal values in either the placebo or active-treatment groups. *Figure 32* shows bronchial chloride responses using the mean of all available interpretable tracings for each patient; a negative value indicates a non-CF direction. The placebo group ($n = 7$) had a median pre–post trial change of +3.1 mV (range +9.3 to −1.2 mV) and the active-treatment group ($n = 10$) −1.3 mV (range +4.0 to −5.8 mV) ($p = 0.032$). Five out of 10 active-treatment patients had values more negative than the largest placebo response. *Figure 32b* shows the same analysis with only the most negative value recorded for each patient at any time point. The placebo group showed a median pre–post trial change of +2.6 mV (range +9.3 to −1.2 mV) and the active group −2.8 mV (range + 4.0 to −16.8 mV) ($p = 0.087$). Six out of 10 active-treatment patients had values more negative than the largest placebo response.
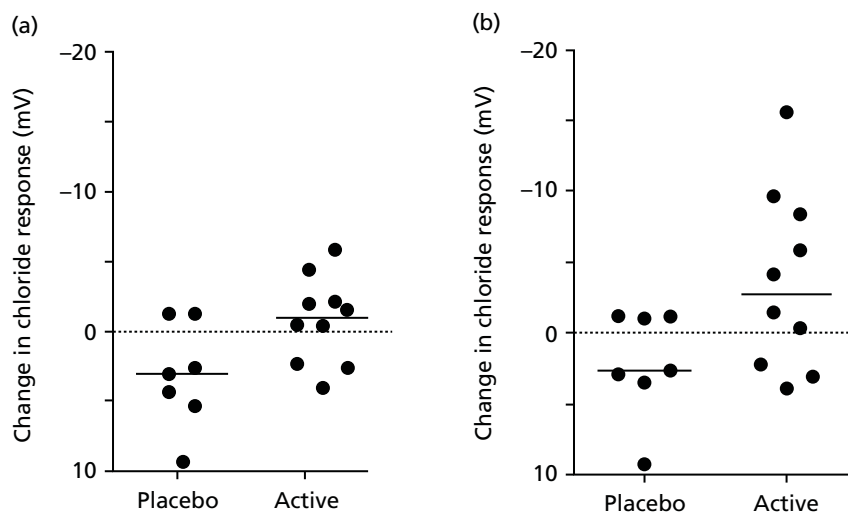
FIGURE 32 Change from pretreatment in the response of the bronchial epithelium to perfusion with a zero chloride solution containing isoproterenol (10 μM). (a) The response of the bronchial epithelium to perfusion with a zero chloride solution containing isoproterenol (10 μM). Each symbol indicates the change in this response from trial start to finish for the relevant treatment in an individual patient. The plotted value is the mean of all interpretable recordings (range 1–3) at each time point for that patient. A more negative value is in the non-CF direction. The placebo group had a median pre–post trial change of +3.1 mV (range +9.3 to −1.2 mV) and the active group −1.0 mV (range +4.0 to −5.8 mV) ($p = 0.032$). (b) The response of the bronchial epithelium to perfusion with a zero chloride solution containing isoproterenol (10 μM). Each symbol indicates the change in this response from trial start to finish for the relevant treatment in an individual patient. The plotted value is the most negative value obtained from all interpretable recordings (range 1–3) at each time point for that patient. A more negative value is in the non-CF direction. The placebo group showed a median pre–post trial change of +2.6 mV (range +9.3 to −1.2 mV) and the active group −2.8 mV (range + 4.0 to −15.6 mV) ($p = 0.087$).

Chapter 5 Safety and adverse events

The trial was monitored at intervals by the independent DMEC, who scrutinised the unblinded group data and provided written confirmation to the TSC that the trial could continue with no modifications. Overall, the drug was well tolerated and AEs were of the nature expected in CF trials. In this section we describe the findings from the early safety cohort, AEs including a detailed description of serious AEs (SAEs) and present results of safety assays throughout the study.

Early safety cohort

The early safety cohort was designed to include 20 patients receiving three doses under intensified monitoring conditions ahead of enrolment of the complete cohort. Two patients who had been successfully screened became unwell before their planned first dose and could not be included without delaying timelines, so were excluded from this subgroup. The data presented to the DMEC came from 18 patients (eight of whom received placebo), 17 of whom had received three doses and one two doses. In addition to the procedures outlined in the general protocol, they had been seen for symptom review, lung function and blood tests (inflammatory markers, liver and renal function) at day 2 following each dose. The data from this cohort were reviewed by the Data Monitoring Safety Board at a meeting in September 2012.

Relevant data have been extracted from the closed report, to which the investigators have been unblinded only since database lock; any amendments to the original text have been made only for typographic or language errors, otherwise text and tables were as presented.

Throughout this section, the placebo group is referred to as group A and the active-treatment group as group B.

Adverse events

The AEs are presented as an aggregate number of events and also are listed by site and treatment group. The total numbers of AEs are presented in *Tables 3* and *4*.

Table 3 presents the total number of AEs that were observed initially in the trial, we observed that that there was only one severe AE.

Table 4 presents the number of AEs that were observed 2 days after dosing the subjects. No SAEs were observed 2 days after dosing the subjects. *Table 5* presents changes in spirometric indices.

We observe that, overall, there were no SAEs occurring during the trial.

TABLE 3 Early safety cohort: number of AEs

	Total			London			Edinburgh		
	Overall	Group A	Group B	Overall	Group A	Group B	Overall	Group A	Group B
Number of AEs	130	38	92	103	33	70	27	5	22
Frequency									
Single episode	108	32	76	87	27	60	21	5	
Intermittent	17	6	11	11	6	5	6	0	
Severity									
Mild	104	23	72	82	24	55	22	5	17
Moderate	12	4	8	7	4	3	5	0	5
Severe	1	1	0	1	1	0	0	0	0
Life-threatening	0	0	0	0	0	0	0	0	0
Number of expected AEs	84	22	62	66	18	48	18	4	14
Expected for study drug	41	16	25	32	12	20	9	4	5
Action taken									
None	121	35	86	94	30	64	27	5	22
Dose adjusted	0	0	0	0	0	0	0	0	0
Dose interrupted	1	1	0	1	1	0	0	0	0
Discontinued	0	0	0	0	0	0	0	0	0
Medication used									
Yes	24	10	14	18	10	8	6	0	6
No	98	28	70	77	23	54	21	5	16
Outcome									
Resolved	105	30	75	83	26	57	22	4	18
Unresolved	9	5	4	5	5	0	4	0	0
Fatal	0	0	0	0	0	0	0	0	0
Relation to study?									
Unrelated	19	8	11	11	7	4	8	1	7
Unlike	25	8	17	23	8	15	2	0	2
Possible	39	11	28	25	7	18	14	4	10
Probable	35	6	29	32	6	26	3	0	3
Almost certain	9	5	4	9	5	4	0	0	0

TABLE 4 Adverse events: 2 days after dosing – number of AEs

	Total			London			Edinburgh		
	Overall	Group A	Group B	Overall	Group A	Group B	Overall	Group A	Group B
Number of AEs	10	4	6	10	4	6	0	0	0
Frequency									
Single episode	10	4	6	10	4	6	0	0	0
Intermittent	0	0	0	0	0	0	0	0	0
Severity									
Mild	9	4	5	9	4	5	0	0	0
Moderate	1	0	1	1	0	1	0	0	0
Severe	0	0	0	0	0	0	0	0	0
Life-threatening	0	0	0	0	0	0	0	0	0
Number of expected AEs	6	3	3	6	3	3	0	0	0
Expected for study drug	4	2	2	4	2	2	0	0	0
Action taken									
None	10	4	6	10	4	6	0	0	0
Dose adjusted	0	0	0	0	0	0	0	0	0
Dose interrupted	0	0	0	0	0	0	0	0	0
Discontinued	0	0	0	0	0	0	0	0	0
Medication used									
Yes	0	0	0	0	0	0	0	0	0
No	10	4	6	10	4	6	0	0	0
Outcome									
Resolved	5	1	4	5	1	4	0	0	0
Unresolved	0	0	0	0	0	0	0	0	0
Fatal	0	0	0	0	0	0	0	0	0
Relation to study?									
Unrelated	5	1	4	5	1	4	0	0	0
Unlike	0	0	0	0	0	0	0	0	0
Possible	1	1	0	1	1	0	0	0	0
Probable	2	0	2	2	0	2	0	0	0
Almost certain	2	2	0	2	2	0	0	0	0

Spirometry data

TABLE 5 Changes in spirometric values [mean (range)] from screening to dosing periods in the early safety cohort. A negative change indicates a decrease in the spirometry parameters

	Dosing period 1			Dosing period 2			Dosing period 3		
	All	Group A	Group B	All	Group A	Group B	All	Group A	Group B
FEV_1 (l)	0.05 (−0.20 to 0.29) n=17	0.00 (−0.12 to 0.17) n=8	0.14 (−0.20 to 0.29) n=9	0.00 (−0.28 to 0.55) n=18	−0.07 (−0.22 to 0.06) n=8	0.22 (−0.28 to 0.55) n=10	−0.04 (−0.49 to 0.43) n=18	−0.10 (−0.31 to 0.04) n=8	0.27 (−0.49 to 0.43) n=10
Percentage predicted FEV_1	1.15 (−7.01 to 7.57) n=18	−0.03 (−3.87 to 6.24) n=8	3.91 (−7.01 to 7.57) n=10	−0.12 (−8.79 to 15.06) n=18	−2.26 (−4.99 to 1.62) n=8	6.46 (−8.79 to 15.06) n=10	−0.92 (−11.28 to 12.47) n=18	−2.92 (−6.65 to 0.42) n=8	7.42 (−11.28 to 12.47) n=10
FVC (l)	0.03 (−0.23 to 0.42) n=18	−0.04 (−0.23 to 0.25) n=8	0.18 (−0.17 to 0.42) n=10	0.05 (−0.28 to 0.89) n=18	0.02 (−0.28 to 0.57) n=8	0.32 (−0.25 to 0.89) n=10	−0.01 (−0.44 to 0.37) n=17	−0.11 (−0.44 to 0.10) n=7	0.21 (−0.32 to 0.37) n=10
MEF_{25} (l)	0.07 (−0.13 to 0.45) n=18	0.06 (−0.13 to 0.42) n=8	0.16 (−0.10 to 0.45) n=10	0.03 (−0.18 to 0.42) n=18	−0.03 (−0.13 to 0.03) n=8	0.18 (−0.18 to 0.42) n=10	−0.04 (−0.42 to 0.21) n=18	−0.04 (−0.17 to 0.10) n=8	0.20 (−0.42 to 0.21) n=10
MEF_{50} (l)	0.15 (−0.26 to 0.52) n=18	0.14 (−0.14 to 0.47) n=8	0.25 (−0.26 to 0.52) n=10	−0.12 (−0.98 to 0.75) n=18	−0.33 (−0.98 to 0.10) n=8	0.39 (−0.61 to 0.75) n=10	−0.05 (−0.87 to 1.14) n=18	−0.14 (−0.37 to 0.17) n=8	0.64 (−0.87 to 1.14) n=10
MEF_{75} (l)	0.07 (−0.62 to 0.63) n=17	0.05 (−0.62 to 0.60) n=8	0.26 (−0.19 to 0.63) n=9	0.17 (−1.29 to 1.84) n=17	−0.10 (−1.29 to 0.69) n=8	0.88 (−0.86 to 1.84) n=9	−0.33 (−2.16 to 1.00) n=17	−0.32 (−0.95 to 0.08) n=8	1.01 (−2.16 to 1.00) n=9

Clinical examination

Table 6 presents the change in clinical examination parameters from screening day to dosing day. The changes in the clinical examination are presented as a number of cases that change from a positive result to a negative result; change from a negative result to a positive result; and do not change.

TABLE 6 Clinical examination findings: *n* (%)

Change in clinical findings	Dosing period 1			Dosing period 2			Dosing period 3		
	All	Group A	Group B	All	Group A	Group B	All	Group A	Group B
Ears									
Normal to abnormal	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
No change	18 (100.0)	8 (100.0)	10 (100.0)	18 (100.0)	8 (100.0)	10 (100.0)	17 (100.0)	8 (100.0)	9 (100.0)
Abnormal to normal	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Eyes									
Normal to abnormal	1 (5.6)	0 (0.0)	1 (10.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
No change	17 (94.4)	8 (100.0)	9 (90.0)	18 (100.0)	8 (100.0)	10 (100.0)	17 (100.0)	8 (100.0)	9 (100.0)
Abnormal to normal	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Nose									
Normal to abnormal	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	3 (17.7)	1 (12.5)	2 (22.2)
No change	18 (100.0)	8 (100.0)	10 (100.0)	18 (100.0)	8 (100.0)	10 (100.0)	14 (82.4)	7 (87.5)	7 (77.8)
Abnormal to normal	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Throat									
Normal to abnormal	0 (0.0)	0 (0.0)	0 (0.0)	1 (5.6)	1 (12.5)	0 (0.0)	2 (11.8)	1 (12.5)	1 (11.1)
No change	17 (94.4)	8 (100.0)	9 (90.0)	16 (88.9)	7 (87.5)	9 (90.0)	14 (82.4)	7 (87.5)	7 (77.8)
Abnormal to normal	1 (5.6)	0 (0.0)	1 (10.0)	1 (5.6)	0 (0.0)	1 (10.0)	1 (5.9)	0 (0.0)	1 (11.1)
Skin									
Normal to abnormal	0 (0.0)	0 (0.0)	0 (0.0)	2 (11.1)	0 (0.0)	2 (20.0)	2 (11.8)	1 (12.5)	1 (11.1)
No change	18 (100.0)	8 (100.0)	10 (100.0)	14 (77.8)	8 (100.0)	6 (60.0)	13 (76.5)	7 (87.5)	6 (66.7)
Abnormal to normal	0 (0.0)	0 (0.0)	0 (0.0)	2 (11.1)	0 (0.0)	2 (20.0)	2 (11.8)	0 (0.0)	2 (22.2)

TABLE 6 Clinical examination findings: *n* (%) (*continued*)

Change in clinical findings	Dosing period 1			Dosing period 2			Dosing period 3		
	All	Group A	Group B	All	Group A	Group B	All	Group A	Group B
Heart									
Normal to abnormal	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
No change	17 (94.4)	7 (87.5)	10 (100.0)	17 (94.4)	7 (87.5)	10 (100.0)	16 (94.1)	7 (87.5)	9 (100.0)
Abnormal to normal	1 (5.6)	1 (12.5)	0 (0.0)	1 (5.6)	1 (12.5)	0 (0.0)	1 (5.9)	1 (12.5)	0 (0.0)
Breath sound left lung									
Normal to abnormal	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
No change	18 (100.0)	8 (100.0)	10 (100.0)	18 (100.0)	8 (100.0)	10 (100.0)	17 (100.0)	8 (100.0)	9 (100.0)
Abnormal to normal	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Breath sound right lung									
Normal to abnormal	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
No change	18 (100.0)	8 (100.0)	10 (100.0)	18 (100.0)	8 (100.0)	10 (100.0)	17 (100.0)	8 (100.0)	9 (100.0)
Abnormal to normal	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Crackles left lung									
Absent to present	0 (0.0)	0 (0.0)	0 (0.0)	1 (5.6)	0 (0.0)	1 (10.0)	1 (5.9)	1 (12.5)	0 (0.0)
No change	18 (100.0)	8 (100.0)	10 (100.0)	16 (88.9)	8 (100.0)	8 (80.0)	15 (88.2)	7 (87.5)	8 (88.9)
Present to absent	0 (0.0)	0 (0.0)	0 (0.0)	1 (5.6)	0 (0.0)	1 (10.0)	1 (5.9)	0 (0.0)	1 (11.1)
Crackles right lung									
Absent to present	2 (11.1)	1 (12.5)	1 (10.0)	3 (16.7)	2 (25.0)	1 (10.0)	1 (5.9)	0 (0.0)	1 (11.1)
No change	15 (83.3)	6 (75.0)	9 (90.0)	15 (83.3)	6 (75.0)	9 (90.0)	15 (88.2)	8 (100.0)	7 (77.8)
Present to absent	1 (5.6)	1 (12.5)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (5.9)	0 (0.0)	1 (11.1)
Wheeze left lung									
Absent to present	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Not change	18 (100.0)	8 (100.0)	10 (100.0)	18 (100.0)	8 (100.0)	10 (100.0)	17 (100.0)	8 (100.0)	9 (100.0)
Present to absent	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)

TABLE 6 Clinical examination findings: *n* (%) (*continued*)

Change in clinical findings	Dosing period 1			Dosing period 2			Dosing period 3		
	All	Group A	Group B	All	Group A	Group B	All	Group A	Group B
Wheeze right lung									
Absent to present	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
No change	18 (100.0)	8 (100.0)	10 (100.0)	18 (100.0)	8 (100.0)	10 (100.0)	17 (100.0)	8 (100.0)	9 (100.0)
Present to absent	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Other respiratory problems									
Absent to present	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	2 (11.8)	1 (12.5)	1 (11.1)
No change	18 (100.0)	8 (100.0)	10 (100.0)	18 (100.0)	8 (100.0)	10 (100.0)	15 (88.2)	7 (87.5)	8 (88.9)
Present to absent	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Abdomen									
Normal to abnormal	1 (5.6)	1 (12.5)	0 (0.0)	1 (5.6)	1 (12.5)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
No change	16 (88.9)	7 (87.5)	9 (90.0)	16 (88.9)	7 (87.5)	9 (90.0)	16 (94.1)	8 (100.0)	8 (88.9)
Abnormal to normal	1 (5.6)	0 (0.0)	1 (10.0)	1 (5.6)	0 (0.0)	1 (10.0)	1 (5.9)	0 (0.0)	1 (11.1)
Liver									
Normal to abnormal	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
No change	18 (100.0)	8 (100.0)	10 (100.0)	18 (100.0)	8 (100.0)	10 (100.0)	17 (100.0)	8 (100.0)	9 (100.0)
Abnormal to normal	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Spleen									
Normal to abnormal	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
No change	18 (100.0)	8 (100.0)	10 (100.0)	18 (100.0)	8 (100.0)	10 (100.0)	17 (100.0)	8 (100.0)	9 (100.0)
Abnormal to normal	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)

Current symptoms

Table 7 presents the symptoms expressed as change from the dosing day to 2 days after dosing.

TABLE 7 Reported symptoms: *n* (%)

Symptom	Dosing period 1			Dosing period 2			Dosing period 3		
	All	Group A	Group B	All	Group A	Group B	All	Group A	Group B
Breathlessness									
Absent to present	1 (5.6)	0 (0.0)	1 (10.0)	2 (11.1)	0 (0.0)	2 (20.0)	0 (0.0)	0 (0.0)	0 (0.0)
No change	16 (88.9)	8 (100.0)	8 (80.0)	13 (72.2)	8 (100.0)	5 (50.0)	15 (88.2)	7 (100.0)	8 (80.0)
Present to absent	1 (5.6)	0 (0.0)	1 (10.0)	3 (16.7)	0 (0.0)	3 (30.0)	2 (11.8)	0 (0.0)	2 (20.0)
Increase cough									
Absent to present	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	4 (23.5)	0 (0.0)	4 (40.0)
No change	17 (94.4)	8 (100.0)	9 (90.0)	12 (66.7)	7 (87.5)	5 (50.0)	9 (52.9)	6 (85.7)	3 (30.0)
Present to absent	1 (5.6)	0 (0.0)	1 (10.0)	6 (33.3)	1 (12.5)	5 (50.0)	4 (23.5)	1 (14.3)	3 (30.0)
Wheeze									
Absent to present	0 (0.0)	0 (0.0)	0 (0.0)	1 (5.6)	0 (0.0)	1 (10.0)	0 (0.0)	0 (0.0)	0 (0.0)
No change	18 (100.0)	8 (100.0)	10 (100.0)	16 (88.9)	8 (100.0)	8 (80.0)	17 (100.0)	7 (100.0)	10 (100.0)
Present to absent	0 (0.0)	0 (0.0)	0 (0.0)	1 (5.6)	0 (0.0)	1 (10.0)	0 (0.0)	0 (0.0)	0 (0.0)
Haemoptysis									
Absent to present	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
No change	18 (100.0)	8 (100.0)	10 (100.0)	18 (100.0)	8 (100.0)	10 (100.0)	17 (100.0)	7 (100.0)	10 (100.0)
Present to absent	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Chest pain									
Absent to present	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
No change	18 (100.0)	8 (100.0)	10 (100.0)	18 (100.0)	8 (100.0)	10 (100.0)	17 (100.0)	7 (100.0)	10 (100.0)
Present to absent	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)

TABLE 7 Reported symptoms: *n* (%) *(continued)*

Symptom	Dosing period 1			Dosing period 2			Dosing period 3		
	All	Group A	Group B	All	Group A	Group B	All	Group A	Group B
Cardiovascular									
Absent to present	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (5.9)	0 (0.0)	1 (10.0)
No change	18 (100.0)	8 (100.0)	10 (100.0)	17 (94.4)	7 (87.5)	10 (100.0)	15 (88.2)	7 (100.0)	8 (80.0)
Present to absent	0 (0.0)	0 (0.0)	0 (0.0)	1 (5.6)	1 (12.5)	0 (0.0)	1 (5.9)	0 (0.0)	1 (10.0)
Gastrointestinal									
Absent to present	1 (5.6)	1 (12.5)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	2 (11.8)	0 (0.0)	2 (20.0)
No change	16 (88.9)	7 (87.5)	9 (90.0)	17 (94.4)	8 (100.0)	9 (90.0)	12 (70.6)	6 (85.7)	6 (60.0)
Present to absent	1 (5.6)	0 (0.0)	1 (10.0)	1 (5.6)	0 (0.0)	1 (10.0)	3 (17.7)	1 (14.3)	2 (20.0)
Throat									
Absent to present	2 (11.1)	0 (0.0)	2 (20.0)	1 (5.6)	0 (0.0)	1 (10.0)	1 (5.9)	0 (0.0)	1 (10.0)
No change	13 (72.2)	7 (87.5)	6 (60.0)	17 (94.4)	8 (100.0)	9 (90.0)	14 (82.4)	6 (85.7)	8 (80.0)
Present to absent	3 (16.7)	1 (12.5)	2 (20.0)	0 (0.0)	0 (0.0)	0 (0.0)	2 (11.8)	1 (14.3)	1 (10.0)
Eye									
Absent to present	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
No change	18 (100.0)	8 (100.0)	10 (100.0)	18 (100.0)	8 (100.0)	10 (100.0)	17 (100.0)	7 (100.0)	10 (100.0)
Present to absent	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Ears									
Absent to present	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
No change	18 (100.0)	8 (100.0)	10 (100.0)	18 (100.0)	8 (100.0)	10 (100.0)	17 (100.0)	7 (100.0)	10 (100.0)
Present to absent	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Nose									
Absent to present	2 (11.1)	0 (0.0)	2 (20.0)	1 (5.6)	0 (0.0)	1 (10.0)	0 (0.0)	0 (0.0)	0 (0.0)
No change	15 (83.3)	8 (100.0)	7 (70.0)	16 (88.9)	7 (87.5)	9 (90.0)	17 (100.0)	7 (100.0)	10 (100.0)
Present to absent	1 (5.6)	0 (0.0)	1 (10.0)	1 (5.6)	1 (12.5)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)

TABLE 7 Reported symptoms: *n* (%) (*continued*)

Symptom	Dosing period 1			Dosing period 2			Dosing period 3		
	All	Group A	Group B	All	Group A	Group B	All	Group A	Group B
Neurological examination									
Absent to present	1 (5.6)	0 (0.0)	1 (10.0)	1 (5.6)	1 (12.5)	0 (0.0)	3 (17.7)	1 (14.3)	2 (20.0)
No change	14 (77.8)	7 (87.5)	7 (70.0)	13 (72.2)	5 (62.5)	8 (80.0)	13 (76.5)	6 (85.7)	7 (70.0)
Present to absent	3 (16.7)	1 (12.5)	2 (20.0)	4 (22.2)	2 (25.0)	2 (20.0)	1 (5.9)	0 (0.0)	1 (10.0)
Skin									
Absent to present	2 (11.1)	0 (0.0)	2 (20.0)	1 (5.6)	0 (0.0)	1 (10.0)	1 (5.9)	0 (0.0)	1 (10.0)
No change	16 (88.9)	8 (100.0)	8 (80.0)	17 (94.4)	8 (100.0)	9 (90.0)	15 (88.2)	6 (85.7)	9 (90.0)
Present to absent	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (5.9)	1 (14.3)	0 (0.0)

Vital signs

Table 8 presents the change from screening day to dosing day. A negative change indicates a decrease in vital sign parameters.

Biochemistry parameters

Table 9 presents the change from screening day to dosing day. A negative change indicates a decrease in the biochemistry parameters.

Haematology parameters

Table 10 presents the change from screening day to dosing day. A negative change indicates a decrease in the haematology parameters.

Gas transfer

Table 11 presents the change from the screening day to 2 days after dosing. A negative change indicates a decrease in the gas transfer.

In summary, the DMEC did not consider there was a safety signal on reviewing these results and wrote approving continuation of full recruitment into the trial at the 5-ml dose.

TABLE 8 Vital signs: mean (range)

Sign	Dosing period 1			Dosing period 2			Dosing period 3		
	All	Group A	Group B	All	Group A	Group B	All	Group A	Group B
Diastolic BP (mmHg)	3.83 (–8.00 to 21.00) n=18	5.88 (–6.00 to 20.00) n=8	2.20 (–8.00 to 21.00) n=10	3.44 (–13.00 to 22.00) n=18	3.13 (–4.00 to 12.00) n=8	3.70 (–13.00 to 22.00) n=10	4.82 (–15.00 to 31.00) n=17	5.63 (–4.00 to 17.00) n=8	4.11 (–15.00 to 31.00) n=9
Systolic BP (mmHg)	–0.56 (–13.00 to 14.00) n=18	1.00 (–13.00 to 14.00) n=8	–1.80 (–13.00 to 8.00) n=10	1.78 (–10.00 to 16.00) n=18	3.25 (–10.00 to 16.00) n=8	0.60 (–7.00 to 13.00) n=10	0.24 (–15.00 to 16.00) n=17	2.50 (–9.00 to 16.00) n=8	–1.78 (–15.00 to 8.00) n=9
Pulse (b.p.m.)	2.11 (–16.00 to 24.00) n=18	4.63 (–8.00 to 24.00) n=8	0.10 (–16.00 to 14.00) n=10	5.94 (–27.00 to 28.00) n=18	4.38 (–27.00 to 23.00) n=8	7.20 (–18.00 to 28.00) n=10	12.06 (–6.00 to 29.00) n=17	6.88 (–6.00 to 25.00) n=8	16.67 (0.00 to 29.00) n=9
Respiratory rate (b.p.m.)	–0.50 (–12.00 to 6.00) n=16	–2.86 (–12.00 to 2.00) n=7	1.33 (–2.00 to 6.00) n=9	0.13 (–10.00 to 8.00) n=16	–1.71 (–10.00 to 8.00) n=7	1.56 (–4.00 to 6.00) n=9	1.56 (–6.00 to 10.00) n=16	0.25 (–6.00 to 10.00) n=8	2.88 (–3.00 to 8.00) n=8
Transcutaneous oxygen saturation (%)	0.22 (–2.00 to 4.00) n=18	–0.13 (–2.00 to 3.00) n=8	0.50 (–1.00 to 4.00) n=10	0.06 (–3.00 to 4.00) n=18	0.63 (–2.00 to 4.00) n=8	–0.40 (–3.00 to 2.00) n=10	0.35 (–2.00 to 3.00) n=17	0.63 (–2.00 to 3.00) n=8	0.11 (–1.00 to 2.00) n=9
Temperature (°C)	–0.01 (–0.60 to 1.00) n=16	–0.14 (–0.60 to 0.70) n=7	0.09 (–0.50 to 1.00) n=9	0.04 (–0.70 to 1.10) n=16	–0.24 (–0.70 to 0.40) n=7	0.26 (–0.60 to 1.10) n=9	0.13 (–0.60 to 0.90) n=16	0.02 (–0.40 to 0.70) n=8	0.23 (–0.60 to 0.90) n=8

b.p.m., beats per minute.

TABLE 9 Biochemical tests: mean (range)

Variable	Dosing period 1			Dosing period 2			Dosing period 3		
	All	Group A	Group B	All	Group A	Group B	All	Group A	Group B
Alanine transaminase (units/l)	2.44 (–2.00 to 14.00) n=18	1.50 (–2.00 to 5.00) n=8	3.20 (0.00 to 14.00) n=10	1.83 (–7.00 to 36.00) n=18	3.50 (–7.00 to 36.00) n=8	0.50 (–3.00 to 5.00) n=10	1.47 (–14.00 to 43.00) n=17	3.38 (–14.00 to 43.00) n=8	–0.22 (–9.00 to 9.00) n=9
Albumin (mmol/l)	–0.78 (–4.00 to 3.00) n=18	–0.88 (–4.00 to 3.00) n=8	–0.70 (–3.00 to 2.00) n=10	–2.06 (–7.00 to 3.00) n=18	–2.50 (–7.00 to 3.00) n=8	–1.70 (–3.00 to –1.00) n=10	–2.18 (–5.00 to 1.00) n=17	–1.63 (–5.00 to 1.00) n=8	–2.67 (–5.00 to –1.00) n=9
Alkaline phosphatise (units/l)	–4.08 (–22.00 to 2.00) n=12	–1.75 (–7.00 to 2.00) n=4	–5.25 (–22.00 to 2.00) n=8	–5.67 (–26.00 to 12.00) n=15	2.20 (–9.00 to 12.00) n=5	–9.60 (–26.00 to 5.00) n=10	–1.93 (–32.00 to 41.00) n=14	–0.40 (–9.00 to 12.00) n=5	–2.78 (–32.00 to 41.00) n=9
Aminotransferase (units/l)	1.38 (–2.00 to 5.00) n=16	1.25 (–2.00 to 3.00) n=8	1.50 (–1.00 to 5.00) n=8	2.41 (–6.00 to 32.00) n=17	3.63 (–6.00 to 32.00) n=8	1.33 (–5.00 to 4.00) n=9	0.88 (–12.00 to 10.00) n=17	0.13 (–12.00 to 10.00) n=8	1.56 (–4.00 to 7.00) n=9
Amylase (units/l)	–2.94 (–28.00 to 12.00) n=16	–4.75 (–28.00 to 12.00) n=8	–1.13 (–10.00 to 7.00) n=8	–2.88 (–19.00 to 44.00) n=17	0.88 (–19.00 to 44.00) n=8	–6.22 (–16.00 to 7.00) n=9	–1.63 (–24.00 to 26.00) n=16	–1.00 (–24.00 to 26.00) n=7	–2.11 (–18.00 to 23.00) n=9
Bilirubin (g/l)	0.92 (–2.00 to 6.00) n=13	1.20 (–2.00 to 6.00) n=5	0.75 (–2.00 to 5.00) n=8	0.27 (–6.00 to 6.00) n=15	2.20 (–2.00 to 6.00) n=5	–0.70 (–6.00 to 3.00) n=10	0.36 (–7.00 to 8.00) n=14	3.20 (–1.00 to 8.00) n=5	–1.22 (–7.00 to 8.00) n=9
Calcium (mmol/l)	–0.03 (–0.20 to 0.10) n=18	–0.04 (–0.20 to 0.00) n=8	–0.03 (–0.20 to 0.10) n=10	–0.06 (–0.30 to 0.10) n=17	–0.06 (–0.20 to 0.10) n=7	–0.06 (–0.30 to 0.00) n=10	–0.04 (–0.20 to 0.10) n=16	–0.03 (–0.20 to 0.10) n=8	–0.05 (–0.20 to 0.10) n=8
Corrected calcium (mmol/l)	–0.03 (–0.20 to 0.10) n=18	–0.04 (–0.20 to 0.10) n=8	–0.02 (–0.20 to 0.10) n=10	–0.03 (–0.20 to 0.10) n=16	–0.05 (–0.20 to 0.10) n=6	–0.01 (–0.20 to 0.10) n=10	–0.01 (–0.20 to 0.10) n=16	–0.01 (–0.10 to 0.10) n=8	–0.01 (–0.20 to 0.10) n=8
Creatinine (μmol/l)	2.00 (–10.00 to 10.00) n=18	–0.88 (–10.00 to 9.00) n=8	4.30 (–3.00 to 10.00) n=10	0.44 (–18.00 to 14.00) n=18	–2.25 (–18.00 to 6.00) n=8	2.60 (–5.00 to 14.00) n=10	0.65 (–11.00 to 13.00) n=17	–1.75 (–11.00 to 5.00) n=8	2.78 (–9.00 to 13.00) n=9

Variable	Dosing period 1			Dosing period 2			Dosing period 3		
	All	Group A	Group B	All	Group A	Group B	All	Group A	Group B
CRP (mg/l)	-0.94 (-12.00 to 6.00) n=16	0.43 (-5.00 to 6.00) n=7	-2.00 (-12.00 to 1.00) n=9	-0.29 (-14.00 to 6.00) n=17	0.86 (-1.00 to 5.00) n=7	-1.10 (-14.00 to 6.00) n=10	0.06 (-14.00 to 13.00) n=16	1.71 (-6.00 to 13.00) n=7	-1.22 (-14.00 to 4.00) n=9
Glucose (mmol/l)	0.10 (-1.60 to 4.00) n=9	0.70 (-1.60 to 4.00) n=4	-0.38 (-1.60 to 1.00) n=5	-0.48 (-2.00 to 0.70) n=9	-0.22 (-2.00 to 0.70) n=4	-0.68 (-1.70 to 0.50) n=5	0.36 (-2.00 to 4.10) n=9	-0.03 (-2.00 to 2.00) n=4	0.66 (-1.20 to 4.10) n=5
γ-Glutamyl transferase (units/l)	-0.35 (-4.00 to 3.00) n=17	-1.00 (-4.00 to 1.00) n=7	0.10 (-2.00 to 3.00) n=10	-0.44 (-4.00 to 3.00) n=18	-0.63 (-3.00 to 3.00) n=8	-0.30 (-4.00 to 3.00) n=10	1.06 (-5.00 to 21.00) n=17	0.63 (-3.00 to 10.00) n=8	1.44 (-5.00 to 21.00) n=9
Inorganic phosphate (mmol/l)	-0.02 (-0.40 to 0.50) n=18	-0.04 (-0.40 to 0.50) n=8	0.00 (-0.40 to 0.20) n=10	-0.03 (-0.20 to 0.20) n=16	-0.01 (-0.20 to 0.20) n=7	-0.04 (-0.20 to 0.20) n=9	-0.08 (-0.50 to 0.30) n=15	-0.18 (-0.50 to 0.10) n=8	0.03 (-0.20 to 0.30) n=7
Magnesium (mmol/l)	0.01 (-0.10 to 0.10) n=14	0.03 (-0.10 to 0.10) n=7	-0.01 (-0.10 to 0.00) n=7	0.01 (-0.20 to 0.20) n=13	0.03 (-0.20 to 0.20) n=6	-0.01 (-0.10 to 0.00) n=7	0.04 (-0.20 to 0.20) n=11	0.04 (-0.20 to 0.20) n=7	0.03 (-0.10 to 0.10) n=4
Potassium (mmol/l)	0.09 (-0.10 to 0.40) n=17	0.18 (-0.10 to 0.40) n=8	0.02 (-0.10 to 0.30) n=9	0.03 (-0.40 to 0.50) n=18	0.04 (-0.40 to 0.40) n=8	0.03 (-0.20 to 0.50) n=10	0.06 (-0.50 to 0.30) n=17	0.09 (-0.20 to 0.30) n=8	0.03 (-0.50 to 0.30) n=9
Total protein (g/l)	-1.55 (-8.00 to 2.00) n=11	-2.75 (-8.00 to 0.00) n=4	-0.86 (-4.00 to 2.00) n=7	-2.43 (-7.00 to 3.00) n=14	-3.80 (-7.00 to 1.00) n=5	-1.67 (-7.00 to 3.00) n=9	-1.77 (-4.00 to 1.00) n=13	-2.20 (-4.00 to 1.00) n=5	-1.50 (-4.00 to 1.00) n=8
Sodium (mmol/l)	-0.28 (-5.00 to 3.00) n=18	-0.63 (-4.00 to 2.00) n=8	0.00 (-5.00 to 3.00) n=10	-0.28 (-7.00 to 3.00) n=18	0.13 (-2.00 to 3.00) n=8	-0.60 (-7.00 to 3.00) n=10	1.00 (-2.00 to 4.00) n=17	1.50 (-1.00 to 4.00) n=8	0.56 (-2.00 to 4.00) n=9
Urea (mmol/l)	-0.13 (-1.20 to 2.70) n=18	-0.53 (-1.20 to 0.60) n=8	0.19 (-1.00 to 2.70) n=10	-0.44 (-2.00 to 0.70) n=18	-0.69 (-2.00 to 0.70) n=8	-0.24 (-1.40 to 0.50) n=10	-0.15 (-3.30 to 3.00) n=17	-0.44 (-1.70 to 1.30) n=8	0.11 (-3.30 to 3.00) n=9

TABLE 10 Haematology: mean (range)

Variable	Dosing period 1			Dosing period 2			Dosing period 3		
	All	Group A	Group B	All	Group A	Group B	All	Group A	Group B
Basophils	0.01 (−0.10 to 0.10) n=17	0.00 (−0.10 to 0.10) n=8	0.01 (−0.10 to 0.10) n=9	0.02 (−0.10 to 0.10) n=17	0.03 (0.00 to 0.10) n=8	0.02 (−0.10 to 0.10) n=9	0.01 (−0.10 to 0.10) n=17	0.01 (0.00 to 0.10) n=8	0.00 (−0.10 to 0.10) n=9
Eosinophils	0.01 (−0.12 to 0.11) n=17	−0.03 (−0.12 to 0.11) n=8	0.04 (−0.01 to 0.10) n=9	0.07 (−0.10 to 0.61) n=17	0.12 (−0.10 to 0.61) n=8	0.02 (−0.10 to 0.10) n=9	0.06 (−0.12 to 0.36) n=17	0.09 (−0.10 to 0.36) n=8	0.04 (−0.12 to 0.17) n=9
Erythrocyte sedimentation rate (mm/hour)	−1.93 (−10.00 to 4.00) n=15	−2.14 (−10.00 to 4.00) n=7	−1.75 (−7.00 to 3.00) n=8	−1.60 (−12.00 to 7.00) n=15	−1.14 (−12.00 to 7.00) n=7	−2.00 (−12.00 to 4.00) n=8	0.40 (−15.00 to 20.00) n=15	−1.86 (−15.00 to 5.00) n=7	2.38 (−9.00 to 20.00) n=8
Lymphocytes	−0.07 (−0.90 to 0.70) n=17	−0.01 (−0.90 to 0.70) n=8	−0.12 (−0.40 to 0.40) n=9	0.00 (−0.60 to 0.60) n=17	0.09 (−0.60 to 0.60) n=8	−0.08 (−0.40 to 0.50) n=9	0.10 (−0.80 to 2.40) n=17	0.03 (−0.70 to 0.70) n=8	0.17 (−0.80 to 2.40) n=9
Mean corpuscular haemoglobin (pg)	−0.19 (−1.40 to 1.50) n=17	−0.45 (−1.40 to 0.30) n=8	0.03 (−0.80 to 1.50) n=9	−0.46 (−1.40 to 1.10) n=17	−0.68 (−1.20 to −0.10) n=8	−0.28 (−1.40 to 1.10) n=9	3.12 (−1.80 to 61.30) n=17	−0.67 (−1.80 to 0.50) n=8	6.49 (−1.20 to 61.30) n=9
Mean corpuscular haemoglobin concentration (g/dl)	−0.50 (−2.00 to 2.00) n=10	−1.00 (−2.00 to 0.00) n=5	0.00 (−2.00 to 2.00) n=5	−0.10 (−2.00 to 2.00) n=10	−0.60 (−2.00 to 0.00) n=5	0.40 (−2.00 to 2.00) n=5	0.25 (−2.00 to 2.00) n=8	−0.25 (−2.00 to 1.00) n=4	0.75 (−1.00 to 2.00) n=4
Mean corpuscular volume (fl)	0.29 (−3.00 to 3.00) n=17	0.25 (−2.00 to 3.00) n=8	0.33 (−3.00 to 3.00) n=9	−1.12 (−4.00 to 3.00) n=17	−1.00 (−3.00 to 3.00) n=8	−1.22 (−4.00 to 1.00) n=9	−1.31 (−6.00 to 1.00) n=16	−1.25 (−4.00 to 1.00) n=8	−1.38 (−6.00 to 1.00) n=8
Monocytes	0.01 (−0.20 to 0.20) n=17	0.00 (−0.20 to 0.14) n=8	0.01 (−0.16 to 0.20) n=9	0.00 (−0.30 to 0.30) n=17	−0.02 (−0.30 to 0.14) n=8	0.01 (−0.30 to 0.30) n=9	0.01 (−0.20 to 0.40) n=17	−0.01 (−0.10 to 0.10) n=8	0.03 (−0.20 to 0.40) n=9
Neutrophils	−0.53 (−4.70 to 2.30) n=17	−0.65 (−3.30 to 2.20) n=8	−0.42 (−4.70 to 2.30) n=9	−0.84 (−5.80 to 2.00) n=17	−0.85 (−5.80 to 2.00) n=8	−0.83 (−3.30 to 0.20) n=9	−0.58 (−4.90 to 2.10) n=17	−1.34 (−4.20 to 0.70) n=8	0.10 (−4.90 to 2.10) n=9
Platelets	−4.53 (−74.00 to 32.00) n=17	−9.13 (−74.00 to 32.00) n=8	−0.44 (−31.00 to 31.00) n=9	−10.94 (−93.00 to 84.00) n=17	−14.50 (−93.00 to 84.00) n=8	−7.78 (−58.00 to 39.00) n=9	−5.53 (−97.00 to 59.00) n=17	−6.00 (−63.00 to 59.00) n=8	−5.11 (−97.00 to 35.00) n=9

TABLE 11 Gas transfer: mean (range)

Variable	Dosing period 1			Dosing period 2			Dosing period 3		
	All	Group A	Group B	All	Group A	Group B	All	Group A	Group B
KCO (mmol)	-0.02 (-0.15 to 0.18) n = 18	0.01 (-0.06 to 0.13) n = 8	-0.05 (-0.15 to 0.18) n = 10	0.00 (-0.14 to 0.16) n = 17	0.00 (-0.11 to 0.16) n = 8	-0.01 (-0.14 to 0.12) n = 9	0.00 (-0.38 to 0.21) n = 16	0.04 (-0.06 to 0.21) n = 6	-0.03 (-0.38 to 0.13) n = 10
KCOc	0.00 (-0.12 to 0.16) n = 18	0.03 (-0.06 to 0.14) n = 8	-0.03 (-0.12 to 0.16) n = 10	0.00 (-0.12 to 0.16) n = 18	0.03 (-0.06 to 0.14) n = 8	-0.03 (-0.12 to 0.16) n = 10	0.03 (-0.35 to 0.25) n = 16	0.08 (-0.02 to 0.25) n = 6	0.00 (-0.35 to 0.17) n = 10
TLCO (mmol)	-0.06 (-1.19 to 1.48) n = 18	0.15 (-0.29 to 0.53) n = 8	-0.22 (-1.19 to 1.48) n = 10	0.03 (-1.09 to 1.60) n = 17	0.04 (-0.75 to 1.01) n = 8	0.03 (-1.09 to 1.60) n = 9	0.03 (-1.90 to 1.48) n = 16	0.13 (-1.04 to 1.14) n = 6	-0.02 (-1.90 to 1.48) n = 10
TLCOc	0.02 (-1.10 to 1.52) n = 18	0.24 (0.03 to 0.56) n = 8	-0.15 (-1.10 to 1.52) n = 10	-0.49 (-8.90 to 1.68) n = 18	0.02 (-0.92 to 0.93) n = 8	-0.89 (-8.90 to 1.68) n = 10	-0.29 (-7.31 to 1.77) n = 17	-0.77 (-7.31 to 1.29) n = 7	0.04 (-1.75 to 1.77) n = 10
VA (l)	0.04 (-0.39 to 1.02) n = 18	0.08 (-0.17 to 0.39) n = 8	0.02 (-0.39 to 1.02) n = 10	0.02 (-0.57 to 0.95) n = 17	0.01 (-0.25 to 0.32) n = 8	0.03 (-0.57 to 0.95) n = 9	0.02 (-0.64 to 0.65) n = 16	-0.01 (-0.45 to 0.37) n = 6	0.04 (-0.64 to 0.65) n = 10

Adverse events and serious adverse events

General adverse events

All recorded AEs were categorised as in *Table 12*, and the frequency of their occurrence expressed proportional to the number of patients in each group. All patients in both groups reported AEs, as would be expected in a population of CF patients monitored for 12 months. However, the majority of AEs were of a nature expected in this clinical context and frequencies were similar between groups; there were no statistically significant differences in number overall or once they were broken down into the categories as tabulated.

One patient in the placebo group (fatigue and increased respiratory symptoms) and one in the active-treatment group (flu-like symptoms) discontinued study treatment because of AEs.

Serious adverse events

There were no deaths during the study. Six SAEs were documented, all in the active-treatment group (*Table 13*). Neither the DMEC nor the TSC considered any SAE was related to the trial medication, but that one (case 2) was probably related to a trial procedure (bronchoscopy).

TABLE 12 Adverse event frequency in the ITT and PP populations expressed separately for active-treatment and placebo groups. Numbers represent the total number of AEs in each category proportional to the numbers of patients in the group. There were no differences seen

Category	ITT population		PP population	
	Placebo ($n = 60$)	Active treatment ($n = 76$)	Placebo ($n = 54$)	Active treatment ($n = 62$)
Lower airway respiratory symptoms	7.6	8.0	7.9	9.0
Gastrointestinal symptoms	2.0	1.7	2.1	1.8
Fever or flu-like symptoms	1.1	1.4	1.1	1.4
Headache	1.2	1.1	1.2	1.1
Upper airway symptoms	2.2	3.1	2.3	3.4
Elevated liver function tests	0.3	0.4	0.3	0.4
Haematuria	0.2	0.2	0.2	0.2
Isolated raised inflammatory markers	0.8	0.6	0.8	0.7
Other	3.0	3.0	3.2	3.3
All	18.2	19.6	19.1	21.2

TABLE 13 Description of SAEs

Case	Description
1	Admission to hospital with acute pancreatitis: 47-year-old pancreatic-sufficient male, with multiple previous episodes of acute, non-alcohol-related pancreatitis. Managed conservatively. 4 weeks after last dose
2	Admission to hospital with severe headache, fever, pulmonary exacerbation and new isolate of MRSA: 28-year-old male with flu-like illness within 24 hours of trial bronchoscopy. Hospital admission for intravenous antibiotics. 5 weeks after last dose
3	Pneumothorax after removal of indwelling intravenous access device: 23-year-old female with semi-elective admission for removal and replacement of malfunctioning portacath. Small pneumothorax induced during surgery requiring no intervention. 4 days after last dose
4	Post-surgical infection: 46-year-old female undergoing laparoscopic Nissen's fundoplication for severe reflux. 10 days post surgery, admitted with pain and fever secondary to surgical site abscess, treated 20 days after last dose
5	Admission to hospital with headache, vomiting and viral upper respiratory tract infection symptoms: 25-year-old male/female. Overnight admission with conservative management. 11 days after previous dose
6	Admission to hospital with minor vomiting illness: 14-year-old male with diabetes. Admitted for assistance with diabetic control. 16 days after previous dose

Other safety assays

There were no clinically relevant changes in haematology (*Figure 33*), biochemistry (*Figures 34* and *35*), urinary markers (*Table 14*), histology (*Figure 36*), or lipid staining in sputum (*Table 15*) or biopsy cells (*Table 16*) during the trial. There was no immunological evidence to suggest the development of anti-DNA antibodies (*Table 17*) or anti-CFTR T cells (*Table 18*). There were no treatment-related differences in change in weight or BMI (*Figure 37*). *Figures 33–37* present longitudinal values as mean (standard error of the mean; SEM), although in many cases error bars are small and not visible outside the mean point.
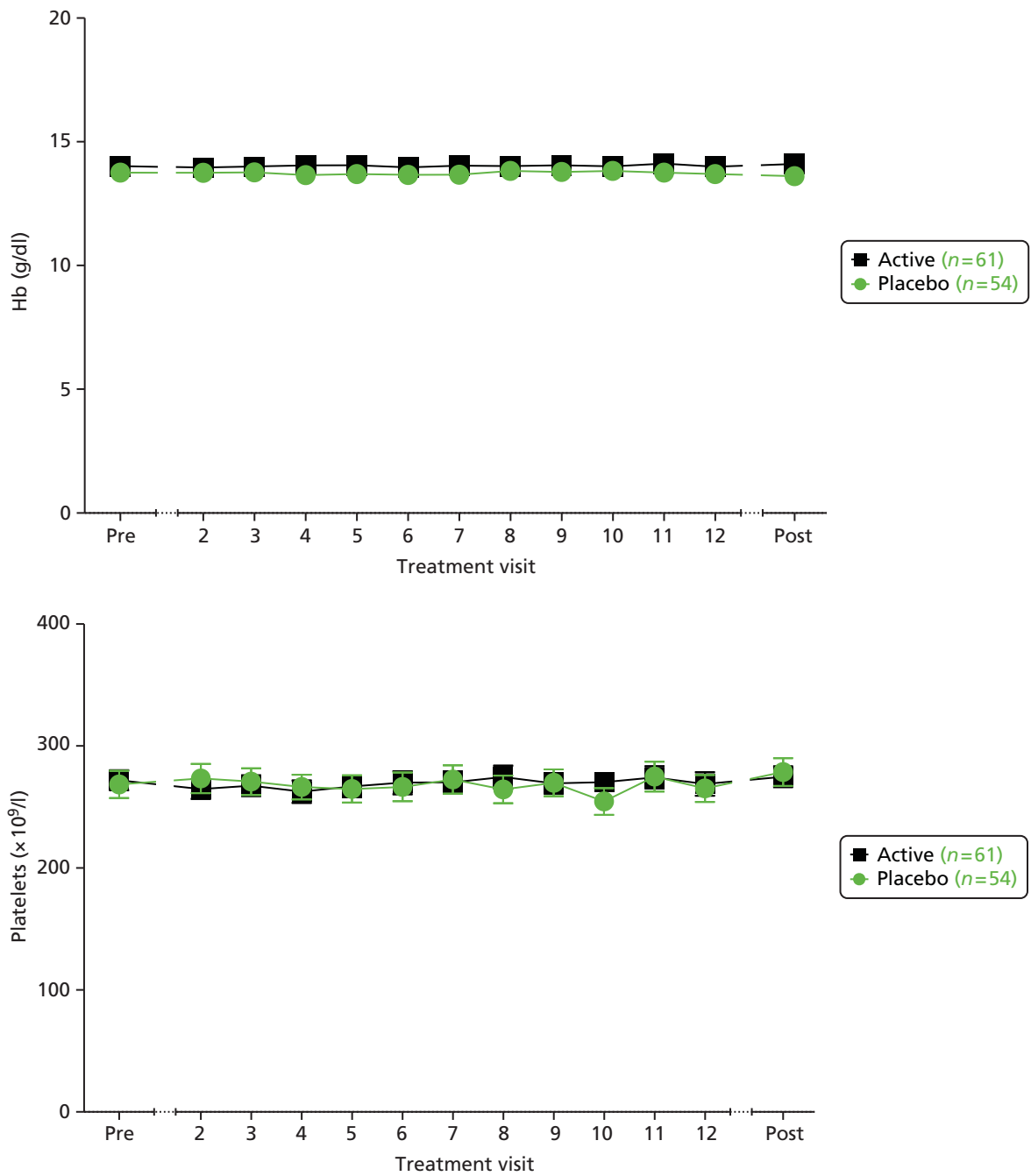
FIGURE 33 Haematological parameters. (*continued*)
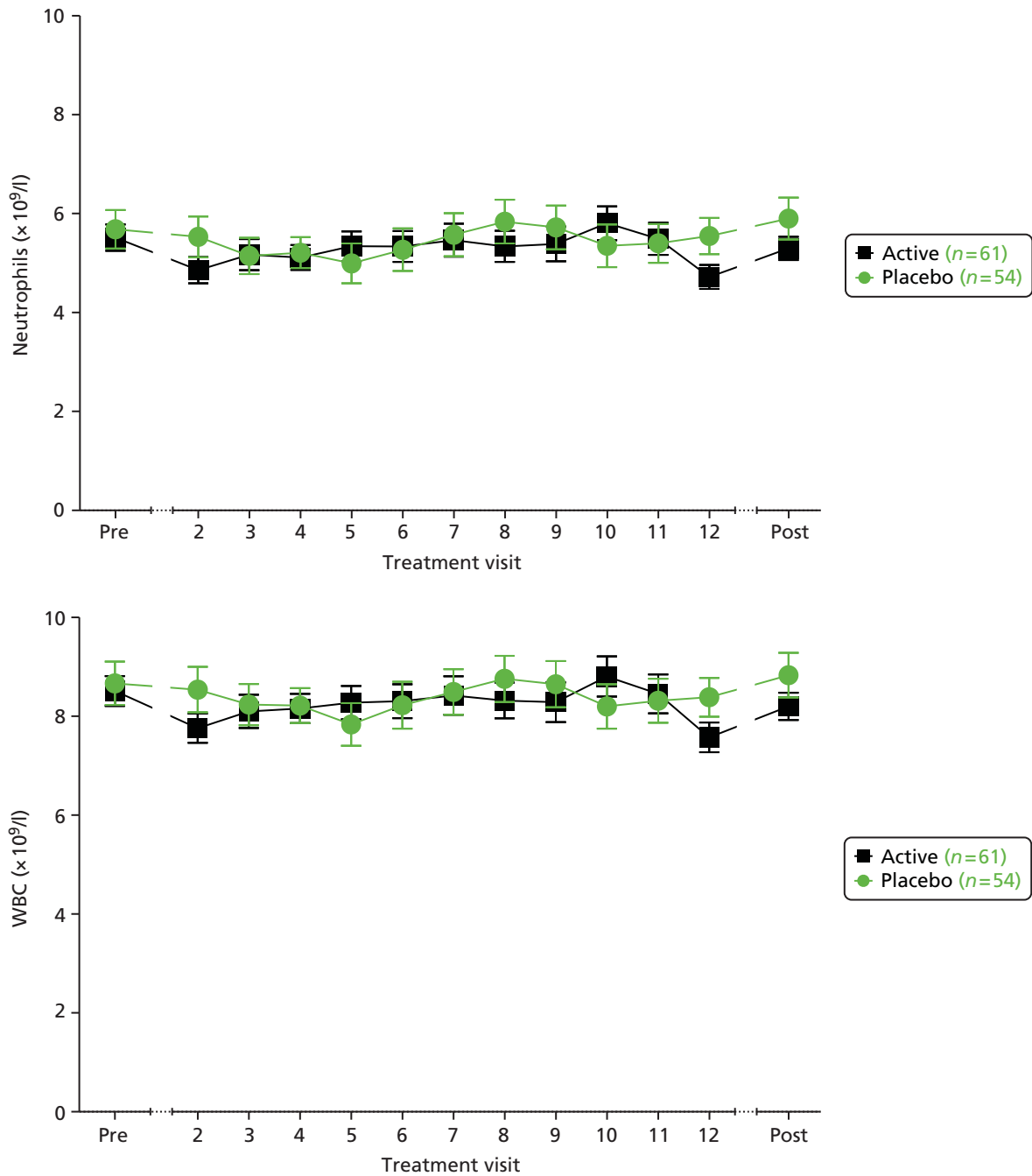
FIGURE 33 Haematological parameters.
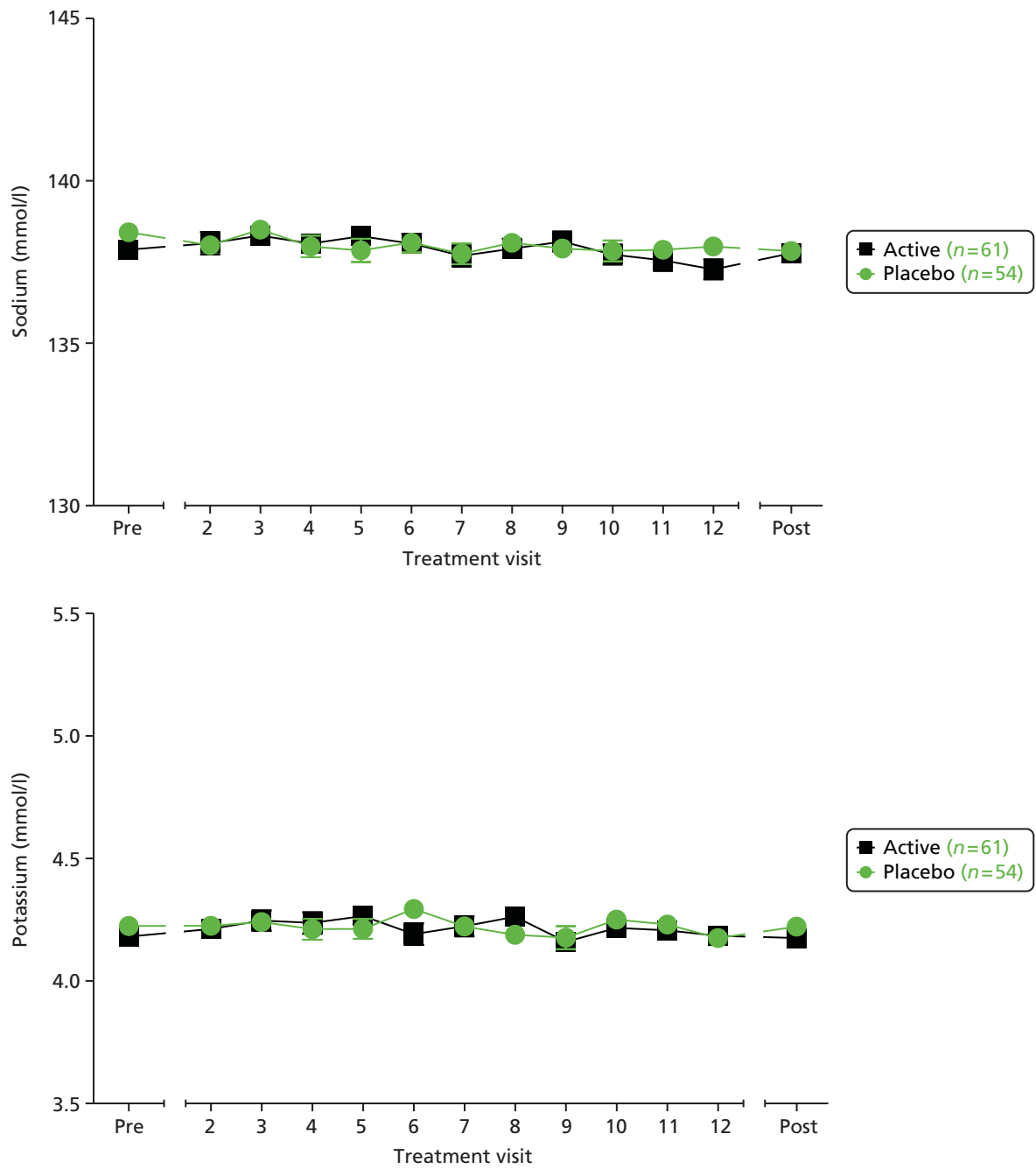
FIGURE 34 Blood biochemistry and renal markers. (*continued*)
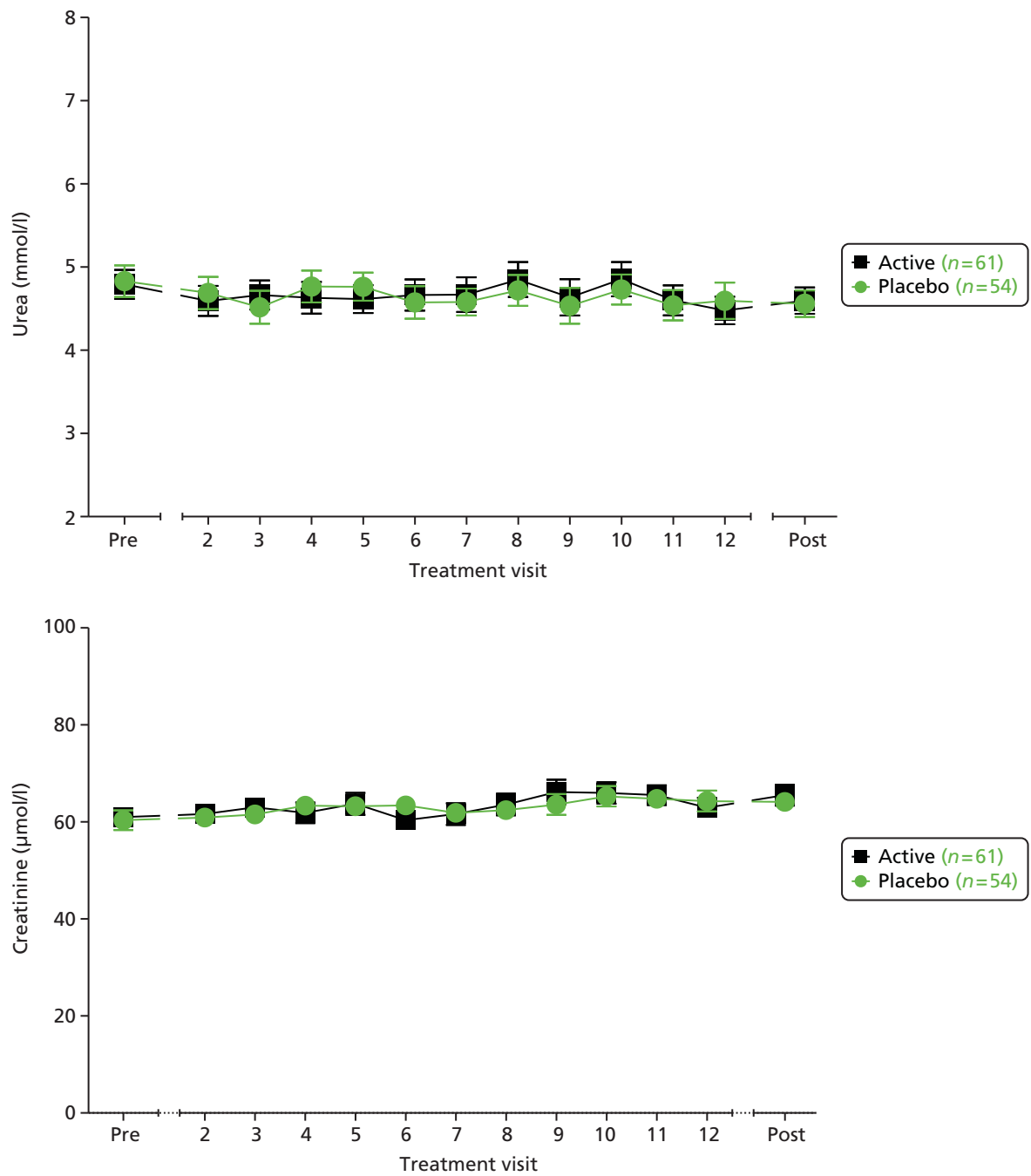
FIGURE 34 Blood biochemistry and renal markers.
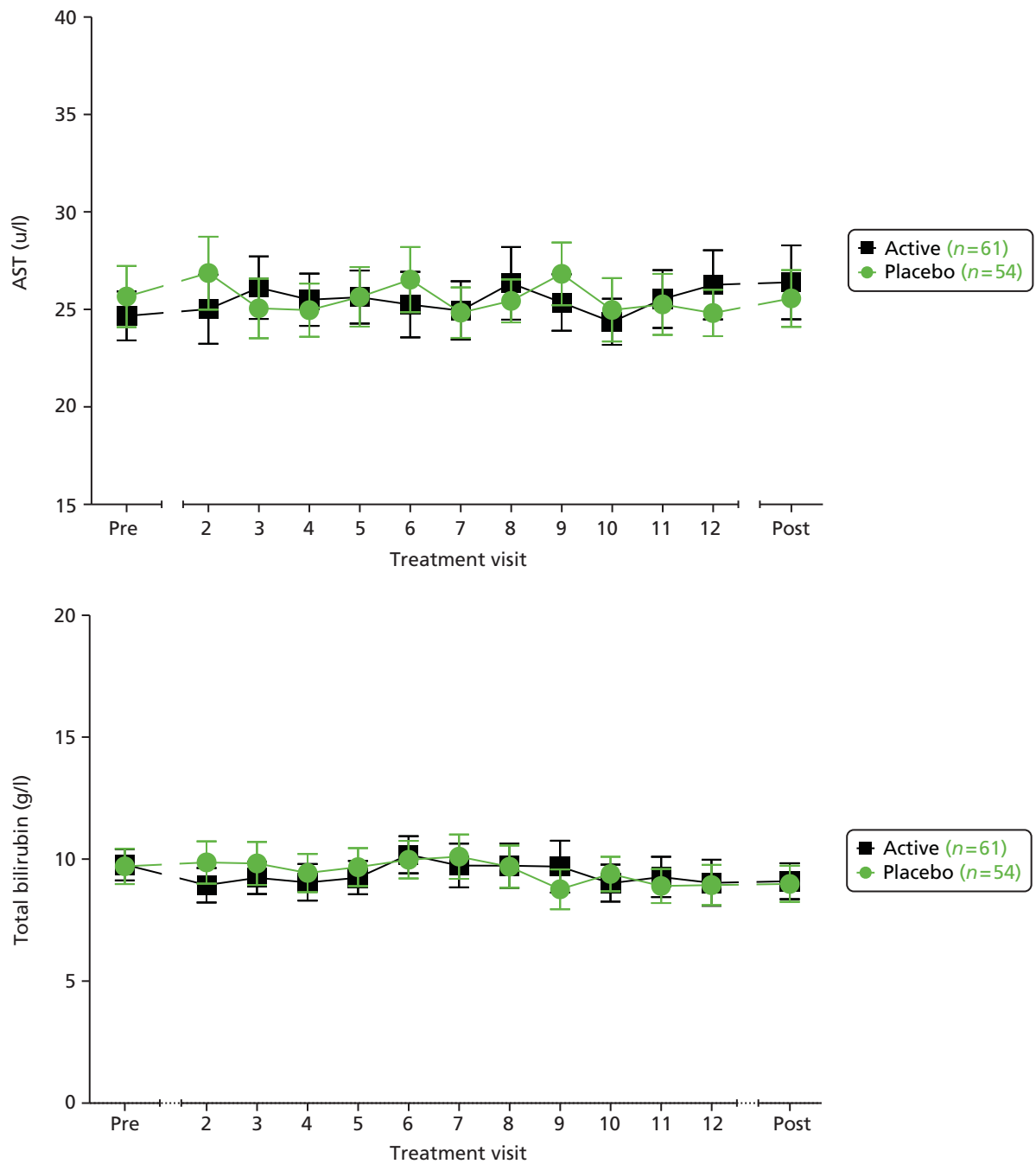
FIGURE 35 Liver and pancreatic function. (*continued*)
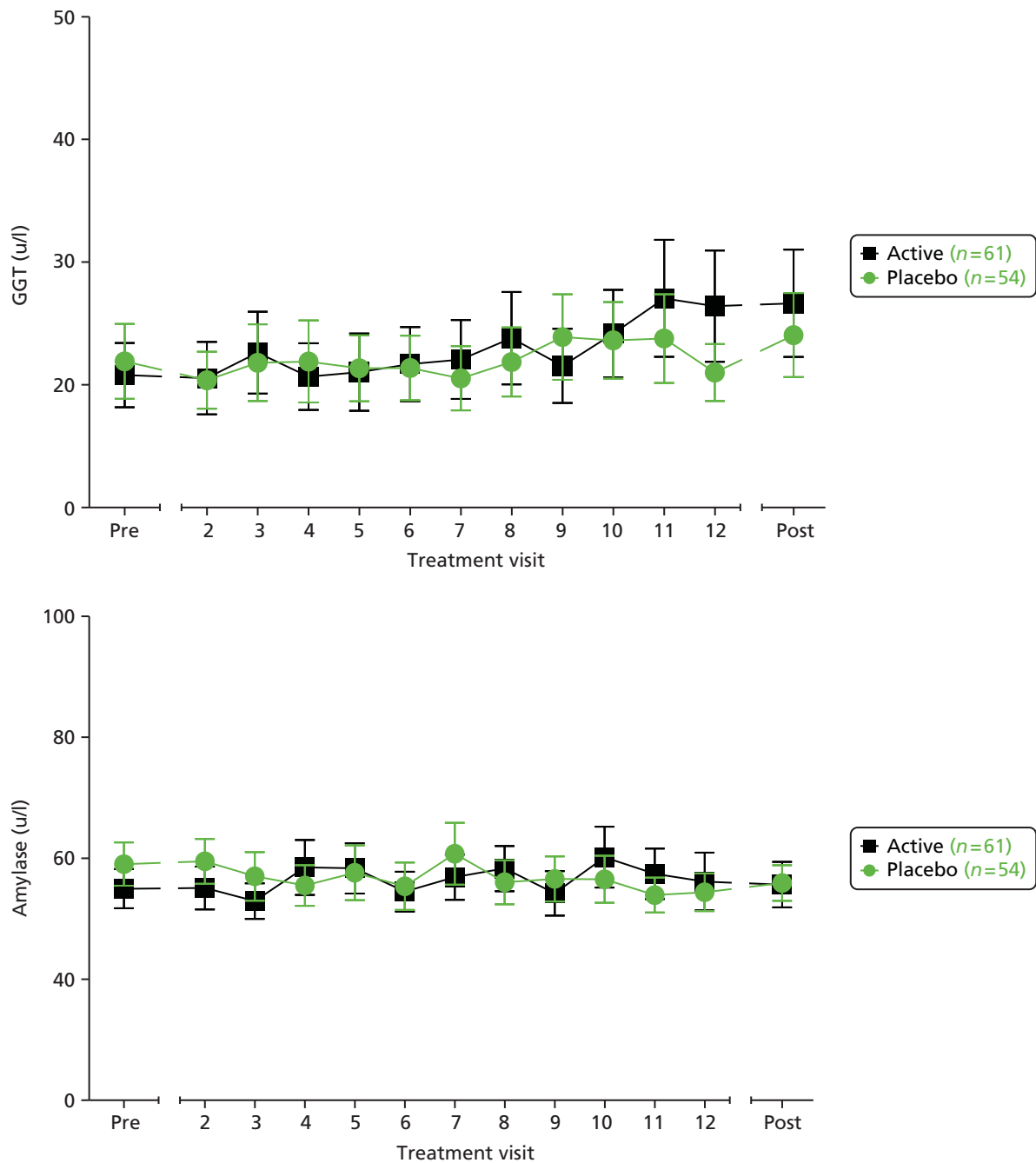
FIGURE 35 Liver and pancreatic function.

TABLE 14 Urinalysis assessed by dipstick for protein, blood and glucose

Urinalysis[a]	Placebo (n = 54)		Active (n = 62)	
	Pretreatment	Post-treatment	Pretreatment	Post-treatment
Urine protein[b]				
Negative	52	47	62	56
Trace	2	6	0	5
+ Positive	0	0	0	0
++ Positive	0	1	0	0
+++ Positive	0	0	0	0
++++ Positive	0	0	0	0
No result	0/54	0/54	0/62	1/62
Negative then positive	2	5	0	4
Positive then negative	0	4	0	2
Urine blood[c]				
Negative	48	47	57	49
Trace	3	1	1	3
+ Positive	0	2	1	1
++ Positive	0	1	3	4
+++ Positive	3	3	0	4
++++ Positive	0	0	0	0
No result	0/54	0/54	0/62	1/62
Negative then positive	2	3	2	4
Positive then negative	4	6	4	5
Urine glucose[d]				
Negative	50	51	55	54
Trace	0	0	1	0
+ Positive	0	1	1	1
++ Positive	1	0	0	0
+++ Positive	1	2	1	2
++++ Positive	2	0	4	4
No result	0/54	0/54	0/62	1/62
Negative then positive	1	1	3	2
Positive then negative	0	1	3	1

a The number of + symbols indicates the level/intensity of positivity based on colour change.
b Analysis is based on two pretreatment and two post-treatment urine samples (collected on different days). In a small number of patients, analysis of the duplicate samples led to different results. For example, two placebo patients were negative for the first pretreatment sample, but positive for the second pretreatment sample. Five placebo patients were negative for the first post-treatment sample, but positive for the second pretreatment sample. These results reflect slight variability in the assays.
c Analysis is based on two pretreatment and two post-treatment urine samples (collected on different days). In a small number of patients analysis of the duplicate samples led to different results. For example, two placebo patients were negative for the first pretreatment sample, but positive for the second pretreatment sample. Four placebo patients were positive for the first pretreatment sample, but negative for the second pretreatment sample. These results reflect slight variability in the assays.
d Analysis is based on two pretreatment and two post-treatment urine samples (collected on different days). In a small number of patients analysis of the duplicate samples led to different results. For example, one-placebo patients were negative for the first pretreatment sample, but positive for the second pretreatment sample. One-placebo patients were negative for the first post-treatment sample, but positive for the second post-treatment sample. These results reflect slight variability in the assays.
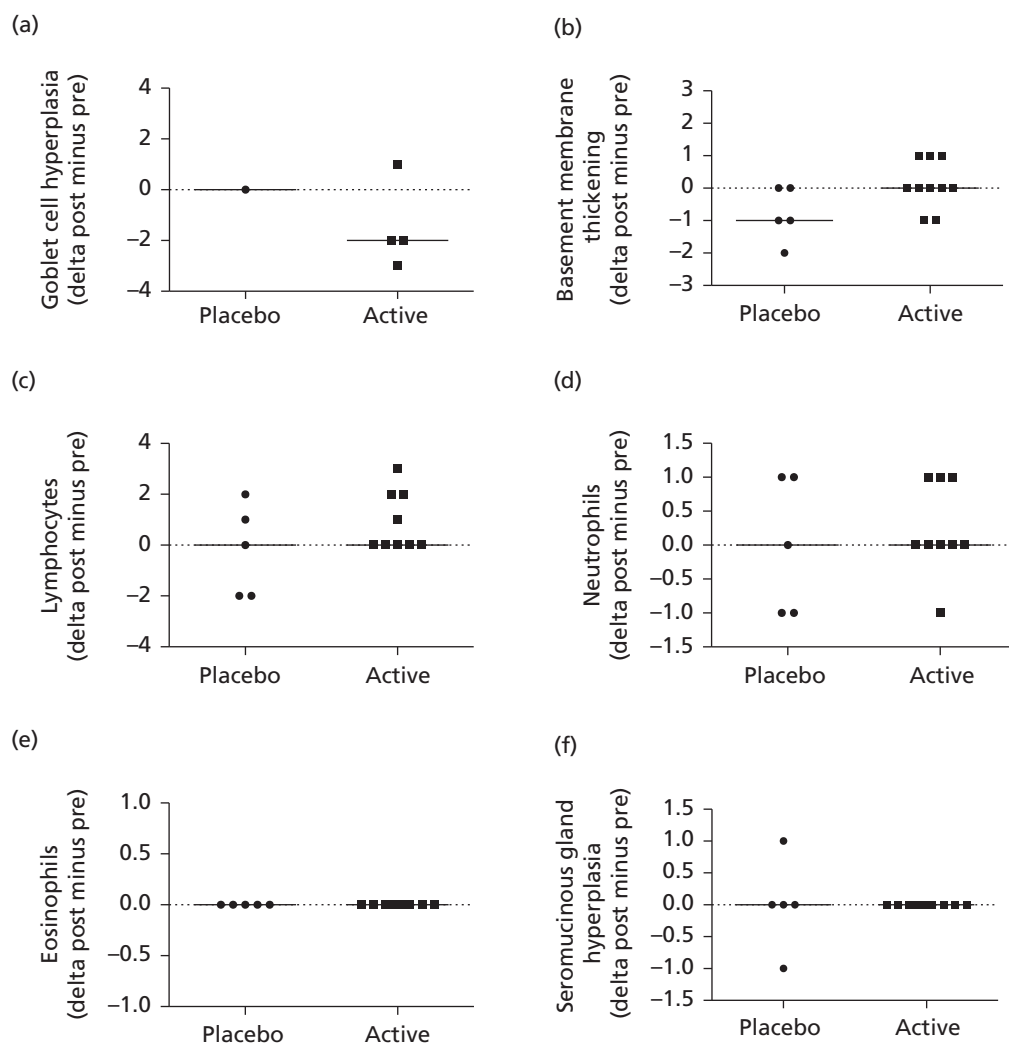
FIGURE 36 Histological assessment of bronchial biopsies. Bronchial biopsies were collected before the first dose (pre) and 28 ± 5 days after the last dose (post). Sections were stained with H&E. Out of the 50 possible biopsies, 39 (78%) were analysed. The remaining 11 biopsies were not analysable because of poor sample quality (n = 10) or because the patient withdrew from the trial (n = 1). Sections were scored semiquantitatively using a scale from 0 to 6, except for seromucinous gland hyperplasia, which was scored as absent (0)/present (1). All data are expressed as the difference between pre and post treatment, with a positive score indicating an increase in that parameter following treatment. Dots represent scores for individual biopsies. The horizontal bar indicates the median. (a) Goblet cell hyperplasia; (b) basement membrane thickening; (c) numbers of lymphocytes; (d) numbers of neutrophils; (e) numbers of eosinophils; and (f) seromucinous gland hyperplasia.

TABLE 15 Differential counts of lipid-staining cells present in sputum samples from placebo and active-treatment subjects

Lipid-staining sputum cells	Placebo, mean[a] (95% CI), n	Active treatment, mean[a] (95% CI), n	TE, mean[a] (95% CI)	p-value
Macrophages	−3.27 (−6.60 to −0.05), 24	−2.13 (−5.45 to 1.20), 24	1.14 (−3.63 to 5.92)	0.631
Neutrophils	−0.23 (−1.62 to 1.16), 19	0.45 (−0.87 to 1.77), 21	0.69 (−1.27 to 2.64)	0.480
Squamous cells	−18.24 (−44.26 to 7.78), 5	−3.49 (−21.23 to 14.25), 9	14.75 (−20.87 to 50.37)	0.360

a   ANCOVA-adjusted.

TABLE 16 Quantification of lipid-laden macrophages in bronchial biopsies[a]

	Placebo		Active treatment	
Lipid-stained macrophages	Pretreatment	Post-treatment	Pretreatment	Post-treatment
0	3	3	9	6
1–6	2	2	1	6
7–14			2	
≥ 15	1		1	

a Bronchial biopsies were collected before the first dose (pretreatment) and 28 ± 5 days after the last dose (post-treatment). Lipid-laden macrophages were quantified in 38 out of the 50 possible biopsies (76%). The remaining 12 biopsies were not analysable because of poor sample quality ($n = 7$) or because the patients withdrew from the trial ($n = 5$). Tissue sections were scored semiquantitatively as containing 0, 1–6, 7–14 or ≥ 15 lipid-laden macrophages per section.

TABLE 17 Quantification of anti-dsDNA antibodies

	Placebo		Active	
Result	Pretreatment	Post-treatment	Pretreatment	Post-treatment
Nuclei anti-nuclear antibody[a]				
Negative	45	43	51	45
+ Diffuse				1
++ Diffuse		1		1
+++ Diffuse				
+ Speckled	5	7	5	7
++ Speckled	1	1	4	3
+++ Speckled				
No result	3	2	2	5
Nucleoli anti-nuclear antibody[b]				
Negative	50	50	60	56
+ Nucleolar	1	2		1
++ Nucleolar				
+++ Nucleolar				
+ Centromere				
++ Centromere				
+++ Centromere				
No result	3	2	2	5

Anti-double-stranded (ds) DNA antibodies were quantified in serum before the first dose (pretreatment) and 4 weeks after the last dose (post-treatment). Samples were classified as positive or negative.
a Quantification of anti-dsDNA antibodies in nuclei.
b Quantification of anti-dsDNA antibodies in nucleoli.

TABLE 18 Quantification of CFTR-specific T cells in the PP population[a]

| | PP population | | | |
| | Placebo (*n* = 54) | | Active (*n* = 62) | |
CFTR-specific T cells	Pre dose	Follow-up	Pre dose	Follow-up
Negative	33	36	37	49
Positive	4	8	1	2
Not determined	17	10	24	11
T-cell conversion		1[b]	1[c]	

a PBMCs were extracted before the first dose (pre-dose) and 2 or 4 weeks after the last dose (follow-up) and a human interferon-γ enzyme-linked immunospot assay was carried out. Samples were classified as positive or negative for the presence of CFTR-specific T cells. Where recovery of viable PBMCs was too low to perform analysis, samples were classified as 'Not determined'.
b Indicates a placebo subject negative for CFTR-specific T cells before dosing and T cell converted to positive after the last dose.
c indicates an active-treatment subject positive for CFTR-specific T cells before dosing and T-cell converted to negative after the last dose.
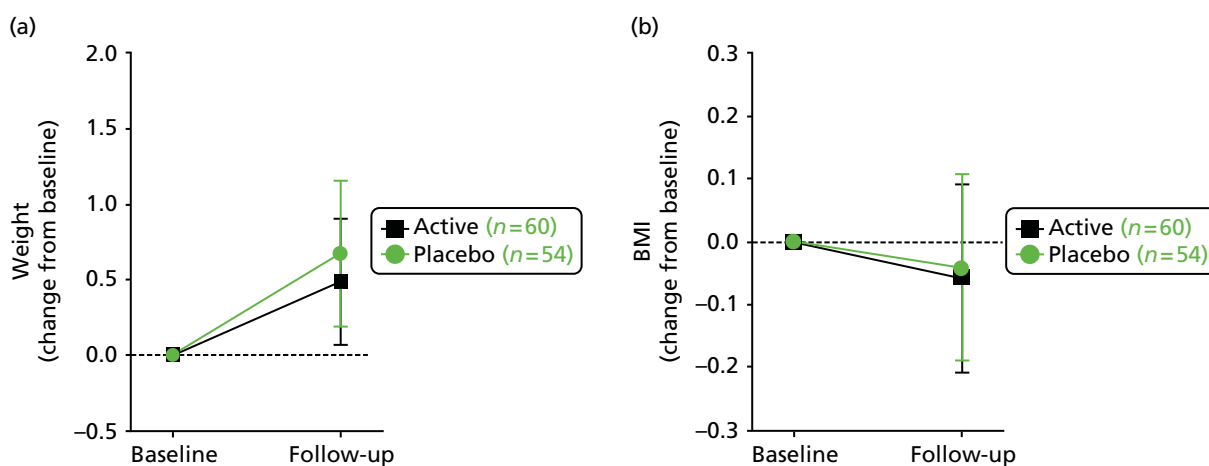
FIGURE 37 Change from baseline: (a) weight; and (b) BMI.

Chapter 6 Discussion and conclusions

In this, the first trial of non-viral-based gene therapy for CF powered to detect clinically relevant changes, the primary outcome was met. Following 12 monthly nebulised doses of pGM169/G67A, there was a significant but modest (3.7%, 95% CI 0.1% to 7.3%; $p < 0.05$) TE in FEV_1 favouring active treatment over placebo. Changes in this outcome were supported by trends in all the secondary outcomes, reaching statistical significance for FVC and gas trapping on CT scan. Underscoring the mechanism of action, there was a significant difference in lower airway CFTR-mediated chloride secretion assessed by PD. No clinically important AEs attributable to the active treatment were seen.

Although these findings are encouraging, it is important to place them in perspective. The active-treatment group demonstrated stabilisation of FEV_1 rather than an improvement, while the placebo group declined. The reduction in FEV_1 in the placebo group was within the range seen in other trials, but greater than has been reported from registry data.[21] We consider that there are two factors which likely explain this. First, there was a requirement for patients to (a) be clinically stable and (b) have a $FEV_1 > 50\%$ predicted at screening. The enthusiasm of patients to pass screening may have led to their being in optimal health at that time and, thereafter, regressing to the mean. Second, registry data are most commonly obtained from annual assessment visits, when patients are well; in our clinic, as in most of the UK, annual assessments are deferred if patients are unwell or experiencing an exacerbation. In this trial, we captured data from all patients irrespective of whether they were stable or exacerbating; this could lead to an apparent steeper rate of decline in the placebo group than has been published from registry data sets.

We acknowledge the relatively modest TE in the primary outcome, but would caution against the bar being set too high for novel drugs in CF, based on comparison with the large response in FEV_1 to ivacaftor in patients with class 3 mutations. Correction of misfolded CFTR is recognised to be a much more difficult task, as suggested by the recent ivacaftor/lumacaftor trials, with FEV_1 outcomes similar to those reported here. Disease stabilisation would be a worthy addition to the CF armoury, particularly if this could be extended to disease prevention if treatment was started early on.

The response observed was heterogeneous, with obvious responders and non-responders. Encouragingly, the data suggest that an approximate doubling of TE (6.4%) could be achieved in the more severe half of patients stratified by FEV_1 and reproduced across a wide range of clinically relevant assays. We consider the simplest hypothesis to relate to deposition: in patients with lower baseline FEV_1 the relatively more obstructed smaller airways will lead to a larger proportion of the administered dose being deposited proximally, in the larger airways. In preparation for this trial we assessed deposition in CF patients of varying FEV_1 severity using technetium-99m-labelled human serum albumin delivered via a different nebuliser system, which has a similar droplet size (3–4 μm) to the pGM169/GL67A formulation. Of the delivered dose deposited in airway generations 2–8, 2.9% (SEM 0.2%) was in those with 70–90% predicted FEV_1 ($n = 33$), whereas approximately twice as much (6.0%, SEM 1.0%) was delivered to this region in patients with 50–70% predicted FEV_1 ($n = 23$). These data are in keeping with the approximately twofold increase in efficacy we noted in the respective severity groups.

There may also be an influence of the increased mitotic rate of cells in more severely inflamed tissues,[97] thereby decreasing the proportion of time that the nuclear membrane is intact, the latter acting as a barrier to pDNA entry to the nucleus. It will be important to verify or refute these data in a larger trial with a stratified trial entry design, powered to assess subgroups and that addresses the mechanisms of response heterogeneity.

The change in lower airway PD supports our clinical outcomes and suggests that the changes observed relate to CFTR expression. However, we have also considered the possibility that such clinical changes could be the result of a non-specific response to the active-treatment formulation. This is difficult to rule out for two reasons. First, the placebo was 0.9% saline rather than the ideal comparator of a scrambled CFTR plasmid/liposome complex. Saline was chosen in part based on ethical considerations (not wishing to expose well-controlled CF patients to first-in-man repeated pulmonary dosing of an untested product as a placebo), in part on pragmatic financial consideration, and finally a wish to compare therapy against the natural history of the disease. We know of no evidence suggesting that monthly nebulisation of 0.9% saline is deleterious to lung function, liposome alone has shown no evidence for producing physiological improvements in either non-CF (Chadwick *et al.*[62]) or CF subjects (Alton *et al.*[58]), and pDNA is generally associated with deleterious rather than non-specific beneficial effects. Furthermore, it was neither noted evidence for pathophysiological changes in the airways, such as inflammation, remodelling or lipid accumulation, nor changes in bacterial species, that might explain the changes. Nevertheless, it cannot be excluded that DNA–liposome complexes induce a previously undocumented augmentation of host defences, stimulate mucus clearance by non-specific mechanisms or produce bacterial killing not detected on semiquantitative routine culture. Second, it would have been reassuring additional evidence to demonstrate more robust evidence for molecular CFTR surrogates. We have repeatedly noted the limited sensitivity of assays assessing vector-specific mRNA from human samples *in vivo*.[55,58] The ratio of area sampled to area dosed, is extremely small, and in ovine studies we have shown that a 20-ml nebulised dose, fourfold greater than that used in the current study, represents the lower threshold for reproducible detection with this assay in airway tissue samples.[98] We have consistently noted that electrophysiological changes are more readily detectable than mRNA, supported by findings in this study. Although we saw significant chloride secretory changes in the bronchial but not in the nasal epithelium, we caution against placing undue weight on either observation. The mechanistic subgroups were opportunistically recruited to, and were not powered to, detect significant changes at the level of correction observed. Rather we would conclude that modest, inconsistent changes can be demonstrated in assays that remain suboptimal to detect low levels of CFTR function when assessed in vivo in man; improvements in these, or other assays, are needed.

Although we are encouraged by the first demonstration of a significant change in lung function associated with gene therapy in CF patients, the improvement was modest and at the lower end of the spectrum seen in clinical trials resulting in changes in patient-related care. Although we did not formally assess infective exacerbations given the relatively small numbers in our study, using antibiotic courses as a surrogate, we saw no obvious changes. It is unlikely, therefore, that, without further improvement in efficacy and response consistency, the current formulation will be suitable for immediate clinical care given the already high treatment cost in CF. Rather, we believe that a follow-on study should examine dose escalation, frequency of dosing, and the potentially attractive combination of gene therapy with a CFTR potentiator. Furthermore, our current study should encourage the introduction of more potent viral vectors into early-phase trials. We suggest the data reported here provide proof of concept that repeated administration of *CFTR* gene therapy can alter clinically relevant parameters, providing another step along the path of translational CF gene therapy.

Implications for practice

Although encouraging proof of principle that *CFTR* gene therapy can lead to changes in a clinically relevant outcome, the modest effect of these to date means that further work is necessary before this becomes a clinical therapy.

Recommendations for areas requiring future research

- Seeking further understanding of responders/non-responders.
- Seeking to maximise benefit by:

  - increasing dose
  - increasing dosing frequency
  - co-administration of a potentiator drug

- The development of safe and efficacious viral vectors.

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David K Armstrong, **Katie J Bayfield**, **Diana Bilton**, **Paula Carvelli**, **Gwyneth Davies**, **Maria H Dewar**, **Natalie S Dwyer**, **Hala I Elgmati**, **Rosanna F Featherstone**, **Jemyr Gavino**, **James SR Gibson**, **David M Hansell**, **Katharine Harman**, **Samantha L Hodges**, **Joseph Jacob**, **Brian F Keogh**, **Michael McGovern**, **Emma K Punch**, **Alexandra L Quittner**, **Clare J Saunders**, **Sarah Sheard**, **Nicholas J Simmonds**, **Stephen N Smith**, **Najwa Soussi**, **Emma J Spearing**, **Rosa P Ureta** and **Michael D Waller** assessed patient outcomes and/or undertook and analysed individual in vivo assays.

Deborah Ashby and **Gordon D Murray** designed and co-ordinated data collection and statistical analysis.

Emily V Bloomfield, MD, and **Samia Soussi** co-ordinated and undertook the administration of the trial.

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Publications

Alton EW, Boyd AC, Cheng SH, Cunningham S, Davies JC, Gill DR, *et al.* A randomised, double-blind, placebo-controlled phase IIB clinical trial of repeated application of gene therapy in patients with cystic fibrosis. *Thorax* 2013;**68**:1075–7.

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Data sharing statement

Data are available from Professor Eric Alton.

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Appendix 1 Patient information sheets

Adult patient information sheet

ADULT PATIENT INFORMATION SHEET

Study title: A randomised, double-blind, placebo-controlled Phase 2B clinical trial of repeated application of gene therapy in patients with cystic fibrosis

Short title: Repeated application of gene therapy in CF patients

Invitation paragraph

You are being invited to take part in a research study. Before you decide you need to understand why the research is being done and what it would involve for you. Please take time to read the following information carefully. Talk to others about the study if you wish.

Please ask us if there is anything that is not clear or if you would like more information. Please take as much time as you need to decide whether or not you wish to take part.

Introduction

There is currently no treatment for Cystic Fibrosis (CF) that completely halts the progression of lung damage; all the available therapies help to slow the rate of decline in lung function at best. Because of this, we have formed the UK CF Gene Therapy Consortium, (scientists, doctors & nurses based at Imperial College/ Royal Brompton Hospital, Oxford & Edinburgh Universities & Hospitals, www.**cfgenetherapy**.org.**uk**/) to develop further gene therapy for CF. Gene therapy uses healthy copies of the *CFTR* gene to replace the damaged versions within the nose and lungs of patients with CF. Each of these three centres has previously been involved in clinical gene therapy trials which were designed simply to prove that we could deliver healthy genes in this way. Together, we have designed a programme of research to lead to a large, multi-dose clinical trial. This will be the first trial to give multiple, repeated doses and look for clinical benefit. The gene therapy will consist of the healthy *CFTR* gene, which will be carried into the cells of the airways by a liposome (a fatty substance), called GL67A. This is the same liposome which we used in clinical trials in the 1990s and we, therefore, have results on its safety and efficacy. The gene, however, has been substantially improved and has recently been used in a pilot clinical trial to ensure its safety and to see how long each dose lasts. The gene therapy can cause a brief change in lung function and mild feverish symptoms. We have therefore decided to use a dose of 5mls (a

teaspoonful, delivered by inhalation of a nebulised aerosol) which was found to be the best tolerated in the pilot trial.

What is the purpose of the study?

The purpose of this study is to assess for the first time whether repeated doses of gene therapy administered to the lungs of CF patients can lead to clinical improvement.

Why have I been invited?

You have been invited because you are an adult with CF and you attend the Royal Brompton Hospital, one of the hospitals within the Scottish Adult Cystic Fibrosis Service, or one of our collaborating patient identification centers. You are also relatively well, with mild or moderate lung involvement, however not so well that we will struggle to measure any improvement. This will mean that the nebulised gene therapy has a good chance of reaching the cells lining your airways without being blocked by excessive mucus, and that we will sample from your airways more easily. If you are a patient from one of our collaborating participant identification centres you will attend the Royal Brompton Hospital, Western General Hospital Edinburgh or Royal Hospital for Sick Children Edinburgh for all trial related visits but continue your clinical care at your own centre. Trial visits are not a substitute for your usual clinic visits and we will not make decisions related to your general care, although we will communicate closely with your local team.

If you have been involved in the Run-In study you will be invited for a single screening visit to determine whether you will fit the criteria to participate in the multi-dose trial. If you have been newly recruited, i.e. not participated in the Run-In trial, you will be invited for an introductory visit to talk to you further about the research and assess your eligibility as well as a screening visit.

We think that you may fulfill all of the Inclusion & Exclusion criteria although we will need to go through these lists in detail with you; in particular we will be looking for lung function within the range of 50-90% and for you to be in a stable condition with no recent changes to your CF treatments. If your recent lung function is close to the cut-offs for being included you may wish to perform spirometry to see if you are eligible before discussing all the details of the trial with us, as this is quite time consuming. In this case, we will ask you simply to sign a form consenting to lung function, which in no way commits you to taking part in the trial. If you are unwell at the time you are seen, we may be able to reschedule your visit for once you have recovered and are once again stable.

Do I have to take part?

It is completely up to you to decide whether or not to take part. If you do decide to take part you will be given this information sheet to keep and be asked to sign a consent form. If you decide to take part you are still free to withdraw at any time and without giving a reason. This will not affect the standard of care you receive.

What will happen to me if I take part?

After the screening visit, all patients volunteering for the main part of this study will receive nebulised doses of either the gene therapy (active) product or placebo (sometimes called the 'dummy drug') at 4 week intervals over a 48 week period. Once you have agreed to take part in the study, you will be assigned by chance (like the flip of a coin, but done by a computer) to receive either gene therapy or placebo. A placebo contains no active ingredient. The probability that you will receive gene therapy is 1 in 2 i.e. there is a 50% chance that you will receive the gene therapy throughout the trial and a 50% chance that you will receive the placebo throughout the trial. Neither you nor your study doctor will know whether you are receiving gene therapy or placebo. This is termed a 'double-blind' study and is extremely important for generating results that are meaningful and which will be believed by the wider scientific community. However, in the event of an emergency, the identity of the nebuliser solution can be determined rapidly.

The first 20 patients (10 active treatment; 10 placebo) will receive a nebulised dose (5ml) on 3 occasions at 4-weekly intervals before any further patients are dosed. This is a safety measure to allow the Data Monitoring and Ethics Committee (DMEC, a panel of medical experts) to review the clinical and laboratory findings to ensure that everything is satisfactory after repeated doses of the active treatment/placebo. If this proves to be the case these patients will continue with subsequent visits and the remaining patients will begin their dosing schedule. Because of the large number of patients (130) involved in the study and the restriction on the facilities (special gene therapy cubicles) used we will have to stagger the dosing visits so it is likely to take at least 5-6 months before all patients have started their dosing schedule.

Were the DMEC to have any concerns with the clinical and laboratory findings, they could recommend that we reduce the dose of the gene therapy product. In this event, and if you belonged to the initial 20 patient group, you would unfortunately have to withdraw from the trial. A further group of 20 patients would then be recruited to receive the reduced dose (2.5ml) of active treatment/placebo on 3 occasions at 4-weekly intervals. The DMEC would again meet to review the clinical and laboratory findings to ensure that everything is

satisfactory. If this proves to be the case these patients will continue with subsequent visits and the remaining patients will begin their dosing schedule. If the DMEC were to have any concerns with the 2.5ml dose the trial would be halted as we do not consider it feasible to administer a smaller dose successfully to the lower airways via nebuliser.

A subgroup (at least 24 patients at the London site) will be asked to undergo nasal dosing. This will involve some additional tests and up to 5 additional short study visits (although we will limit these as much as possible). *This will be explained in detail on another sheet if you wish to consider this (see Appendix 1).*

A subgroup (at least 24 patients at the London site) will be asked to undergo a flexible bronchoscopy (a test to look inside the lungs using a flexible telescope) under general anaesthetic on two occasions (prior to the first dose of gene therapy/placebo and between 29 and 35 days after the 12th dose of gene therapy/placebo). This will involve two additional study visits. *This will be explained in detail on another sheet if you wish to consider this (see Appendix 2).*

You will be monitored before and after each nebulised dose (dosing visit) during 12 scheduled dosing visits. The schedule of visits is 4 weeks +/- 5 days for 48 weeks with further details given below. You will be given a flow chart summarising the sequence of study visits:

1. Introductory visit –new patients only (i.e. those not previously involved in Run-In study) (3-4 hours). This is to help us get to know you and for you to familiarise yourself with the tests before the trial starts. However, it is not essential and, particularly if you have a long distance to travel or other time commitments, we can discuss omitting this visit with you.

2. Eligibility and Consent visit (selected subgroup patients only- see below)* (2-3 hours)

3. Screening visit – all patients (3-4 hours)

4. Pre-dosing nasal PD visits up to 3 (nasal subgroup only)

5. Pre-dosing bronchoscopy (bronchoscopy subgroup only)

6. Dosing visits x 12 (4-6 hours)

7. Post-dosing day 2 visits (1st 3 doses in 1st 20 patients only) (2 hours)

8. Follow up visits on Days 14 and 28 (+/-2days) post-dosing (3-4 hours)

9. Post-dosing bronchoscopy Days 27-36 after dose 12 (bronchoscopy subgroup only)

*As the screening visit must occur within 28 days before the first dosing visit, we will offer people taking part in the nasal or bronchoscopy subgroups the opportunity to plan the additional investigation visits earlier if convenient. This will require an initial, short visit, at

which we assess eligibility, perform a physical examination and limited tests and you will be asked to sign consent for the trial.

Should there be any safety concerns, we have the flexibility to see patients for additional visits at any time during the study; such visits will of course be kept to an absolute minimum.

The procedures performed at each of these visits are summarised below. If for any reason we are unable to perform a test at a certain visit (eg. if you miss a visit or equipment fails), the protocol allows us to consider performing that test at a future visit. A full description of each test follows this summary of visits.

PRE-DOSING VISITS

Introductory visit – if considered necessary for new patients only (i.e. those not previously involved in Run-In study). This visit will take around 3-4 hours and will involve:

- explanation of study
- full medical history
- confirmation that you are medically suitable for the trial
- sign informed consent
- a simple (bedside) examination of your chest, blood pressure, temperature, pulse, and oxygen levels (with a finger probe)
- blood sampling
- urine sampling
- Sputum sample (induction with 7% hypertonic saline if required)
- spirometry (lung function tests, as usually done in clinic)
- lung clearance index (LCI)
- 24 hour sputum weight
- nasal potential difference measurements may be conducted (nasal PD subgroup only)
- we will give you a diary card to take home and instructions on how to complete it
- we will give you a hand-held PiKo-6 device for you to carry out blowing tests (lung function) at home
-

Screening visit (all patients)

This visit will take around 3-4 hours and will involve:

- explanation of the study
- confirmation that you are medically suitable for the trial
- sign informed consent (if not already signed at introductory visit)

- quality of life questionnaire

- full medical history and physical examination

- a simple (bedside) examination of your chest, blood pressure, temperature, pulse, and oxygen levels (with a finger probe)

- blood sampling

- urine sampling

- spirometry (lung function tests, as usually done in clinic)

- lung clearance index (LCI)

- exercise bike test

- body worn activity monitor (which we will show you how to use)

- chest CT scan

- gas transfer test

- Sputum sample (induction with 7% hypertonic saline if required)

- 24 hour sputum weight

- nasal potential difference measurements may be conducted (nasal PD subgroup only)

- we will give you a diary card to take home and instructions on how to complete it

- we will give you a hand-held PiKo-6 device for you to carry out blowing tests (lung function) at home

DOSING VISITS

Each of the 12 dosing visits will take around 4-6 hours. If you normally take Pulmozyme, you will be asked to withhold treatment for 24 hours prior to each visit and for 24 hours after dosing.

Predosing, the following will be performed at every visit:

- history (we will ask you how well you have been since the last visit and if any of your medicines have been changed)

- a simple (bedside) examination of your chest, blood pressure, temperature, pulse, and oxygen levels (with a finger probe)

- blood sampling

- spirometry (lung function tests, as usually done in clinic)

At specified visits, the following will also be performed

- lung clearance index (LCI)

- urine sampling

- quality of life questionnaire

- exercise bike test

- body worn activity monitor

- chest CT scan

- gas transfer

- 24 hour sputum weight and collection of fresh sample

- nasal PD (subgroup only)

- bronchial blood flow measurement

Dosing

Approximately 20 minutes before the nebulised dose is administered, you will receive 200-400 µg (2-4 puffs) of inhaled salbutamol to prevent airway narrowing and wheeze, which may be associated with the gene therapy agent / placebo. The nebulised dose will then be given via a mouthpiece from a clinical nebuliser. You will be asked to wear a nose clip for the duration of the   nebulisation (3 minutes on, 2 minutes off for 8 cycles) which usually takes around 40 minutes.. The nebuliser will be administered whilst you are sitting in a cubicle to prevent contamination of the immediate environment. You will have contact throughout and will be able to communicate with your research nurse through a glass window. You will be asked to report any new symptoms. Although this environment is not spacious, we will do our best to make you as comfortable as possible. We can provide music or a DVD player should you wish. You will be able to leave the cubicle as required for a toilet break, although we will ask that you wear a mask over your mouth and nose during this time and for 30 minutes after dosing is complete.

If you agree to take part in the nasal dosing subgroup (London site only), we will ask you to administer a nasal spray during the 'off' nebuliser period, with one spray being delivered to each nostril at the beginning and end of the first 6 two minute'off' periods which will deliver a dose of approximately 2 ml.

You will be observed for a minimum of 30 minutes after dosing before leaving the hospital. We will listen to your chest and perform pulse oximetry after the dosing and prior to you leaving the hospital. . If either you or we have concerns about your level of well-being after treatment, we may suggest that you stay in hospital overnight, although we are not expecting this to be the case. We will ask you to take a standard 1g dose of paracetamol immediately after dosing and you will be given another 1g dose of paracetamol to take at home approximately 6 hours after dosing. This is to prevent any mild feverish symptoms that could possibly occur after dosing.

Day 2 post-dosing visits (first 20 patients after each of the first three doses only)

The following will be performed:

- history (we will ask you how you have been since your last visit and if any of your medicines have been changed)
- a simple (bedside) examination of your chest, blood pressure, temperature, pulse, and oxygen levels (with a finger probe)
- spirometry ((lung function tests, as usually done in clinic)
- gas transfer
- blood sampling
- bring in sputum coughed up over last 24 hours and collect fresh sample

Follow up visits

There will be two follow up (F/U) visits that will occur at 14 (+/-2) days and 28 (+/-2) days after your last dosing visit.

At F/U **day 14** we will do the following:

- history (we will ask you how well you have been since the last visit and if any of your medicines have been changed)
- a simple (bedside) examination of your chest, blood pressure, temperature, pulse, and oxygen levels (with a finger probe)
- quality of life questionnaire
- spirometry
- blood sampling
- lung clearance index (LCI)
- nasal PD (subgroup only)
- urine sampling
- bring in sputum coughed up over last 24 hours and collect fresh sample
- diary card check
- download hand-held PiKo-6 device on which you carried out blowing tests

At F/U **day 28** we will do the following:

- history (we will ask you how well you have been since the last visit and if any of your medicines have been changed)
- all the tests performed at the screening visit
- collection of diary card and hand-held PiKo-6 device

Long term follow up: you will be followed up by your clinical team at scheduled CF clinic appointments approximately every 3 months for 2 years.

Description of Tests

- *Full medical history:* we will ask about your medical condition(s) including history of medications (specifically any additional antibiotics required for your chest), operations and allergies plus anything else relevant to the safety of the trial.

- *Clinical examination*: we will listen to your lungs using a stethoscope and measure your height, weight, temperature, pulse, blood pressure and respiratory rate

- *Pulse oximetry (finger probe):* a measurement of the oxygen level in your blood using a finger probe

- *Blood test:* (at each visit where blood is required, up to 25 mls, (5 teaspoons), will be taken for a variety of tests including routine clinical tests e.g. liver and kidney function, full blood count, electrolyte (salt) levels and C-reactive protein (a marker of inflammation). Samples will be stored for future measurement of inflammatory markers and possibly for end-products of gene therapy breakdown. If you wish, we will arrange for you to have local anaesthetic, either cream or spray. If you have a portacath or similar device and your clinical team is happy for this to be used for blood sampling, this may be possible as an alternative. At one single visit at any time during the trial, we will take a sample of blood for DNA (genetic) research. The only tests we will perform on this sample will be:

  - *Testing for your CF gene(s) if these are not known; we will be happy to let you know the results of this test.* **This sample will not be used to test for any other genetic diseases**

  - *Testing for common, naturally-occurring changes in genes which don't cause disease but might be involved in how individuals respond to gene therapy, eg, inflammatory response genes. This will be most useful in the event that we see that some people respond to gene therapy and others do not, to help us understand this better.*

- *Urine sample*: routine testing with a dipstick for protein, sugar etc; we will also perform a pregnancy test if you are female.Samples will be stored for future measurement of inflammatory proteins and possibly for end-products of gene therapy breakdown

- *Spirometry* (blowing tests, as usually done in clinic).

- *Lung clearance index (LCI)*: this test demonstrates how evenly gas is distributed in the lungs and is a sensitive marker of airway disease. You will be asked to breathe a

tracer gas (SF6) which has no smell or taste and is completely harmless, over a period of several minutes. Once levels in your lungs are stable, you will switch to breathing air and the time taken for the SF6 to leave the lungs is used to calculate the LCI. We will perform the test 3 times, each one taking about 10 minutes.

- *Exercise bike test:* this test will be performed on an exercise bike and is incremental (gets more difficult to pedal as it goes on). Exercise is an event that involves a large number of physiological processes from the heart and lungs, to the blood circulation and the muscle itself. This test will enable us to calculate your exercise capacity. During the test we will record a breathlessness score and a probe will be attached to your finger or ear lobe to measure the oxygen levels in your blood. You will be wearing a nose clip and breathing through a mouthpiece. You will **be** asked to pedal at a comfortable speed and maintain this speed throughout the test. The resistance to pedalling will automatically increase each minute and you will be encouraged to continue until you either cannot keep up with the speed or feel you have exercised as much as you are able; we will ask you to stop if your oxygen level falls. Once the test is complete you will have a 2 minute cool down period, and we will continue to monitor your oxygen levels until they return to baseline.

- *Sweat test:* Although the diagnosis of CF is not in doubt, some patients, particularly those who were diagnosed many years ago, do not have documented confirmation of a diagnostic test in their notes. If you have neither a confirmed genetic diagnosis (2 mutations) nor a sweat test result, we would like to perform a sweat test as part of the trial. An area of the skin on your forearm will be cleaned and 2 electrodes will be attached with straps. One of these contains a gel which stimulates sweat and the medication is applied to the skin by a weak current; you will feel a little tingling but this is not painful. Following this a collection device will be attached to the skin surface and the sweat collected over a 30 minute period. This will then be analysed by the laboratory for the high levels of salt seen in CF.

- *Activity monitor:* a small band worn on your upper arm that collects step and movement data. You will be asked to wear this for 7 full days after specific study visits.

- *Chest computed tomographic (CT) scan:* Computed tomography uses x-rays to create detailed images of the lungs. It is a good measure of how CF affects the shape and structure of the airways. For each scan you will be asked to lie still on a table which will move slowly through the centre of a large x-ray machine but at no time will you be in an enclosed space. You will be able to communicate with the CT staff if you need to. You will be given breathing instructions at the time of the scan. No

injections are involved and the procedure is completely painless. As x-rays are involved, you will be exposed to a small amount of radiation. However, we will be using as low a dose as is possible for your scans. The estimated maximum dose of all three scans over the entire study period amounts to 4.5 mSV, which is similar to the radiation you would be exposed to as part of natural background radiation over a 2 year period. Because even low levels of radiation can be dangerous to an unborn child, it is a requirement that all females undergo a urine pregnancy test on the day of the procedure.

- *Transfer factor (TLCO)*: this is a lung function test which measures the rate at which gas travels across the lung tissue (alveolus) into the blood stream. Gas transfer is usually normal in patients with CF. We are using this test as a safety measure and in order to assess whether the gene transfer has an effect on this, we need to make a baseline measurement. You will be asked to wear a nose-clip and to breathe out as far as you can through a mouth-piece. You will then breathe in as far as possible, a mixture of air and low concentration of a tracer gas (less than 0.3% carbon monoxide) and hold your breath for 10 seconds before breathing out as far as possible. This will be repeated up to 5 times with rest periods in between tests. This concentration of CO is completely harmless and this is a well-established test used routinely in clinical practice.

- *Sputum sampling:* we will ask you to try and cough up around a teaspoon of sputum; if this is not possible for you, we will obtain secretions for microbiology by asking you to cough onto a sterile swab or brush . At either your introductory or screening visit and at either of your follow up day 14 or day 28 visits if you are unable to cough up a sputum sample, we will use a well-established nebulisation technique to induce sputum. To prevent wheeze (a common side effect), you will be given 200 mcg of salbutamol (Ventolin) or an equivalent drug to open up the airways. After 15 minutes you will receive a 7% saline nebuliser for 5 minutes, and this will be repeated up to 3 times. We will monitor your lung function (FEV_1) throughout, and should we see a significant drop, the test will be stopped. If sputum induction fails, we will obtain secretions for microbiology by asking you to cough onto a sterile swab or brush Samples will be tested for infection and stored for future measurement of inflammatory proteins and possibly for end-products of gene therapy breakdown.

- *CF specific quality of life questionnaire:* this will take a total of 10 minutes to complete.

- *Diary card:* You will be given a diary card which you will be asked to complete for the duration of the study. This will record new symptoms, changes in routine treatments etc.

- *Home lung function:* You will be taught how to use a small lung function device (PiKo-6) and asked to make regular recordings on it at home for the duration of the study period. The machine stores all the readings which will be downloaded onto a computer at each visit.

- *Bronchial blood flow measurement:* (London site only) This test is designed to look at the blood flow to the airways which is often increased in patients with airway disease, probably reflecting inflammation. The test takes about 45 minutes to do and consists of 10 breath-holding manoeuvres of either 8 or 16 seconds. For each manoeuvre you will be seated, wearing a noseclip and breathing in and out through a mouthpiece. After several normal breaths you will need to breathe in a small amount of test gas, which is safe and enriched with oxygen, and hold this breath for 8 or 16 seconds before breathing out again slowly. Your heart rate and oxygen saturation will also be monitored during the test. There is a gap (3-4 min) between each of the manoeuvres to save the data and calibrate the equipment. As the blood flow to the airways may be affected by alcohol and caffeine, it is requested that you do not consume alcohol the night before the test and do not have any caffeine on the day of the test. This test will be undertaken after all other assays / tests if time allows. If you are too tired to do the procedure it will not be done or if during the procedure you feel too tired to continue we will stop the test at any time.

- *Nasal PD and nasal brushings: see Appendix 1* (London site only)

- *Bronchoscopy: see Appendix 2* (London site only)

What else will I have to do?

In addition to the study visits and home monitoring outlined above, we will ask that if you are sexually active, you agree to take contraceptive precautions from enrolment into the study until 3 months after completion. This is a requirement of the Medicines and Healthcare products Regulations Agency (MHRA) and the Gene Therapy Advisory Committee (GTAC) for all clinical trials involving gene therapy. Approved (reliable) methods of contraception include:

- 'the pill'

- long-acting injections or implants

- placement of an intrauterine device (IUD; sometimes called a 'coil') or system (IUS)

- condom or occlusive cap with spermicide

Exceptions to this can be made in the case of:

- male trial participants with CF-related infertility, which has been confirmed on semen testing

- male trial participants who have undergone a vasectomy followed by confirmation of success

- female trial participants whose only male sexual partner has undergone a vasectomy followed by confirmation of success

If you do not already fall into one of these groups, we will ask you to attend your General Practitioner of local Family Planning Clinic to discuss options. You may also find it useful to discuss these issues with your CF Consultant and / or Nurse Specialist who will provide you with information relevant to patients with CF.

There will be no other changes made to your routine clinical care.

We ask that you consent to our informing your General Practitioner about your involvement in the study, including these requirements for contraception.

What is the drug or procedure that is being tested?

We are testing a formulation consisting of a healthy copy of the *CFTR* gene mixed with a fatty substance which helps the gene enter the cells of the airway. The *CFTR* gene has been changed since our original trial in ways that we believed would make it likely to cause less inflammation and last for longer. We did see some flu-like responses and drops in lung function in some people in the Pilot study but by giving a smaller dose (5ml) we were able to reduce these side effects. The research team will be happy to provide you with more specific scientific details should you wish.

What are the alternatives for diagnosis or treatment?

As you will know, conventional treatments for CF have improved greatly over the last few decades. However, they do not correct the basic defect in your cells, which is what we are aiming to do with gene therapy. In general therefore, conventional treatments slow the natural progression of lung disease, rather than stop it altogether. Should you wish not to take part, your standard clinical care will continue as usual.

What are the possible disadvantages and risks of taking part?

We are asking for a large time commitment from you over a period of a year. The study requires you to make a minimum of 15 visits and perform multiple tests. As described above, most of these are straightforward and many of them will be familiar to you from clinics (and, for many of you, your participation in the Run-in).

The risks that we consider likely are as follows:

1. Either the gene therapy or the placebo could cause wheeze or increased cough. To limit this, we will administer salbutamol, to relax the airways, prior to nebulisation. In our previous study, some of the single dose patients had a fall in their lung function several hours after dosing but this was well-tolerated and, for most patients receiving 5 ml, was not associated with any increased symptoms. Lung function had resolved in all dosing groups within 2 days of the dosing visit.

2. In the single dose study, some patients developed a temperature and a mild flu-like illness within a few hours of dosing. These side effects were minimal with the 5 ml dose that we have chosen for this trial and appear to be responsive to paracetamol, which we will ask you to take on two occasions (2hrs and 8 hrs) post-dose whether you have side effects or not. All side effects should have disappeared within 2 days of the dosing visit.

3. The CT scans involve exposure to a small amount of radiation, which in common with all radiation does carry a small risk of causing cancer. We plan to do a type of CT scan using lower radiation doses than a standard CT. The estimated maximum dose of all three scans over the entire study period amounts to around 4.5 mSv. To put this in context, the maximum amount of radiation from all three scans over the entire study period is equivalent to the amount of natural radiation to which everyone is exposed from environmental sources over a period of 2 years. Female patients of a child-bearing age will be required to have a negative pregnancy test on the day of each scan.

4. Please see Appendices 1 and 2 for disadvantages associated with nasal PD and bronchoscopy. (These tests are only being performed at the London site.)

What are the possible benefits of taking part?

Because we are the first research group in the world to administer multiple doses of this gene therapy product, and CF is such a chronic disease, we cannot be sure that there will be any immediate or long-term benefits of participating in the study. You will be helping to advance the field of gene therapy by agreeing to take part. Should this study be successful, we will undertake further trials with a view to developing gene therapy as a treatment.

What happens when the research study stops?

When you have completed your study visits, you will continue to receive your routine clinical care. If you wish, we will keep you informed of any developments that arise from the results of this study.

What if there is a problem?

Throughout the study, we will be happy to talk to you or see you at any point should you have concerns. Should you develop any health problems, we will investigate and treat these after discussion with your usual medical team.

An independent Data Monitoring and Ethics Committee (DMEC) will oversee the safety of the trial as it progresses. Any significant adverse events will be reported to the DMEC.

Complaints : If you have a concern about any aspect of the study, you should ask to speak to a member of your clinical research team (see contact numbers at the end of this Information Sheet) who will do their best to answer your questions. If you remain unhappy and wish to complain about any aspect of the way you have been approached or treated during the course of this study, the normal National Health Service complaints mechanisms are available to you. Details can be obtained from the hospital.

Harm: In the event that something does go wrong and you are harmed during the research due to someone's negligence, then you may have grounds for legal action or compensation, but you may have to pay your legal costs. The normal National Health Service complaints mechanisms will still be available to you (if appropriate).

Imperial College London holds Public Liability ("negligent harm") and Clinical Trial ("non-negligent harm") insurance policies which apply to this trial. Liability for the gene therapy product design will be accepted by Oxford University and Imperial College London. Liability for the protocol design will be accepted collectively by Oxford University, Edinburgh University and Imperial College London. Clinical negligence insurance / indemnity is provided by the NHS.

Will my taking part in the study be kept confidential?

Yes.

All information collected during the course of the study will be coded and kept strictly confidential. Clinical results and analysis will be collated by the Imperial College Trials Unit. All samples will be stored indefinitely in a coded fashion to preserve confidentiality. Codes will be held by a single investigator at each site. If you consent to take part in the research, any of your medical records may be inspected for purposes of analysing the results. They may also be looked at by people from regulatory authorities to check that the study is being carried out correctly. Your name, however, will not be disclosed outside the hospital.

If you agree to take part, your GP will be informed and your hospital notes will be flagged to ensure that other members of the clinical team are also informed.

What if relevant new information becomes available?

Sometimes during the course of a research project, new information becomes available about the test/treatment that is being studied. If this happens, your research doctor will tell you about it and discuss with you whether you want to continue in the study. If you decide to withdraw, your research doctor will make arrangements for your normal clinical care to continue. If you decide to continue in the study you will be asked to sign an updated consent form.

Also, on receiving new information your research doctor might consider it to be in your best interest to withdraw you from the study. He/she will explain the reasons and arrange for your normal clinical care to continue.

What will happen if I don't want to carry on with the study?

You will be free to discontinue the study at any time, without giving a reason, and this will not affect your clinical care in any way. For patients who have commenced dosing we would be grateful if you could attend at least a 14 and 28 day follow up appointment as part of the trial. Samples already obtained will be used for this and other ethically approved studies unless you specifically request samples to be destroyed. In addition we would like to discuss your progress with your clinical team after your routine clinic visits to gather as much information as possible for the trial and ensure continuing patient safety.

What will happen to any samples I give?

Samples will be stored in a coded fashion so that, with the exception of one or two key people, the researchers will not be able to identify individual patients. Many of the samples will be frozen and stored indefinitely. We are working closely with the other members of the UK Gene Therapy Consortium in Oxford, Edinburgh and Southampton Universities and will be sending coded samples to them for analysis and storage. We will also be sending coded blood samples to colleagues at the University of Pennsylvania, USA. We would wish that any spare samples left over could be used in other ethically-approved research protocols and would ask you to agree to this by gifting your samples to us as part of consenting to this study. All of our storage conditions comply with the regulations of the Human Tissue Act and/ or the Human Tissue Act (Scotland) 2006.

What will happen to the results of the research study?

At the end of the study, all results will be analysed, and will be reported at conferences and published in the Medical press. Results of the study will also be made available to study participants on request. You will not be identified in any report/publication.

Will my expenses for taking part in the study be reimbursed?

Yes. We understand the inconvenience involved in taking part in such a study and will be happy to reimburse travel expenses for the visits you will be asked to make.

Who is organising and funding the research?

The study is being funded by the National Institute for Health Research & Medical Research Council. There is no financial gain to either the Hospital or the staff for including and looking after patients in this study.

Who has reviewed this study?

This study has been submitted for review by the Gene Therapy Advisory Committee, the national body charged with assessing the ethical aspects of any clinical trial involving gene therapy.

Thank you for considering taking part in this study

We very much hope that after reading this information and talking to the research team, you feel able to take part, but please be assured that should you not wish to, this will in no way affect your clinical care.

For further information please contact:

Royal Brompton Hospital, London

Clare Saunders (Clinical Trial Co-ordinator) – Tel: ███████████

cfgenetherapy@rbht.nhs.uk

Prof Jane Davies - Tel: ████████

Prof Eric Alton - Tel: ██████████

Western General Hospital, Edinburgh

Dr J A Innes – Tel: ████████

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Maria Dewar (Research Manager) – Tel: ████████

Lothian.cfgtscotland@nhs.net

Appendix 1

Nasal dosing Subgroup (London site only)

A group of at least 24 patients in the trial will be asked to undergo nasal dosing and have nasal potential difference (nPD) measured and nasal brushings taken. Because the nostrils are relatively easy to access, and these measurements can therefore be made repeatedly, this offers us a valuable opportunity to study how well the gene is expressing at different time points during the study. This is optional and you do not have to take part in this additional testing if you do not wish to. If you do agree and are considered suitable, there will be a 2 in 3 chance that you will be randomized to receive the active gene therapy rather than placebo.

The section below explains what you will be asked to do in addition to the visits already described in the main Information Sheet

Additional pre-1st dose visits:

Eligibility and Consent (E&C) visit

In certain cases to ease scheduling, we will schedule the nPD tests several months ahead of your planned dosing dates. In this case, the E&C visit will take place, which will include:

- We will confirm that you are eligible to take part and ask you to sign the Consent Form
- We will take a full medical history and perform a standard (bedside) examination including heart rate, breathing rate, blood pressure, pulse oximetry, temperature, and listen to your chest
- blood sampling
- urine sampling
- Spirometry
- Nasal potential difference measurement may be performed at this visit or on separate occasion

Nasal PD visits

You will be asked to have 3 nPD measurements before receiving your first dose of gene therapy. A maximum of 2 measurements can be performed at a visit. These may be performed at E&C, Introductory or Screening visits, or may be scheduled separately to suit you. On a single occasion, either with or after your 3rd nPD, you will undergo a nasal brushing test.

Nasal Dosing: You will be taught how to use the nasal spray and will be asked to administer a nasal spray during the 'off' nebuliser period, with one spray being delivered to each nostril at the beginning and end of the first 6 two minute 'off' periods which will deliver a dose of approximately 2 ml.

***Nasal potential difference (nPD)*:** We will perform nasal PD on three occasions before the first dose (this is because we know the measurements are quite variable and want to be as certain as possible that any changes we see are due to the gene therapy and not just this variability), and after the sixth dose and at two time points after the twelfth doseWe may do additional nasal PDs post dose 3, 6 and 9 if opportunity arises and you are agreeable. This test is a sensitive way of measuring salts moving in and out of the cells lining the nose. The readings are different in patients with CF from those without and this test has been used clinically for many years in difficult diagnoses. As gene therapy is directed at correcting these cells, this test can help tell us how well the gene therapy is working. A small abrasion is made with a sterile device on the skin of the forearm; this is not painful. An electrode wire is attached to the abraded skin and a small volume of cream is applied and kept in place with a piece of tape. A soft catheter is inserted about 5-7 cm into one nostril until a stable reading is obtained. This will then be taped in place. A series of solutions is perfused into the nostril; you will be asked to lean forward so that drops fall into a bowl rather than go down the back of your throat. The response to these solutions is measured on a portable computer. The whole test takes approximately 20 minutes. We may wish to perform this on both nostrils (one after the other) depending on the results.

The more common side effects include:
- A slight salty taste from the solutions in your nose
- Sneezing or mild discomfort during insertion of the catheter
- Mild discomfort on your arm for a short while following skin abrasion

***Nasal brushing*:** We will obtain cells lining the nose by inserting a small, sterile brush into the nostrils, one at a time. The procedure is not particularly pleasant and makes the eye on that side water slightly. It is however very quick, each nostril taking only approximately 5 seconds. We will perform nasal brushing on one occasion at or after your third nasal PD visit and again at the end of the trial.

Rarely, patients are unhappy to have tests performed on their nose as they find this unpleasant; if you feel this way and are also in the bronchoscopy subgroup, we can include you in the nasal dosing group and perform only a single nPD and brushing pre and post-dosing whilst you are under anaesthetic.

Appendix 2

Bronchoscopy Subgroup (London site only)

A group of at least 24 patients in this trial will be asked to undergo two bronchoscopies, one before the first dose of gene therapy / placebo and the second between day 29 and 35. After the 12th dose of gene therapy / placebo. The purpose of this is to make measurements of the levels and function of the CFTR gene in the hope that we can learn how much gene we need to replace in order to achieve health benefits. As with the nasal group, this is optional and you do not need to take part in this subgroup if you do not want to. If you do agree and are considered suitable, there will be a 2 in 3 chance that you will be randomised to receive the active gene therapy rather than placebo.

The section below explains what you will be asked to do in addition to the visits already described in the main Information Sheet

Additional visits:

Eligibility and Consent (E&C) visit
In certain cases to ease scheduling, we will schedule the bronchoscopy several months ahead of your planned dosing dates. In this case, the E&C visit will take place, which will include:

- We will confirm that you are eligible to take part and ask you to sign the Consent Form
- We will take a full medical history and perform a standard (bedside) examination including heart rate, breathing rate, blood pressure, pulse oximetry, temperature, and listen to your chest
- blood sampling
- urine sampling
- Spirometry

Flexible bronchoscopy under general anaesthetic (GA):
This will be performed once before you start dosing and again at the end, between 29 and 35 days after your 12th dosing visit.
The bronchoscope allows us a view down into your airways; once you have been anaesthetized and are fully asleep, we will make a general inspection of lung inflammation, plugging etc and will then take 2 biopsies and 10 airway wall brushings from one lung. These are safe procedures, although a small amount of airway wall bleeding is common and you

may notice some blood in your sputum for a few days afterwards. We will also make PD measurements down the bronchoscope onto the airway surface in a fashion similar to that described for the nPD (see Appendix 1). We will, as is usual clinical practice, record the entire procedure onto a DVD, which you may view afterwards with us should you wish. The samples that we obtain will be tested using:

      i.   Molecular tests to tell us whether the gene has reached these parts of the lung and successfully entered the airway lining cells

     ii.   Stains to demonstrate whether the CFTR protein has been produced

    iii.   Routine histology (examination under a microscope)

We estimate that the whole procedure will take approximately 45-60 minutes. At the end of the procedure, we will give you a single dose of the antibiotic(s) most suited to the bacteria found most recently in your lungs (sputum or cough swab) to limit the chance of a fever after the procedure.

You will have the opportunity to meet and talk to both the Consultant Anaesthetist and Consultant Respiratory Physician performing the procedure beforehand and ask any additional questions you may have. All staff are fully trained and experienced in these procedures, which are performed regularly at this hospital as part of routine clinical management.

You will be monitored and observed for at least 4 hours after the bronchoscopy. We anticipate that most patients will recover fully within this time period and feel able to be discharged. Should this not be the case, we will ask that you remain in hospital overnight for further observation. You will not be able to drive and so should it be more convenient for your travel arrangements, we will arrange for you to stay overnight either at the hospital or at a local hotel.

In summary, bronchoscopy is generally a safe procedure and performed regularly in hospitals.

The more common side effects are;

- Drowsiness after the anaesthetic – this should wear off after a few hours
- A mild, sore throat for a day or so
- Fever (this will be made less likely with the use of an IV antibiotic and the end of the procedure)
- A small amount of blood (specks) may come up when you cough for a day or so due to the biopsies

Rarely, an anaesthetic can lead to an increase in symptoms such as cough and sputum production. Major blood loss or a chest infection after bronchoscopy are very rare.

Many thanks for considering these additional tests; please feel free to ask any questions you may have.

Parent information sheet

PARENT INFORMATION SHEET

Study title: A randomised, double-blind, placebo-controlled Phase 2B clinical trial of repeated application of gene therapy in patients with cystic fibrosis

Short title: Repeated application of gene therapy in CF patients

Invitation paragraph

Your child/ ward (hereafter referred to as 'child') is being invited to take part in a research study and has been given an Information sheet similar to this one. This is specifically for parents/ carers, to help you decide whether you wish your child to participate. Before you decide you need to understand why the research is being done and what it would involve for you both. Please take time to read the following information carefully. Talk to others about the study if you wish.

Please ask us if there is anything that is not clear or if you would like more information. Please take as much time as you need to decide with your child whether or not you and they wish to take part.

Introduction

There is currently no treatment for Cystic Fibrosis (CF) that completely halts the progression of lung damage; all the available therapies help to slow the rate of decline in lung function at best. Because of this, we have formed the UK CF Gene Therapy Consortium, (scientists, doctors & nurses based at Imperial College/ Royal Brompton Hospital, Oxford & Edinburgh Universities & Hospitals, www.**cfgenetherapy**.org.**uk**/) to develop further gene therapy for CF. Gene therapy uses healthy copies of the *CFTR* gene to replace the damaged versions within the nose and lungs of patients with CF. Each of these three centres has previously been involved in clinical gene therapy trials which were designed simply to prove that we could deliver healthy genes in this way. Together, we have designed a programme of research to lead to a large, multi-dose clinical trial. This will be the first trial to give multiple, repeated doses and look for clinical benefit. The gene therapy will consist of the healthy *CFTR* gene, which will be carried into the cells of the airways by a liposome (a fatty substance), called GL67A. This is the same liposome which we used in clinical trials in the 1990s and we, therefore, have results on its safety and efficacy. The gene, however, has been substantially improved and has recently been used in a pilot clinical trial to ensure its safety and to see how long each dose lasts. The gene therapy can cause a brief change in lung

function and mild feverish symptoms. We have therefore decided to use a dose of 5mls (a teaspoonful, delivered by inhalation of a nebulised aerosol) which was found to be the best tolerated in the pilot trial.

What is the purpose of the study?

The purpose of this study is to assess for the first time whether repeated doses of gene therapy administered to the lungs of CF patients can lead to clinical improvement.

Why has my child been invited?

He/she has been invited because they are have CF and attend the Royal Brompton Hospital, one of the hospitals within the Scottish Paediatric Cystic Fibrosis Service, or one of our collaborating patient identification centres. They are also relatively well, with mild or moderate lung involvement, however not so well that we will struggle to measure any improvement. This will mean that the nebulised gene therapy has a good chance of reaching the cells lining your child's airways without being blocked by excessive mucus, and that we will sample from their airways more easily. If your child is a patient from one of our collaborating participant identification centres they will attend the Royal Brompton Hospital or Western General Hospital / Royal Hospital for Sick Children Edinburgh for all trial related visits but continue their clinical care at their own centre. Trial visits are not a substitute for usual clinic visits and we will not make changes to your child's usual treatment. We will communicate closely with your local team and let them know of anything that might influence treatment decisions.

If your child has been involved in the Run-In study they will be invited for a single screening visit to determine whether they will fit the criteria to participate in the multi-dose trial. If your child has been newly recruited, i.e. not participated in the Run-In trial, they will be invited for an introductory visit to talk to you and your child further about the research and assess their eligibility, as well as attending a subsequent screening visit.

We think that your child may fulfill all of the Inclusion & Exclusion criteria although we will need to go through these lists in detail with you; in particular we will be looking for lung function within the range of 50-90% and for your child to be in a stable condition with no recent changes to their CF treatments. If your childs recent lung function is close to the cut-offs for being included we may wish to perform spirometry to see if they are eligible before discussing all the details of the trial with us, as this is quite time consuming. In this case, we will ask you both simply to sign a form consenting to lung function, which in no way

commits you to taking part in the trial. If your child is unwell at the time they are seen, we may be able to reschedule their visit for when they have recovered and are once again stable.

Does my child have to take part?

It is completely up to you to decide with your child whether or not they wish take part. If he/she does decide to take part you will be given this information sheet to keep and be asked to sign a consent form. Your child will also be asked to sign a separate form agreeing to take part. If you and your child decide to take part you are still free to withdraw at any time and without giving a reason. This will not affect the standard of care your child receives.

What will happen if we take part?

After the screening visit, all patients volunteering for the main part of this study will receive nebulised doses of either the gene therapy (active) product or placebo (sometimes called the 'dummy drug') at 4 week intervals over a 48 week period. Once you have agreed for your child to take part in the study, they will be assigned by chance (like the flip of a coin, but done by a computer) to receive either gene therapy or placebo. A placebo contains no active ingredient. The probability that you will receive gene therapy is therefore 1 in 2 i.e. there is a 50% chance that your child will receive the gene therapy throughout the trial and a 50% chance that your child will receive the placebo throughout the trial. Neither you nor your child's study doctor will know whether your child are receiving gene therapy or placebo. This is termed a 'double-blind' study and is extremely important for generating results that are meaningful and which will be believed by the wider scientific community. However, in the event of an emergency, the identity of the nebuliser solution can be determined rapidly.

When would our child take part? The first 20 patients (10 active treatment; 10 placebo) will receive a nebulised dose (5ml) on 3 occasions at 4-weekly intervals before any further patients are dosed. This is a safety measure to allow the Data Monitoring and Ethics Committee (DMEC, a panel of medical experts) to review the clinical and laboratory findings to ensure that everything is satisfactory after repeated doses of the active treatment/placebo. If this proves to be the case these patients will continue with subsequent visits and the remaining patients will begin their dosing schedule. Because of the large number of patients (130) involved in the study and the restriction on the facilities (special gene therapy cubicles) used we will have to stagger the dosing visits so it is likely to take at least 5-6 months before all patients have started their dosing schedule.

Were the DMEC to have any concerns with the clinical and laboratory findings, they could recommend that we reduce the dose of the gene therapy product. In this event, and if your

child belonged to the initial 20 patient group, they would unfortunately have to withdraw from the trial. A further group of 20 patients would then be recruited to receive the reduced dose (2.5ml) of active treatment/placebo on 3 occasions at 4-weekly intervals. The DMEC would again meet to review the clinical and laboratory findings to ensure that everything is satisfactory. If this proves to be the case these patients will continue with subsequent visits and the remaining patients will begin their dosing schedule. If the DMEC were to have any concerns with the 2.5ml dose the trial would be halted as we do not consider it feasible to administer a smaller dose successfully to the lower airways via nebuliser.

A subgroup (at least 24 patients at the London site) will be asked to undergo nasal dosing. This will involve some additional tests and up to 3 additional short study visits (although we will limit these as much as possible) . *This will be explained in detail on another sheet if you wish to consider this (see Appendix 1).*

A subgroup (at least 24 patients at the London site) will also be asked to undergo a flexible bronchoscopy (a test to look inside the lungs using a flexible telescope) under general anaesthetic on two occasions (prior to the first dose of gene therapy/placebo and between 29 and 35 days after the 12th dose of gene therapy/placebo). This will involve two additional study visits. *This will be explained in detail on another sheet if you wish to consider this (see Appendix 2).*

How often will we need to visit the hospital for this study?

There will be a minimum of 15 visits over one year. Your child will be monitored before and after each nebulised dose (dosing visit) during 12 scheduled dosing visits. The schedule of visits is 4 weeks +/- 5 days for 48 weeks with further details given below. You will be given a flow chart summarising the sequence of study visits:

1. Introductory visit – new patients only (i.e. those not previously involved in Run-In study) (3-4 hours) This is to help us get to know you and your child and for you both to familiarise yourself with the tests before the trial starts. However, it is not essential and, particularly if you have a long distance to travel or other time commitments, we can discuss omitting this visit with you.

2. Eligibility and Consent visit (selected subgroup patients only- see below)* (2-3 hours)

3. Screening visit – all patients (3-4 hours)

4. Pre-dosing nasal PD visits up to 3 (nasal subgroup only)

5. Pre-dosing bronchoscopy (bronchoscopy subgroup only)

6. Dosing visits x 12 (3-4 hours)

7. Post-dosing day 2 visits (1st 3 doses in 1st 20 patients only) (2 hours)
8. Follow up visits on Days 14 and 28 (+/-2days) post-dosing (3-4 hours)
9. Post-dosing bronchoscopy Days 27-36 after dose 12 (bronchoscopy subgroup only)

*As the screening visit must occur within 28 days before the first dosing visit, we will offer people taking part in the nasal or bronchoscopy subgroups the opportunity to plan the additional investigation visits earlier if convenient. This will require an initial, short visit, at which we assess eligibility, perform a physical examination and limited tests and you will be asked to sign consent for the trial.

Should there be any safety concerns, we have the flexibility to see patients for additional visits at any time during the study; such visits will of course be kept to an absolute minimum.

The procedures performed at each of these visits are summarised below. If for any reason we are unable to perform a test at a certain visit (eg. if you miss a visit or equipment fails), the protocol allows us to consider performing that test at a future visit. A full description of each test follows this summary of visits.

PRE-DOSING VISITS

Introductory visit – if considered necessary for new patients only (i.e. those not previously involved in Run-In study). This visit will take around 3-4 hours and will involve:

- explanation of study
- full medical history
- confirmation that your child is medically suitable for the trial
- sign informed consent
- a simple (bedside) examination of your child's chest, blood pressure, temperature, pulse, and oxygen levels (with a finger probe)
- blood sampling
- urine sampling
- Sputum sample (induction with 7% hypertonic saline if required)
- spirometry (lung function tests, as usually done in clinic)
- lung clearance index (LCI)
- 24 hour sputum weight
- nasal potential difference measurements may be conducted (nasal PD subgroup only)
- we will give your child a diary card to take home and instructions on how to complete it
- we will give your child a hand-held PiKo-6 device for you to carry out blowing tests (lung function) at home

Screening visit (all patients)

This visit will take around 3-4 hours and will involve:

- explanation of the study

- confirmation that your child is medically suitable for the trial

- sign informed consent (if not already signed at introductory visit)

- quality of life questionnaire

- full medical history and physical examination

- a simple (bedside) examination of your child's chest, blood pressure, temperature, pulse, and oxygen levels (with a finger probe)

- blood sampling

- urine sampling

- spirometry (lung function tests, as usually done in clinic)

- lung clearance index (LCI)

- exercise bike test

- body worn activity monitor (which we will show you and your child how to use)

- chest CT scan

- gas transfer test

- Sputum sample (induction with 7% hypertonic saline if required)

- 24 hour sputum weight

- nasal potential difference measurements may be conducted (nasal PD subgroup only)

- we will give your child a diary card to take home and instructions on how to complete it

- we will give your child a hand-held PiKo-6 device for you to carry out blowing tests (lung function) at home

DOSING VISITS

Each of the 12 dosing visits will take around 3-4 hours. If your child normally takes Pulmozyme (DNase), they will be asked to withhold treatment for 24 hours prior to each visit and for 24 hours after dosing.

Predosing, the following will be performed at every visit:

- history (we will ask your child how well they have been since the last visit and if any of their medicines have been changed)

- a simple (bedside) examination of your child's chest, blood pressure, temperature, pulse, and oxygen levels (with a finger probe)

- blood sampling

- spirometry (lung function tests, as usually done in clinic)

At specified visits, the following will also be performed

- lung clearance index (LCI)
- urine sampling
- quality of life questionnaire
- exercise bike test
- body worn activity monitor
- chest CT scan
- gas transfer
- 24 hour sputum weight and collection of fresh sample
- nasal PD (London site subgroup only)
- bronchial blood flow measurement (London site subgroup only)

Dosing

Approximately 20 minutes before the nebulised dose is administered, your child will receive 200-400 □g (2-4 puffs) of inhaled salbutamol to prevent airway narrowing and wheeze, which may be associated with the gene therapy agent / placebo. The nebulised dose will then be given via a mouthpiece from a clinical nebuliser. Your child will be asked to wear a nose clip for the duration of the nebulisation (3 minutes on, 2 minutes off for 8 cycles) which usually takes around 40 minutes.. The nebuliser will be administered whilst your child is sitting in a cubicle to prevent contamination of the immediate environment. Your child will have contact throughout and will be able to communicate with a research nurse through a glass window. You will be able to wait nearby, but will not be able to be in the cubicle while your child is receiving his/her nebulised dose. Your child will be asked to report any new symptoms. Although this environment is not spacious, we will do our best to make your child as comfortable as possible. We can provide music or a DVD player should they wish. They will be able to leave the cubicle as required for a toilet break, although we will ask that they wear a mask over their mouth and nose during this time and for 30 minutes after dosing is complete.

If you agree for your child to take part in the nasal dosing subgroup (London site only), we will ask your child to administer a nasal spray during the 'off' nebuliser period, with one spray being delivered to each nostril at the beginning and end of the first 6 two minute'off' periods which will deliver a dose of approximately 2 ml.

Your child will be observed for a minimum of 30 minutes after dosing before leaving the hospital. We will listen to your child's chest and perform pulse oximetry before they leave the hospital. If there are any concerns about your child's level of well-being after treatment, we

may suggest that they stay in hospital overnight, although we are not expecting this to be the case. We will ask your child to take a standard dose of paracetamol immediately post-dosing and you will be given another dose of paracetamol for your child to take at home approximately 6 hours after dosing. This is to prevent any mild feverish symptoms that could possibly occur after dosing.

Day 2 post-dosing visits (first 20 patients after each of the first three doses only)

The following will be performed:

- history (we will ask your child how they have been since their last visit and if any of their medicines have been changed)
- a simple (bedside) examination of your child's chest, blood pressure, temperature, pulse, and oxygen levels (with a finger probe)
- spirometry ((lung function tests, as usually done in clinic)
- gas transfer
- blood sampling
- bring in sputum coughed up over last 24 hours and collect fresh sample

Follow up visits

There will be two follow up (F/U)visits that will occur at 14 (+/-2) days and 28 (+/-2) days after your child's last dosing visit.

At F/U **day 14** we will do the following:

- history (we will ask your child how well you have been since the last visit and if any of their medicines have been changed)
- a simple (bedside) examination of your child's chest, blood pressure, temperature, pulse, and oxygen levels (with a finger probe)
- quality of life questionnaire
- spirometry
- blood sampling
- lung clearance index (LCI)
- nasal PD (subgroup only)
- urine sampling
- bring in sputum coughed up over last 24 hours and collect fresh sample
- diary card check
- download hand-held PiKo-6 device on which your child carried out blowing tests

At F/U **day 28** we will do the following:

☐   history (we will ask your child how well they have been since the last visit and if any of their medicines have been changed)

☐   all the tests performed at the screening visit

☐   collection of diary card and hand-held PiKo-6 device

Long term follow up: your child will be followed up by their clinical team at scheduled CF clinic appointments approximately every 3 months for 2 years.

Description of Tests

☐   *Full medical history:* we will ask about your child's medical condition(s) including history of medications (specifically any additional antibiotics required for their chest), operations and allergies plus anything else relevant to the safety of the trial.

☐   *Clinical examination*: we will listen to your child's lungs using a stethoscope and measure their height, weight, temperature, pulse, blood pressure and respiratory rate

☐   *Pulse oximetry (finger probe):* a measurement of the oxygen level in your child's blood using a finger probe

☐   *Blood test:* (at each visit where blood is required, up to 25 mls, (5 teaspoons), will be taken for a variety of tests including routine clinical tests e.g. liver and kidney function, full blood count, electrolyte (salt) levels and C-reactive protein (a marker of inflammation). Samples will be stored for future measurement of inflammatory proteins and possibly for end-products of gene therapy breakdown. If you or your child wish, we will arrange for your child to have local anaesthetic, either cream or spray. If your child has a portacath, we may be able to use this to take blood samples if they would prefer. At a single visit at any time during the trial, we will take a sample of blood for DNA (genetic) research. The only tests we will perform on this sample will be:

     o   *Testing for your child's CF gene(s) if these are not known; we will be happy to let you know the results of this test.* **This sample will not be used to test for any other genetic diseases**

     o   *Testing for common, naturally-occurring changes in genes which might be involved in how people respond to gene therapy, eg, inflammatory response genes. We will do this testing in the event that we see that some people respond to gene therapy and others do not, to help us understand this better.*

☐   *Urine sample*: routine testing with a dipstick for protein, sugar etc; we will also perform a pregnancy test if your child is female   Samples will be stored for future

measurement of inflammatory proteins and possibly for end-products of gene therapy breakdown.

☐ *Spirometry* (blowing tests, as usually done in clinic).

☐ *Lung clearance index (LCI)*: this test demonstrates how evenly gas is distributed in the lungs and is a sensitive marker of airway disease. Your child will be asked to breathe a tracer gas (SF6) which has no smell or taste and is completely harmless, over a period of several minutes. Once levels in your child's lungs are stable, they will switch to breathing air and the time taken for the SF6 to leave the lungs is used to calculate the LCI. We will perform the test 3 times, each one taking about 10 minutes.

☐ *Exercise bike test:* this test will be performed on an exercise bike and is incremental (gets more difficult to pedal as it goes on). Exercise is an event that involves a large number of physiological processes from the heart and lungs, to the blood circulation and the muscle itself. This test will enable us to calculate your child's exercise capacity. During the test we will record a breathlessness score and a probe will be attached to your child's finger or ear lobe to measure the oxygen levels in their blood. Your child will be wearing a nose clip and breathing through a mouthpiece. They will be asked to pedal at a comfortable speed and maintain this speed throughout the test. The resistance to pedalling will automatically increase each minute and your child will be encouraged to continue until they either cannot keep up with the speed or feel they have exercised as much as they are able; we will ask your child to stop if their oxygen level falls. Once the test is complete your child will have a 2 minute cool down period, and we will continue to monitor their oxygen levels until they return to baseline.

☐ *Sweat test:* Although the diagnosis of CF is not in doubt, some patients, particularly those who were diagnosed many years ago, do not have documented confirmation of a diagnostic test in their notes. If your child has neither a confirmed genetic diagnosis (2 mutations) nor a sweat test result, we would like to perform a sweat test as part of the trial. An area of the skin on your childs forearm will be cleaned and 2 electrodes will be attached with straps. One of these contains a gel which stimulates sweat and the medication is applied to the skin by a weak current; there will be a little tingling but this is not painful. Following this a collection device will be attached to the skin surface and the sweat collected over a 30 minute period. This will then be analysed by the laboratory for the high levels of salt seen in CF*Activity monitor:* a small band worn on your child's upper arm that collects step and movement data. Your child will be asked to wear this for 7 full days after specific study visits.

☐ *Chest computed tomographic (CT) scan:* Computed tomography uses x-rays to create detailed images of the lungs. It is a good measure of how CF affects the shape and structure of the airways. For each scan your child will be asked to lie still on a table which will move slowly through the centre of a large x-ray machine but at no time will they be in an enclosed space. Your child will be able to communicate with the CT staff if they need to. They will be given breathing instructions at the time of the scan. No injections are involved and the procedure is completely painless. As x-rays are involved, your child will be exposed to a small amount of radiation. However, we will be using as low a dose as is possible for your child's scans. The estimated maximum dose of all three scans over the entire study period amounts to 4.5 mSV, which is similar to the radiation your child would be exposed to as part of natural background radiation over a 2 year period. Because even low levels of radiation can be dangerous to an unborn child, it is a requirement that all females undergo a urine pregnancy test on the day of the procedure.

☐ *Transfer factor (TLCO)*: this is a lung function test which measures the rate at which gas travels across the lung tissue (alveolus) into the blood stream. Gas transfer is usually normal in patients with CF. We are using this test as a safety measure and in order to assess whether the gene transfer has an effect on this, we need to make a baseline measurement. Your child will be asked to wear a nose-clip and to breathe out as far as they can through a mouth-piece. They will then breathe in as far as possible, a mixture of air and low concentration of a tracer gas (less than 0.3% carbon monoxide) and hold their breath for 10 seconds before breathing out as far as possible. This will be repeated up to 5 times with rest periods in between tests. This concentration of CO is completely harmless and this is a well-established test used routinely in clinical practice.

☐ *Sputum sampling:* we will ask your child to try and cough up around a teaspoon of sputum; if this is not possible , we will obtain secretions for microbiology by asking your child to cough onto a sterile swab or brush . At either your child's introductory or screening visit and at either of their follow up day 14 or day 28 visits if your child is unable to cough up a sputum sample, we will use a well-established nebulisation technique to induce sputum. To prevent wheeze (a common side effect), your child will be given 200-400 µg (2-4 puffs)of salbutamol (Ventolin) or an equivalent drug to open up the airways. After 15 minutes your child will receive a 7% saline nebuliser for 5 minutes, and this will be repeated up to 3 times. We will monitor your child's lung function (FEV_1) throughout, and should we see a significant drop, the test will be stopped. If sputum induction fails, we will obtain secretions for microbiology by

asking your child to cough onto a sterile swab or brush. Samples will be tested for infection and stored for future measurement of inflammatory proteins and possibly for end-products of gene therapy breakdown. *CF specific quality of life questionnaire:* this will take a total of 10 minutes to complete.

☐ *Diary card:* Your child will be given a diary card which they will be asked to complete for the duration of the study. This will record new symptoms, changes in routine treatments etc.

☐ *Home lung function:* You and your child will be taught how to use a small lung function device (PiKo-6) and asked to make regular recordings on it at home for the duration of the study period. The machine stores all the readings which will be downloaded onto a computer at each visit.

☐ *Bronchial blood flow measurement: (London site only)* This test is designed to look at the blood flow to the airways which is often increased in patients with airway disease, probably reflecting inflammation. The test takes about 45 minutes to do and consists of 10 breath-holding manoeuvres of either 8 or 16 seconds. For each manoeuvre your child will be seated, wearing a noseclip and breathing in and out through a mouthpiece. After several normal breaths they will need to breathe in a small amount of test gas, which is safe and enriched with oxygen, and hold this breath for 8 or 16 seconds before breathing out again slowly. Their heart rate and oxygen saturation will also be monitored during the test. There is a gap (3-4 min) between each of the manoeuvres to save the data and calibrate the equipment. As the blood flow to the airways may be affected by caffeine, it is requested that your child does not consume any caffeine on the day of the test. This test will be undertaken after all other assays / tests if time allows. If your child is too tired to do the procedure it will not be done or if during the procedure they feel too tired to continue we will stop the test at any time.

☐ *Nasal PD and nasal brushings: see Appendix 1 (London site only)*

☐ *Bronchoscopy: see Appendix 2 (London site only)*

What else will we have to do?

In addition to the study visits and home monitoring outlined above, we will ask that if your child is sexually active, they agree to take contraceptive precautions from enrolment into the study until 3 months after completion. This is a requirement of the Medicines and Healthcare products Regulations Agency (MHRA) and the Gene Therapy Advisory Committee (GTAC) for all clinical trials involving gene therapy. We will talk to them and you about approved (reliable) methods of contraception.

There will be no other changes made to your child's routine clinical care.

We ask that you consent to our informing your child's General Practitioner about their involvement in the study, including these requirements for contraception.

What is the drug or procedure that is being tested?

We are testing a formulation consisting of a healthy copy of the *CFTR* gene mixed with a fatty substance which helps the gene enter the cells of the airway. The *CFTR* gene has been changed since our original trial in ways that we believed would make it likely to cause less inflammation and last for longer. We did see some flu-like responses and drops in lung function in some people in the Pilot study but by giving a smaller dose (5ml) we were able to reduce these side effects. The research team will be happy to provide you and your child with more specific scientific details should you wish.

What are the alternatives for diagnosis or treatment?

As you and your child will know, conventional treatments for CF have improved greatly over the last few decades. However, they do not correct the basic defect in your child's cells, which is what we are aiming to do with gene therapy. In general therefore, conventional treatments slow the natural progression of lung disease, rather than stop it altogether. Should you or child wish not to take part, your child's standard clinical care will continue as usual.

What are the possible disadvantages and risks of taking part?

We are asking for a large time commitment from you and your child over a period of a year. The study requires you and your child to make a minimum of 15 visits and perform multiple tests. As described above, most of these are straightforward and many of them will be familiar to you and your child from clinics (and also if your child participated in the Run-in). The risks that we consider likely are as follows:

1. Either the gene therapy or the placebo could cause wheeze or increased cough. To limit this, we will administer salbutamol, to relax the airways, prior to nebulisation. In our previous study, some of the single dose patients had a fall in their lung function several hours after dosing but this was well-tolerated and, for most patients receiving 5 ml, was not associated with any increased symptoms. Lung function had resolved in all dosing groups within 2 days of the dosing visit.

2. In the single dose study, some patients developed a temperature and a mild flu-like illness within a few hours of dosing. These side effects were minimal with the 5 ml dose that we have chosen for this trial and appear to be responsive to paracetamol, which we will ask your child to take on two occasions (2hrs and 8 hrs) post-dose whether they have side effects or not. All side effects should have disappeared within 2 days of the dosing visit.

3. The CT scans involve exposure to a small amount of radiation, which in common with all radiation does carry a small risk of causing cancer. We plan to do a type of CT scan using lower radiation doses than a standard CT. The estimated maximum dose of all three scans over the entire study period amounts to around 4.5 mSv. To put this in context, the maximum amount of radiation from all three scans over the entire study period is equivalent to the amount of natural radiation to which everyone is exposed from environmental sources over a period of 2 years. Female patients of a child-bearing age will be required to have a negative pregnancy test on the day of each scan.

4. Please see Appendices 1 and 2 for disadvantages associated with nasal PD and bronchoscopy. (These tests are only being performed at the London site.)

What are the possible benefits of taking part?

Because we are the first research group in the world to administer multiple doses of this gene therapy product, and CF is such a chronic disease, we cannot be sure that there will be any immediate or long-term benefits of participating in the study. Your child will be helping to advance the field of gene therapy by agreeing to take part. Should this study be successful, we will undertake further trials with a view to developing gene therapy as a treatment.

What happens when the research study stops?

When your child has completed their study visits, they will continue to receive their routine clinical care. If you wish, we will keep you and your child informed of any developments that arise from the results of this study.

What if there is a problem?

Throughout the study, we will be happy to talk to you and your child, or see you both at any point should you have concerns. Should your child develop any health problems, we will investigate and treat these after discussion with your usual medical team.

An independent Data Monitoring and Ethics Committee (DMEC) will oversee the safety of the trial as it progresses. Any significant adverse events will be reported to the DMEC.

Complaints : If you have a concern about any aspect of the study, you should ask to speak to a member of your child's clinical research team (see contact numbers at the end of this Information Sheet) who will do their best to answer your questions. If you remain unhappy and wish to complain about any aspect of the way you or your child have been approached or

treated during the course of this study, the normal National Health Service complaints mechanisms are available to you. Details can be obtained from the hospital.

Harm: In the event that something does go wrong and your child is harmed during the research due to someone's negligence, then you may have grounds for legal action or compensation, but you may have to pay your legal costs. The normal National Health Service complaints mechanisms will still be available to you (if appropriate).

Imperial College London holds Public Liability ("negligent harm") and Clinical Trial ("non-negligent harm") insurance policies which apply to this trial. Liability for the gene therapy product design will be accepted by Oxford University and Imperial College London. Liability for the protocol design will be accepted collectively by Oxford University, Edinburgh University and Imperial College London. Clinical negligence insurance / indemnity is provided by the NHS.

Will my child's participation in the study be kept confidential?
Yes.
All information collected during the course of the study will be coded and kept strictly confidential. Clinical results and analysis will be collated by the Imperial College Trials Unit. All samples will be stored indefinitely in a coded fashion to preserve confidentiality. Codes will be held by a single investigator at each site. If you consent to your child taking part in the research, any of their medical records may be inspected for purposes of analysing the results. They may also be looked at by people from regulatory authorities to check that the study is being carried out correctly. Your child's name, however, will not be disclosed outside the hospital.

If you agree to your child taking part, their GP will be informed and your hospital notes will be flagged to ensure that other members of your child's clinical team are also informed.

What if relevant new information becomes available?
Sometimes during the course of a research project, new information becomes available about the test/treatment that is being studied. If this happens, your child's research doctor will tell you about it and discuss with you and your child whether you want your child to continue in the study. If you decide to withdraw your child, your child's research doctor will make arrangements for their normal clinical care to continue. If you decide that your child can continue in the study you will be asked to sign an updated consent form.

Also, on receiving new information your child's research doctor might consider it to be in your child's best interest to withdraw them from the study. He/she will explain the reasons and arrange for your child's normal clinical care to continue.

What will happen if I don't want my child to carry on with the study?

Your child will be free to discontinue the study at any time, without giving a reason, and this will not affect their clinical care in any way. For patients who have commenced dosing we would be grateful if they could attend at least a 14 and 28 day follow up appointment as part of the trial. Samples already obtained will be used for this and other ethically approved studies unless you specifically request samples to be destroyed. In addition we would like to discuss your child's progress with their clinical team after their routine clinic visits to gather as much information as possible for the trial and ensure continuing patient safety.

What will happen to any samples my child gives?

Samples will be stored in a coded fashion so that, with the exception of one or two key people, the researchers will not be able to identify individual patients. Many of the samples will be frozen and stored indefinitely. We are working closely with the other members of the UK Gene Therapy Consortium in Oxford, Edinburgh and Southampton Universities and will be sending coded samples to them for analysis and storage. We will also be sending coded blood samples to colleagues at the University of Pennsylvania, USA. We would wish that any spare samples left over could be used in other ethically-approved research protocols and would ask you to agree to this by gifting your samples to us as part of consenting to this study. All of our storage conditions comply with the regulations of the Human Tissue Act and/ or the Human Tissue Act (Scotland) 2006.

What will happen to the results of the research study?

At the end of the study, all results will be analysed, and will be reported at conferences and published in the Medical press. Results of the study will also be made available to study participants on request. Your child will not be identified in any report/publication.

Will my expenses for my child's taking part in the study be reimbursed?

Yes. We understand the inconvenience involved in taking part in such a study and will be happy to reimburse travel expenses for the visits you will be asked to make with your child.

Who is organising and funding the research?

The study is being funded by the National Institute for Health Research & Medical Research Council. There is no financial gain to either the Hospital or the staff for including and looking after patients in this study.

Who has reviewed this study?

This study has been submitted for review by the Gene Therapy Advisory Committee, the national body charged with assessing the ethical aspects of any clinical trial involving gene therapy.

Thank you for considering taking part in this study

We very much hope that after reading this information and talking to the research team, you and your child feel able to take part, but please be assured that should you or your child not wish to, this will in no way affect your child's clinical care.

For further information please contact:

Royal Brompton Hospital, London

Clare Saunders (Clinical Trial Co-ordinator) – Tel: 0207 352 8121 ext. 3516

cfgenetherapy@rbht.nhs.uk

Prof Jane Davies - Tel: ██████████

Prof Eric Alton - Tel: ████████████████

Royal Hospital for Sick Children, Edinburgh

Dr Steve Cunningham – Tel: ███████████

Dr David Armstrong – Tel: ████████████████

Kay Riding (Senior Research Nurse)- Tel: ██████████

Maria Dewar (Research Manager) – Tel: ████████

Lothian.cfgtscotland@nhs.net

Appendix 1

Nasal dosing Subgroup (London site only)

A group of at least 24 patients in the trial will be asked to undergo nasal dosing and have nasal potential difference (nPD) measured and nasal brushings taken. Because the nostrils are relatively easy to access, and these measurements can therefore be made repeatedly, this offers us a valuable opportunity to study how well the gene is expressing at different time points during the study. This is optional and you do not have to take part in this additional testing if you do not wish to. If you do agree and are considered suitable, there will be a 2 in 3 chance that you will be randomised to receive the active gene therapy rather than placebo.

The section below explains what you will be asked to do in addition to the visits already described in the main Information Sheet

Additional pre-1st dose visits:

Eligibility and Consent (E&C) visit

In certain cases to ease scheduling, we will schedule the nPD tests several months ahead of your planned dosing dates. In this case, the E&C visit will take place, which will include:

- We will confirm that you are eligible to take part and ask you to sign the Consent Form
- We will take a full medical history and perform a standard (bedside) examination including heart rate, breathing rate, blood pressure, pulse oximetry, temperature, and listen to your chest
- blood sampling
- urine sampling
- Spirometry
- Nasal potential difference measurement may be performed at this visit or on separate occasion

Nasal PD visits

You will be asked to have 3 nPD measurements before receiving your first dose of gene therapy. A maximum of 2 measurements can be performed at a visit. These may be performed at E&C, Introductory or Screening visits, or may be scheduled separately to suit you. On a single occasion, either with or after your 3rd nPD, you will undergo a nasal brushing test.

Nasal Dosing: You will be taught how to use the nasal spray and will be asked to administer a nasal spray during the 'off' nebuliser period, with one spray being delivered to each nostril at the beginning and end of the first 6 two minute 'off' periods which will deliver a dose of approximately 2 ml.

Nasal potential difference (nPD): We will perform nasal PD on three occasions before the first dose (this is because we know the measurements are quite variable and want to be as certain as possible that any changes we see are due to the gene therapy and not just this variability), and after the sixth dose and at two time points after the twelfth dose. We may do additional nasal PDs post dose 3, 6 and 9 if opportunity arises and you are agreeable This test is a sensitive way of measuring salts moving in and out of the cells lining the nose. The readings are different in patients with CF from those without and this test has been used clinically for many years in difficult diagnoses. As gene therapy is directed at correcting these cells, this test can help tell us how well the gene therapy is working. A small abrasion is made with a sterile device on the skin of the forearm; this is not painful. An electrode wire is attached to the abraded skin and a small volume of cream is applied and kept in place with a piece of tape. A soft catheter is inserted about 5-7 cm into one nostril until a stable reading is obtained. This will then be taped in place. A series of solutions is perfused into the nostril; you will be asked to lean forward so that drops fall into a bowl rather than go down the back of your throat. The response to these solutions is measured on a portable computer. The whole test takes approximately 20 minutes. We may wish to perform this on both nostrils (one after the other) depending on the results.

The more common side effects include:
- A slight salty taste from the solutions in your nose
- Sneezing or mild discomfort during insertion of the catheter
- Mild discomfort on your arm for a short while following skin abrasion

Nasal brushing: We will obtain cells lining the nose by inserting a small, sterile brush into the nostrils, one at a time. The procedure is not particularly pleasant and makes the eye on that side water slightly. It is however very quick, each nostril taking only approximately 5 seconds. We will perform nasal brushing on one occasion at or after your third nasal PD visit and again at the end of the trial.

Rarely, patients are unhappy to have tests performed on their nose as they find this unpleasant; if your child feels this way they and are also in the bronchoscopy subgroup, we can include them in the nasal dosing group and perform only a single nPD and brushing pre and post-dosing whilst they are under anaesthetic.

Appendix 2

Bronchoscopy Subgroup (London site only)

A group of at least 24 patients in this trial will be asked to undergo two bronchoscopies, one before the first dose of gene therapy / placebo and the second between day 29 and 35. after the 12th dose of gene therapy / placebo. The purpose of this is to make measurements of the levels and function of the CFTR gene in the hope that we can learn how much gene we need to replace in order to achieve health benefits. As with the nasal group, this is optional and you do not need to take part in this subgroup if you do not want to. If you do agree and are considered suitable, there will be a 2 in 3 chance that you will be randomised to receive the active gene therapy rather than placebo.

The section below explains what you will be asked to do in addition to the visits already described in the main Information Sheet

Additional visits:

Eligibility and Consent (E&C) visit

In certain cases to ease scheduling, we will schedule the bronchoscopy several months ahead of your planned dosing dates. In this case, the E&C visit will take place, which will include:

- We will confirm that you are eligible to take part and ask you to sign the Consent Form
- We will take a full medical history and perform a standard (bedside) examination including heart rate, breathing rate, blood pressure, pulse oximetry, temperature, and listen to your chest
- blood sampling
- urine sampling
- Spirometry

*Flexible bronchoscopy under general anaesthetic (GA)***:**

This will be performed once before you start dosing and again at the end, between 29 and 35 days after your 12th dosing visit.

The bronchoscope allows us a view down into your airways; once you have been anaesthetized and are fully asleep, we will make a general inspection of lung inflammation, plugging etc and will then take 2 biopsies and 10 airway wall brushings from one lung. These

are safe procedures, although a small amount of airway wall bleeding is common and you may notice some blood in your sputum for a few days afterwards. We will also make PD measurements down the bronchoscope onto the airway surface in a fashion similar to that described for the nPD (see Appendix 1). We will, as is usual clinical practice, record the entire procedure onto a DVD, which you may view afterwards with us should you wish.

The samples that we obtain will be tested using:

       i. Molecular tests to tell us whether the gene has reached these parts of the lung and successfully entered the airway lining cells

      ii. Stains to demonstrate whether the CFTR protein has been produced

     iii. Routine histology (examination under a microscope)

We estimate that the whole procedure will take approximately 45-60 minutes. At the end of the procedure, we will give you a single dose of the antibiotic(s) most suited to the bacteria found most recently in your lungs (sputum or cough swab) to limit the chance of a fever after the procedure.

You will have the opportunity to meet and talk to both the Consultant Anaesthetist and Consultant Respiratory Physician performing the procedure beforehand and ask any additional questions you may have. All staff are fully trained and experienced in these procedures, which are performed regularly at this hospital as part of routine clinical management.

You will be monitored and observed for at least 4 hours after the bronchoscopy. We anticipate that most patients will recover fully within this time period and feel able to be discharged. Should this not be the case, we will ask that you remain in hospital overnight for further observation. You will not be able to drive and so should it be more convenient for your travel arrangements, we will arrange for you to stay overnight either at the hospital or at a local hotel.

In summary, bronchoscopy is generally a safe procedure and performed regularly in hospitals.

The more common side effects are;

- Drowsiness after the anaesthetic – this should wear off after a few hours
- A mild, sore throat for a day or so
- Fever (this will be made less likely with the use of an IV antibiotic and the end of the procedure)
- A small amount of blood (specks) may come up when you cough for a day or so due to the biopsies

Rarely, an anaesthetic can lead to an increase in symptoms such as cough and sputum production. Major blood loss or a chest infection after bronchoscopy are very rare.

Many thanks for considering these additional tests; please feel free to ask any questions you may have.

Paediatric information sheet

PAEDIATRIC INFORMATION SHEET

Study title: A randomised, double-blind, placebo-controlled Phase 2B clinical trial of repeated application of gene therapy in patients with cystic fibrosis

Short title: Repeated application of gene therapy in CF patients

Invitation paragraph

We would like to tell you about a research study we are doing. A research study is a way to learn more about something.

Before you decide if you want to join in, it is important to understand why the research is being done and what it would involve for you. So please read this sheet carefully. Talk about it with your family, friends, doctor or nurse if you want to.

Why are we doing this research and what is the medicine being tested?

We are a group of scientists and doctors working together trying to develop a new type of treatment for patients with cystic fibrosis, called gene therapy. We are called the UK CF Gene Therapy Consortium. Cystic fibrosis is caused by a faulty gene. A gene is part of your cells that tells your body how to grow. Gene therapy replaces faulty CF genes with healthy copies within the nose and lungs. We have done research to find the best type of gene therapy, the best dose and the best nebuliser and we know that we can get the gene into the right place within the cells of the airways. This trial is to see whether the medicine can make people with CF better (higher lung function, feeling better, fewer infections etc). We don't know yet if the medicine works.

Why have I been invited to take part?

You have been invited to join this study because you have cystic fibrosis and we think you are likely to be in a group of patients with good (but not perfect) lung function. You may have been kindly helping with our Run-in study, in which case you will probably know quite a lot about gene therapy and the sorts of tests we're doing. If you're new to this research, you will have the opportunity to hear more about it and we will answer any questions you may have. In this study, around 130 people, which includes children (12 years and above), will help test this medicine in our clinical sites based in London and Edinburgh. If you are a patient from one of our collaborating participant identification centres you will attend the Royal Brompton Hospital or the Western General Hospital / Royal Hospital for Sick Children Edinburgh for all trial related visits but continue your clinical care at your own centre. Trial visits are not a substitute for your usual clinic visits and we will not make decisions about your treatment. We will be in close touch with your usual centre and will let them know the results of tests if we think they need to know.

We think you are likely to be in a group of patients with good (but not perfect) lung function. Because the trial itself is relatively long to talk through, we may ask you to perform lung function first to make sure that you will be in the right group to take part. If you fall below this range at the time of your visit we may be able to arrange for you to come again to test you again.

Do I have to be in this study?

No. It is up to you to decide (with your parents). If you do decide to take part, your research doctor will ask you to sign a form giving your agreement to take part. You will be given a copy of this information sheet and your signed form to keep.

You can decide to take part now but change your mind later and stop being in the study without needing to give a reason. If you decide to stop, this will not affect the care you will receive at your normal CF centre.

Talk with your Mum or Dad or guardian before you decide. We will also ask your Mum or Dad or guardian if it is okay for you to be in this study. Even if they say "yes", you have the final say and it is okay for you to say "no".

What will happen to me if I take part?

This is quite a complicated study so we have attached a flow chart to the end of this sheet, which might help explain what's going to happen

Firstly, we will see you at the hospital for one or two visits (2-3 hours each) to check you over, make sure you are fine to take part and do some tests; we call these 'baseline' tests and we will be looking at whether they change once you have had the treatment. If you are suitable and you agree to take part, the next stage is that a computer decides whether you get the gene therapy, or a 'dummy' medicine (half the people will be in each group). Neither you nor the study team will know which group the computer has placed you in. We know this is the best way to be certain whether a new medicine is actually working.

You will then have a visit every month for 12 months. At each visit you will receive a dose of the study medicine (gene therapy or dummy) and we will do some tests too. If you normally take DNase, we will ask you to miss this on the day of dosing and the next day (we will have checked this is okay with your clinic doctors). The tests are explained in detail further down this sheet. First we will give you a few puffs of a blue inhaler (salbutamol/ventolin) to open up your airways and reduce the chance that the medicine will make you wheezy. The gene therapy medicine (or dummy drug) will be in a nebuliser, which you will breathe in for around 40 minutes, with regular breaks. We will ask you to wear a noseclip while using the nebuliser, to make sure that all the medicine is getting into your lungs, but you will be able to take this off during the breaks. The nebuliser pot will be covered in tape so that none of us can see inside., We do all this inside a special cubicle to make sure that the nebulised medicine does not get everywhere; there is a window so you can see chat to the study nurse, and your parent or guardian will just be in the next room. You can come out if you need to, but we will ask you to wear a mask for the first half hour. After you have finished your dosing we will examine you and give you a dose of paracetamol (which should make side effects unlikely). You can then go home and we would like you to take another dose of paracetamol about 6 hours later. At the end of the 12 monthly visits, we will ask you to come back to the hospital for further tests 2 and 4 weeks later. That is the end of the study, but we will keep in touch with your regular clinic team to see how you are doing.

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There are some patients in London who will be asked if they want to do also be part of a smaller study. Twenty four people will be asked to be in a 'nasal subgroup' – these patients will also be asked to spray some medicine up their nose during their nebuliser breaks. We will show them how to do them. There is more information about this test in Appendix 1 at the bottom of this test.

Another small study will also be the 'bronchoscopy subgroup', where 24 patients will also have a camera look into their lungs. There is more information about this test in Appendix 2 at the bottom of this sheet.

If you are asked to be in either of these groups, you can say no and still carry on with the rest of the study.

What are the tests I will have at the study visits?
You can see which tests are done at each visit on the sheet your parent/guardian has been given.

- *Full medical history:* we will ask about your general health, whether you've had operations and which medicines you take normally; this will be very like your clinic doctors do in out-patients.
- *Clinical examination*: we will listen to your lungs using a stethoscope and measure your height, weight, temperature, pulse, blood pressure and respiratory rate (you will have had all of this done in clinic too).
- *Pulse oximetry (finger probe):* we will measure the oxygen level in your blood using a finger clip.
- *Blood test:* There are quite a few blood tests needed in this trial, so we can be certain the medicine is safe. We will only let a very experienced person take your blood. If you like you may have anaesthetic cream or spray. If you have a portacath, and you would prefer that we used this to take blood samples, we may be able to use it instead.
- *Urine sample*: we will ask you to pee into a pot and give us a sample for testing. If you are a girl and you have started your periods, we will also do a pregnancy test, because we are not allowed to test this medicine on pregnant women.
- *Spirometry* (blowing tests, as usually done in clinic).
- *Lung clearance index (LCI)*: this test shows how evenly gas is distributed in the lungs and is a good marker of how healthy your lungs are. You will be asked to breathe a special gas mixture for a few minutes. You can't smell or taste this gas and it has no side effects. Once levels in your lungs are stable, we will switch you to breathing normal air and the time taken for the gas to leave the lungs is used to calculate the LCI. We will do the test 3 times, each one taking about 10 minutes.
- *Exercise bike test:* This test allows us to see how much exercise you can do and involves pedalling a bike which is fixed to the ground, whilst we monitor your heart rate and oxygen levels (finger clip). We will ask you to breathe through a mouth piece and wear a nose clip. You will be asked to pedal at a comfortable speed and to keep it up. The pedalling will get harder and harder and we will ask you to carry on until you either cannot keep up or you feel exhausted. We will ask you to stop if your oxygen level falls. Once the test is complete you will have a 2 minute cool down period, and we will continue to monitor your oxygen levels until they return to back to normal.

- *Sweat Test:* Although we are not in doubt about your diagnosis, if we can not find a document showing your blood test results, or your sweat test result we would like to perform one as part of the trial. An area of the skin on your arm will be cleaned and 2 electrodes will be attached with straps. One of these contains a gel which stimulates sweat and the medication is applied to the skin by a weak current; you will feel a little tingling but this is not painful. Following this a collection device will be attached to the skin surface and the sweat collected over a 30 minute period. This will then be analysed by the laboratory for the high levels of salt seen in CF.

- *Activity monitor:* this is a small band worn on your upper arm that collects step and movement data. You will be asked to wear this for 7 full days after 3 of the study visits.

- *Chest computed tomographic (CT) scan:* CT uses x-rays to create detailed images of the lungs. It is a good measure of how CF affects the shape of the airways. For each scan you will be asked to lie still on a table which will move slowly through the centre of a large x-ray machine, but at no time will you be in an enclosed space. You will be able to talk to the CT staff if you need to. You will be given breathing instructions at the time of the scan. No injections are involved and the test is completely painless.

- *Transfer factor (TLCO)*: this is a lung function test which measures how well gas travels across the lungs into the blood. You will probably have had this test before, as part of your annual assessment. You will be asked to wear a nose-clip and to breathe out as far as you can through a mouth-piece. You will then breathe in, as far as possible, a mixture of air and a low concentration of a 'tracer' gas and hold your breath for 10 seconds, before breathing out as far as possible. This will be repeated up to 3 times with rest periods in between tests.

- *Sputum sampling:* we will ask you to try and cough up around a teaspoon of sputum. At one of your first visits and one of your follow-up if you can't cough up sputum we will ask you to breathe in a salty solution which makes this easier. We will give you some ventolin beforehand to stop you becoming wheezy but if you feel tight, tell us. We will check your lung function through the test and stop if it falls. If we don't manage to get any sputum at the end of this, we will ask you to do a cough swab. For some visits, we will ask you to collect all your sputum for the 24 hours before you come and bring this with you.

- *CF specific quality of life questionnaire:* we will ask you to complete some questions about how you are feeling, which takes around 10 minutes.

- *Diary card:* we will ask you to write down at home when you are feeling unwell or when your treatments change. You may want to do this with a parent.

- *Home lung function:* we will show you how to use a small lung function machine at home which stores the results inside. We can then put the results onto a computer the next time you come to the hospital.

- *Bronchial blood flow measurement:* This test will just be for the people in London only. It is designed to look at blood flow to the airways, which is often increased in people with lung disease. It involves you breathing a small amount of a special gas combination (once again, you cannot smell this and it is harmless). You will be sitting down, wearing a nose clip and breathing in and out through a mouthpiece. After several normal breaths you will be asked to hold your breath for a fixed period (either 8 or 16 seconds) before breathing out again slowly. This will be repeated 10 times with a gap (3-4min) between each to allow us to save the data

and reset the equipment. Your heart rate and oxygen levels will be monitored during the test. The whole test takes about 45 minutes to complete

☐ *Nasal potential difference (Nasal PD) and nasal brushings: London subgroup only - see Appendix 1*

☐ *Bronchoscopy: London subgroup only - see Appendix 2*

What other medicines can I have instead?

You probably know a lot about your CF and the types of medicines you normally take. You will continue on these whether or not you decide not to take part in this trial. Any other decisions about changing your medicines will be made by your usual CF team.

What are the side effects of the medicines and might I get some if I take part in the research?

We have chosen a dose that we hope has the least chance of side effects, but at higher doses this medicine did make some people feel a little fluey- they had a temperature for a few hours and felt a bit shivery. This is why we ask all patients to take some paracetamol after receiving the medicine. Most people also have a slight drop in their lung function, but they felt fine in themselves at this dose. If you do get any side effects, please tell your Mum or Dad, who will report them to the study team. Do not worry about telling us about any side effects, it will not mean that we will automatically take you off the trial.

What are the possible benefits of taking part?

We don't know if being in this study will help you, but what we find out will help other people with cystic fibrosis. If we show that gene therapy can make people with CF better, we will work hard towards future trials which could lead to it becoming available as a treatment.

What happens if the research project stops?

When the study stops you will be looked after as usual by your hospital CF team and continue to take your usual treatments.

What happens if new information about the research medicine comes along?

Sometimes during research, new things are found out about the research medicine. Your doctor will tell you all about it if this happens. What is best for you might be:

• To carry on as before
• To stop taking part and go back to your usual treatment

What if there is a problem or something goes wrong?

If you have any concern about any aspect of this study you should speak to your study doctor who will do their best to answer your questions.

Will anyone else know I am doing this?

We will keep all your information confidential. This means that we will only tell those people who have a right or need to know. Wherever possible, we will only send out information that has your name and address removed. We will let your family doctor (GP) know that you are taking part in the study.

What will happen to any samples I give?

Samples such as blood, sputum, urine will be labelled with a special code, used for testing and stored for future tests. Some of the time we will be sending the samples out to other groups working with us; they will only have the code numbers and would not be able to tell that the sample was from you.

Who is organizing the research?

This study is being organized by the UK CF Gene Therapy Consortium.

Who has reviewed the study?

Before any research goes ahead it has to be checked by a Research Ethics Committee. They make sure the research is fair. This project has been checked by the Gene Therapy Advisory Committee, which specialises in trials such as this.

Can I ask questions?

You can ask questions about the study now. You can ask questions about the study whenever you want to. If you forget to ask a question and think of it later, you can call the doctor or ask your Mum, Dad or guardian to call the phone numbers below. You can ask the next time you see your doctor if you want to.

For further information please contact:

Royal Brompton Hospital, London
Clare Saunders (Clinical Trial Co-ordinator) – Tel: ███████████████
cfgenetherapy@rbht.nhs.uk
Prof Jane Davies - Tel: ████████████
Prof Eric Alton - Tel: ██████████████████

Royal Hospital for Sick Children, Edinburgh
Dr Steve Cunningham – Tel: ████████████
Dr David Armstrong – Tel: ██████████████████
Kay Riding (Senior Research Nurse)- Tel: ██████████████
Maria Dewar (Research Manager) – Tel: ██████████
Lothian.cfgtscotland@nhs.net

Appendix 1
Nasal dosing Subgroup

A group of at least 24 patients in the trial will be asked to have doses sprayed into their nose as well as the nebulisers. Because the nose is easy to measure from, we can get extra valuable information from this. *This is optional and you do not have to take part in this substudy if you do not wish to.*

Nasal Dosing: You will be taught how to use the nasal spray and will be asked to squirt one spray into each nostril every 5 minutes; the entire 2 mls will take around 1 hour to administer and will be given at the same time as your nebulised dose, during the 2 minute breaks.

Nasal potential difference (nPD): These are measurements made in the nostril and help us to know how well the gene therapy is working. The test does not hurt; some children have this done if we are finding it difficult to decide whether they have CF and they don't usually mind. We will do this three times before your first dose (this is because we know the measurements can change quite a bit and we want to be as certain as possible that any changes we see are due to the gene therapy and not just these normal changes), and after the sixth and twelfth doses. We would also like to do tests after the third, sixth and ninth doses if you have time and don't mind. We rub a small area on your arm and then attach a wire with some cream and tape it on. A soft tube is then put into your nostril and taped in place. Whilst you lean forward over a bowl, some salty solutions pass through the tube and out of your nose; we watch the response of your nose to this on a portable computer. The whole test takes approximately 20 minutes. We may wish to perform this on both nostrils (one after the other) depending on the results. There are no serious side effects but you might get a slight salty taste from the solutions in your nose or feel like sneezing.

Nasal brushing: On two visits only, we will take some cells from the lining of your nose with a small brush. This is not particularly nice but only takes about 5 seconds. We can get a million cells by doing this, which really helps us to find out how well the gene therapy is working.

If you don't want to have the nasal test but would not mind taking part in the bronchoscopy subgroup we can still include you in the nasal dosing group and perform only a single nasal test and brushing, once before and once after dosing whilst you are under anaesthetic.

Appendix 2
Bronchoscopy Subgroup

A bronchoscope is a flexible telescope which allows the doctor to look down into your lungs, take samples and make measurements. It is done whilst you are asleep under a general anaesthetic. In this trial a group of at least 24 patients will be asked to have two of these tests, one before the first dose of gene therapy / placebo and the second at the end of the trial. The tests will allow us to learn how much gene we need to replace in order to achieve health benefits. *As with the nasal group, this is optional and you do not need to take part in this subgroup if you do not want to.*

We will take 2 small biopsies (pieces of the lining of the breathing tubes, about the size of a pinhead) and 10 airway wall brushings from one lung; we will also make measurements with a salty solution that will tell us how well the gene therapy is working. You will not feel any of this as you will be asleep and they are very safe procedures. You might notice you cough up tiny flecks of blood in your sputum afterwards for a day or two. This is nothing to worry about.

You will be asleep for around 45-60 minutes. Afterwards we will give you a dose of antibiotics through a cannula that you will have had put in whilst you're asleep. When you're fully awake, this can come out.

We will keep an eye on you for 4 hours afterwards. Most likely you will then feel fine and be able to go home. The side effects you might have are
- Drowsiness after the anaesthetic – this should wear off after a few hours
- A mild, sore throat for a day or so
- A temperature (this will be made less likely with the use of an IV antibiotic at the end of the procedure)
- A small amount of blood (specks) may come up when you cough for a day or so due to the biopsies

Many thanks for considering these additional tests; please feel free to ask any questions you may have.

Appendix 2 Dose preparation sheet

Investigator's Brochure Appendix　　　　　　　**EudraCT: 2011-004761-33**

Investigator's Brochure Appendix VII

Investigational product dose preparation sheets can be found below:

Investigational Product   **pGM169/GL67A**

EudraCT: 2011-004761-33   **Dose Preparation Overview v01**

Dose Preparation Overview

pGM169/GL67A or 0.9% (w/v) NaCl placebo will be administered to the nose and/or the lungs of trial subjects. Randomisation of trial subjects to pGM169/GL67A or 0.9% (w/v) NaCl placebo is co-ordinated by the Imperial College Trials Unit.

For nasal administration, the nominal dose administered will be 2mL pGM169/GL67A or placebo. For lung administration, the nominal dose administered will be 5mL of pGM169/GL67A or placebo.

Sterile 0.9% (w/v) NaCl saline solution for intravenous infusion is supplied by pharmacy and used to prepare nasal and lung placebo doses as appropriate.

pGM169/GL67A is supplied by the sponsor in single-use packages sufficient to prepare a 10mL aliquot of pGM169/GL67A.

One 10mL aliquot of pGM169/GL67A is used to fill a nasal spray device sufficient to deliver the 2mL necessary for nasal administration.

One 10mL aliquot of pGM169/GL67A is used to prepare up to two (2) 5mL lung doses. Thus up to two lung doses may be prepared from one single-use package.

Investigational Product   pGM169/GL67A   Patient ID: _____

EudraCT: 2011-004761-335 Preparation of 10mL pGM169/GL67A Worksheet v01 Date: _____

Preparation of 10mL pGM169/GL67A Worksheet

Final Material For Clinical trial use only

Date:		Patient ID:	
Pharmacy Batch Number:		10ml Aliquot Number:	

Data entry by:	Data checked by:

Time box removed from freezer: _____hrs	
Time recorded by:	Time checked by:

Raw Material	Manufacturer	Form	Batch number	Retest or expiry date	Quantity	Assembled by	Check by
pGM169/GL67A	NHSBT/CBC	Pouch					
pGM169/GL67A	NHSBT/CBC	Box					
pGM169 (Blue crimp)	VGX	vial					
GL67A (Red crimp)	Octoplus	vial					
Water For Injection		Ampoule					
Container	**Manufacturer**	**Form**	**Batch number**	**Expiry date**	**Quantity**	**Assembled by**	**Check by**
5ml Syringe	BD	Syringe					
1ml Syringe	BD	Syringe					
Interim container Double lumen syringe		Barrel					
		Plunger					
		Mixer					
Final container		Universal tube (Sterilin)					

Cleaning log checked by:	Temperature log checked by:
Approval to proceed: Pharmacist/Senior Technician	

Isolator is clean and free of items not required for this procedure.

Signed:_____

Investigational Product   pGM169/GL67A   Patient ID: _____

EudraCT: 2011-004761-335 Preparation of 10mL pGM169/GL67A Worksheet v01 Date: _____

Method Step 1- Prepare Pneumatic mixer

1) Follow SOP CFGT/SOP/601/SD LMD2 Pneumatic mixer: Open cylinder valve with gas spanner to pressurise regulator. Ensure adequate cylinder pressure (>70 psi) before commencing studies.

Pressure adequate   Yes   _____check

                                                           No   _____check

Method Step 2- Hydration of GL67A

2a) Allow vials to warm to room temperature for 10-15 minutes.

Time vials at room temperature: Time removed from freezer (from page 1):   _____ hrs

                                                       Time ready to start: _____ hrs

Timings recorded by:	Timings checked by:

If time ready to start <10minutes then wait until 10 minutes has elapsed.

2b) Add 5.25 ml of sterile water for injection to the GL67A vial through the septum using a sterile green 21G butterfly needle and a 5 ml and 1 ml syringe.

Volume measured by _____ Volume checked by_____

2c) As per SOP CFGT/SOP/603/SD GL67A Hydration, place the GL67A vial firmly in rack of the vortexer. Set the speed to 2500 and the time to 40 minutes and then switch on the vortexer.

2d) Vortex the vial of GL67A on a setting of 2500 for forty (40) minutes.

2e) During hydration of GL67A (red crimp vial) in the vortexer, occasionally gently shake the vial of pGM169 (blue crimp vial) to encourage thawing.

2f) Remove vial of GL67A (red crimp vial) from vortexer and visually inspect to confirm that the material is completely dissolved (no white residues on the bottom of the vial, no visible lumps of material in suspension).

Note that in some cases it may take longer than 40 min for the lipid to dissolve completely. If lipid is not completely dissolved, use the vortexer as described for additional 10 minute cycles until the material is completely dissolved. If total vortexing material exceeds 100 minutes, discard GL67A

Additional Vortex start time: _____ am / pm

Final Vortex stop time: _____ am / pm

Total vortexing time: _____ minutes

Timings recorded by:	Timings checked by:

Investigational Product   pGM169/GL67A   Patient ID: _____

EudraCT: 2011-004761-335 Preparation of 10mL pGM169/GL67A Worksheet v01 Date: _____

Method Step 3- Formation of pGM169/GL67A

3a) Visually inspect appearance of solution in pGM169 (blue crimp) vial to confirm that the material is completely thawed (no visible lumps of material in suspension).

Note that in some cases the pGM169 may not be completely thawed. If pGM169 is not completely thawed, gently shake vial to encourage thawing until there are no visible lumps of material in suspension.

GL67A and pGM169 solution homogeneous by visual inspection:

Assessed by:	Checked by:

3b) Check calibration of the pneumatic mixer is within acceptable limits (SOP CFGT/SOP/601/SD LMD2 Pneumatic mixer).

Adjustment required: _____ Yes

                                                        _____ No

Assessed by:	Checked by:

3c) Fill double lumen syringe as described in SOP CFGT/SOP/602/SD Preparation of pGM169/GL67A complexes with five (5) ml of solution from GL67A (red crimp) vial through the septum using a sterile 16 gauge needle and sterile 5 ml syringe and transfer to lumen two (2) of double lumen syringe.

Volume measured by:_____ Volume checked by:_____

3d) Fill double lumen syringe as described in SOP CFGT/SOP/602/SD with five (5) ml of solution from pGM169 (blue crimp) vial through the septum using a sterile 16 gauge needle and sterile 5 ml syringe and transfer to lumen one (1) of double lumen syringe.

Volume measured by:_____ Volume checked by:_____

3e) Insert double lumen syringe into pneumatic mixer as described in SOP CFGT/SOP/602/SD, place 20 ml steralin immediately below the protruding static mixer element to collect mixed formulation and operate as described in SOP CFGT/SOP/602/SD.

Activate static mixer: _____ Check

3e) Record time preparation was completed: _____ hrs

Time recorded by:	Time checked by::

Method Step 4- Labelling of pGM169/GL67A investigational product

4a) Calculate and record dosage expiry time.

Dosage expiry time = time preparation was completed (recorded at 3e) plus 4 hours.

Record dosage expiry time: _____ hrs

Time calculated by:	Time checked by:

Investigational Product   pGM169/GL67A   Patient ID: _____

EudraCT: 2011-004761-335 Preparation of 10mL pGM169/GL67A Worksheet v01 Date: _____

4b) Label Sterilin Universal tube containing final dosage form with label adhering to following format:

Master Label:	Sample Label:
pGM169/GL67A Batch: 10mL Patient ID: Expiry time: Date: 10ml Aliquot number: Room temperature storage Clinical trial use only	

4c) Confirm final dosage form labelling and release:

Labelling

Number of labels issued		*Number of units expected*	
Labels issued by		Units prepared by	
Labels checked by		Units checked by	
Number of labels used		Number of units prepared	
Number of samples used		Number of units issued	
Discrepancy		Discrepancy	
Explanation for discrepancy		Explanation for discrepancy	
Label reconciliation Completed by		Product reconciliation Completed by	

Product Release

Number of units for inspection	
Number of units rejected	
Discrepancy	
Comment	

Pharmacists inspection completed: Name	Date
Releasing Pharmacists sign off: Name	Date

Investigational Product   **pGM169/GL67A**
**EudraCT: 2011-004761-33**   **CFGT/SOP/601/MD UKCFGT LMD-2 Pneumatic Mixer v01**

SOP: CFGT/SOP/601/MD

LMD-2 Pneumatic mixer

Introduction

The LMD-2 pneumatic mixer device is a specialised piece of equipment designed specifically for use within the UK Cystic Fibrosis Gene Therapy Consortium to enable rapid and reproducible mixing of lipid and DNA components for clinical applications. The device consists of a fully adjustable pneumatic piston that can be used in conjunction with a dual lumen syringe and static mixer to achieve controlled and reproducible mixing of plasmid DNA and GL67A components of gene transfer formulations.
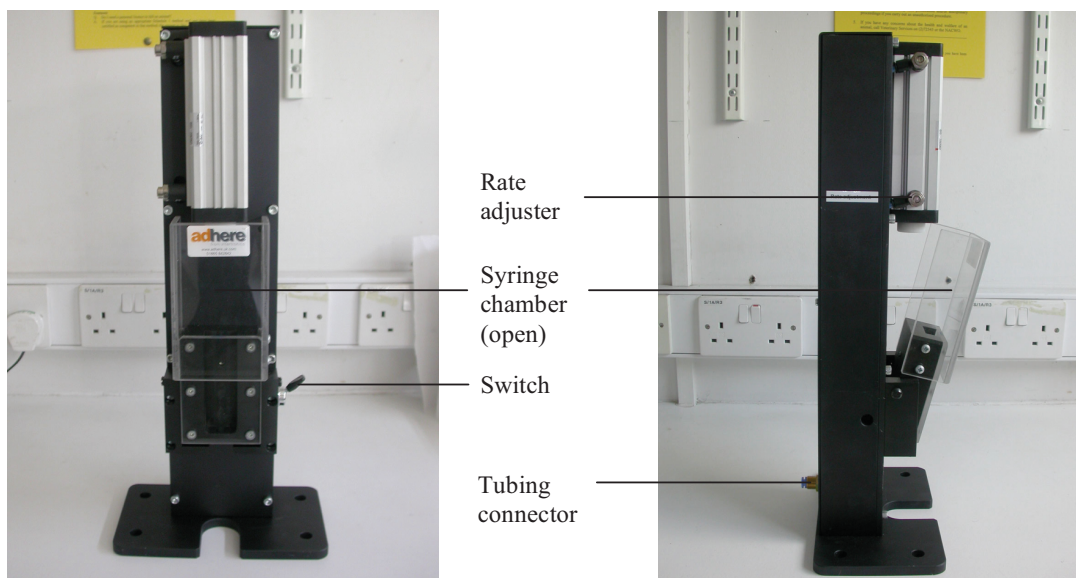
Procedure
1. LMD-2 Setup

1.1   Required apparatus

LMD-2 pneumatic mixing device (Intertronics Ltd)
Compressed air/nitrogen cylinder capable of 50 psi output pressure
Suitable gas regulator with G3/8 male output connector
G3/8 to G1/4 steel sleeve connector (RS Components Cat 367-5590)
G1/4 to 6 mm push-fit straight connector (RS Components Cat 726-718)
6 mm nylon pressure tubing (RS Components Cat 726-718)
Gas joint sealing tape (RS Components Cat 231-964)

Setup

Attach gas regulator to suitable compressed air/nitrogen cylinder and open cylinder valve with gas spanner to pressurise regulator. Ensure adequate cylinder pressure (>200 psi) on the right hand dial before commencing studies. If pressure is too low then replace cylinder.

Connect 6mm pressure tubing to regulator via G1/4 to 6 mm push-fit connector and G3/8 to G1/4 steel sleeve connector.
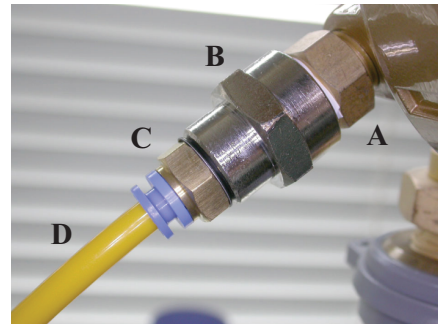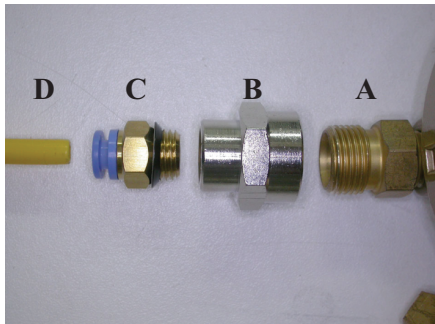
Wrap one layer of gas joint sealing tape around G3/8 male thread on regulator outlet connector (A)

Thread G3/8 to G1/4 steel sleeve connector (B) onto regulator outlet (A) and tighten into place with 24 mm spanner

Thread G1/4 to 6 mm push-fit connector (C) into G3/8 to G1/4 steel sleeve connector (B) and tighten into place with 16 mm spanner

Insert length of 6mm pressure tubing (D) into G1/4 to 6 mm push-fit connector (C). Push firmly into place and check that it is secure by gently trying to pull the tubing free

If the tubing needs to be removed, press firmly down on the blue securing ring at the connector outlet whilst pulling the tube free. This does not require a great deal of force and if there is considerable resistance ensure that the securing ring is depressed sufficiently. Do not try to force the tubing out as this will result in damage to the connector.

Insert the other end of the 6mm pressure tubing into the 6 mm push fit connector located at the base of the rear of the LMD-2 (see below). Ensure secure connection as above.
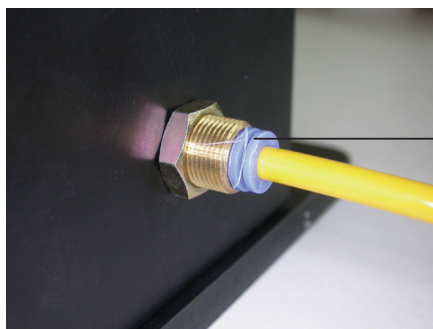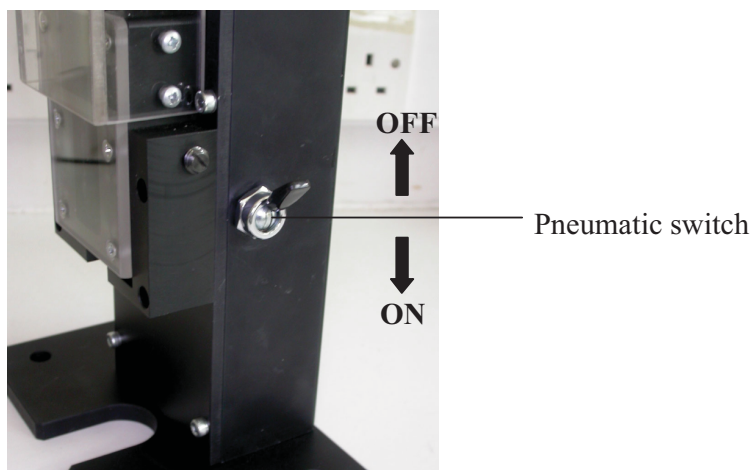

Rear tubing connector

Close syringe chamber door on front of LMD-2 by pushing it flat against the body of the device and ensure pneumatic switch is in the OFF (switch upwards) position (see below).

Investigational Product　　**pGM169/GL67A**
EudraCT: 2011-004761-33　　**CFGT/SOP/601/MD UKCFGT LMD-2 Pneumatic Mixer v01**
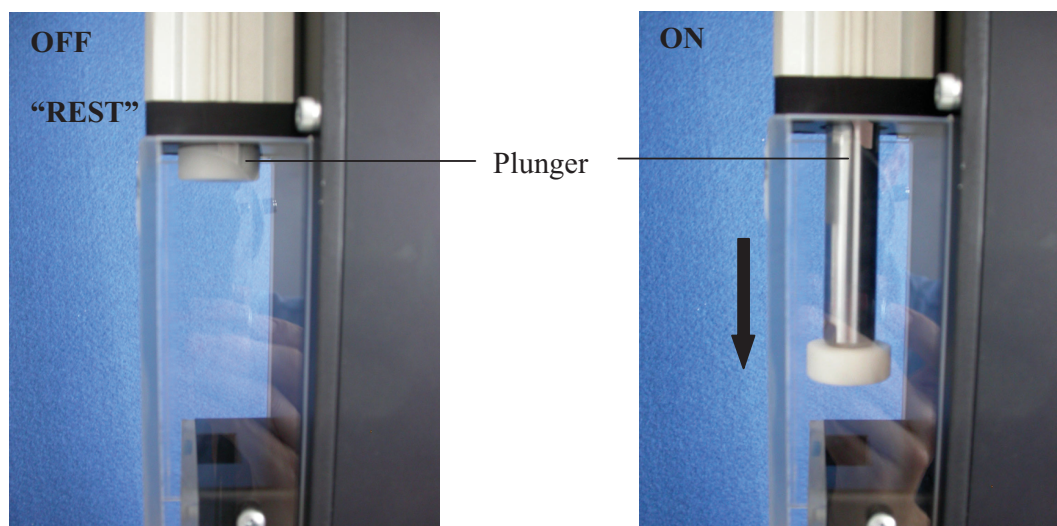

Pneumatic switch

pressurise LMD-2 by opening gas regulator tap (turn clockwise) and turning until output pressure on regulator reads 50 ± 5 psi (left hand dial).

Check for any leaks evident as "hissing" or obvious escape of gas from connections and tubing. If leaks are present, turn off the regulator (turn handle anti-clockwise), allow any gas within the system to escape and then tighten affected connector or apply sealing tape where necessary. Re-pressurise system.

LMD-2 is now charged and ready to use

2. Operation and calibration of LMD-2

2.1　The LMD-2 device utilises gas pressure from the compressed air cylinder to drive a plunger that in turn depresses the plunger on the dual syringe mixing device.

2.2　With the switch in the OFF position the pneumatic plunger remains in its "REST" position at the top of the syringe chamber (see below).

Plunger

2.3   To activate the device, ensure that the syringe door is closed and depress the switch to the ON position (down). The plunger will descend through the syringe chamber until it reaches the bottom.

2.4   IMPORTANT – the plunger will not automatically return to the top of the chamber. Once the plunger reaches the bottom of the chamber you MUST immediately deactivate the device by lifting the switch to the OFF position (up) to prevent damage to the device. This will also return the plunger to the "REST'' position.

2.5   A flow regulator valve positioned on the left hand side of the mixer unit determines plunger speed. Regulator valve is labelled "Rate adjustment >" (see below) and is adjusted using a flat screwdriver.

Rate adjustment valve

2.6   To adjust the plunger speed insert screwdriver into slot on regulator valve and turn clockwise for SLOWER and anti-clockwise for FASTER plunger movement.

2.7   To determine the plunger velocity use a stopwatch accurate to $1/100^{th}$ second to time a full depression from the top of the chamber to the bottom with no mixer syringe inserted into device.

2.8   With plunger in the "REST" position and switch turned OFF. Activate device and start stopwatch. When plunger reaches the bottom of the chamber stop stopwatch and record time. Turn switch OFF to return plunger to "REST" position.

2.9   Adjust regulator valve until desired plunger velocity is achieved. A turn to clockwise will slow the velocity.

2.10   All mixer studies should be carried out with plunger taking 4 ± 0.5 s to travel from the top of the chamber to the bottom. This range is equivalent to mixing speeds of 2.1 to 2.7 ml/s/syringe.

2.11   Plunger velocity should be confirmed and adjusted, if necessary, prior to each use.

3. Disassembly of the LMD-2

3.1 The LMD-2 can be left pressurised between mixing runs. However, it is recommended that it should be depressurised if it is to be left unused for periods greater than 2 hr.

Investigational Product   pGM169/GL67A
EudraCT: 2011-004761-33   CFGT/SOP/601/MD UKCFGT LMD-2 Pneumatic Mixer v01

3.2 To depressurise the LMD-2 turn off the gas regulator on the cylinder by turning the handle anti-clockwise.

3.3 NOTE – output pressure on regulator will still read approximately 50 psi as the tubing between the regulator and the mixing device REMAINS PRESSURISED.

3.4 To release pressure in tubing repeatedly activate/deactivate the LMD-2 several times.

3.5 Upon each activation the measured pressure on the regulator output dial will fall as gas within the system is purged.

3.6 Repeat until regulator output dial reads 0 psi.

3.7 It is now safe to disconnect tubing if required or simply re-pressurise system for next mixing experiments.

SOP: CFGT/SOP/602/MD

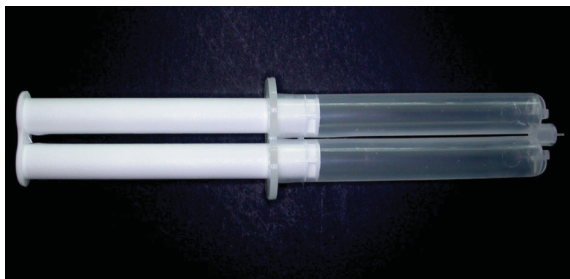**Preparation of pGM169/GL67A complexes with the LMD-2**

Required apparatus
LMD-2 pneumatic mixing device setup and calibrated as described above
Dual lumen syringe barrels (Plas-Pak Industries Cat 14B35(S505A)
Dual lumen syringe plungers (Plas-Pak Industries Cat 14C35(S446)
Eight element static mixers (Plas-Pak Industries Cat 003M08B005-3(S732)
16G disposable needles with Luer fitting hub (Sigma Cat Z118036)
5 ml sterile disposable syringes with Luer fitting hub
Thawed vial of pGM169 plasmid
Thawed and re-suspended vial of GL67A
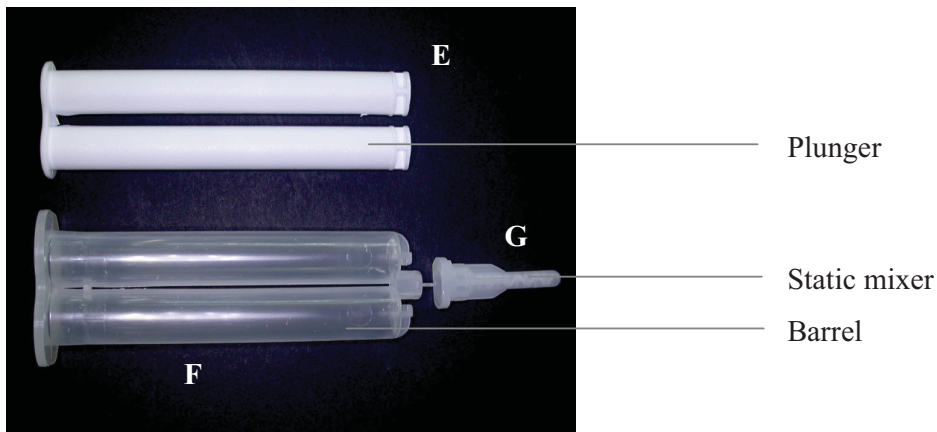
Method
1. For preparation of 10 ml aliquot of pGM169/GL67A complexes.
2. Fully insert a single dual lumen syringe plunger (E) into a single dual lumen syringe barrel (F).
3. Withdraw plunger until end of plunger approaches the end of the syringe barrels. At this point there will be resistance to any further withdrawal of the plungers and the syringe barrels will be filled with air (see below).

4. Hold dual lumen syringe assembly with the syringe barrels uppermost and the plunger pointing downwards. Support in this inverted position.

Investigational Product   pGM169/GL67A
EudraCT: 2011-004761-33   CFGT/SOP/602/MD Preparation of pGM169/GL67A Complexes v01

5   Attach a 16G needle to a disposable 10ml syringe and take up 5ml of room air into the syringe barrel.

6   Holding a vial of pGM169 upright, insert the needle through the top of the rubber stopper on the vial and inject the 5 ml of air into the vial. Hold the needle firmly in place to resist the increased pressure in the vial trying to push it out.

7   With the syringe now emptied, carefully invert the vial with the needle and syringe still *in situ*. Keeping the tip of the needle below the surface of the DNA solution, withdraw 5 ml of pGM169 into the syringe.

8   Remove the needle and syringe from the DNA vial and insert into one barrel of the dual lumen syringe assembly via the small hole at the tip of the barrel (see below).


16G syringe needle

Syringe exit hole
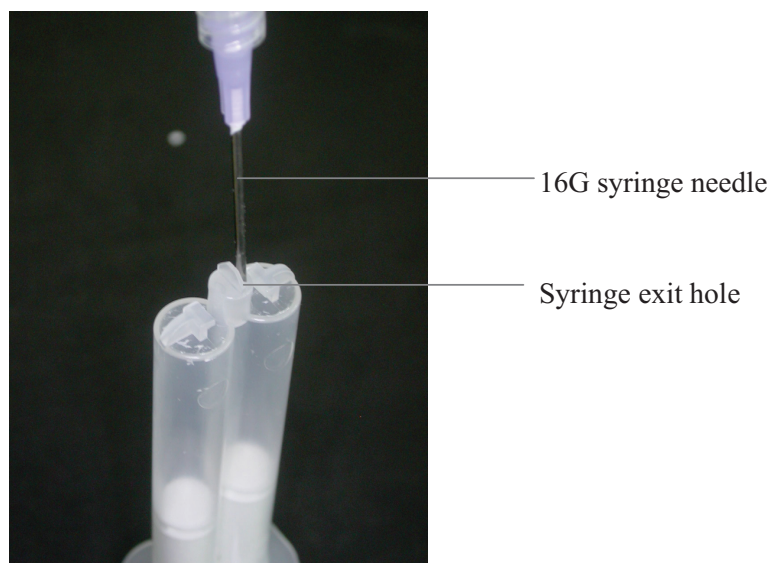
9   Carefully inject 5 ml of pGM169 into the syringe barrel.
10   Remove and discard 5 ml syringe and 16G needle.
11   Repeat the procedure using a fresh 16G needle and 5 ml syringe to transfer 5 ml of re-suspended GL67A from the vial to the other barrel on the dual lumen syringe.
12   Whilst keeping the dual lumen syringe inverted, attach the 8 element static mixer (G) to the end of the dual lumen syringe by pushing into place and then rotating the mixer 90° clockwise to lock into position beneath plastic retainers.
13   It is now safe to hold the dual lumen syringe assembly containing 5ml of pGM169 and 5ml of GL67A vertically with the static mixer pointing downwards. Some air (1-2ml) will remain within the syringe barrels. This is normal and important to allow complete emptying of the syringes upon activation of the mixer.
14   Check calibration of LMD-2 is within acceptable limits (see setup and calibration section of SOP: CFGT/SOP/601/MD).
15   Open the syringe chamber door of the pressurised and calibrated (see SOP: CFGT/SOP/601/MD) LMD-2 by pulling the top of the anterior clear perspex panel gently forwards – you will hear a small escape of gas as you open the door. This is normal and prevents activation of the device whilst the door is open.

16   Insert the dual syringe assembly into the syringe chamber such that the tip of the static mixer protrudes through the hole at the bottom of the chamber (see below).

Syringe chamber door
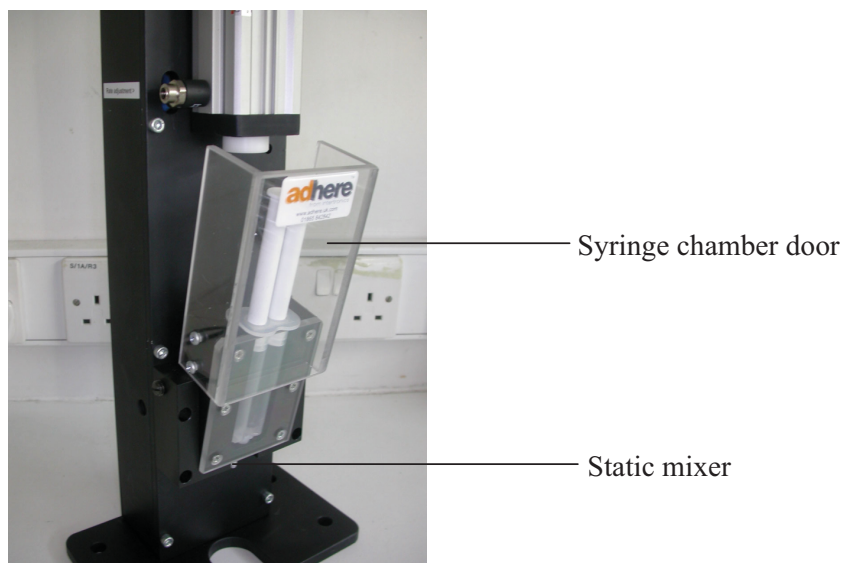
Static mixer

17   Close the syringe door by pushing it gently backwards until it clicks into place. The device is now primed and ready for operation.

18   Place a sterile 20ml Sterilin tube immediately below the protruding static mixer element to collect mixed formulation (see below).
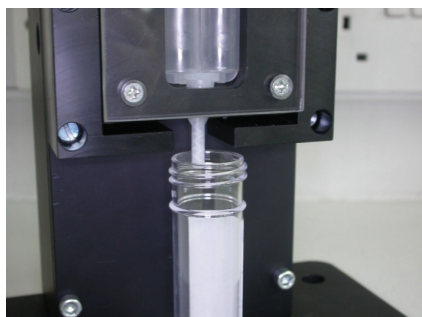
19   Activate pneumatic mixer by turning the switch on the right hand side of the device to the ON position (down). The pneumatic plunger will descend and push the dual plunger of the dual lumen syringe assembly. Mixed pGM169/GL67A complexes will be ejected from the static mixer into the 20 ml Sterilin collection tube.

20   When the dual syringe has been fully depressed, quickly return the switch to the OFF position (up) to return the pneumatic plunger to the "REST" position.

21   Screw cap into place on 20 ml Sterilin tube and store at room temperature for 20 min before use.

22   Open syringe chamber door and remove and discard empty dual lumen syringe. Removal is aided by pushing the static mixer vertically upwards from beneath.

23   Mixer is now ready for next sample.

Investigational Product	**pGM169/GL67A**
EudraCT: 2011-004761-33	**CFGT/SOP/603/MD Preparation of pGM169/GL67A**
Complexes v01	

SOP: CFGT/SOP/603/MD

GL67A Hydration

Required apparatus
VWR-DVX2500 vortexer
Foam rack

Method

1. Place the vial containing GL67A and water (red crimp vial) into the foam rack (Figure 1)
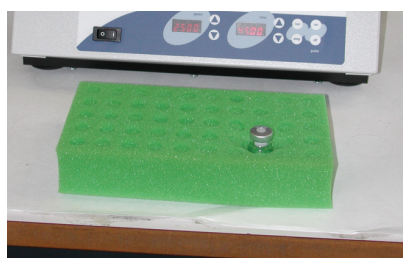

Figure 1

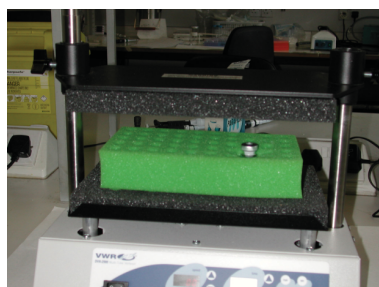2. Place the foam rack into the VWR-DVX2500 vortexer (Figure 2)

Figure 2

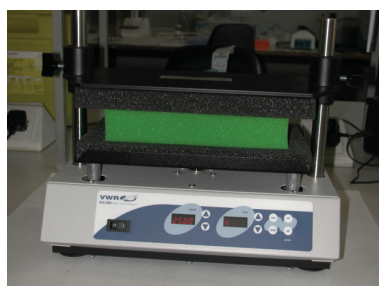3. Lower the top plate onto the foam rack and tighten screws on both sides (Figure 3)

Figure 3

4. Set to maximum speed (2500 rpm) for 40 min and press run button

Investigational Product   **pGM169/GL67A**
EudraCT: 2011-004761-33   **CFGT/SOP/603/MD Preparation of pGM169/GL67A**
Complexes v01

5. After required time unscrew top plate and lift up, remove vial and check that lipid has completely resolved (no white residues on the bottom of the vial, no visible lumps of material in suspension).

6. If the lipid is not dissolved repeat steps 1-3. Set to maximum speed (2500 rpm) for 10 min and press run button

7. Repeat step 5 and check that lipid has completely resolved (no white residues on the bottom of the vial, no visible lumps of material in suspension).

8. If the lipid is not dissolved repeat steps 1-3. Set to maximum speed (2500 rpm) for 10 min and press run button.

9. Repeat step 5 and check that lipid has completely resolved (no white residues on the bottom of the vial, no visible lumps of material in suspension).

10. If the lipid is not dissolved repeat steps 1-3. Set to maximum speed (2500 rpm) for 10 min and press run button.

11. Repeat step 5 and check that lipid has completely resolved (no white residues on the bottom of the vial, no visible lumps of material in suspension).

12. If the lipid is not dissolved repeat steps 1-3. Set to maximum speed (2500 rpm) for 10 min and press run button.

13. Repeat step 5 and check that lipid has completely resolved (no white residues on the bottom of the vial, no visible lumps of material in suspension).

14. If the lipid is not dissolved repeat steps 1-3. Set to maximum speed (2500 rpm) for 10 min and press run button.

15. Repeat step 5 and check that lipid has completely resolved (no white residues on the bottom of the vial, no visible lumps of material in suspension).

16. If the lipid is not dissolved repeat steps 1-3. Set to maximum speed (2500 rpm) for 10 min and press run button.

17. Repeat step 5 and check that lipid has completely resolved (no white residues on the bottom of the vial, no visible lumps of material in suspension).

18. Discard GL67A, if the total vortexing time exceeds 100 min.

Appendix 3 Blinding protocol

Blinding Procedure – How to tape the nebulisers
SOP Reference: CFGT/SOP/638/MDT
Version Number: Final Version 2; 01 Jun 2013
Effective Date: 01 Jun 2013
Last Review: 01 Jun 2013
Author: Katie Bayfield, Paula Carvelli, David Armstrong
Approved by (Edinburgh):
Signature:
Date:
Approved by (Imperial):
Signature:
Date:
Approved by (Oxford):
Signature:
Date:

Amended Version	Date (DD/MON/YY)	Outline of Change	Page with change
1	17 Apr 2012	Slight change of method of taping	3

CFGT/SOP/638/MDT version 2

CFGT SOP

OUTLINE OF PROCEDURE

PRINCIPLE
The multidose trial (MDT) will be placebo controlled; therefore all aspects of the trial where a patient or staff member could be un-blinded to the product or placebo must be monitored and controlled, including delivery of the pots to and from pharmacy. This protocol also reduces infection risk for the patient.

OBJECTIVE
1) To correctly and appropriately tape the nebuliser pots so that a patient or staff member cannot be un-blinded to product or placebo inside.
2) To ensure the nebulisers and nasal spray pots are infection free.
3) To ensure that the nasal pot does not leak.

SECTION 1 Summary of health and safety

GENERAL The main risks involved in this protocol are:
N/A

PROTECTIVE EQUIPMENT
N/A

EQUIPMENT
- Nebuliser packing (nebuliser pot + nebuliser tubing)
- White electrical tape
- Gloves
- Lab Coat
- Extractor Hood
- Scissors
- Filter paper
- Falcon pot – Blue max 17 x 120mm style
- Antibacterial wipes

SPILLAGE PROCEDURES
N/A

WASTE DISPOSAL
General waste

CFGT/SOP/638/MDT version 2

SECTION 2 Protocol

1. Put on all personal protective equipment.
2. Switch on the extractor hood.
3. Ensure all of the equipment required is at hand.
4. Wipe the surface with an antibacterial wipe and cover with a sterile utility drape/clean surface cover.
5. Open the nebuliser packaging and remove the nebuliser.
6. Dispose of the mouthpiece and retain the packing with nebuliser tubing in a box (the Pari Filter/Ventil set will be used to dose the patients – not this mouthpiece).
7. Wipe the nebuliser with antibacterial wipes and leave to dry.
8. Fold a piece of opaque circular filter paper into 4 'pie' segments and cut the end off to reveal a hole in the middle (see figure 2).
9. Place the filter paper into the end of the nebuliser.
10. Tape the bottom of the nebuliser with 4 strips of tape ensuring that a hole is left in the middle (see figure 3).
11. Prepare strips of tape or wind the tape around the nebuliser pot starting at the bottom and working up the pot until the pot is fully covered (see figure 1). **Ensure that the tape strips always overlap, that the end of the nebuliser pot is neat and that there are no gaps**.
12. Clean the sterile tube lid (see figure 4) with the antibacterial wipe; place the tube lid into the hole of the nebuliser.
13. Complete the quality control sheet (see appendix 1) with another person and amend the nebuliser pot if necessary.
14. Place into a sealable bag and send to pharmacy.

Figure 1.   Figure 2.

Figure 3.   Figure 4.

CFGT/SOP/638/MDT version 2

Appendix 1
Quality Control of nebuliser pot taping

Lot Number	Nebuliser Box number	Person completing	Date	Reviewer	Date	Satisfactory?	Re-taped?
						Y/N	Y/N/n/a
						Y/N	Y/N/n/a
						Y/N	Y/N/n/a
						Y/N	Y/N/n/a
						Y/N	Y/N/n/a
						Y/N	Y/N/n/a
						Y/N	Y/N/n/a
						Y/N	Y/N/n/a
						Y/N	Y/N/n/a
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						Y/N	Y/N/n/a
						Y/N	Y/N/n/a
						Y/N	Y/N/n/a
						Y/N	Y/N/n/a
						Y/N	Y/N/n/a

CFGT/SOP/638/MDT version 2

Training Log

BLINDING PROCEDURE – TAPING THE NEBULISER POTS CFGT/SOP/638/MDT

Local Lead Investigators: Paula Carvelli, David Armstrong

Date	Version	Competent	Name of Investigator	Signature of investigator	Next review	Name of Reviewer	Signature of reviewer
		Yes/No					
		Yes/No					
		Yes/No					
		Yes/No					
		Yes/No					
		Yes/No					
		Yes/No					
		Yes/No					
		Yes/No					
		Yes/No					
		Yes/No					
		Yes/No					
		Yes/No					
		Yes/No					

CFGT/SOP/638/MDT version 2

Appendix 4 Cystic Fibrosis Questionnaire – Revised quality-of-life questionnaire

CFQ-UK

Adolescents and Adults (Patients 14 Years Old and Older)

CYSTIC FIBROSIS QUESTIONNAIRE - REVISED

Understanding the impact of your illness and treatments on your everyday life can help your healthcare team keep track of your health and adjust your treatments. For this reason, this questionnaire was specifically developed for people who have cystic fibrosis. Thank you for your willingness to complete this form.

Instructions: The following questions are about the current state of your health, as you perceive it. This information will allow us to better understand how you feel in your everyday life.

Please answer all the questions. There are **no** right or wrong answers! If you are not sure how to answer, choose the response that seems closest to your situation.

Section I. Demographics *Please fill-in the information or tick the box indicating your answer.*

A. What is your date of birth?

Date ☐☐ ☐☐ ☐☐☐☐
    Day Month Year

B. What is your gender?
☐ Male   ☐ Female

C. During the **past two weeks**, have you been on holiday or out of school or work for reasons **NOT** related to your health?

☐ Yes   ☐ No

D. What is your current marital status?
☐ Single/never married
☐ Married
☐ Widowed
☐ Divorced
☐ Separated
☐ Remarried
☐ With a partner

E. Which of the following best describes your racial background?
☐ White - UK
☐ White - other
☐ Indian/ Pakistani
☐ Chinese/ Asian
☐ African
☐ Caribbean
☐ Other [not represented above or people whose predominant origin cannot be determined/ mixed race]
☐ Prefer not to answer this question

F. What is the highest level of education you have completed?
☐ Some secondary school or less
☐ GCSEs/ O-levels
☐ A/AS-levels
☐ Other higher education
☐ University degree
☐ Professional qualification or post-graduate study

G. Which of the following best describes your current work or school status?
☐ Attending school outside the home
☐ Taking educational courses at home
☐ Seeking work
☐ Working full or part time (either outside the home or at a home-based business)
☐ Full time homemaker
☐ Not attending school or working due to my health
☐ Not working for other reasons

CFQ-UK

CYSTIC FIBROSIS QUESTIONNAIRE - REVISED

Adolescents and Adults (Patients 14 Years Old and Older)

Section II. Quality of Life

Please tick the box indicating your answer.

*During the past **two weeks**, to what extent have you had difficulty:*

	A lot of difficulty	Some difficulty	A little difficulty	No difficulty
1. Performing vigorous activities such as running or playing sports......................	☐	☐	☐	☐
2. Walking as fast as others	☐	☐	☐	☐
3. Carrying or lifting heavy things such as books, shopping, or school bags........	☐	☐	☐	☐
4. Climbing one flight of stairs	☐	☐	☐	☐
5. Climbing stairs as fast as others..	☐	☐	☐	☐

During the past two weeks, indicate how often:

	Always	Often	Sometimes	Never
6. You felt well	☐	☐	☐	☐
7. You felt worried	☐	☐	☐	☐
8. You felt useless	☐	☐	☐	☐
9. You felt tired	☐	☐	☐	☐
10. You felt full of energy...	☐	☐	☐	☐
11. You felt exhausted	☐	☐	☐	☐
12. You felt sad	☐	☐	☐	☐

Please circle the number indicating your answer. Please choose only one answer for each question.

*Thinking about the state of your health over the last **two weeks**:*

13. To what extent do you have difficulty walking?
   1. You can walk a long time without getting tired
   2. You can walk a long time but you get tired
   3. You cannot walk a long time because you get tired quickly
   4. You avoid walking whenever possible because it's too tiring for you

14. How do you feel about eating?
   1. Just thinking about food makes you feel sick
   2. You never enjoy eating
   3. You are sometimes able to enjoy eating
   4. You are always able to enjoy eating

15. To what extent do your treatments make your daily life more difficult?
   1. Not at all
   2. A little
   3. Moderately
   4. A lot

CFQ-UK

Adolescents and Adults (Patients 14 Years Old and Older)

CYSTIC FIBROSIS QUESTIONNAIRE - REVISED

16. How much time do you currently spend each day on your treatments?
1. A lot
2. Some
3. A little
4. Not very much

17. How difficult is it for you to do your treatments (including medications) each day?
1. Not at all
2. A little
3. Moderately
4. Very

18. How do you think your health is now?
1. Excellent
2. Good
3. Fair
4. Poor

Please select a box indicating your answer.

*Thinking about your health during the past **two weeks**, indicate the extent to which each sentence is true or false for you.*

	Very true	Somewhat true	Somewhat false	Very false
19. I have trouble recovering after physical effort	☐	☐	☐	☐
20. I have to limit vigorous activities such as running or playing sports	☐	☐	☐	☐
21. I have to force myself to eat	☐	☐	☐	☐
22. I have to stay at home more than I want to	☐	☐	☐	☐
23. I feel comfortable discussing my illness with others	☐	☐	☐	☐
24. I think I am too thin	☐	☐	☐	☐
25. I think I look different from others my age	☐	☐	☐	☐
26. I feel bad about my physical appearance	☐	☐	☐	☐
27. People are afraid that I may be contagious	☐	☐	☐	☐
28. I get together with my friends a lot	☐	☐	☐	☐
29. I think my coughing bothers others	☐	☐	☐	☐
30. I feel comfortable going out at night	☐	☐	☐	☐
31. I often feel lonely	☐	☐	☐	☐
32. I feel healthy	☐	☐	☐	☐
33. It is difficult to make plans for the future (for example, going to college, getting married, getting promoted at work, etc.)	☐	☐	☐	☐
34. I lead a normal life	☐	☐	☐	☐

CFQ-UK

CYSTIC FIBROSIS QUESTIONNAIRE - REVISED

Adolescents and Adults (Patients 14 Years Old and Older)

Section III. School, Work, or Daily Activities

Questions 35 to 38 are about school, work, or other daily tasks.

35. To what extent did you have trouble keeping up with your schoolwork, professional work, or other daily activities during the past **two weeks**?
    1. You have had no trouble keeping up
    2. You have managed to keep up but it's been difficult
    3. You have been behind
    4. You have not been able to do these activities at all

36. How often were you absent from school, work, or unable to complete daily activities during the last two weeks because of your illness or treatments?
    ☐ Always ☐ Often ☐ Sometimes ☐ Never

37. How often does CF get in the way of meeting your school, work, or personal goals?
    ☐ Always ☐ Often ☐ Sometimes ☐ Never

38. How often does CF interfere with getting out of the house to run errands such as shopping or going to the bank?
    ☐ Always ☐ Often ☐ Sometimes ☐ Never

Section IV. Symptom Difficulties

Please select a box indicating your answer.

*Indicate how you have been feeling during the past **two weeks**.*

	A great deal	Somewhat	A little	Not at all
39. Have you had trouble gaining weight?	☐	☐	☐	☐
40. Have you been congested?	☐	☐	☐	☐
41. Have you been coughing during the day?	☐	☐	☐	☐
42. Have you had to cough up mucus?	☐	☐	☐	☐
				Go to Question 44

43. Has your mucus been mostly: ☐ Clear ☐ Clear to yellow ☐ Yellowish-green ☐ Green with traces of blood ☐ Don't know

*How often during the past **two weeks**:*

	Always	Often	Sometimes	Never
44. Have you been wheezing?	☐	☐	☐	☐
45. Have you had trouble breathing?	☐	☐	☐	☐
46. Have you woken up during the night because you were coughing?	☐	☐	☐	☐
47. Have you had problems with wind?	☐	☐	☐	☐
48. Have you had diarrhoea?	☐	☐	☐	☐
49. Have you had abdominal pain?	☐	☐	☐	☐
50. Have you had eating problems?	☐	☐	☐	☐

Please make sure you have answered all the questions.

THANK YOU FOR YOUR COOPERATION!

Appendix 5 Statistical Analysis Plan

Gene Therapy CF MDT

Imperial Clinical Trials Unit

MDT GT-CF
A randomised, double-blind, placebo-controlled Phase 2B clinical trial of repeated application of gene therapy in patients with cystic fibrosis

Short title: Repeated application of gene therapy in CF patients

Chief Investigator: Professor Eric Alton

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Statistical Analysis Plan (SAP)

Non-confidential

SAP Working Group:

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Gene Therapy CF MDT

1.   Approvals

This SAP is approved by:

Name	Role	Signature	Date
10.5 Prof. Eric Alton	**10.6** Chief Investigator		
10.7 Prof. Jane Davies	**10.8** Co-investigator		
10.9 Prof. Gordon Murray	**10.10** Senior Statistician		
10.11 Dr. George Bouliotis	**10.12** Trial Statistician		
10.13 Dr Stephen Hyde	**10.14** Trial Statistician		

Gene Therapy CF MDT

Revision History

Version	Reason for Update	Date	Author
V0.0	Creation of new statistical analysis plan	13-03-2014	Gordon Murray
V0.1	Revision following teleconference on 9th April 2014 between E Alton, J Davies, U Griesenbach and G Murray	09-04-2014	Gordon Murray
V1.0	Revision following undertaking statistical analysis by George Bouliotis	20-06-2014	Gordon Murray & George Bouliotis
V 1.1	Revision with comments from both statisticians GM and GB	10-07-2014	Gordon Murray & George Bouliotis
V1.2	Revision with comments from investigators	15-07-2014	Gordon Murray & George Bouliotis
V2.0	Finalised SAP	23-07-2014	Gordon Murray & George Bouliotis
V2.1	Revision following unblinding & trial data analysis including (i) correct protocol number; (ii) clarification of the ITT and randomised populations (p.10); (iii) clarification of STATS software used (p.11); (iv) change >4 doses to ≥4 doses (Appendix B, p.16).	27-02-2015	Gordon Murray & Stephen Hyde

Gene Therapy Cystic Fibrosis MDT Trial

Gene Therapy CF MDT

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    6.3.   Demographic Characteristics

    6.4.   Extent of Exposure and Treatment Compliance

7.   Analysis Plan Details

    7.1.   Primary Outcome Measure

    7.2.   Secondary Outcome Measures

8.   Safety/Adverse Events

9.   Subgroup Analyses

10.   Changes to the Planned Analyses

    *Any item marked with an asterisk is for future 'AUC' type longitudinal analysis

    Statistical Analysis Of Nasal & Bronchial Brushing DNA & RNA Samples

Gene Therapy CF MDT

2. Study Objectives / Hypotheses Testing

2.1. Primary Objectives

   i)   To assess the clinical benefit of repeated doses of pGM169/GL67A administered to the lungs of patients with CF over a period of 48 weeks

   ii)   To assess the safety and tolerability of repeated doses of pGM169/GL67A administered to the lungs of patients with CF over the same period

   iii)   To assess gene expression of the formulation over the same period

2.2. Secondary Objectives

   i)   To assess a number of secondary outcomes related to the efficacy, safety and tolerability of the active treatment (see appendices A & B)

   ii)   To explore relationships between gene expression data (PD and mRNA) with clinical outcomes

Gene Therapy CF MDT

3.   Background / Introduction

3.1. Introduction

The Cystic Fibrosis gene therapy multidose randomised trial recruits patients in three clinical centres: *Royal Brompton Hospital* (London) and *Western General Hospital and Royal Hospital for Sick Children* (Edinburgh). The aim of this study is to investigate safety/tolerability as well as efficacy of repeated doses of pGM169/GL67A administered to the lungs of patients with CF over a period of 48 weeks, and also to assess gene expression of the formulation over the same period. The purpose of this Statistical Analysis Plan is to give a more detailed and comprehensive description of the methods for the analysis of the data, to enhance robustness, and to avoid post hoc decisions that may affect the interpretation of the results of the statistical analysis.

3.2. Study Design

This is a randomised, double-blind placebo-controlled study. Randomisation will be on a 1:1 basis, stratified for centre, age and FEV1, in multiple UK centres. There are two sub-studies integrated into the main trial, where patients are randomised 2:1 in favour of active treatment.

3.3. Study Endpoints (see appendix A for details)

3.3.1.   Primary endpoints:

3.3.1.1.   Secondary endpoints (Efficacy):

3.3.1.2.   Secondary endpoints (Safety):

3.3.1.3.   Gene expression outcomes (Nasal & Bronchoscopy Sub-studies *only*)

3.4. Treatment Groups

Administration of 5 ml pGM169/GL67A or placebo (0.9% saline) via nebuliser to the lungs every 4 weeks for 12 doses

Administration of 2 ml pGM169/GL67A or placebo (0.9% saline) via nasal spray to the nose every 4 weeks for 12 doses *(nasal sub-study only)*

Two sub-studies are proposed: one undergoing nasal dosing and assessment and the other undergoing bronchoscopic assessment. Subgroups of patients at the London site will be enrolled for gene expression measurement in both nose (Nasal sub-study: n=24) and lower airway via bronchoscopy (Bronchoscopy sub-study: n=24).

3.5. Study Population

Children (12 years and above) and adults with cystic fibrosis confirmed on standard diagnostic criteria, attending or referred into the study sites and fulfilling the inclusion/ exclusion criteria.

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3.6. Intervention being tested

pGM169/ GL67A in 5 ml dose via nebuliser every 28 +/-5 days; same as 2 ml nasal spray (subgroup only).

3.7. Sample Size

This study plans to enrol at least 130 subjects;

3.8. Schedule of Time and Events

All subjects will receive 12 doses of nebulised gene therapy at intervals of 4 weeks over a 48 week period. After dose 12 there will be 2 formal follow-up visits, at 14 and 28 days post-dose. Evaluation for all participants will be made at baseline and subsequently on a ***monthly*** *basis*, at 48 weeks and they will be followed long term.

3.9. Randomisation

Randomisation will be on a 1:1 basis, stratified for centre, age and FEV1. Randomisation in the nasal and bronchoscopy sub-studies will be 2:1 in favour of active treatment, to enrich power for the actively-treated patients. The randomisation algorithm has been developed by the Imperial CTU.

4. Analysis Datasets & Derived Variables

The primary analysis will be on a per-protocol basis. In a subsidiary intention to treat analysis, participants will be analysed in accordance with the treatment to which they are randomised, regardless of the treatment that they actually receive. The baseline will be defined as the mean of Screening and Pre-dose 1 values, and the end value will be defined as the mean of the measures obtained 14 and 28 days after the final dose

4.1. Derived Variables
4.1.1. Primary-Outcome Variables

- FEV1 percent predicted

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4.1.2.   Secondary-Outcome Variables (see appendix A & B)

4.2. Safety Variables (see appendix B)

Adverse Events

  ☐ Any adverse event listed in the related forms

Serious Adverse Events

  ☐ Serious Adverse Events (death, life threatening, prolongation of existing inpatient hospitalisation, persistent or significant disability or incapacity)

5. Statistical Analysis

5.1. Exploratory and descriptive analyses

Where possible, the relationship between the outcomes and other variables will be explored graphically. Histograms and box-plots will be used to assess the distributional assumptions and to check for possible outliers. Appropriate transformations (logarithms) will be applied, where this is useful, in order to satisfy distributional assumptions (normality). Categorical variables (binary, ordered and multinomial) will be presented in terms of frequencies and percentages, whilst continuous variables will be presented using the mean, standard deviation (SD), median, lower and upper quartiles, minimum, maximum and number of patients with an observation (n). In general, minimum and maximum will be quoted to the number of decimal places as recorded in the CRF or other appropriate source data. Means, medians, quartiles and SDs will be quoted to one further decimal place. Percentages will be rounded to two decimal places.

All applicable statistical tests will be 2-sided and will be performed using a 5% significance level, leading to 95% (2-sided) confidence intervals (CIs). No formal adjustment will be made to significance levels to allow for multiplicity, as there is a single pre-specified primary outcome measure. However, a very large number of secondary analyses (of exploratory value) are planned, and so p-values for these secondary analyses will need to be interpreted cautiously. A bootstrapped CI will be presented in some occasions, where this is useful and appropriate.

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5.2. Associational analyses/Modelling

As this is a classic multicentre placebo-controlled trial, the primary analysis will compare the two randomised groups in terms of the *mean percent change* in percent predicted FEV1 from baseline to end of treatment. We are aware of various analyses commonly employed for this task. Among them, the analysis of covariance (ANCOVA) is well established and reliable. The ANCOVA model will include baseline percent predicted FEV1 together with the other variables used in the randomisation algorithm as covariates. 'Baseline' will be taken as the average of the FEV1 values from the two pre-treatment assessments. 'End of treatment' will be taken as the average of the values taken at 14 and 28 days after final treatment. The treatment effect will be presented as an adjusted difference in mean percent change along with its corresponding 95% confidence interval. Also within-group (FEV1) change over time will be investigated. No interim efficacy analyses are planned.

5.3. Managing Missing Values

The per-protocol primary analysis of the primary endpoint will be replicated using imputation methods to allow for missing data. This will serve as a sensitivity analysis if less than 10% of outcome values are missing, or as the primary analysis if more than 10% is missing. The analysis of all other secondary outcome measures will be based only on patients with complete data.

5.4. Analysis Populations

The intention to treat (ITT) population will comprise all randomised subjects with usable follow-up (i.e. post-randomisation) data.

The per-protocol (PP) population will comprise those members of the ITT population who completed the study without a major protocol violation and who complied adequately with the randomised treatment i.e. received ≥9 doses. The PP population will be confirmed before database lock. The primary and major secondary efficacy analyses will be performed using the PP population.

The safety population will comprise all recruited patients who received at least one dose of study medication.

5.5. Baseline characteristics

These will be presented in the form of summary tables

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5.6. Quality Control (QC) of Statistical Analysis

Isolated data errors detected in the database as a result of the QC checks that are deemed significant will be submitted for enquiry to the trial manager or designee. Systematic data errors in the data reporting will be investigated further; the data will be corrected if necessary, and the appropriate table then re-checked.

A random selection of unique analysis and summary tables will be checked and validated using manual methods (e.g. comparison by a calculator, spreadsheet, database output or any alternative summarisation tool) and with differing statistical programming (e.g. Stata approaches "collapsing" Vs. "reshaping"). QC of statistical analyses will be performed by peer review of program code, log and output. The primary analysis will be replicated independently and the reasons for any discrepancies identified and resolved.

5.7. Software and Programming

Data management will use Excel; descriptive statistics and standard analyses will be performed using STATA, Prism, Minitab and/or IBM Statistics SPSS. More complex models will be fitted using R.

6. Patient Disposition, Demographics, Baseline/Clinical Characteristics

No formal statistical testing will be performed on patient disposition, or on demographic or baseline/clinical, or concomitant medication data. Summaries of patient disposition will be based on all patients and summaries of all other data described in this section will be based on the ITT population, unless otherwise stated.

6.1. Patient Disposition and Withdrawals

The number and percentage of patients randomised, dosed, completed and discontinued will be presented by treatment and overall. The number of patients discontinued early from the study will be summarised by reason for withdrawal and treatment.

6.2. Analysis Populations

A summary table will be produced detailing the number and percentage of patients in each population for each treatment and overall. The reasons for exclusion from the PP population will be included in the summary.

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6.3. Demographic Characteristics

Demographic data will be reported overall and by treatment group, stratified by whether or not included in a sub-study. Summary statistics (mean, SD, median, lower and upper quartiles, minimum, maximum and n) will be presented for age, height, weight, BMI % predicted FEV1 and FVC. Number and percentage of patients will be presented for gender, centre, CFTR mutation (classified as on pg 15) pre-specified treatments (pg 15), *P. aeruginosa* infection status (pg 15), pancreatic insufficiency, CF related diabetes and smoking history.

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6.4. Extent of Exposure and Treatment Compliance

Tabulate by treatment group (stratified by sub-study inclusion) the number of patients receiving a total of 0, 1, 2, …, 12 doses.

7. Analysis Plan Details
7.1. Primary Outcome Measure

The primary analysis will compare the two randomised groups in terms of the mean percent change in percent predicted FEV1 from baseline to end of treatment. So for example, a patient with a baseline mean percent predicted FEV1 of 60% increasing to 66% by the end of the study would be analysed as having had a 10% increase from baseline. An analysis of covariance (ANCOVA) model will be used to compare the two randomised groups with this percent increase in predicted FEV1 as the response variable. Baseline percent predicted FEV1 will be used as a covariate in the model together with the variables used in the randomisation algorithm (Age [<18 years versus ≥ 18 years on day of randomisation], Centre [Edinburgh versus London], and Stratum [Included in one or both of the gene expression sub-studies versus not], but not categorised FEV1 as this is already included as a continuous covariate).

'Baseline' will be taken as the average of the percent predicted FEV1 values from the two pre-treatment assessments (Screening and Pre-dose 1). 'End of treatment' will be taken as the average of the values taken at 14 and 28 days after the final treatment. Should any patients only have a single baseline value, or a single end of treatment value, then this value will be used in place of the mean of the two relevant values. The treatment effect will be presented as an adjusted difference in mean percent change along with its corresponding 95% confidence interval.

The primary analysis will be performed on the per-protocol (PP) population, so that all included patients will have received 9 or more doses of trial treatment. If any patients in the PP population have missing outcome data then the above analyses will be repeated after the missing values have

Gene Therapy CF MDT

been estimated using an imputation technique. The imputation will be based on the matrix of the 15 percent predicted FEV1 values (Screening; Pre-dose 1; Pre-dose 2; ...; Pre-dose 12; 14 days post-dose 12; 28 days post-dose 12) for the patients in the PP population, and will use single imputation, with the EM approach. If more than 10% of the patients in the PP population have missing outcome data then the analysis based on imputation will be taken as the primary analysis.

As a sensitivity analysis the above analyses will be repeated, but with the logarithm (base 10) of the end of treatment percent predicted FEV1 taken as the response variable, and the logarithm of the baseline percent predicted FEV1 included as a covariate in place of its raw value. The other covariates will be as described above.

As a further sensitivity analysis the primary analysis will be repeated after having excluded the small number of patients who were suffering from an acute exacerbation of their condition at the time of their end of study assessments. These patients will be identified ahead of database lock.

An exploratory analysis will compare the two groups in terms of the evolution of FEV1 over the duration of the trial. For descriptive purposes, mean, SD and n for percent predicted FEV1, stratified by treatment group and sub-study stratum, for Screening; Pre-dose 1; Pre-dose 2; ...; Pre-dose 12; 14 days post-dose 12; 28 days post-dose12 will be tabulated for both the ITT and PP populations. Profile plots will be used to display the evolution of percent predicted FEV1 for individual patients (using the ITT population).

A more formal comparison of the groups in terms of the evolution of percent predicted FEV1 over the duration of the trial will be based on an 'area under the curve' (AUC) approach. Baseline FEV1 will be defined as previously as the mean of the Screening and Pre-dose 1 values. The 'area under the curve' value will be taken as the area under the curve, using the trapezoidal rule, based on whichever of the following values are available: Pre-dose 2, Pre-dose 3, ..., Pre-dose 12; 14 days post-dose 12; 28 days post-dose 12. Where appropriate, '14 days post-dose 12' and '28 days post-dose 12' will be replaced by '14 days post-final dose' and '28 days post-final dose' respectively. The area under the curve value will be divided by the total length of time between the first and last values used for the AUC calculation, to give a mean on treatment percent predicted FEV1.

The randomised groups will then be compared formally in terms of percent change from baseline to on treatment percent predicted FEV1, using the same ANCOVA approach as was used in the primary analysis. This analysis will be performed for both the ITT and PP populations.

Finally and in addition to this and as a model validation task only for the primary outcome, we are intended to repeated the analysis but this time reflecting the repeated-measurement nature of the

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study by using the well-established random-effects models. Among various considerable benefits, repeated-measures regressions can accommodate the repeated visits, will strengthen comparisons and will allow robust predictions (e.g. random effects for sites) avoiding possibly unattainable assumptions of ANCOVA (homogeneity of slopes, compound symmetry within (measurements) correlation etc). Models' fit will be visually compared and statistically tested. Such modelling approach will help us to estimate the treatment effect more precisely after adjusting for a series of relevant covariates, both continuous and categorical and finally report estimates with reasonable confidence intervals. We anticipate that the study is not powerful enough to support those prespecified subgroup analyses and thus, mixed-effects models will provide a reasonable alternative to fixed-effects models. In any case, a trade-off between statistical robustness, estimates' precision, and simplicity will guide our model selection process. Finally, both fixed and mixed effects models will include interactions for exploring the action-mechanism of the intervention.

7.2. Secondary Outcome Measures

In general, unless specified otherwise below, the secondary outcome measures will be summarized per visit and analysed formally using analysis of covariance to compare mean values at end of treatment adjusted for baseline values, together with the variables used in the randomisation algorithm (Age [<18 years versus ≥ 18 years on day of randomisation], Centre [Edinburgh versus London], percent predicted FEV1 on day of Screening [<70% versus ≥70%] and Stratum [Included in one or both of the gene expression sub-studies versus not]).

Effects on longitudinal data will also be explored; the outcomes for which this would be relevant are marked with an asterisk in Appendices A, B & C

Variables' transformation and missing-values imputation will be applied where this is required (e.g. when severe deviation from gaussianity, and/or considerable missingness is observed) and analyses will be re-run and compared as an additional "security" procedure, especially for LCI, CT parameters and CFQR.

8. Safety/Adverse Events

These analyses will be performed on the Safety Population. As well as summarising and analysing data as for the secondary outcome measures, where appropriate outlying values of clinical relevance will be flagged. Should a safety signal be observed, AEs will be explored in more detail.

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9.　　Subgroup Analyses

As set out in Section 2 above, the study is not adequately powered to explore subgroup effects for the primary outcome measure, although we shall look at the stability of treatment effect over subgroups defined by the covariates included in the ANCOVA model (Age, Centre, Baseline percent predicted FEV1 and sub-group stratum). A formal analysis will be performed by including interaction terms in the model. A similar approach will be used with certain secondary outcome measures which are closer to the direct mechanism of action of the study intervention, as there is likely to be more statistical power with such variables to explore subgroup effects which could support a 'stratified medicine' approach to the use of gene therapy.

Predefined subgroup analysis for response:

Subjects randomised to active treatment will be further explored using the following categories for differences in response of the primary outcome :

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A. ENTRY CHARACTERISTICS

1. Severity of lung physiology at baseline (mean of pre-treatment values as used for primary outcome analysis):
    a. Mean pre-dose FEV1%:
        i. upper and lower half cut off at median
        ii. top vs bottom quartiles
    b. Mean pre-dose LCI (groups as above)
2. Predominantly small vs large airways disease as defined on pre-dosing CT:
    a. Small: air trapping + small plugs > score for bronchiectasis (severity and extent) + large plugs
3. *P. aeruginosa* positive vs negative at entry (on any of pre-dosing cultures)
4. CFTR mutations in 6 separate groups:
    a. F508del/F508del
    b. F508del heterozygotes:
        i. Class 1
        ii. Class 2
        iii. Classes 3-6
    c. Class 1 heterozygote (2nd allele anything other than F508del)
    d. Homozygous or compound het for 2 mutations from classes 3-6
5. Gender
6. Age: <18 vs 18 yrs +
7. Con meds at baseline (no adjustment for introduction of drugs during trial):
    a. DNase (also called pulmozyme or dornase alpha)
    b. Corticosteroids, inhaled or oral (any of: flixotide, seretide, fluticasone, fluticasone/salmeterol, clenil, beclomethasone, pulmicort, budesonide, symbicort, budesonide/fornoterol, prednisolone)
    c. Azithromycin (also called Zithromax)
    d. Hypertonic saline (also called nebusal or mucoclear)

B. TREATMENT ASSOCIATED AEs

8. Presence or absence of acute post-dosing (on day of dosing or within next 2 days) adverse events on ≥4 doses:
    a. Systemic (any of: headache, tiredness, lethargy, flu-like, or fever)
    b. Lung (any of: increased cough, wheeze, shortness of breath, increased sputum)

TOTAL number of subgroups: 24

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Gene expression and clinical outcomes

Clinical outcomes for patients in the nasal and/ or bronchoscopic subgroups will be further explored on the basis of whether there is evidence of molecular (RNA or DNA positive or negative) or electrophysiological evidence of CFTR expression. This will initially focus on FEV1, LCI, CT and CFQR, but may be expanded to include all listed outcomes.

For this analysis, the above outcomes will initially be compared for the actively treated group on the basis of:

　　a)Molecular: positive versus negative:
　　　　a.　RNA
　　　　b.　DNA
　　b) Electrophysiological response:
　　　　a.　Nasal: total Cl- secretion at end of trial (mean of F/U1 and F/U2 nPD values) greater (more negative) than at start (mean of all pre-dosing values)- yes/ no
　　　　b.　Bronchial: mean ZCI on bronchoscopy 2 greater (more negative) than at bronchoscopy- 1 yes/ no

Subsequent analyses may also be performed by categorising response as detailed in Appendix B, Secondary Outcome Full Analysis.

10.　Changes to the Planned Analyses

Any changes to the planned analyses will be fully documented and explained

Gene Therapy CF MDT

APPENDIX A: Secondary Endpoints – Efficacy

Relative change in other spirometric measures

FEV1 Z score

FVC Z score

FEV1/FVC ratio

MEF 25-75

Lung clearance index

Mean LCI*(end offset)

Mean FRC(end offset)

Chest CT scan

Extent bronchiectasis

severity bronchiectasis

wall thickness

small mucus plugs

large mucus plugs

air trapping

consolidated lung

Total CT score

Quality of Life Questionnaires (%)

Physical*

respiratory symptoms *

Exercise capacity

VO2 at AT*

VO2max*

Activity monitoring

average mins/ day spent at >3 METs*
(moderate, vigorous, very vigorous)

Serum inflammatory markers

Serum calprotectin (ug/ml)*   Please include an analysis of pre-dosing
                                               vs D2 for any patients where this is available

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Sputum culture (to include aspirates from bronchoscopy)

Pseudomonas aeruginosa (all)*

Pseudomonas aeruginosa

non mucoid

Pseudomonas aeruginosa mucoid

Staphylococcus aureus *

Aspergillus fumigatus*

Non tuberculous mycobacteria* (NTM)
termed 'Mycobacterium....'

Sputum weight, cell counts and inflammatory markers

24 hour weight*

Total cell count*

Neutrophils*

lipid laden sputum macrophages*

lipid laden sputum epithelial cells

solid content

Calprotectin*

IL8*

Extracellular DNA

Frequency of antibiotics for increased chest symptoms (days and courses); hospital admissions for chest symptoms (days and episodes)

cumulative tally over on-Rx period

number of courses

period between dose 1 and 1st treatment course

AEs including SAEs

Con meds:

Increase/ decrease in standard CF Rx over trial period

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Electrophysiological measures: nasal and lower airway potential difference (subgroups only; per protocol population only)

Nasal:

> basal
>
> delta amiloride
>
> delta zero chloride
>
> delta zero chloride isoprenaline
>
> total chloride secretion
>
> (sum of delta ZC + delta ZCI)

Bronchial:

> mean of 4 carinal basal measurements
>
> mean of distal basals 1-3
>
> delta zero chloride isoprenaline 1
>
> delta zero chloride isoprenaline 2
>
> delta zero chloride isoprenaline 3
>
> maximal ZCI
>
> mean ZCI

Gene expression on nasal and bronchial brushings

(mRNA; subgroups only- see appendix for details)

> DNA TaqMan (nasal)
>
> Consensus RNA Score (nasal)
>
> Consensus DNA Score (bronch)
>
> Consensus RNA Score (bronch)

Urine biomarkers

> Protein
>
> Glucose
>
> N-acetyl-b-d glucosaminidase (NAG)

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12.　APPENDIX B: Secondary Endpoints – Safety

Sputum producer: yes, no, variable?	
Clinical examination parameters: temperature, HR, RR, BP, chest sounds	
Transcutaneous oxygen saturation	
Serum inflammatory markers (Hb, platelets, CRP*, white blood cell count*, neutrophil count, ESR, IL-6*,)	IL-6: Please include an analysis of pre-dosing vs D2 for any patients where this is available
Renal and hepatic function (U, Cr, AST, gGT, bili, amylase)	
Gas transfer	

KCOc

Immune response markers (anti-nuclear double-stranded DNA antibodies, CFTR-specific T cell responses):	
anti-DNA antibodies – nuclei	
anti-DNA antibodies-nucleoli	
anti-double stranded DNA	
CFTR-specific T cells	
Biopsy: inflammation, remodelling (all-0-4 scores)	
Goblet cell hyperplasia	
basement membrane thickening	
lymphocytes/plasma cells	
neutrophils	
eosinophils	
lipid laden macrophages	
lipid laden epithelial cells	
other lipid laden cells	
lipid (present/absent)	
Other outcomes that need reporting:	
Protocol deviations (have been grouped into categories listed in comments box on relevant page)	
number of doses received	
early termination numbers and reasons	

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Safety report as presented to DMC for early 'intensively monitored' cohort- does not require re-analysis	The only exception to this is serum IL-6 and calprotectin which were not available at that time and should be examined now

*Any item marked **with an asterisk is for future 'AUC' type longitudinal analysis**

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Statistical Analysis Of Nasal & Bronchial Brushing DNA & RNA Samples

These outcomes look indicate successful delivery and expression of the transgene; DNA (DNA TaqMan) and RNA (consensus RNA score) will be recorded only for the two subgroups (nasal and bronchoscopy). Treatment and placebo groups will be compared with regard to post-treatment results. Pre-treatment results are present as a control and should all be negative; any positive values are likely to indicate contamination and will need to be reported.

Nasal Brushing

"Post" nasal brushing data can be found at one of: F/U2, Bronch2 or unscheduled

"Pre" nasal brushing @ visit Screening, Screening2, Unscheduled

One

Data to be analysed is within NB N tab for "Pre"

Line 5   DNA TaqMan

Line 9   Consensus RNA Score

Data to be analysed is within NB N tab for "Post"

Line 5   DNA TaqMan

Line 9   Consensus RNA Score

Bronchial Brushing

"Pre" bronchial brushing @ visit Bronch

"Post" bronchial brushing @ visit Bronch2

Data to be analysed is within BRO BRU 1 tab for "Pre"

Line 9   Consensus DNA Score & Consensus RNA Score

Data to be analysed is within BRO BRU 2 tab for "Post"

Line 13   Consensus DNA Score & Consensus RNA Score

Possible Scores

Each score has ONE of FIVE possible values, charted on a Y axis in this order:

%VE   (Absolute % vector/endogenous CFTR DNA or mRNA))

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PBNQ   (Positive But Not Quantifiable–typically trace vector & some endogenous)

Zero   (Zero %VE CFTR DNA or mRNA–no vector & some endogenous)

ND   (Not Determined–no vector & no endogenous)

NS   (No Sample)

%VE is entered numerically and has an expected range of 1E-4 to 1E6

PBNQ, ND & NS are entered as radio buttons

Zero is entered as 0 within the %VE numerical range

Data Coding (Post-eCRF Pre-Stats Analysis)

Positive %VE values are accepted. The expected range is 1E-4 to 1E6

Determine minimum value of ALL %VE values.

If minimum value ≥ 1E-4 then use category values described below

Else adjust category values described below

PBNQ values are coded 1E-5

%VE values of 0 are coded 1E-6

ND values are coded 1E-7

NS values are coded 1E-8

Analysis

Will be categorical in the first instance comparing proportions of positive and negative samples in the two groups:

All samples that have a non-zero %VE value OR a PBNQ value are positive.

All samples that have a zero %VE value are negative

Samples that have a ND or NS value have effectively failed the analysis and should be ignored (there are very few, possibly none of these, so they won't greatly skew any analysis)

Subsequently, a Mann-Whitney test will be performed on the following basis:

1) include all positive %VE and zero %VE data

2) exclude ND and NS samples

3) recode PBNQ samples to a %VE value below lowest positive %VE (e.g. 1e-5 assuming lowest %VE is 1e-4)

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13. APPENDIX C: Secondary outcome full analysis

Category 1: Spirometric values: FEV1 (L), FVC (L), Piko FEV1 and FEV_6

Category 2: Quality of Life: Role, Vitality, Emotion, Social, Health perception, Body image, eating disturbances, treatment burden, weight, digestive symptoms

Category 3: Exercise capacity: VE max, VO2/Kg at AT, VO2/kg at max

Category 4: Infection: Candida species, Burkholderia cepacia complex (any organism that starts Burkholderia), Methicillin resistant Staphylococcus aureus (MRSA), Stenotrophomonas maltophilia, Haemophilis influenza, Alcaligenes xylosoxidans

Category 5: Sputum markers: viable cell count, macrophages, lymphocytes, eosinophils, other lipid laden sputum cells

Category 6: Safety

a)　　　Post-dosing-pre-dosing delta: RR, SaO2

b)　　　Serum biochemistry: ALT, Alk phos, alb, Ca

c)　　　Urine: Blood (is the patient menstruating?- yes and no, collected separately), leucocytes, bilirubin

d)　　　CT scans: dose 4 CT scan worse/ better?

e)　　　Gas transfer: TLCOc

Category 7: Electrophysiology

In the initial analysis patients in the actively treated groups are defined as demonstrating evidence of chloride secretion if the mean post-end of trial total Cl- secretion is greater (more negative) than the mean pre-dosing values.

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In this more detailed analysis we may also look at all or any of the following methods of categorising subjects:

a) Any one on-treatment trace demonstrating total Cl- greater (more negative) than:

    a. pre-dosing mean: yes/ no

    b. pre-dosing maximal (most negative): yes/ no

b) Any one on-treatment trace demonstrating total Cl- greater (more negative) by at least 5mV than:

    a. pre-dosing mean: yes/ no

    b. pre-dosing maximal (most negative): yes/ no

c) End of trial mean Cl- secretion of -5mV or more: yes/ no

d) As above a-c) for ZC and ZCI phases independently

e) Changes in basal values

f) Changes in amiloride response

g) For any of the above, during-trial measurements may be taken into account with an area under the curve analysis