

Clinical study protocol

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

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Sponsor

Imperial College is the main research sponsor for this study. For further information regarding the sponsorship conditions, please contact the Research Governance Manager at:

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This protocol describes '**A randomised, double-blind, placebo-controlled Phase 2B clinical trial of repeated application of gene therapy in patients with cystic fibrosis**'. This trial will adhere to the principles outlined in the Medicines for Human Use (Clinical Trials) Regulations 2004 (SI 2004/1031) and the International Conference on Harmonisation Good Clinical Practice (ICH GCP) guidelines. It will be conducted in compliance with the protocol, the Data Protection Act and other regulatory requirements as appropriate.

Amendments to previous version of this protocol:

Version 3 of this protocol was finalised on 26th March 2012, replacing Version 2 of this protocol. To assist in document review, a list of substantive changes is presented below:

Substantive changes between V2 and V3

- The sponsor requires that the Chief Investigator is also the Principal Investigator at site; Dr Davies' name has been removed from PI at the Royal Brompton site and added to Co-Investigators (pg 8)
- Nasal and bronchoscopy subgroups increased to at least 24 patients (pg 12 & 17)
- The timing of the post-dose bronchoscopy has been moved to uncouple it from either of the two follow-up visits; is now D29-35 post-dose 12 (pg 12)
- Section on 'Procedures and sequence of study visits' has been restructured for clarity (pg 13)
- Dosing window has been increased to +/- 5 days (pg 15)
- Pre-dosing salbutamol can be administered by any licensed spacer; reference to 'large volume spacer' has been removed. Dose has been adjusted to 200-400 micrograms to allow for an increase if required (still at low end of standard clinical dose) (pg 16)
- Dosing: nebulisation will be conducted for 8 cycles whilst the subjects wear a nose clip. Nasal doses will be administered at the start and end of the first 6 'off-nebuliser' periods (pg 16)
- Pre-dose nPD or bronchoscopies can be conducted outside the 4 week window allowed between screening and 1st dose (ie. earlier) to aid flexibility in planning. In this case, subjects will first attend an eligibility and consent visit

(limited to the investigations listed in Table 1) and will undergo the rest of the tests in a subsequent screening visit, within 4 weeks prior to 1st dose (pg 18).

- Flexibility of timing of investigations: attempts will be made to ensure that the tests and samples listed are obtained on the visits indicated, but where this has not been possible, for example because of a missed visit or equipment failure, they may be performed at an alternative visit (pg 13)
- On one occasion a sample of blood will be obtained for genomic (human) DNA: this may only be used to test for CFTR mutations, where unknown, or to look for polymorphisms in potential modifier genes to explain response differences (pg 18)
- Blood to be sampled via indwelling portacath if permitted by local clinicians and SOPs adhered to (pg 19)
- All subjects will use the CFQR for adolescents and adults rather than the child version (pg 19)
- Inconsistency noted between protocol and GTAC form regarding total radiation dose during the trial; this has been amended (pg 20).
- Cycle ergometry will be performed with breath-by-breath analysis (pg 21)
- Details of funding and regulatory approval have been added (pg 32)
- Risk assessment by Imperial College Trials Unit added to Audits and Inspection section (pg 32)
- Figure 1 has been restructured for clarity and amended to include changes outlined above and renamed Table 1 (pg 34)
- Addition of Table 2: assays performed on samples at specific visits. For ease these have been removed from the text (pg 36)
- Figure 2 has been renamed figure 1 (pg 37)

Substantive changes between v1 and v2

- Randomisation in the nasal and bronchoscopic subgroups will be 2:1 in favour of active treatment, rather than 1:1 as for the trial overall
- Reference to a separate consent form for new participants has been removed and therefore also possible assessment of eligibility and completion of consent form at screening visit

- Bronchial blood flow measurements will be made on Royal Brompton Hospital patients only due to equipment availability. The protocol has been amended to reflect this
- Bronchoscopies will be performed on Royal Brompton Hospital patients only, due to local expertise in bronchial PD measurements. The protocol has been amended to reflect this
- A new section 'Recruitment of participants' has been introduced
- Withdrawal criteria have been expanded to include missing more than 3 doses of study drug; withdrawal related to a life-threatening or serious adverse event placing a subject at immediate risk will lead to mandatory withdrawal.
- Clarification that there is no intended subgroup analysis of the paediatric patients
- Several assessments were omitted from Follow-up V1 in Fig 1. There was also mention of nasal biopsy, which was unintended; the table has been replaced with an updated version to correct this

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KEYWORDS

Cystic fibrosis, gene therapy, repeated dose

STUDY SUMMARY

Title	A randomised, double-blind, placebo-controlled Phase 2B clinical trial of repeated application of gene therapy in patients with cystic fibrosis
Design	Double-blind, placebo-controlled, randomised study
Study Phase	2B
Aims	1. 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 2. To assess the safety and tolerability of repeated doses of pGM169/GL67A administered to the lungs of patients with CF over the same period 3. To assess gene expression of the formulation over the same period
Outcome measures	Primary outcome: • relative change in percent predicted FEV1 after 12 doses Secondary outcomes- Efficacy: • relative change in other spirometric measures • lung clearance index • change in body weight • chest CT scan • Quality of Life Questionnaires • exercise capacity • activity monitoring • serum calprotectin • sputum culture • sputum weight, cell counts and inflammatory markers • frequency of additional antibiotics for increased respiratory symptoms Secondary outcomes- Safety: • the above efficacy measures

- clinical examination
- transcutaneous oxygen saturation
- serum inflammatory markers (CRP, white blood cell count, IL-6)
- renal and hepatic function
- gas transfer
- bronchial blood flow
- immune response markers (anti-nuclear and double-stranded DNA antibodies, CFTR-specific T cell responses)
- endobronchial histology (subgroup only)

Gene expression outcomes (subgroups only):

- transgene mRNA expression in nasal and lower airway brushing samples
- potential difference measurements in nose and bronchi

Population	Cystic fibrosis
Eligibility	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
Treatment	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 (subgroup only)
Number of subjects	This study plans to enrol 130 subjects; an additional 20 subjects may be recruited under the adaptive design if the safety data from 5 ml necessitate switching to a 2.5 ml dose.
Duration	Total estimated duration of study 24 months
Chief Investigator	Eric Alton, Imperial College London
Principal Investigators	Alastair Innes, Western General Hospital, Edinburgh; Steve Hyde, Oxford University

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1. INTRODUCTION

1.1 BACKGROUND

Cystic fibrosis (CF), a common, genetically inherited disease, is caused by mutations in the cystic fibrosis transmembrane conductance regulator (*CFTR*) gene. This gene encodes the CFTR protein, which is expressed on the apical surface of epithelial cells, and which has many functions, the most important of which is thought to be ion transport. Abnormal ion transport leads to thick secretions in the airways, infection, inflammation and eventually irreversible lung damage. There is currently no treatment that halts the natural progression of the disease; all available successful therapies merely slow the rate of decline in clinical condition.

The UK Cystic Fibrosis Gene Therapy Consortium (Imperial College, University of Edinburgh and University of Oxford) has been working, for over a decade, towards this repeated-dose clinical trial of *CFTR* gene therapy. Studies to date have provided proof-of-principle for gene transfer to the airways, but there has been no attempt to ameliorate disease. The aim of this trial is to allow us to assess, for the first time, whether *CFTR* gene therapy can lead to clinical improvement.

1.2 RATIONALE FOR CURRENT STUDY

1. Choice of formulation

As CF is a lifelong disease and respiratory epithelial cells have a limited life span, we reason any gene therapy with a chance of clinical success will require repeated application. As, to date, no viral gene transfer agents retain efficacy upon repeated administration, due to immune responses, we have conducted extensive work to identify the best currently available non-viral gene transfer agent. Preclinical assessment indicated that GL67A was superior to others in both small and large animal models. GL67A has been used both in healthy volunteers and in previous CF

clinical trials, including a single dose nebuliser study 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). For the latter and to be taken forward into the current protocol, we made extensive changes to the *CFTR* plasmid, which was specifically designed with the following features:

- a) The promoter was changed from the commonly used CMV viral promoter to a human elongation factor 1 alpha promoter. Preclinical studies demonstrated more sustained levels of expression (up to 2 months) with this new promoter, which could lead to the requirement for a much less frequent dosing regime. The gene expression profile observed in the recent single dose study was in support of this, with relatively few changes observed at early time points and several subjects demonstrating functional changes in nasal PD several months after dosing.
- b) The DNA usually used in gene therapy trials is generally rich in unmethylated CpG dinucleotides, which are likely recognised by humans as foreign and to which an inflammatory response is mounted. Such a response is thought to have resulted in the flu-like illness, reported in our previous trial in the group receiving DNA/ lipid but not lipid alone. The new plasmid has been rendered CpG-free in an attempt to reduce such an inflammatory response.

2. Choice of dose

Despite depletion of pro-inflammatory CpG motifs, 20 ml nebulised doses led to systemic inflammatory responses and acute reduction in pulmonary function which, whilst largely well tolerated, were considered unacceptable for a multidose trial. These 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. This dose has therefore been chosen for the repeated dose trial. In addition, the administration of standard doses of the antipyretic agent, paracetamol, post-dosing appeared to further reduce both symptoms and inflammatory markers and will be used here at 2 and 8 hours post-dosing, with a specific aim to ensure patient blinding.

3. Choice of study design (outcomes, power, dosing interval etc)

This is the first non-viral study to be powered for and designed to detect clinical benefit. We consider therefore that a placebo-controlled, parallel group study is the optimal trial design. FEV₁ is the major CF outcome acceptable to regulatory agencies and was shown, in our longitudinal study of outcome measures (Run-in study) to be the most powerable assay; this has therefore been chosen as primary outcome. A number of secondary outcomes are included to assess both efficacy and safety. In addition, we wish to correlate these measures with assays of transgene expression and function in two subgroups, one undergoing nasal dosing and assessment and the other undergoing bronchoscopic assessment. Preclinical data indicate sustained gene expression out to 2 months, which has led to our choice of dosing interval of 28 days. We hope to achieve sustained levels of expression, or even to build on efficacy with subsequent doses. Data from our extensive preclinical toxicology study in mice would support the possibility that this is likely; a minority of mice had measureable levels of transgene mRNA after single dose, most after 6 doses and all after 12 doses.

2. STUDY OBJECTIVES

The study objectives are to assess clinical efficacy, safety & tolerability and gene expression of repeated doses of gene therapy. A detailed description of all outcome measures is given below.

3. 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. An adaptive design (see Fig 2) will allow the early identification of cumulative side effects: 20 subjects (10 active treatment; 10 placebo) will receive 3 doses at 4-weekly intervals before any further subjects are dosed. In addition to the visits described below undertaken by the entire cohort, they will be seen on day 2 post each dose. Clinical examination findings, lung function, gas transfer and systemic inflammatory markers will be reviewed in an unblinded fashion by the DMEC.

- Should data prove satisfactory, these subjects will continue with subsequent visits and the remainder of the cohort will begin dosing; logistics necessitate

that this happens in a staggered fashion and will take place over the next 12-14 weeks.

- Should the data be of sufficient concern to the DMEC that progression is unacceptable, a second cohort of 20 patients will receive 3 doses of the formulation at a 2.5 ml dose, followed by DMEC review in a similar fashion.
 - o If these data are acceptable, the trial will continue with all subjects receiving a 2.5 ml dose of either gene therapy or placebo. In this instance, the initial cohort will be discontinued and an additional 20 'naïve' subjects recruited
 - o Should the DMEC consider these data unacceptable, the trial will be halted as we do not consider it feasible to administer a smaller dose successfully to the lower airway via nebuliser.

All subjects will receive 12 doses of nebulised gene therapy at intervals of 4 weeks over a 48 week period. The majority of subjects have been participating in our Run-in study and have extensive pre-dosing data; these patients will undergo a single screening visit prior to dosing. Subjects who are new to the programme will undergo an initial Introductory visit at which suitability will be assessed prior to the formal screening visit. After dose 12 there will be 2 formal follow up visits, at 14 and 28 days post-dose. In addition, patients will be followed up long-term. The first 3 month post-trial clinical visit will be attended by a member of the site trial team to aid with consistency of assessments and thereafter close communication with the clinical team will be maintained.

Subgroups of patients at the London site will be enrolled for gene expression measurement in both nose (at least n=24) and lower airway via bronchoscopy (at least n=24). To enrich for actively-treated patients, these subgroups will be randomised 2:1. A subject could be included in both of these subgroups if suitable and willing. In addition to the visits above they will undergo:

- Nasal subgroup: up to 3 additional pre-dosing nasal PD visits
- Bronchoscopy subgroup: two additional visits for bronchoscopy (pre-dosing and post-12th dose day 29-35 to avoid any impact on outcomes assessed at F/U days 14 and 28)

- Pre-dose nPD or bronchoscopies may be conducted outside the 4 week window allowed between screening and 1st dose (ie. earlier) to aid flexibility in planning. In this case, subjects will first attend an eligibility and consent visit (limited to the investigations listed in Table 1) and will undergo the full screening visit within 4 weeks prior to 1st dose.

3.1 PROCEDURES AND SEQUENCE OF STUDY VISITS (see appendix 1)

Pre-dosing visits (all patients or selected ones as indicated)

- Eligibility & Consent (E&C) visit (selected nPD or bronchoscopy subjects only)
- Introductory visit (new, non-Run In study subjects only) Screening visit (all subjects)
- Pre-dosing nasal PD visits x up to 3 (nasal subgroup only- may be before or after Screening; if before, must have undergone E&C or Introductory visit)
- Pre-dosing bronchoscopy (bronchoscopy subgroup only- may be before or after Screening; if before, must have undergone E&C visit)

Dosing visits

Dosing visits 1-12 (all)

Follow up visits

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

Follow up day 14 (all)

Follow up day 28 (all)

Post-dosing bronchoscopy (subgroup only): days 29-35 post 12th dose [Long term follow up post end of study will be conducted at hospital clinic attendances approximately every 3 months for 2 years]

***Part I: Intensively monitored initial cohort**

N= 20 patients will receive the first 3 doses of gene therapy or placebo with increased post-dose monitoring before the rest of the cohort is dosed. They will undergo the following:

Introductory Visit (new, non-Run in study subjects only)

This will involve:

- full medical history
- confirmation that patient fulfils inclusion/ exclusion criteria
- sign informed consent
- physical examination including heart rate, respiratory rate, blood pressure, pulse oximetry, temperature, chest auscultation
- blood sampling
- Urine sampling
- Spirometry
- Lung clearance index
- Sputum sample (induction with 7% hypertonic saline if required)
- 24 hour sputum weight
- Patient given daily symptom/ treatment diary to take home
- Patient given training in the use of hand-held lung function device (PiKo-6) and a device to take home on which to record daily lung function for the duration of the study
- Nasal potential difference measurements (may be conducted- nasal subgroup only)

Screening visit

This will involve:

- confirmation that patient fulfils inclusion/ exclusion criteria
- informed consent signed (if no Introductory Visit)
- quality of life questionnaire
- full medical history and physical examination including heart rate, respiratory rate, blood pressure, pulse oximetry, temperature, chest auscultation
- blood sampling
- Urine
- Spirometry
- Lung clearance index
- Cycle ergometry
- Body worn activity monitor: subjects instructed for use
- CT chest

- Gas transfer
- Sputum sample (induction with 7% hypertonic saline if required)
- 24 hour sputum weight
- Nasal potential difference measurements (may be conducted- nasal subgroup only)
- Patient given daily symptom/ treatment diary to take home
- Patient given training in the use of hand-held lung function device (PiKo-6) and a device to take home on which to record daily lung function for the duration of the study

Dosing Visits

Dosing visits will be performed at 28 (+/- 5 days) intervals; all dosing visit windows will be calculated with reference to visit 1 to avoid any cumulative time shifts. Any patient normally receiving rhDNase (Pulmozyme) 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:

- Recent history, adverse events & medication record
- Vital signs and physical examination as above
- Pulse oximetry
- Blood sampling
- Spirometry

At specified visits (see table 1), the following will also be performed

- LCI
- Urinalysis
- Quality of life questionnaires
- Cycle ergometry
- Body worn activity monitor
- CT chest
- Gas transfer
- Sputum (weight of 24 hour sample) and collection of fresh specimen
- Bronchial blood flow measurements
- Nasal PD

Dosing

Approximately 20 minutes before dosing patients will receive 200-400 micrograms of inhaled salbutamol via metered dose inhaler and spacer to limit any bronchoconstriction induced by the hypotonic gene therapy formulation. 5 ml doses of pGM169/GL67A or placebo (0.9% saline) will be delivered via an AeroEclipse, breath-actuated nebuliser in cubicles designed to comply with local NHS requirements. Nebuliser pots will be masked with tape to prevent either patient or study team members visualising the fluid within (active agent appears milky white) and will have been filled by pharmacy staff holding the randomisation code. Nebulisation will be performed in a 3 minutes 'on', 2 minutes 'off' fashion' for 8 cycles ie. approximately 40 minutes. During 'on' periods, subjects will wear nose clips to aid mouth-breathing. Patients will be observed for 2 hours post-dosing before discharge. Spirometry will not be performed unless clinically indicated (new symptoms reported and at discretion of medical personnel). Chest auscultation and transcutaneous oxygen saturation will be recorded at between 90 and 120 minutes post end of dose. A standard 1g dose of paracetamol will be administered at 2 hours post-dosing and will be given to subjects to take at home approximately 8 hours after dosing to mitigate against the possibility of a mild inflammatory reaction leading to fever, which would be apparent to the patient, and could lead to unblinding. Nasal dosing (subgroup only) will occur 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.

Day 2 post-dosing visits

The following will be performed:

- History (symptoms and adverse events)
- Clinical examination
- Transcutaneous oxygen saturation
- Spirometry
- Gas transfer
- Blood sampling
- Sputum sample collection
- 24 hour sputum weight

- Urine collection

The DMEC will examine results from this cohort before allowing the remaining patients to begin dosing in the full trial. Should there be any safety concerns, we have built an adaptive design into this protocol to allow the above to be repeated with a 2.5 ml dose. In this instance, the initial 5 ml dosing cohort will exit the trial.

Part II: Full trial

All visits will be as for Part I although no post-dosing day 2 visits will be performed

Nasal or bronchoscopy subgroups: additional pre-1st dose visits:

Eligibility and Consent (E&C) visit

[This may be conducted to ease scheduling if nPD or bronchoscopy can be performed earlier than allowed Screening window]:

- confirmation that patient fulfils inclusion/ exclusion criteria
- informed consent signed
- full medical history and physical examination including heart rate, respiratory rate, blood pressure, pulse oximetry, temperature, chest auscultation
- blood sampling
- urine pregnancy test
- Spirometry
- Nasal potential difference measurement may be performed at this visit or on separate occasion
- Nasal subgroup (at least n=24) may undergo up to three further visits:
 1. for nasal potential difference measurements only (x 2 visits)*
 2. for nasal PD and nasal brushing** (one from each nostril) for quantification of transgene mRNA (1 visit)

*These procedures may be performed at E&C, Introductory or Screening visits, or may be scheduled separately. **This may be performed at Screening visit or scheduled separately (must be performed after *).

- Bronchoscopy subgroup (at least n=24) will undergo one further visit for flexible bronchoscopy under general anaesthesia to include:
 1. bronchial brushings (n=10)

2. airway wall biopsies (n=2)
3. bronchial PD measurements

After the procedure a single dose of the most suitable antibiotic(s) (based on recent culture and sensitivity data) will be administered intravenously to reduce the chance of a post-procedure fever.

Subjects will be monitored for 6 hours after the procedure with recording of vital signs, temperature and saturations. Provision will be made for an overnight stay should observations for a longer period be considered desirable or should subjects have any symptoms or logistic considerations.

Follow up visits

Follow up will be conducted formally at days 14 & 28 (+/- 2) and will include the tests outlined in Figure 1. Long term follow up will also occur at patients' scheduled clinical appointments approximately every 3 months for 2 years.

Whenever possible, the investigations above will be conducted on the study visit indicated in Table 1, but in the event that a visit is missed, or for any other reason an investigation cannot be conducted, investigations can be conducted at another visit and recorded in the electronic CRF as an 'additional investigation'.

On one occasion (any visit, pre or post-dosing), blood will be obtained for extraction of genomic (human) DNA. This may be used for CFTR (the gene causing cystic fibrosis) genotyping in patients who lack a fully informative genotype. In the event that responders and non-responders are observed amongst the actively treated group in the trial, a further understanding of this will be sought by analysing naturally occurring genetic variations in genes such as those involved in inflammation and host defence, so-called 'modifier' genes. This DNA will not be further tested for any known disease-causing genetic mutations.

Unscheduled visits

These will be scheduled if either subject or trial staff consider them indicated, eg. telephone report of AE.

3.2 DETAILED DESCRIPTION OF INTERVENTIONS

Clinical examination

This will include recordings of heart rate, blood pressure, respiratory rate, temperature and pulse oximetry, height and weight, lung auscultation.

Spirometry

Spirometry will be performed on the Easyone spirometer with disposable mouthpiece and filter. Three measurements will be made and the best of these will be recorded in absolute values. Three tests will also be performed on the PIKo-6 machine for comparison purposes and the best recorded.

Venepuncture for blood sampling

This will be performed by an experienced practitioner after the application of topical anaesthetic cream, if desired by the patient, via a peripheral vein. If local protocols allow, blood may be sampled from an indwelling central venous access device such as a portacath; this will only be performed by trained and experienced personal and will adhere to local SOPs.

Urine sampling

Urine will be collected into a sterile container. Where indicated (see fig 1) a pregnancy test will be performed. Otherwise, urine will be dipstick tested and frozen for future analysis.

Quality of life questionnaire

The validated CFQ-R (Quittner) for adolescents and adults will be completed by all subjects (including children <14 years of age) at the start of visits, before any study investigations are performed. An exception will be made for the Screening visit, when eligibility and consent will be conducted first.

Lung CT scan

Each subject will undergo a total of three high resolution CT scans; two of these (pre-dosing and end of trial) will be volumetric and the third, performed for safety reasons

immediately prior to the 4th dose, will be interspaced. Total radiation is estimated at 4.5 mSv, which equates to natural background radiation over 2 years.

T_LCO

Single breath carbon monoxide diffusion capacity (transfer factor) will be measured according to the clinical protocol of the Lung Function Laboratory at the Royal Brompton Hospital; three measurements will be obtained at each time point.

Nasal brushings

Samples will be obtained from the middle or inferior turbinate of both nostrils using a sterile, single use, dental brush or rhinoprobe.

Sputum sampling

Sputum samples will be collected into sterile containers. Unless patients are able to expectorate sputum spontaneously, we will, on the visits indicated, induce sputum with 7% hypertonic saline, according to standard clinical protocols using a deVilbiss 2000 or equivalent nebuliser. This is a safe procedure which is used in routine clinical practice, although bronchoconstriction is a common side effect; this will be minimized by routine pre-treatment with an inhaled bronchodilator (200 micrograms of salbutamol or equivalent, 15 mins before test) and subjects will have FEV₁ monitored carefully throughout according to the Standard Operating Procedure. If the subject is unable to expectorate sputum, a cough swab will be performed for microbiological culture.

Lung Clearance Index (LCI)

LCI involves the inhalation of an inert, odourless tracer gas (0.2% SF₆) to steady state. Once this has been achieved the patient then breathes room air until the gas is completely removed from the lungs as measured by the concentration of gas in the exhalate. This procedure is performed in triplicate and each test will take approximately 15 minutes. SF₆ is a completely safe and inert gas and has been used previously by ourselves and other research groups in CF patients.

Cycle ergometry

This test will be performed on a stationary exercise bike with breath-by-breath analysis. The subjects will be asked to pedal at a set speed of 70 revolutions per minute (rpm) and maintain this speed throughout the test. The subject will wear a nose clip and a mouth piece attached to a gas analyser. The resistance will automatically increase each minute and they will be asked to continue to pedal for as long as they can. The starting workload and increase in workload is dependent on the patient's height, and calculated using the Godfrey protocol.

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

The subjects' heart rate, oxygen saturations and Borg scale will be measured at rest and each minute during exercise. This is a symptom limited test and patients will be encouraged to exercise to their maximum capacity. The test will be stopped by the operator if the patient's oxygen saturation falls below 80%, if they are unable to maintain the required pedaling rate or if there is any concern about the patients' condition. Once the test is complete they will have a 2 minute cool down period and be monitored until their oxygen saturation returns to baseline.

Body worn activity monitoring

An activity monitor will be worn on the upper arm for a period of a week at intervals during the study; data will be downloaded on site.

Bronchial blood flow measurement (Royal Brompton Hospital patients only)

This test is designed to look at the blood flow to the airways, which is often increased in patients with airway disease, most likely reflecting inflammation. The test takes about 45 minutes to do and consists of 10 breath-holding manoeuvres of either 8 or 16 seconds duration. For each of the manoeuvres, the subject will be seated wearing a noseclip and breathing in and out through a mouthpiece. After several normal breaths they will breathe in a small amount of test gas (which includes acetylene (C₂H₂) 0.3%, dimethylether (DME) 1%, sulphur hexafluoride (SF₆) 5%, oxygen (O₂) 35%, and nitrogen (N₂) balance). This gas mixture is of medicinal quality, non-explosive and is

oxygen enriched to prevent hypoxia in patients with severe respiratory disease. Subjects perform 10 breath-hold manoeuvres in random order, followed by slow exhalation. Each test is performed 3 minutes apart. Heart rate and oxygen saturation will be monitored throughout. Gas measurements are made using an Amis 2000 mass spectrometer (Innovision, Denmark). Ensuring no contamination from alveolar gas and allowing for any dilution using the insoluble sulphur hexafluoride, the difference in DME in the exhaled gas between the 2 breath-hold times represents dissolution of DME into the bronchial wall circulation assuming dissolution into the bronchial mucosa is complete by 8 seconds. Animal data suggests this occurs within 2 seconds.

Nasal potential difference

Nasal potential difference (PD) will be measured in the nostril. Reference will be to an electrode placed on the forearm after gentle, localised dermal abrasion. Baseline PD during perfusion with Ringers solution will be measured, as will responses to amiloride, a low chloride solution and isoprenaline. In this study, pharmacological solutions are designated non-investigational medicinal products (NIMP) for which no CTA is required. Full details of the composition of solutions is provided in Appendix 1.

Bronchoscopy and associated procedures (Royal Brompton Hospital patients only)

A subgroup of at least 20 patients will undergo two flexible bronchoscopies, one pre-first treatment and a second at between days 29 and 35 post-treatment 12 (ie. after follow up visits 1&2). These will be performed under general anaesthetic, administered by a consultant anaesthetist, as in our previous trials, to limit the detrimental effect of cough encountered in CF patients and to allow the PD measurements to be taken (local anaesthesia interferes with these measurements). Patients will be endotracheally intubated for the duration of the procedure. A semi-quantitative assessment of severity of lung involvement will be performed as in our previous study.

Sampling will be limited to one lung only for safety reasons.

Samples will include:

a) Endobronchial (**not** transbronchial) biopsies. Two biopsies will be obtained using clinically available cup forceps to optimise epithelial integrity.

b) Bronchial brushings. Up to 10 will be obtained from geographically diverse areas in the same lung using sterile, single use, sheathed cytology brushes.

c) Visible secretions will be aspirated

Bronchial potential difference (PD) measurements will be made at baseline and after perfusion with a series of pharmacological solutions described above and used in our previous trial². The measurements will take between 10 and 30 minutes.

3.3 ASSAYS PERFORMED ON SAMPLES

a) Blood Samples

Blood will be sent to the clinical laboratories in the Royal Brompton Hospital for full blood count, C-reactive protein, ESR, renal and liver function tests and coagulation at the time points indicated in Tables 1 & 2. Whole blood or serum will be frozen in aliquots for future analysis of inflammatory proteins and may be used for lipid/ DNA degradation products at Imperial College and Edinburgh University. At the time points indicated, blood will be tested for anti-nuclear and anti-double stranded DNA antibodies in the Clinical Microbiology Laboratory, Royal Brompton Hospital. Samples which are positive will be further tested for a clinical panel of auto-antibodies. Lymphocytes will be removed from whole blood and sent to the University of Pennsylvania for assessment of anti-CFTR T-cell responses. On one occasion (any visit, pre or post-dosing) blood will be obtained for extraction of genomic (human) DNA for CFTR genotyping or polymorphism analysis of possible modifier genes.

b) Sputum (and aspirated bronchial secretions)

A sample will be sent to the site Clinical Microbiology laboratory for quantitative culture on the standard CF agar panel. Aliquots will be saved for possible future analysis of lipid/ DNA, solid content, 16SrRNA for microbiological analysis. Aliquots treated with the mucolytic agent, DTT will be used for total and differential cell counts and the supernatant frozen for analysis of inflammatory proteins. Analysis will be performed at Imperial College and Edinburgh University. On several visits, subjects will be asked to collect all sputum for a 24 hour time period immediately before the visit, which will then be weighed and discarded.

c) Urine

Urine will be dipsticked for the presence of protein, blood, glucose etc and assayed for cotinine. Aliquots will be frozen for future analysis of inflammatory mediators or relevant breakdown products. In addition, where indicated, urine will be tested with a commercially available pregnancy detection system (Clearview).

d) Nasal and bronchial brushings

Samples from either nose or lower airways will be pooled and assessed for endogenous and transgene mRNA detection at Oxford University.

e) Bronchial biopsies

Samples will be fixed and embedded for conventional histological analysis. Samples will be analysed at Imperial College, Edinburgh University and Oxford University.

3.4 STUDY OUTCOME MEASURES

Primary outcome:

- relative change in percent predicted FEV₁ after 12 doses The **baseline** will be defined as the mean of Screening and Pre-dose 1 values, whilst the **end value** will be the mean of measures obtained 14 and 28 days after the 12th dose.

Secondary outcomes- Efficacy:

- relative change in other spirometric measures
- lung clearance index
- chest CT scan
- Quality of Life Questionnaires
- exercise capacity
- activity monitoring
- serum calprotectin
- sputum culture
- sputum weight, cell counts and inflammatory markers
- frequency of antibiotics for increased chest symptoms

Secondary outcomes- Safety:

- the above efficacy measures
- clinical examination
- transcutaneous oxygen saturation
- serum inflammatory markers (CRP, white blood cell count, cytokines)
- renal and hepatic function
- gas transfer
- immune response markers (anti-nuclear and double-stranded DNA antibodies, CFTR-specific T cell responses)
- endobronchial histology (subgroup only)

Gene expression outcomes (subgroups only):

- transgene mRNA expression in nasal and lower airway brushing samples
- potential difference measurements in nose and bronchi

4. PARTICIPANT ENTRY

4.1 INCLUSION CRITERIA

1. Cystic fibrosis confirmed by sweat testing or genetic analysis
2. Males and females aged 12 years and above
3. Forced expiratory volume in the 1st second (FEV₁) between 50 & 90% predicted inclusive (Stanojevic reference equations).
4. Clinical stability at screening defined by:
 - a. Not on any additional antibiotics (excluding routine, long-term treatments) for the previous 2 weeks
 - b. No increase in symptoms such as change in sputum production/colour, increased wheeze or breathlessness over the previous 2 weeks
 - c. No change in regular respiratory treatments over the previous 4 weeks
 - d. 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 (pulmozyme) is willing, and considered able by independent medical carers, to withhold treatment for 24 hours before and 24 hours after the gene therapy dose (nebulised doses only)
7. Written informed consent obtained
8. Permission to inform GP of participation in study

4.2 EXCLUSION CRITERIA (JUSTIFICATION)

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. Chloride secretory response on nasal PD of > 5 mV (nasal cohort only; will only be known after first measurement)
4. Acute upper respiratory tract infection within the last 2 weeks (entry can be deferred)
5. Previous spontaneous pneumothorax without pleurodesis (bronchoscopic subgroup only)
6. Recurrent severe haemoptysis (bronchoscopic subgroup only)
7. Current smoker
8. Significant comorbidity including:
 - a. Moderate/severe CF liver disease (varices or significant, sustained elevation of transaminases: ALT/ AST>100 IU/l)
 - b. Significant renal impairment (serum creatinine > 150 micromol/l)
 - c. Significant coagulopathy (bronchoscopic group only)
9. Receiving 2nd line immunosuppressant drugs such as methotrexate, cyclosporine, intravenous immunoglobulin preparations
10. Pregnant or breastfeeding

4.3 RECRUITMENT OF PARTICIPANTS

The majority of patients required for this trial have been participating in an observational study (the Run-in) designed to identify optimal outcome measures and participants for this protocol. They are known to fulfil eligibility criteria, although these will be reconfirmed at screening. 'New' patients, ie those who have not participated will be identified from clinical records at each site and approached with information about the trial. Subjects from outside the trial sites may also be

considered if their local centre refers them for the purpose of the trial; clinical care will remain with their local CF centre. Any patient fulfilling the additional inclusion criteria (listed above) required for the nasal PD or bronchoscopy subgroups will be asked to consider participation in one or both of these. Should they agree, they will be included as long as the relevant logistic demands can be met; patients in both these subgroups will be randomized 2:1 active treatment: placebo. Should a subject in the nasal PD subgroup demonstrate significant residual chloride secretion (> 5 mV) ie. be atypical for CF, they will be discontinued from this subgroup on the grounds that a transgene-specific response might be more difficult to observe post-treatment.

4.4 WITHDRAWAL CRITERIA

Subjects may be withdrawn from the trial under the following circumstances; a) Development of one or more of the exclusion criteria during the course of the study; b) Inability or unwillingness to comply with the study protocol; c) Prolonged interruption of study drug due to concomitant illness; d) Missing more than 3 doses of study drug; e) Development of a life-threatening or serious adverse event which places them at immediate risk will mandate withdrawal. Any subject who has been dosed will undergo two follow up visits, at days 14 and 28 days post-last dose and will be followed up long term as above.

4.5 CONTRACEPTIVE ADVICE

Patients wishing to take part in the trial will be informed during the consent process of the requirement to agree to use an acceptable form of contraception during the study period and for 3 months thereafter. Acceptable forms of contraception include: oral, injected or implantable methods; placement of an intrauterine device (IUD) or intrauterine 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. Subjects wishing to be considered for the study who do not at that time fulfil one of these criteria, will be asked to attend their local Family Planning Clinic or General Practitioner for advice. They may also wish to consult their CF Physician.

This information will be clearly stated on the Patient Information Sheet and subjects will be asked to confirm on the Consent Form that they have read and understood this. This information will also be included on the letter that will be sent to the subject's General Practitioner, to which they will also consent.

5. TREATMENTS

pGM169/ GL67A or placebo (0.9% saline) in 5 ml dose via nebuliser every 28 +/- days; same as 2 ml nasal spray (subgroup only).

5.1 DOSE MODIFICATIONS FOR TOXICITY

The first 20 patients will receive 3 doses of 5 ml and be monitored closely. Unblinded data will be made available to the DMEC. Any major safety concerns can be addressed by a further cohort of 20 patients being dosed in the same fashion with 2.5 ml; this will then be used for the remainder of the trial if safety is acceptable. If this dose also gives rise to major safety concerns, the trial will be halted, as we do not consider it possible to nebulise a volume smaller than this in a consistent fashion.

5.2 PRE/ADDITIONAL MEDICATION

20 minutes before the nebulised dose is administered, patients will receive 200-400 micrograms of inhaled salbutamol to prevent bronchoconstriction which may be associated with the hypotonic DNA/ lipid/ formulation. Should symptomatic wheeze be a problem during or after the trial treatment, further doses of salbutamol may be administered. This information will be included in that presented to the DMEC. All subjects will also receive a standard 1 g dose of paracetamol at 2 and 8 hours post each dose to mitigate for the possibility of drug-related fever unblinding the subjects.

5.3 INTERACTION WITH OTHER DRUGS

We have insufficient preclinical data to be confident that nebulised rhDNase does not adversely affect gene transfer and will therefore request that patients withhold treatment for 24 hours before and after dosing.

6. PHARMACOVIGILANCE

Definitions of adverse events and reactions and assessment of causality will be as described by the MHRA.

All non-serious adverse events (AE) or reactions (AR), whether expected or not, will be recorded in the toxicity section of the relevant case report form. Fatal or life threatening SAEs and SUSARs will be reported on the day that the event occurs and the DSMB informed. We will notify the MHRA, GTAC and R&D office of all SUSARs occurring during the study according to the following timelines; fatal and life-threatening within 7 days of notification and non-life threatening within 15 days.

7. ASSESSMENT AND FOLLOW-UP

Described in Section 3, Study Design and in Figure 1.

8. STATISTICS AND DATA ANALYSIS

The sample size derivation has been based on outcomes of previous clinical trials in Cystic Fibrosis and available data on what is widely considered the minimum clinically meaningful difference. Using our extensive pre-trial monitoring data from the Run-in Study, we have estimated the standard deviation (SD) of the percent change in percent predicted FEV₁ over 12 months to be 10.0%. This is based on using the mean of two measurements at baseline and the mean of two measurements at 12 months. Using duplicate measurements substantially reduces the variability, and hence increases the power of the trial. The corresponding SD using only single measurements is 12.2%. Data from the Run-in Study also demonstrated that analysis of percent predicted FEV₁ is more sensitive than analysis of absolute FEV₁, with the corresponding SD for percent change from baseline based on duplicate measurements being 11.6% for absolute FEV₁. With the SD of 10.0%, a total sample of 120 evaluable patients will give us 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 130 subjects, we will have a safety margin for individuals leaving the study prior to completion. This number also allows us to be powered at 80% or above to detect changes in the secondary outcomes LCI and CT scan parameters which are

smaller than we have previously observed in a study of intravenous antibiotics. The above power calculation is conservative in that covariate adjustment can be anticipated to increase the statistical power (see Section 10 below). The sample size for the measurements of molecular efficacy are based on our extensive experience in the previous proof-of-concept studies, and the current Pilot Study. Three clinical centres will be involved; Royal Brompton Hospital, London, Western General Hospital, Edinburgh, and Royal Hospital for Sick Children, Edinburgh. The majority of patients are already recruited and have been extensively followed-up in the Run-in Study noted above. These patients will enrol in the trial whilst the remaining patients are being sought and recruited. The adaptive design of the study will lead to an approximately 4-5 month window being available for this recruitment, which we are comfortable, on the basis of previous recruitment rates, will be more than adequate.

The primary analysis will compare the two randomised groups in terms of the mean percent change in percent predicted FEV_1 from baseline to end of treatment. An analysis of covariance (ANCOVA) model will include baseline percent predicted FEV_1 together with the other variables used in the randomisation algorithm as covariates. 'Baseline' will be taken as the average of the FEV_1 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 the final treatment. The treatment effect will be presented as an adjusted difference in mean percent change along with its corresponding 95% confidence interval. No interim efficacy analyses are planned.

As a sensitivity analysis the above analysis will be repeated, but with the logarithm of the end of treatment percent predicted FEV_1 taken as the response variable, and the logarithm of the baseline percent predicted FEV_1 included as a covariate in place of its raw value. An exploratory analysis will compare the two randomised groups in terms of the evolution of FEV_1 over the 12 months of treatment. The study is not adequately powered to explore subgroup effects for the primary outcome measure and is not designed to study paediatric patients separately, although we shall look at the stability of the treatment effect over subgroups defined by the covariates included in the ANCOVA model. The formal analyses 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. A Statistical Analysis Plan will be finalised ahead of study close to specify fully all aspects of the primary, secondary and exploratory analyses.

9. REGULATORY ISSUES

9.1 CTA

We have received Clinical Trials Authorisation from the UK Competent Authority, MHRA.

9.2 ETHICS APPROVAL

We have received approval from the Gene Therapy Advisory Committee and have submitted Site Specific Assessments (SSA) at the Royal Brompton & Harefield NHS Trust, Edinburgh Royal Infirmary and the Royal Hospital for Sick Children, Edinburgh. The study will be conducted in accordance with the recommendations for physicians involved in research on human subjects adopted by the 18th World Medical Assembly, Helsinki 1964 and later revisions and in accordance with the protocol and Good Clinical Practice (ICH GCP E6 guideline).

9.3 CONSENT

Consent to enter the study will be sought from each participant only after a full explanation has been given, an information sheet given and at least 1 week allowed for consideration. Signed consent will be obtained. All participants are free to withdraw at any time from the protocol without giving reasons and without prejudicing further treatment.

9.4 CONFIDENTIALITY

We will preserve the confidentiality of participants taking part in the study. Imperial College is registered under the Data Protection Act.

9.5 INDEMNITY

Imperial College 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. Liability for the protocol design will be accepted collectively by Oxford University, Edinburgh University and Imperial College. Clinical negligence insurance / indemnity is provided by the NHS.

9.6 SPONSOR

Imperial College London will act as the sponsor for this study.

9.7 FUNDING

Funding for the study has been obtained from the NIHR/MRC EME board.

Investigators will not receive any financial incentive for recruitment or participation in this study over and above their normal conditions of employment. Participants will be offered reimbursement of travel expenses (and overnight accommodation if required) and compensation for loss of earnings.

9.8 AUDITS AND INSPECTIONS

The study may be subject to inspection and audit by Imperial College London under their remit as sponsor, the Study Coordination Centre and other regulatory bodies to ensure adherence to GCP. A risk assessment will be performed by the Imperial College trials unit to establish a monitoring plan for the study. Ten percent of all data will be audited by independent staff in the Imperial College Trials Unit.

10. TRIAL MANAGEMENT

The day-to-day management of the trial will be co-ordinated by a Trial Steering Committee (membership to be determined) with the Imperial College Trials Unit. The DMEC will be provided with unblinded data from the first 20 subjects after 3 doses and again when 40 patients have received 6 doses of drug.

11. PUBLICATION POLICY

All publications and presentations relating to the study will be authorised by the UK CF Gene Therapy Consortium Strategy Group.

TABLE 1

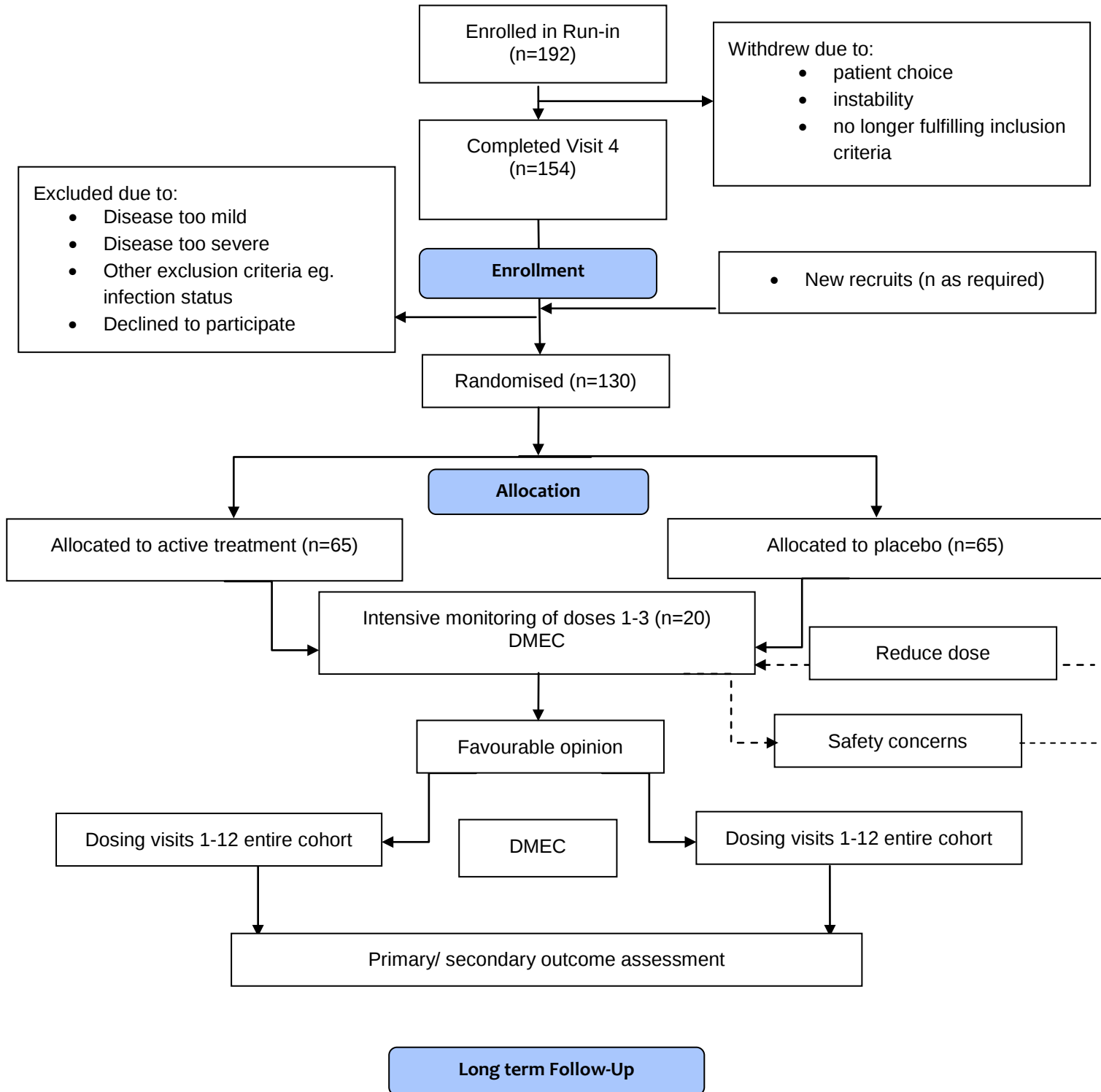
Week	Summary of Study Assessments																												49
Visit name	E&C	Intro	Screen	nPD1 ^E	nPD2 ^E	nPD3 ^E	Bronch ^E	dose1	D2a	dose2	D2b	dose3	D2c	dose4	dose5	dose6	dose7	dose8	dose9	dose10	dose11	dose12	F/U1	F/U2	Bronch				
CONSENT/ HISTORY/ EXAMINATION																													
Informed Consent	X ^A	X ^A	X ^A				X ^H																						
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-UK)			X											X			X			X			X	X					
Vital signs ^F	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 ^A	X ^A	X ^A																										
Concomitant medications			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	X				
Adverse events								X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X				
Download PiKo-6 device			X ^K					X		X		X		X	X	X	X	X	X	X	X	X	X	X	X				
SAMPLES ^F																													
Blood sampling	X	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	X				
Sputum ^G		X ^I	X ^I					X	X	X ^N	X	X	X	X		X		X				X ^L	X ^L						
24 hour sputum weight		X	X					X	X	X	X	X	X	X				X					X	X					
INVESTIGATIONS ^F																													
Lung Clearance Index		X	X											X			X			X			X	X					
Exercise Bike test			X											X					X					X					
Activity monitor			X													X								X					
CT scan			X											X ^F										X					
Gas transfer			X					X		X			X	X			X							X					
Bronchial blood flow measurement ^G			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	X				
DOSING																													
Randomisation ^E								X																					
Study dose administration								X		X		X		X	X	X	X	X	X	X	X	X							
NASAL/ BRONCH SUBGROUPS ONLY																													
Nasal potential difference	X ^I	X ^I	X ^I	X ^L	X ^L	X ^L											X						X ^L	X ^L	X ^L				
Nasal brushing			X ^D			X ^D																		X ^N	X ^N				
Bronchoscopy							X																		X				
PRE-DISCHARGE ADMIN																													
Instructions re home spirometry (PiKo-6)		X	X					X		X		X		X	X	X	X	X	X	X	X	X	X	X	X				
Instructions re use of symptom diary		X	X					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	X	X				
Superscripts																													
A- first point of contact only																													
B- to be conducted within week prior to visit																													
C- 3 occasions only																													
D- one occasion only with final nPD																													
E- may occur before Screening																													
F- limited cut																													
G- if available; timing may vary																													
H- bronchoscopy-specific consent only																													
I- on dosing visits, must be pre-dose																													
K- Patients who have undergone Intro visit only																													
L- 2 time points only																													
M- one time point only																													
N - for intensively monitored cohort only																													
P - Can induce at these timepoints; only required once pre and once post dosing																													
Q- if no sputum available, cough swab to be performed																				</									

TABLE 2

Sample collection & assay time-points																													
		week	not defined	not defined	-4						0		4		8		12	16	20	24	28	32	36	40	44	46	48	49	
		visit			1	1a	1b	1c			2	2a	3	3a	4	4a	5	6	7	8	9	10	11	12	13	14	15		
Assay			E&C (select)	Intro (new)	Screen	nPD1	nPD2	nPD3	Bronch	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	14	15	Bronch
24hr sputum weight				x	x					x	x	x	x	x	x	x				x						x	x		
Sputum ^{1,2}	total + diff cell counts			x	x					x	x	x	x	x	x			x								x	x		
	Calprotectin		x	x						x	x	x	x	x	x			x								x	x		
	IL-8		x	x						x	x	x	x	x	x			x								x	x		
	lipid		x	x						x	x	x	x	x	x			x								x	x		
	Microbiology (routine)		x	x						x	x	x	x	x	x			x								x	x		
	plasmid DNA+wet/dry weight		x	x						x	x	x	x	x	x			x								x	x		
	Molecular microbiology		x	x						x	x	x	x	x	x			x								x	x		
Urine	Lipid			x	x					x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x		
	plasmid DNA			x	x					x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x		
	Protein	x	x	x						x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x		
	Ketones	x	x	x						x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x		
	blood	x	x	x						x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x		
	cotinine ^a	x	x	x																									
	pregnancy	x	x	x						x						x											x		
Blood routine clinical	WBC and CRP, ESR, Biochem	x	x	x						x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x		
	coagulation (bronch subgroup only)	x	x ³	x ³																							x		
Blood Research ²	IL8		x	x						x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x		
	IL6		x	x						x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x		
	Calprotectin		x	x						x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x		
	lipid		x	x						x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x		
	plasmid DNA (serum)		x	x						x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x		
	plasmid DNA (separate small sample)		x	x						x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x		
Blood T-Cells and antibodies				x	x												x ⁴	x ⁴	x ⁵							x ⁶	x ⁶		
Blood genomic DNA			at any time-point when convenient - only one sample throughout study																										
Nasal brushing					x ⁷				x ⁷																		x ⁸	x ⁸	
Bronchial samples									x																			x	
x=pretreatment																													
x= 20 intensive patients only																													
x= post-treatment samples from all patients																													
Superscripts																													
1- induce on one pre-dosing visit and one F/U visit only. All other time points, spontaneous only																													
2- refer to SOP for prioritisation of assays																													
3- if not obtained on previous visit																													
4- one time point only needed																													
5- only if not already obtained																													
6- one time point only needed																													
7-one time point only needed																													
8-one time point only needed																													
9- first point of contact only																													

FIGURE 1

A randomised, double-blind, placebo-controlled Phase 2B trial of repeated application of gene therapy in patients with cystic fibrosis
Patient Journey Flow Diagram



Appendix 1: Solutions for measuring airway potential difference (PD)

Reagents

Agent	Source	Catalogue number	Size	Designation
NaCl USP	Spectrum	SO155P	500g	NIMP
CaCl ₂ ·2H ₂ O USP	Spectrum	CA140P	500g	NIMP
MgCl ₂ ·6H ₂ O USP	Spectrum	MA110P	500g	NIMP
K ₂ HPO ₄ USP	Spectrum	PO204P	500g	NIMP
NF KH ₂ PO ₄	Spectrum	PO200P	500g	NIMP
Sodium gluconate USP	Spectrum	S128P	500g	NIMP
Calcium gluconate USP	Spectrum	CA1939	500g	NIMP
Potassium gluconate USP	Spectrum	PO178	500g	NIMP
MgSO ₄ ·7H ₂ O USP	Spectrum	MA135P	500g	NIMP
Amiloride HCl USP	Spectrum	AM122	5g	NIMP
Potassium chloride USP	Spectrum	PO170P	500g	NIMP
Isoprenaline for injection	Hospital Pharmacy		100 µg/ 2ml ampule	NIMP
Water for injection	Hospital Pharmacy			NIMP
			1000ml	

Composition of perfusion solutions:**1. Ringers**

Add to water:

	mM/L	g/L
NaCl	135	7.83
CaCl ₂ ·2H ₂ O	2.25	0.33
MgCl ₂ ·6H ₂ O	1.2	0.24
K ₂ HPO ₄	2.4	0.42
KH ₂ PO ₄	0.4	0.05

pH confirmed between 7.0-7.6. Solution filtered (0.22 micron).

May be refrigerated for 3 months or frozen (-20°C) for 6 months

2. Amiloride hydrochloride in Ringers

On day of use, Amiloride 0.03mg/ml (0.1mM) added to Ringers

3. Zero chloride (ZC) solution

Add to water:

	mM/L	g/L
Sodium gluconate	135	29.43
Calcium gluconate	2.2	0.95
K ₂ HPO ₄ USP	2.4	0.42
KH ₂ PO ₄	0.4	0.05
MgSO ₄ ·7H ₂ O	1.2	0.3

Check that pH between 7.0-7.6. Filter with 0.22 micron filter.

May be refrigerated for 3 months or frozen (-20°C) for 6 months

On day of use Amiloride 0.03mg/ml (0.1mM) added to ZC solution

4. Zero chloride solution with Isoprenaline

On day of use, 0.25mg Isoprenaline added to 100ml of ZC solution