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

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Statistical Analysis Plan (SAP)
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1. Approvals

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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

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*Any item marked with an asterisk is for future 'AUC' type longitudinal analysis

Statistical Analysis Of Nasal & Bronchial Brushing DNA & RNA Samples

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

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.

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

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.

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

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.

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

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

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.

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 :

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

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

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

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

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)

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	

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**

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))

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)

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.

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