Statistical Analysis Plan (SAP)

PAciFy Cough: A multicentre, double blind, placebo controlled, crossover trial of morphine sulfate for the treatment of PulmonAry Fibrosis Cough

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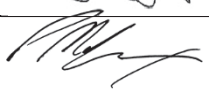
Table of Contents

	Page
1. Approval signatures	3
2. Abbreviations	4
3. Introduction and study summary	5
4. Study objectives	5
5. Study design	8
6. Populations of analysis sets	9
7. Variables of analysis	9
8. Statistical methodology	10
Appendix	15

1. Approval Signatures

Name	Signature	Role	Date
Prof Toby Maher		TSC Chair	26th Sept 2022
Dr Phil Molyneaux		Chief Investigator	17th Oct 2022
Mr Winston Banya		Trial Statistician	17th Oct 2022

2. Abbreviations

AE	adverse events
CTCAE	Common Terminology Criteria for Adverse events
D12	dyspnoea 12
DLCO	diffusion capacity of lung
FEV1	forced expiratory volume in 1 second
FVC	forced vital capacity
GEE	general estimating equation
HADS	hospital anxiety and depression scale
HRCT	high resolution computed tomography
IMP	investigational medicinal product
ITT	intent-to-treat
K-BILD	King's brief interstitial lung disease
LCQ	Leicester cough questionnaire
L-IPF	living with idiopathic pulmonary fibrosis
LME	linear mixed effects
MFT	Manchester University NHS Foundation Trust
MST	morphine sulfate
PROs	patient reported outcomes
SAP	statistical analysis plan
SAS	safety analysis set
VAS	visual analogue scale
WOCBP	woman of childbearing potential

3. Introduction and study summary

This statistical analysis plan (SAP) outlines the data and procedures used for assessing the efficacy, safety, and tolerability of morphine sulfate (MST) from Protocol v4.1 Pacify Cough trial: A multicentre, double blind, placebo controlled, crossover trial of morphine sulfate for the treatment of PulmonAry Fibrosis Cough.

4. Study Objectives / Hypotheses Testing

Due to the crossover design, changes will be compared between day 14 versus 0 for the first treatment period, and between day 36 and 22 for the second period.

4.1 Primary Objective

The primary efficacy endpoint is the percent change in daytime cough frequency (coughs per hour) from baseline as assessed by objective digital cough monitoring following 14 days of treatment.

4.2 Secondary Objectives

- Change from baseline in health-related quality of life scores (L-IPF, HADS, K-BILD)
- Change from baseline in self-reported cough (LCQ & VAS)
- Change from baseline in Dyspnoea (D12)
- Change from baseline in global impression of change in quality of life, cough and breathlessness.
- Proportion of responders with a minimum of 20% decrease from baseline at the end of treatment in awake average cough count.

4.3 Exploratory objectives

- To explore the relationship between activity and cough
- To explore the association between serum biomarkers of fibrosis and response to therapy.

5. Design

5.1 Study Design

This is a randomised, double-blind, placebo controlled two-way crossover study (see Figure 1). Patients will be randomised (1:1) to either placebo twice daily or

MST 5mg twice daily for 14 days. Patients will then crossover after a 7 day wash out period. Those that were randomised to placebo will be given MST 5mg twice daily and those that were randomised to MST will take placebo for 14 days. Cough recordings and quality of life surveys will be conducted pre and post each treatment period. The study will recruit 44 patients with a diagnosis of idiopathic pulmonary fibrosis (IPF).

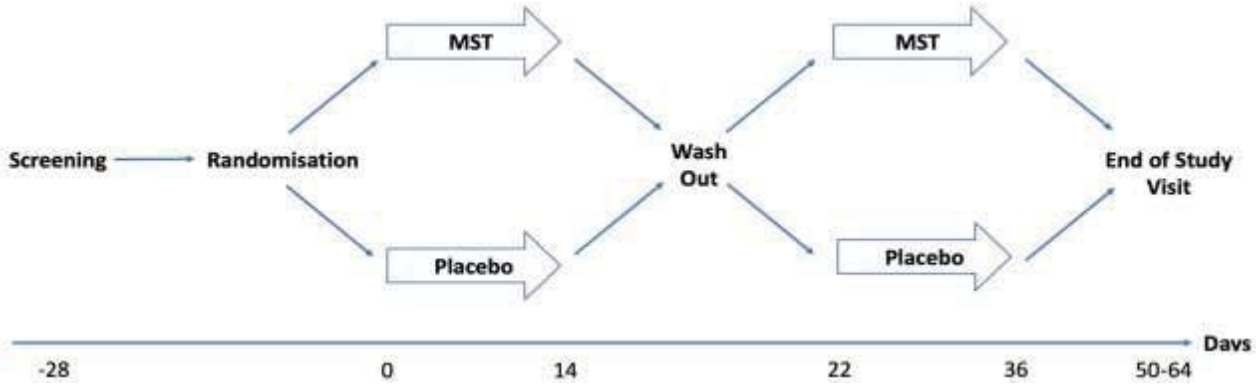


Figure 1. Schematic of trial design

5.2 Treatment Groups

Half of the patients will be given MST for the first treatment period and will crossover to placebo for the second period. The remaining patients will start with placebo and crossover to MST.

5.3 Study Population

Individuals who meet a multi-disciplinary diagnosis of IPF based on established international criteria will be recruited prospectively from the Royal Brompton Hospital, Manchester University NHS Foundation Trust (MFT) and Aintree University Hospital NHS Foundation Trust. We will include subjects with a cough VAS score of 30mm or over.

5.4 Eligibility Criteria

Inclusion criteria

1. Self-reported cough (> 8 weeks) with cough VAS ≥ 30/100
2. A diagnosis of IPF within 5 years prior to the screening visit, as per applicable ATS/ERS/JRS/ALAT guidelines, in line with hospital records
3. Male and female participants aged ≥ 40 – 90 years at the time of signing informed consent
4. Sex:
   4.1 Male participants: A male participant must agree to use contraception

as detailed in Appendix 2 of this protocol during the study and for at least 90 days after the follow-up visit, and refrain from donating sperm during this period

4.2 Female participants: A female participant is eligible to participate if she is not pregnant, not breastfeeding, and not a woman of childbearing potential (WOCBP) as defined in Appendix 2.

5. Meeting all of the following criteria during the screening period: FVC ≥ 45% predicted of normal, Forced expiratory volume in 1 second (FEV1)/FVC ≥0.7, DLCO corrected for Hb ≥30% predicted of normal. Lung function performed within 12 months of the screening period is acceptable.
6. The extent of fibrotic changes is greater than the extent of emphysema on the most recent HRCT scan (investigator determined within 24 months of the study screening visit).
7. Written informed consent

Exclusion criteria

1. Treatment with immunosuppressive therapy or antibiotics within last 4 weeks of screening visit. A stable dose of corticosteroids equivalent to prednisolone of 10 mg per day or less, if used for an indication other than pulmonary disease will be permitted.
2. Current smoker
3. History of alcohol and drug(s) addiction
4. Regular use of sedative therapies
5. Acute IPF exacerbation within 6 months prior to screening and/or during the screening period.
6. Concurrent use of pirfenidone or Nintedanib, unless receiving a stable dose for at least 8 weeks prior to screening
7. Use of ACE inhibitors
8. Patients with co-existent conditions known to be associated with the development of fibrotic lung disease. This includes; connective tissue disease, suspected drug- induced lung disease, asbestosis or other asbestos related disease (pleural plaques, mesothelioma), granulomatous disease including sarcoidosis. Patients with an auto- immune profile considered diagnostic for a specific connective tissue disease will be excluded, even in the absence of systemic symptoms. Non-specific rises in auto antibodies *e.g.* rheumatoid factor, anti-nuclear antibody etc. will not be used to exclude individuals from the study.
9. Significant other organ co-morbidity including hepatic or renal impairment and pulmonary hypertension (investigator determined).
10. Significant coronary artery disease (myocardial infarction within 6 months or ongoing unstable angina within 4 weeks of screening visit) or congestive cardiac failure based on clinical examination

11. Patients at significant risk for side effects, intolerance or allergy to morphine
12. Pregnant and breastfeeding patients, or women of child-bearing potential, not using a reliable contraceptive method. A urine pregnancy test will be performed in females of child-bearing potential at the initial study visit.
13. Unable to provide informed written consent
14. Predicted life expectancy < 6 months
15. Use of long-term oxygen therapy. Use of ambulatory oxygen will be permitted.
16. Current or use of opiates within 14 days of the screening visit.

5.5 Blinding

This will be a double-blind study with both the patient and investigator blinded to study treatment. The investigator or treating physician may un-blind a patient's treatment assignment in the case of an emergency, when knowledge of the study treatment is essential for the appropriate clinical management or welfare of the patient.

5.6 Sample Size

Based on previous repeatability data in IPF patients studied over a 11 day period a sample size of 40 subjects will have 90% power to detect a true difference in 24h cough frequency on the natural log scale -0.132 or 0.132 (equivalent to ~35% change) with a probability (power) of 0.9; assuming a within subject standard deviation of 0.310 (natural log scale) and the standard significance level of 0.05. Allowing for a 10% drop out rate, we will aim to recruit 44 patients for the study.

5.7 Schedule of Time and Events

The pre-screening phase (minimum of 1 day, maximum of 28 days where necessary) will establish baseline characteristics and suitability for enrolment into the study. The screening and baseline visit will be conducted on the same day (day 0). Baseline VAS, 24 hour cough recording and quality of life surveys will be conducted. Subjects will commence the first dose of IMP on day 1 and continue for 14 days. On the second visit (day 14) repeat VAS, cough recording and quality of life surveys will be conducted, in addition to a patient assessed global impression of change. This will be followed by a 7 day washout period.

During the third (crossover) visit (day 22) patients will switch to whichever treatment they did not take during the first period. A recording of VAS, cough count and surveys will be conducted. IMP will commence on day 23, for a duration of 14 days. At the crossover follow up (day 36) repeat VAS, cough recording and quality of life surveys will be conducted, in addition to a patient assessed global impression of change. A fifth and final visit will be conducted via telephone review to ensure safety. During all visits adverse events will be recorded. A summary chart of assessments is in **Appendix 1**.

5.8 Randomization

Subjects will be randomised sequentially to a sequence group defining the order in which active drug and placebo are given, according to a computer-generated schedule (Sealed Envelope EDC). Access to the database at each participating centre will be restricted to authorised study staff.

6. Populations of Analysis Sets

6.1 Intent-to-Treat (ITT) Population

All randomized patients will be included in the intention to treat population. Patients will be analyzed according to the treatment group to which they are randomized.

6.2 Safety population

The safety analysis set (SAS) will consist of all subjects randomized. Subjects will be classified based upon the treatment received.

6.3 Per protocol Population

A per protocol analysis set will consist of all subjects who receive at least 80% of doses for the treatment period and provided a baseline and post-baseline primary endpoint observation for both treatment periods.

7. Variables of Analysis

7.1 Primary Efficacy Variable

Daytime cough frequency (as measured by coughs per hour). Cough recordings consisting of at least 4 hours daytime duration will be eligible for analysis.

7.2 Secondary Efficacy Variables

These will include change from baseline for each treatment period in patient reported outcomes (PROs), namely cough VAS and LCQ; and the quality of life scores L-IPF, HADS, K-BILD, Dyspnoea 12. Changes in global impression as percentage of subjects with stable, improvement or worsening of cough, breathlessness and overall quality of life will be presented. The proportion of subjects classified as responders (minimum of 20% decrease from baseline) in awake average cough count in a 24hr period will be evaluated for each treatment group.

7.3 Tertiary Efficacy Variables

These variables are exploratory and will be analysed according to the data. Activity data is collected as step count every 15 minutes and correlations with cough counts will be assessed.

7.4 Safety Variables

Adverse events (AE) will be recorded according to the Common Terminology Criteria for Adverse events (CTCAE) version 5.0. An overall summary of AEs will be presented. This will include the number and percentage of subjects experiencing any AEs, study drug related AEs, SAEs, study drug related SAEs, discontinued due to AE and deaths. The number and percentage of subjects experiencing each AE, study drug related AE, and SAEs will be summarized by treatment group according to system organ class and preferred CTCAE term. An additional table including only preferred terms summarized by treatment for the number and percentage of subjects experiencing each AE will be presented as well.

7.5 Demographic Variables

Demographic data will include age, gender and ethnicity of the subjects.

8. Statistical Methodology

8.1 General Methodology

For continuous variables, descriptive statistics will include the number of subjects reflected in the calculation (n), mean, standard deviation, median, minimum and maximum. Categorical variables will be summarized using frequency counts and percentages. Treatment differences in continuous data collected over multiple study visits (e.g., changes from baseline in awake cough frequency) will be assessed using the general estimating equation (GEE) model.

Individual subject data obtained from the electronic case report forms (eCRFs), cough monitoring data and any derived data will be presented by subject in data listings to facilitate the investigation of tabulated values and to allow for the clinical review. In general, all tables will be presented by treatment group and listings will be displayed by sorted unique subject identifier and treatment group. A subject identification number is defined as a 3-digit site number followed by a 3-digit sequentially assigned subject id (xxx-xxx). The treatment group in the listings will be based on the planned treatment(s) as allocated by the randomization scheme.

All p-values will be rounded to 4 decimal places. If a p-value is less than 0.0001 it will be displayed as “< 0.0001”, a p-value rounding to 1 will be displayed as “> 0.9999”. Unless otherwise specified all summaries will be performed by treatment group, all efficacy analyses will be based upon the ITT analysis set and all safety analyses will be based upon the SAS. Demographic and baseline characteristic analyses will be based upon the intention to treat analysis set.

The analyses described in this plan are considered a priori, in that they have been defined prior to database lock. Any analyses not described herein performed subsequent to database lock will be considered post hoc and exploratory.

8.2 Data Handling rules for cough analysis

The 24-hour cough frequency (coughs per hour) for a specified visit is calculated as:

24-hour cough frequency = (total number of cough events over 24-hour monitoring period)/ (Total duration (in hours) over 24-hour monitoring period)

The awake cough frequency (coughs per hour) is defined as below:
Awake cough frequency = (total number of cough events during the monitoring period the subjects is awake)/(Total duration (in hours) for the monitoring period the subject is awake)

Awake duration (hours) is time between waking up and sleep during the 24-hour monitoring period.

The cough data will contain all cough events occurring during that 24 hour monitoring period as well as the information about “sleep time” and “awake time”. Any session with duration of recording < 4 hours will be considered as missing. Repeated monitoring may be conducted when there is an equipment failure or an unacceptable evaluation from the initial monitoring. In general, each 24-hour session is composed by an awake monitoring period and a sleep monitoring period. If a subject did not wake up before the end of the recording session, it will be assumed that the subject slept for the rest of the session. The session will have missing awake time, and the rest of session will be considered under the sleep monitoring period. For any session with both sleep time and awake time missing, the entire 24-hour session will be considered under the awake monitoring period, unless the session has early termination of recording.

On each collection day, the cough count, the actual cough monitoring duration (in hours), and the coughs per hour will be derived for the total 24-hour period, the awake period, and the sleep period, respectively.

The percent change in 24-hour coughs per hour at each specific visit is defined as below:

percent change in 24-hour cough frequency =
[(change from baseline in 24-hour cough frequency)/(baseline 24-hour cough frequency) x 100]

8.3 Patient Flow (CONSORT diagram)

A CONSORT diagram (figure 2) will present numbers for enrolment, treatment allocation completion, follow up and analysis.

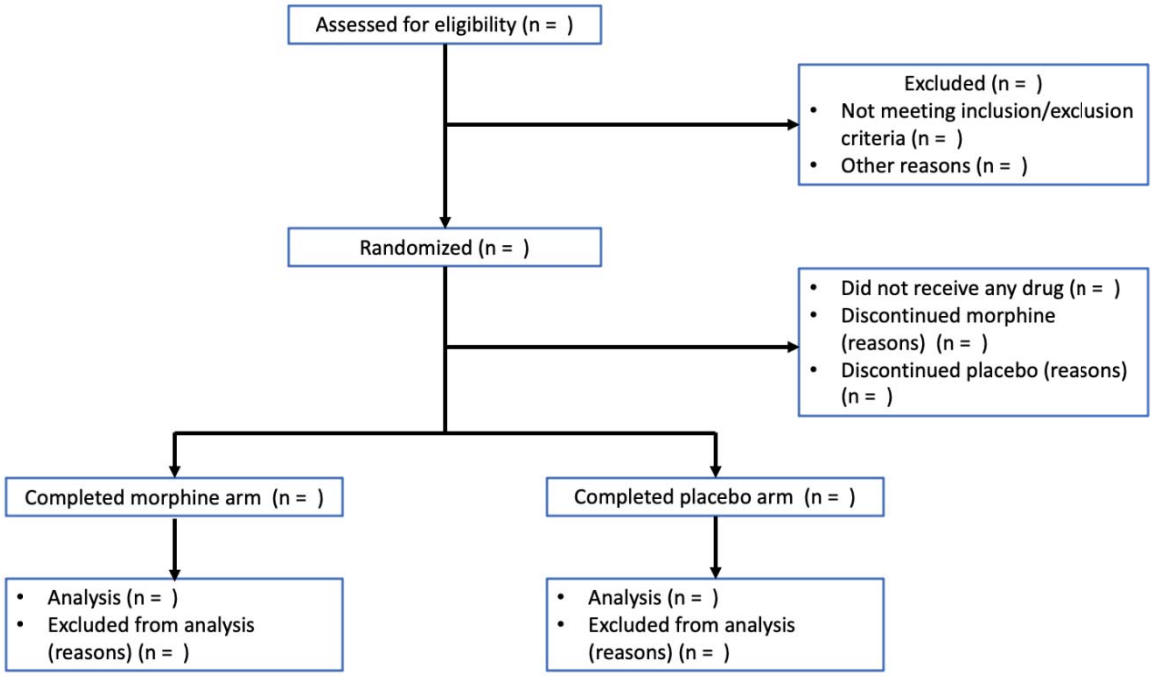


Figure 2. Sample CONSORT diagram

8.4 Primary Efficacy Analysis

The primary efficacy endpoint will be the change from baseline in log-transformed awake average cough count. The primary analysis will be conducted using a general estimating equation (GEE) of log transformed cough counts if levels of missing data permit. Otherwise a Linear Mixed Effects (LME) model will be employed.

As the change in awake cough frequency will have a skewed and wide distribution, the primary analysis for the primary endpoint will be on the log scale of the cough frequency data. The difference between morphine and placebo will be estimated using a GEE model. The model will include fixed effects for period, treatment group, sequence, and all interaction terms, and the log-transformed period-specific baseline value as a covariate. If the fixed effect for sequence should prove to be non-significant (that is, if the p-value > 0.1), the effect may be removed from the model. The GEE model will use all available awake cough frequency data from Days 0, 14, 22, and 36. Contrasts will be constructed to compare the change in cough frequency with morphine treatment to the change in the placebo group. The least-squares (LS) mean change from baseline (on the log scale) with the associated standard errors will be reported for each treatment group. Estimated treatment differences (morphine vs. placebo) along with corresponding 95% CIs and two-sided p-values will also be reported.

In addition, the geometric mean of awake cough frequency will be presented by treatment and by visit. The percent difference change between morphine and placebo will be estimated by 100 (e^{diff} - 1), where diff is the difference provided by the analysis of the log-transformed variable.

If at certain time points zero coughs per hour are recorded, this will be replaced by a cough rate of 0.1 for the calculation of geometric means.

8.5 Secondary Efficacy Analysis

Secondary efficacy endpoints will be summarized using descriptive statistics. For continuous endpoints (*e.g.,* change from baseline), summaries will include mean, median, standard deviation, minimum, and maximum. For discrete variables (*e.g*., frequency), summaries will include number of instances and percentage of total instances for that category or time period.

Analyses for secondary endpoints (change from baseline in L-IPF, HADS, K-BILD, LCQ, VAS, D12) will proceed in a similar manner as the analyses indicated in section 8.4 above for the primary efficacy endpoint. For cough frequency data will be log transformed, however this is unlikely to be necessary for PROs. To explore the correlation between VAS and cough frequency, scatter plots between VAS and objective cough frequency counting will be generated.

8.6 Cough frequency responder analysis

The cough frequency responder endpoint is:

- Proportion of subjects with ≥ 20% reduction in awake objective cough frequency per hour at the end of treatment (i.e., Day 14 and Day 36)

The proportion of participants with ≥ 20% of reduction from baseline in awake cough frequency at each specific visit is the number of participants with ≤ -20% change in awake cough frequency divided by the total number of participants with available data at the specific visit.

8.7 Safety Analysis

The safety analysis set will include all patients who have taken at least one dose of study medication. All AEs will be presented as outlined in section 7.4.

8.8 Sensitivity Analysis

The GEE analyses for assessing awake cough frequency are valid if the missing data are missing at random (MAR), meaning that the probability of a value being missing, conditional on the observed data and factors in the statistical model, is random and not dependent on the unknown value of the missing data point. Therefore, a sensitivity analyses under missingness not at random (MNAR) will be conducted for awake cough frequency to evaluate the robustness of efficacy results and the effect of missing data, as appropriate.

Sensitivity analysis will impute worst change from baseline i.e. the largest increase in cough frequency from baseline. Values will be imputed where day 14/36 data was missing, as long as the baseline data on day 0/14 were available for that period. Hence no values will be imputed where data was missing for both baseline and day 14/36 in a period.

8.9 Exploratory analysis

In addition, the exploratory analyses of ≥ 50% of reduction and ≥ 70% of reduction will be conducted for awake cough frequency.

Appendix 1. Summary chart of study assessments.

	Pre-screening Visit	**Baseline Screening & Randomization Visit (Treatment 1)**		**Follow up Visit 1**			**Crossover Visit (Treatment 2)**		**Follow up Visit 2**		**EOS Visit**	**Unscheduled**
Visit	REMOTE	1	1a	2 REMOTE	2a		3	3a	4	4a	5 REMOTE	
Treatment day	-28 to -1	0	1	14	15		22	23	36	37	50-64	
				(+/- 1 day)			(+/- 1 day)		(+/- 1 day)			
PIS and ICF sent to potentially eligible patients	x					WASH OUT – 7 DAYS						
Verbal consent obtained over the phone	x											
Informed consent		x										
Demographics		x										
Medical history		x										x
Review of concomitant medications		x		x			x		x			x
Adverse events review		x		x			x		x		x	x
Physical examination		x										x
Spirometry and gas		x										x
Cough VAS at screening		x										
Inclusion/exclusion criteria		x										
Vital signs – body temperature, heart rate, blood pressure, O_2		x					x		x			x
ECG (optional)												x
Routine bloods (FBC, renal and liver)		x										x
Blood for DNA, RNA, biomarkers		x										
Pregnancy test α		x							x			
QoL questionnaires β		x		x			x		x			
Global impression of change				x					x			
Randomisation		x										
IMP dispensing		x					x					
Commence IMP			x					x				
IMP reconciliation							x		x			

IMP compliance				x								
Attach cough monitor and		x		x			x		x			
Provide pre-paid envelope		x		x			x		x			
Patient to return cough monitor and Fitbit			x		x			x		x		
Post out cough monitor, Fitbit and pre-paid envelope (after receipt of cough monitor and Fitbit in time for			x									
Telephone call	x		x	x	x			x		x	x	
α- for women of child bearing potential												
β- send electronic link *via* eCRF, includes Cough VAS, L-IPF, K-BILD, Dyspnoea-12, LCQ, HADS												
† - lung function performed within the last 12 months is acceptable												
¥ - for visit 1, 3 & 4 a member of the research team will attach monitor and Fitbit, for visit 2 the patient will attach the devices themselves												