DPM-CF-303 Long Term Administration of Inhaled Mannitol in Cystic Fibrosis - A Safety and Efficacy in Adult Cystic Fibrosis Subjects

Protocol: v2.0 13 October 2014

NCT02134353

Clinical Trial Protocol DPM-CF-303

Version 2.0, 13th October 2014

Long Term Administration of Inhaled Mannitol in Cystic Fibrosis – A Safety and Efficacy Trial in Adult Cystic Fibrosis Subjects

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Phase III

Compound: Dry powder mannitol

Sponsor

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Prepared by

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Confidential Statement

SYNOPSIS

Protocol Version & Date:	V2.0 13th October 2014	**Drug Name:**	Dry powder mannitol
Trial Number:	DPM-CF-303	**Phase:**	III
Indication:	Cystic fibrosis	**Trial Centers:**	Multicenter
Title:	Long Term Administration of Inhaled Mannitol in Cystic Fibrosis – A Safety and Efficacy Trial in Adult Cystic Fibrosis Subjects		
Sponsor:	Pharmaxis Limited, 20 Rodborough Road, Frenchs Forest, NSW 2086 Australia. Tel: +61 2 9454 7200		
Trial Duration	28 weeks	**Number of Subjects**	350 to 440
Trial Design	Randomized, multicenter, double-blind, controlled, parallel group		
Primary Objective(s):	To determine whether inhaled mannitol (400 mg b.i.d.) is superior to control (50 mg b.i.d.) for improving lung function as measured by mean change from baseline FEV_1 (mL) over the 26-week treatment period in adult subjects with cystic fibrosis (CF).		
Secondary Objectives:	*There are hierarchical and non-hierarchical secondary objectives. Analysis of hierarchical secondary objectives will continue until a non-significant p-value (i.e. $p > 0.05$) is returned.* **Hierarchical secondary objectives** 1. To determine whether inhaled mannitol (400 mg b.i.d.) is superior to control for improving lung function as measured by mean change from baseline FVC (mL) over the 26-week treatment period in adult subjects with CF; 2. To determine whether inhaled mannitol (400 mg b.i.d.) is superior to control in increasing the time to first pulmonary exacerbation over the 26-week treatment period in adult subjects with CF; 3. To determine whether in adult subjects with CF, inhaled mannitol (400 mg b.i.d.) is superior to control for reducing the number of days on antibiotics due to pulmonary exacerbation (oral, inhaled or IV); 4. To determine whether in adult subjects with CF, inhaled mannitol (400 mg b.i.d.) is superior to control for decreasing the number of days in hospital due to pulmonary exacerbation; *and* 5. To determine whether inhaled mannitol (400 mg b.i.d.) decreases the rate of pulmonary exacerbations over the 26-week treatment period compared to control in adult subjects with CF.		

	Non-hierarchical secondary objectives To determine whether in adult subjects with CF, inhaled mannitol (400 mg b.i.d.) is superior to control • for decreasing the incidence of exacerbations (i.e. the proportion of subjects with one or more exacerbation); • for improving ease of expectoration; *and* • for improving subject reported respiratory symptoms as measured by CFQ-R respiratory domain.
Primary Endpoint(s):	The primary endpoint is the mean change in FEV_1 (mL) from baseline (Visit 1) over the 26-week treatment period (to Visit 4).
Secondary Endpoints:	• Mean change from baseline FVC (mL) over the 26-week treatment period; • Time to first pulmonary exacerbation over the 26-week treatment period; • Rate of pulmonary exacerbations over the 26-week treatment period; • Number of days in hospital due to pulmonary exacerbation; • The incidence of pulmonary exacerbations; • Number of days on antibiotics (oral, inhaled or IV) due to pulmonary exacerbation; • Ease of expectoration measured using a visual analogue scale; *and* • CFQ-R respiratory domain score.
Hypothesis:	We hypothesize that inhaled mannitol 400 mg b.i.d. will increase the mean change from baseline FEV_1 (mL) compared to control over the 26-week treatment period in adult subjects with cystic fibrosis. For the purposes of determining an appropriate sample size for statistical power while retaining trial feasibility in an orphan disease population the trial is powered to detect a difference of 80 mL, however any improvement in FEV_1 could be considered clinically meaningful.

Trial Population: *(Diagnosis for main criteria for inclusion)*	**Subject Inclusion Criteria** The subject must meet all of the following criteria: 1. Have given written informed consent to participate in this trial in accordance with local regulations; 2. Have a confirmed diagnosis of cystic fibrosis (positive sweat chloride value ≥ 60 mEq/L) and/or genotype with two identifiable mutations consistent with CF, accompanied by one or more clinical features consistent with the CF phenotype); 3. Be aged at least 18 years old; 4. Have FEV_1 > 40 % and < 90% predicted (using NHanes III [1]); 5. Be able to perform all the techniques necessary to measure lung function; 6. Be adherent with maintenance therapies (antibiotics and/or rhDNase), if used, for at least 80% of the time in the two weeks prior to visit 1; *and* 7. If rhDNase and/or maintenance antibiotic are being used treatment must have been established at least 1 month prior to screening (Visit 0). The subject should remain on the rhDNase and / or maintenance antibiotics for the duration of the trial. The subject should not commence treatment with rhDNase or maintenance antibiotics during the trial. **Subject Exclusion Criteria** The subject must NOT meet any of the following criteria: 1. Be investigators, site personnel directly affiliated with this trial, or their immediate families. Immediate family is defined as a spouse, parent, child or sibling, whether biologically or legally adopted; 2. Be considered "terminally ill" or eligible for lung transplantation; 3. Have had a lung transplant; 4. Be using maintenance nebulized hypertonic saline in the 2 weeks prior to visit 1; 5. Have had a significant episode of hemoptysis (> 60 mL) in the three months prior to Visit 0; 6. Have had a myocardial infarction in the three months prior to Visit 0; 7. Have had a cerebral vascular accident in the three months prior to Visit 0; 8. Have had major ocular surgery in the three months prior to Visit 0; 9. Have had major abdominal, chest or brain surgery in the three months prior to Visit 0; 10. Have a known cerebral, aortic or abdominal aneurysm; 11. Be breast feeding or pregnant, or plan to become pregnant while in the trial; 12. Be using an unreliable form of contraception (female subjects at risk of pregnancy only); 13. Be participating in another investigative drug trial, parallel to, or within 4 weeks of screening (Visit 0); 14. Have a known allergy to mannitol; 15. Be using non-selective oral beta blockers; 16. Have uncontrolled hypertension –i.e. systolic BP > 190 and / or diastolic BP > 100; 17. Have a condition or be in a situation which in the Investigator's opinion may put the subject at significant risk, may confound results or may interfere significantly with the subject's participation in the trial; or 18. Have a failed or incomplete mannitol tolerance test (MTT) (as evaluated in Section 8.1.1.1).

Dosage and Administration:	Mannitol (400 mg b.i.d.) or control (50 mg b.i.d.)
Blinding:	Double-blind
Data Collection:	*Refer to the time and events schedule for a schedule of activities*
Efficacy Evaluations:	*Refer to the time and events schedule for a schedule of activities*
Safety Evaluations:	*Refer to the time and events schedule for a schedule of activities*
Statistical Methods:	**Sample size:** The minimum sample size is based on the assumption that the change from baseline in FEV_1 on control is 0 mL and the change from baseline in FEV_1 on mannitol is 80 mL. A sample size of 350 (175 Mannitol : 175 Control) subjects will yield 90 % power at the 0.05 significance level to detect this difference between the treatment groups assuming a pooled standard deviation of 230 mL. **Primary Endpoint:** The mean absolute change from baseline FEV_1 (mL) over weeks 6, 14 and 26 will be compared between the two treatment groups with a restricted maximum likelihood (REML) based repeated measures approach. **Secondary Endpoints:** The mean absolute change in FVC, the changes from baseline in ease of expectoration and the CFQ-R respiratory domain score will be modeled as per the primary endpoint. The time to first pulmonary exacerbation will be explored using Cox Proportional Hazards. Changes in rates of pulmonary exacerbations, antibiotic use and days in hospital will be explored using negative binomial models. Analysis of incidence of pulmonary exacerbation will be completed using logistic regression.

TIME AND EVENTS SCHEDULE

Event	Screening Visit 0	☎	Visit 1	☎	☎	Visit 2	☎	☎	Visit 3	☎	☎	☎	Visit 4	☎	IMP discontinuation visit[a]
Week	**-5 to- 2**	**-1**	**0[b]**	**2**	**4**	**6**	**8**	**12**	**14**	**16**	**20**	**24**	**26**	**27**	
Visit window		**± 1 day**		**± 3 days**	**± 3 days**	**± 7 days**	**± 3 days**	**± 3 days**	**± 7 days**	**± 3 days**	**± 3 days**	**± 3 day**	**- 7 days to + 28 days**	**± 1 day**	**Within 2 weeks of IMP discontinuation**
Informed consent	X														
Inclusion/exclusion criteria	X														
Medical history / demographics	X														
Concomitant medications	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Physical examination/ vital signs	X		X			X			X				X		X
Pulmonary function tests	X		X			X			X				X		X
Urine pregnancy test	X														
Pulmonary exacerbations review			X			X			X				X		X
MTT procedure	X														
Randomize subject			X[c]												
Dispense trial medication & bronchodilator			X			X			X						
Administer treatment dose in clinic			X			X			X				X		
Sputum qualitative microbiology	X														
Screening blood sample	X														

[a] *The IMP discontinuation visit is used for all subjects that discontinue IMP early, but are remaining in the study.*

[b] *Subjects should be stable and clear of pulmonary exacerbations for at least two weeks prior visit 1. If a subject has an exacerbation after Visit 0 (screening), visit 1 should occur 2 to 5 weeks from the end of the treatment of the exacerbation or the end of the adverse event, whichever is later.*

[c] *Randomize eligible subjects if compliance with maintenance therapies (antibiotic & rhDNase) is at least 80% in the two weeks prior to visit 1.*

Event	Screening Visit 0	☎	Visit 1	☎	☎	Visit 2	☎	☎	Visit 3	☎	☎	☎	Visit 4	☎		IMP discontinuation visit[a]
Week	**-5 to- 2**	**-1**	**0[b]**	**2**	**4**	**6**	**8**	**12**	**14**	**16**	**20**	**24**	**26**	**27**		
Visit window		**± 1 day**		**± 3 days**	**± 3 days**	**± 7 days**	**± 3 days**	**± 3 days**	**± 7 days**	**± 3 days**	**± 3 days**	**± 3 day**	**- 7 days to + 28 days**	**± 1 day**		**Within 2 weeks of IMP discontinuation**
Issue subject diary	X															
Review subject diary			X			X			X				X			X
Collect subject diary													X			
Adverse event assessment		X	X	X	X	X	X	X	X	X	X	X	X	X		X
Ease of expectoration VAS			X			X			X				X			X
CFQ-R respiratory domain			X			X			X				X			X
IMP compliance and accountability[d]						X			X				X			
Discuss adherence to treatment (if subject has discontinued IMP, schedule IMP discontinuation visit within 2 weeks of last IMP)				X	X		X	X		X	X	X				
Remind subject of next visit or phone call, withholding periods, to complete subject diary, and to return trial drug (if applicable)[e]		X	X	X	X	X	X	X	X	X	X	X	X			X
Discharge subject from trial														X		

d *IMP accountability and collection must occur at the next scheduled visit for any subjects that withdraw from the study or discontinue from IMP early*

e *The subject diary should be collected for all subjects, including those who withdraw early from the study*

TABLE OF CONTENTS

CONTACT LIST

A full trial contact list will be provided as a separate document.

Changes in the names, addresses or telephone numbers of these contacts will in general not require a protocol amendment. A protocol amendment maybe required for contact information being referenced, for the addition or removal of the primary Principal Investigator, regulatory authorities documented notifications or updates.

ABBREVIATIONS

ADR	adverse drug reaction
AE	adverse event
ATS/ERS	American Thoracic Society / European Respiratory Society
b.i.d.	twice daily
BOCF	baseline observation carried forward
BTPS	body temperature and pressure, saturated
CF	cystic fibrosis
CFTR	cystic fibrosis transmembrane conductance regulator
cGMP	current Good Manufacturing Practice
CRF	case report form
eCRF	electronic case report form
DSMB	data safety monitoring board
FAO	Food and Agriculture Organization of the United Nations
FDA	US Food and Drug Administration
FEF_{25-75}	forced expiratory fraction from 25% to 75% of vital capacity
FEV_1	forced expiratory volume in one second
FVC	forced vital capacity
GCP	Good Clinical Practice
HR	hazard ratio
HRCT	high resolution computed tomography
ICF	informed consent form
ICH	International Conference on Harmonization
ICMJE	international committee of medical journal editors
IEC	Independent Ethics Committee
IRB	Institutional Review Board
IMP	investigational medicinal product
IWRS	interactive web response system
ITT	intent to treat
IUDR	imputation using dropout reason
IV	intravenous
JECFA	The joint FAO / WHO expert committee on food additives
MDI	metered dose inhaler
MedDRA	Medical Dictionary for Regulatory Activities
MI	multiple imputation
MMRM	mixed-effect model repeated measures
MTT	mannitol tolerance test
PEF	peak expiratory flow
PP	per protocol
REML	restricted maximum likelihood

rhDNase	Recombinant human deoxyribonuclease [redacted]
SABA	Short-acting beta$_2$ agonist
SAE	serious adverse event
SAP	statistical analysis plan
SOP	standard operating procedure
SpO_2	blood oxygen saturation
VAS	visual analogue scale
WHO	World Health Organization
WOCF	worst observation carried forward

1 INTRODUCTION

1.1 Background

Cystic fibrosis (CF) is an autosomal recessive inherited disorder caused by mutations in the gene encoding for the cystic fibrosis transmembrane conductance regulator (CFTR) [1]. It is characterized by fat and protein malabsorption, failure to thrive and pulmonary disease [2]. Cystic fibrosis is a life-threatening and chronically debilitating disease that markedly impairs quality of life [1]. To date, there are more than 1,600 specific mutations in the CF gene that are known [3]. The incidence of CF varies by ethnic group, with the greatest incidence occurring in Caucasians (1:3,200). Far less people of African or Asian descent have this disease (incidence between 1:15,000 and 1:31,000) [2]. Despite genetic counseling, the prevalence of CF is likely to increase as the median survival time for subjects with CF increases [2].

Chronic progressive lung disease is the main negative prognostic factor in CF subjects [3]. Subjects have progressive cycles of infection and inflammation, ultimately leading to respiratory failure [2]. The lungs become colonized with bacteria, probably due to the presence of thick, inspissated mucus in the airways, which traps inhaled microbes [2].

Cystic fibrosis has no cure. Current life expectancy is between 30 and 40 years [1], although life expectancy is related to phenotype, with those with severe phenotypes having reduced life expectancy. The goal of therapy is to delay disease progression [1]. The goal of therapy with respect to pulmonary disease is to maintain or improve respiratory function, as assessed by forced expiratory volume in one second (FEV_1) [1]. Slowing the decline of lung function occurs by clearing airways of mucus, by controlling respiratory infections and by improving and maintaining respiratory function [1]. Clearing mucus occurs using physical techniques, such as active cycle of breathing, positive pressure autogenic drainage and physical therapy. Currently available pharmacological treatments include therapy with mucolytic drugs to improve mucociliary clearance of lower-airway secretions and treat chronic obstructive pulmonary disease [1]. Mucus clearance can be increased using hypertonic saline solutions [1], while rhDNase is used as a mucolytic agent. Inhaled mannitol is being developed as a therapeutic agent for the treatment of cystic fibrosis and other diseases characterized by difficult to clear, thickened respiratory mucus. Inhaled mannitol has been shown to increase mucociliary clearance and cough clearance [4, 5]. The exact mechanisms responsible remain unclear, however, due to its osmotic activity a combination of factors including changes in the rheology of mucus favoring ciliary and cough clearance may be responsible [6-8]. Osmotic agents may change the viscoelastic properties of mucus by reducing the number of entanglements that mucin polymers form. While ionic agents may achieve this by shielding the fixed charges along the mucin macromolecules, non-ionic agents such as mannitol may achieve this by disrupting the hydrogen bonds between mucins [6, 9]. Osmotic agents also increase the amount of water in the airway lumen potentially normalizing the airway surface liquid layer. This increased hydration may contribute to an increase in the activity of cilia and transportability of mucus. Additionally, osmotic agents may increase clearance of mucus by stimulating mucus secretion. The effect of osmolarity on mucus secretion could be direct or indirect via release of mediators from epithelial and mast cells and neuropeptides from sensory nerves which are known to be secretagogues. Although an increase in mucus secretion is not seen to be a desirable effect, this stimulation of mucus secretion may help increase clearance of viscous and stagnated mucus.

Mannitol may benefit subjects by reducing the mucus load acutely, or it may have a prolonged beneficial effect on mucociliary clearance. As excessive mucus in the airways may result in some limitation of airflow, a reduction in the mucus load should improve airway function irrespective of age.

While adult subjects (aged at least 18 years) have been studied in previous phase III clinical trials of inhaled mannitol that assessed improvements in lung function [10, 11], there are remaining uncertainties around the effect size of inhaled mannitol 400 mg b.i.d. in this population.

Therefore the primary purpose of this trial is to assess the efficacy of inhaled mannitol compared with control in subjects with cystic fibrosis aged 18 years and above.

1.1.1 Findings from non-clinical trials

Information regarding the findings from Non-Clinical Studies is presented in the *Investigator's Brochure* Section NONCLINICAL STUDIES.

Mannitol is present naturally in plants, algae, fungi and some bacteria. It is used extensively as a food additive. It does not appear to be mutagenic, cytotoxic, carcinogenic or teratogenic. One trial has shown increased incidence of adrenal medullary hyperplasia plus pheochromocytoma in rats. However, these were not dose related and were considered unrelated to the administration of mannitol.

When ingested, mannitol has a laxative effect when given in doses greater than 10 to 20 g. The accepted daily intake of mannitol set by the joint FAO / WHO expert committee on food additives (JECFA) is 50 mg/kg. It is unlikely however, that the inhalation of less than 1g of mannitol per day via inhalation would reach this level.

In animals, mannitol is rapidly absorbed following inhalation (peaking within 1 hour of completion of the dose), with negligible serum levels detected after 24 hours.

1.1.2 Findings from clinical trials

Findings from clinical studies are discussed in detail in the *Investigator's Brochure*, section EFFECTS IN HUMANS.

1.1.3 Pharmacokinetics

Refer to the *Investigator's Brochure (see Sections 5.2, 5.3 and 6.1.2).*

1.1.4 Efficacy

The DPM-CF-301 trial [10] assessed the safety and efficacy of inhaled mannitol 400 mg b.i.d. compared to control in subjects aged six years or more with a diagnosis of cystic fibrosis. This was a multicenter, randomized, controlled, parallel arm, double-blind phase III trial over 26 weeks. It was followed by an optional 26-week open-label extension of the trial during which all subjects received inhaled mannitol 400 mg b.i.d.. The treatment effect of mannitol was superior to that of control ($p < 0.001$, Table 1). Changes in other measures of lung function (FVC and FEF_{25-75}) were consistent with the improvements in FEV_1.

DPM-CF-302 [11] was essentially the same as DPM-CF-301 in design, with only minor differences in the inclusion / exclusion criteria. In this trial, inhaled dry powder mannitol improved absolute change in FEV_1 compared with control over six-month treatment period, although this failed to reach statistical significance ($p = 0.059$, Table 1). However, there were significant improvements in the relative change in FEV_1 when subjects were treated with mannitol were compared to control groups – a difference of 3.75% ($p = 0.029$).

A pooled analysis of DPM-CF-301 and DPM-CF-302 studies found a mean absolute change from baseline in FEV_1 for dry powder mannitol versus control over 6 months was 73.4 mL ($p<0.001$). Overall the studies show that dry powder mannitol improves lung function by six weeks, and the improvement was sustained over 26 weeks. The increase in FEV_1 at six weeks was highly correlated with the improvement over the 26-week treatment period.

Table 1: Treatment effect from the CF-301 and CF-302 studies

Lung function measure	**CF-301** **Mannitol 400 mg b.i.d. vs. control across 26-weeks**	**CF-302** **Mannitol 400 mg b.i.d. vs. control across 26-weeks**
Absolute change in FEV_1 (mL)	94.5 p<0.001	54.1 p=0.059
Absolute change in FVC (mL)	126 p<0.001	71.4 p=0.022

Significant improvements in FEV_1 were observed in subjects aged 18 and above in post-hoc analyses of studies DPM-CF-301 and DPM-CF-302 (Table 2).

Table 2: Treatment effect in adults from the CF-301 and CF-302 studies

Lung function measure	**CF-301** **Mannitol 400 mg b.i.d. vs. control across 26-weeks**	**CF-302** **Mannitol 400 mg b.i.d. vs. control across 26-weeks**
Absolute change in FEV_1 (mL)	108.46 p<0.001	85.94 p=0.038
Absolute change in FVC (mL)	138.71 p<0.001	113.77 p=0.011

1.1.5 Safety

Detailed description on the safety of inhaled dry powder mannitol in subjects with cystic fibrosis can be found in the *Investigator's Brochure*, section SAFETY IN BRONCHITOL CLINICAL TRIALS.

A total of 672 subjects have received mannitol in all the completed mannitol CF studies. The most frequently reported adverse drug reactions (ADRs) were cough (9.23 %), condition aggravated (cystic fibrosis pulmonary exacerbation) (6.10 %) and hemoptysis (5.36 %). Cough was very common (> 1/10), condition aggravated and hemoptysis were common (frequency ≥ 1/100, < 1/10).

Other common ADRs (frequency ≥ 1/100, < 1/10) were: pharyngolaryngeal pain, headache, wheezing, bronchospasm, productive cough, throat irritation, post-tussive vomiting and vomiting, chest discomfort, upper and lower respiratory tract infections, and bacteria identified in the sputum.

Adverse events with a difference of at least 2 % between mannitol 400 mg b.i.d. and control in the combined Phase 3 data (DPM-CF-301 and DPM-CF-302) are listed in Table 3.

Table 3: Adverse events with a difference of at least 2 % between mannitol 400 mg b.i.d. and control in the combined Phase 3 data (DPM-CF-301 and DPM-CF-302)

MedDRA System Organ Class/ Preferred Term	Mannitol 400 mg b.i.d. N=361 n (%)	Control N=239 n (%)
Gastrointestinal Disorders		
Diarrhea	17 (4.7)	6 (2.5)
Vomiting	22 (6.1)	6 (2.5)
General Disorders and Administration Site Conditions		
Condition aggravated	133 (36.8)	96 (40.2)
Infections and Infestations		
Lower respiratory tract infection	22 (6.1)	24 (10.0)
Nervous System Disorders		
Headache	64 (17.7)	50 (20.9)
Reproductive System and Breast Disorders		
Dysmenorrhea	8 (2.2)	0 (0.0)
Respiratory, Thoracic and Mediastinal Disorders		
Cough	76 (21.1)	40 (16.7)
Hemoptysis	34 (9.4)	13 (5.4)
Pharyngolaryngeal pain	44 (12.2)	18 (7.5)

Post-hoc analysis of CF-301 and CF-302 studies found that the incidence of hemoptysis was similar in the mannitol 400 mg and control arms of these studies in adults, although this has not been tested prospectively.

1.2 Rationale for trial

Given the improvements in FEV_1 observed in adult subjects the post-hoc analysis of DPM-CF-301 and DPM-CF-302, this trial (DPM-CF-303) aims to provide prospective evidence of the safety and efficacy of mannitol 400 mg b.i.d. in subjects aged 18 years and above.

2 TRIAL OBJECTIVES AND PURPOSE

2.1 Primary objective

To determine whether inhaled mannitol (400 mg b.i.d.) is superior to control (50 mg b.i.d.) for improving lung function as measured by mean change from baseline FEV_1 (mL) over the 26-week treatment period in adult subjects with CF.

2.2 Secondary objectives

There are hierarchical and non-hierarchical secondary objectives. Analysis of hierarchical secondary objectives will continue until a non-significant p-value (i.e. $p > 0.05$) is returned.

2.2.1 Hierarchical secondary objectives

1. To determine whether inhaled mannitol (400 mg b.i.d.) is superior to control for improving lung function as measured by mean change from baseline FVC (mL) over the 26-week treatment period in adult subjects with CF.
2. To determine whether inhaled mannitol (400 mg b.i.d.) is superior to control in increasing the time to first pulmonary exacerbation over the 26-week treatment period in adult subjects with CF.
3. To determine whether in adult subjects with CF, inhaled mannitol (400 mg b.i.d.) is superior to control for reducing the number of days on antibiotics (oral, inhaled or IV) due to pulmonary exacerbation;
4. To determine whether in adult subjects with CF, inhaled mannitol (400 mg b.i.d.) is superior to control for decreasing the number of days in hospital due to pulmonary exacerbation; *and*
5. To determine whether inhaled mannitol (400 mg b.i.d.) decreases the rate of pulmonary exacerbations over the 26-week treatment period compared to control in adult subjects with CF.

2.2.2 Non-hierarchical secondary objectives

To determine whether in adult subjects with CF, inhaled mannitol (400 mg b.i.d.) is superior to control

- for decreasing the incidence of exacerbations (i.e. the proportion of subjects with one or more exacerbation);
- for improving ease of expectoration; *and*
- for improving subject reported respiratory symptoms as measured by CFQ-R respiratory domain.

2.3 Hypothesis

We hypothesize that inhaled mannitol 400 mg b.i.d. will increase the mean change from baseline FEV_1 (mL) compared to control over the 26-week treatment period in adult subjects with cystic fibrosis. Any improvement in FEV_1 is considered clinically meaningful, however, this trial has set a threshold of 80 mL for the purposes of determining an appropriate sample size for statistical power while retaining trial feasibility in an orphan disease population.

3 COMPLIANCE STATEMENT

The trial will be conducted in accordance with the protocol and the International Conference on Harmonization (ICH) and Good Clinical Practice (GCP) guidelines. In addition, the trial will be conducted in compliance with all applicable laws and regulatory requirements relevant to the use of new therapeutic agents in each participating country. Agreement of the Investigator(s) to conduct and administer this trial in accordance with the protocol and associated regulations will be documented in trial agreements with the Sponsor, and other forms as required by national authorities in the country where the trial center is located.

The Investigator(s) is/are responsible for ensuring the privacy, safety and welfare of the subjects during and after the trial, and must ensure that fully functional resuscitation equipment and personnel trained in its proper use are immediately available in case of a medical emergency. The Investigator(s) must be familiar with the background and requirements of the trial and with the properties of the investigational medication as described in the *Investigator's Brochure.*

The Principal Investigator at each center has the overall responsibility for the conduct and administration of the trial at their center, and for contact with trial center management, the Independent Ethics Committee (IEC) / Institutional Review Board (IRB), and local authorities.

3.1 Variations to the protocol

No changes from the final approved (signed) protocol will be initiated by the Investigator without agreement by the Sponsor, and if required, the ethics committee's prior written approval of favorable opinion of a written amendment, except when necessary to eliminate immediate hazards to the subjects. Changes that are considered only logistical or administrative, do not require prior ethics committee prior approval. The Principal Investigator(s) and the Sponsor must sign any protocol amendment.

4 OVERVIEW OF TRIAL DESIGN

4.1 Trial design

This is a double-blind, randomized, parallel arm, controlled, multicenter, and interventional clinical trial. Potential subjects will sign the informed consent form (ICF) and be assessed for eligibility. After satisfying all inclusion & exclusion criteria, subjects will be given a mannitol tolerance test (MTT). Those subjects that pass the MTT will be randomized to receive inhaled mannitol (400 mg b.i.d.) or control b.i.d. for a period of 26-weeks, in a 1:1 fashion (See trial flowchart in Figure 1). Note: No tests should be performed before consent is obtained (also refer to the TIME AND EVENTS schedule).

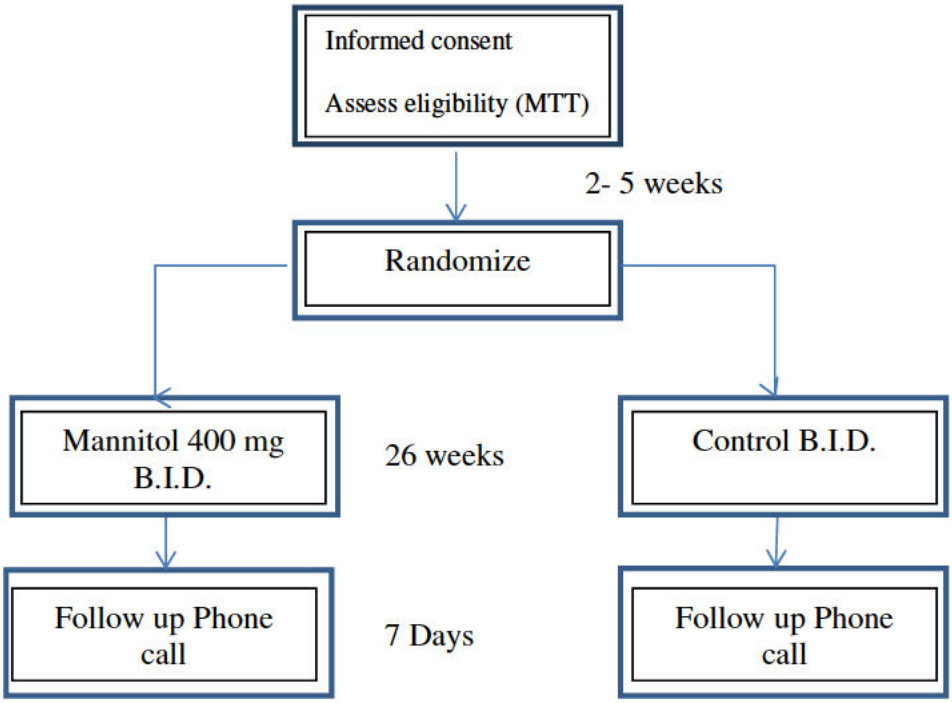


Figure 1: Trial flowchart

4.2 Trial design rationale

In post-hoc analyses of previous double-blind, randomized, parallel arm, controlled, multicenter, and interventional clinical trials, significant improvements in FEV_1 have been observed in subjects with CF aged 18 and over (DPM-CF-301 and DPM-CF-302). However, efficacy in this subgroup has not been assessed prospectively. The DPM-CF-301 and DPM-CF-302 were both randomized studies of mannitol 400 mg b.i.d. and control. Thus the trial design for this trial (DPM-CF-303) reflects the design of the previous studies.

4.2.1 Primary endpoint

The primary endpoint is the mean change in FEV_1 (mL) from baseline (Visit 1) over the 26-week treatment period (to Visit 4).

4.2.2 Secondary endpoints

4.2.2.1 Hierarchical secondary endpoints

1. Mean change from baseline FVC (mL) over the 26-week treatment period.
2. Time to first pulmonary exacerbation over the 26-week treatment period.
3. Number of days on antibiotics (oral, inhaled or IV) due to pulmonary exacerbation;
4. Number of days in hospital due to pulmonary exacerbation;
5. Rate of pulmonary exacerbations over the 26-week treatment period.

4.2.2.2 Non-hierarchical secondary endpoints

- the incidence of pulmonary exacerbations;
- ease of expectoration measured using a visual analogue scale; *and*
- the CFQ-R respiratory domain score.

5 TRIAL POPULATION

5.1 General considerations

Potential subjects must meet all the inclusion, but none of the exclusion criteria, as listed below.

5.2 Subject inclusion criteria

The subject must meet all of the following criteria:

1. Have given written informed consent to participate in this trial in accordance with local regulations;
2. Have a confirmed diagnosis of cystic fibrosis (positive sweat chloride value ≥ 60 mEq/L) and/or genotype with two identifiable mutations consistent with CF, accompanied by one or more clinical features consistent with the CF phenotype);
3. Be aged at least 18 years old;
4. Have FEV_1 > 40 % and < 90% predicted (using NHanes III [1]);
5. Be able to perform all the techniques necessary to measure lung function;
6. Be adherent with maintenance therapies (antibiotics and/or rhDNase), if used, for at least 80% of the time in the two weeks prior to visit 1; *and*
7. If rhDNase and/or maintenance antibiotic are being used treatment must have been established at least 1 month prior to screening (Visit 0). The subject should remain on the rhDNase and / or maintenance antibiotics for the duration of the trial. The subject should not commence treatment with rhDNase or maintenance antibiotics during the trial.

5.3 Subject exclusion criteria

The subject must NOT meet any of the following criteria:

1. Be investigators, site personnel directly affiliated with this trial, or their immediate families. Immediate family is defined as a spouse, parent, child or sibling, whether biologically or legally adopted;
2. Be considered "terminally ill" or eligible for lung transplantation;
3. Have had a lung transplant;
4. Be using maintenance nebulized hypertonic saline including in the 2 weeks prior to visit 1;
5. Have had a significant episode of hemoptysis (>60 mL) in the three months prior to Visit 0;
6. Have had a myocardial infarction in the three months prior to Visit 0;
7. Have had a cerebral vascular accident in the three months prior to Visit 0;
8. Have had major ocular surgery in the three months prior to Visit 0;
9. Have had major abdominal, chest or brain surgery in the three months prior to Visit 0;
10. Have a known cerebral, aortic or abdominal aneurysm;
11. Be breast feeding or pregnant, or plan to become pregnant while in the trial;
12. Be using an unreliable form of contraception (female subjects at risk of pregnancy only);
13. Be participating in another investigative drug trial, parallel to, or within 4 weeks of visit 0;
14. Have a known allergy to mannitol;
15. Be using non-selective oral beta blockers;
16. Have uncontrolled hypertension i.e. systolic BP > 190 and / or diastolic BP > 100;

17. Have a condition or be in a situation which in the Investigator's opinion may put the subject at significant risk, may confound results or may interfere significantly with the subject's participation in the trial;
18. Have a failed or incomplete MTT (as evaluated in Section 8.1.1.1);

6 INVESTIGATIONAL MEDICINAL PRODUCT (IMP)

6.1 Dosage and administration

This is a parallel trial, including 26 weeks of treatment with either inhaled mannitol (400 mg b.i.d.) or control (mannitol 50 mg b.i.d.). The inhalation technique for control and mannitol is identical.

WHEN TO START TREATMENT FOR SUBJECTS WITH A CURRENT PULMONARY EXACERBATION

Subjects should be stable and clear of pulmonary exacerbations for at least two weeks prior to visit 1. If a subject has an exacerbation after Visit 0 (screening), Visit 1 should occur 2 to 5 weeks from the end of the treatment for the exacerbation or the end of the adverse event, whichever is later.

There is no requirement to stop the IMP if a subject has a pulmonary exacerbation during a treatment phase. Subject can receive additional treatment as required.

The IMP is to be administered in a standardized manner (see Figure 2). The Investigator is responsible for instructing the subject on the correct IMP inhalation technique, and on use of the bronchodilator (albuterol/salbutamol metered dose inhaler (MDI) or similar) 5 to 15 minutes prior to the IMP administration. To ensure a good IMP delivery technique, training kits containing empty capsules are available.

The Investigator will prescribe the subject with a bronchodilator (albuterol /salbutamol metered dose inhaler [MDI] or similar) to be used as premedication prior to inhalation of the IMP. This bronchodilator may also be used in the event of post-treatment chest tightness. Premedication (albuterol/salbutamol MDI or preferred alternative) should be recorded in the *Subject Diary*. Use of any bronchodilator following IMP for post-treatment chest tightness should also be recorded in the *Subject Diary*.

Subjects will take IMP twice a day, once in the morning and once in the evening. The evening dose should be administered approximately 2 hours before sleep.

The recommended order of treatment procedures is:

1. bronchodilator;
2. mannitol/control;
3. physiotherapy / exercise*;
4. rhDNase;
5. inhaled antibiotics; *then*
6. inhaled corticosteroids.

The procedures 4, 5 and 6 may not be applicable to all subjects and the order of procedures 3, 4, 5 and 6 listed above is a suggestion only but the order in which they are used should remain consistent throughout the study. Bronchodilator must always precede mannitol/control.

**When possible, individual physiotherapy and exercise (if used as a substitute to physiotherapy) techniques and routines should be kept consistent throughout the study.*

Subjects should record in the *Subject Diary* the date of any missed doses of IMP.

Each kit dispensed contains additional supply to account for the allowed visit windows.

6.1.1 Treatment adherence

Treatment adherence will be determined by returned medication count. If the subject has missed doses of their medication, then they should be counseled about the importance of compliance, and re-educated on investigational medication intake. Any possible treatment discontinuation due to non-adherence should be discussed with the Sponsor's Project Manager and Sponsor's Medical Director.

6.1.2 Investigational medicinal product – formulation and manufacture

Bronchitol has a centralized European marketing authorization for use in Adult patients with cystic fibrosis. However it does not have a marketing authorization or is not available in every participating country therefore it is being treated as Investigational medicinal product. Details on the investigational product can be found in the *Investigator's Brochure*. Trial treatment (mannitol and control) will be provided as indistinguishable red capsules.

6.1.3 Labeling, packaging and storage

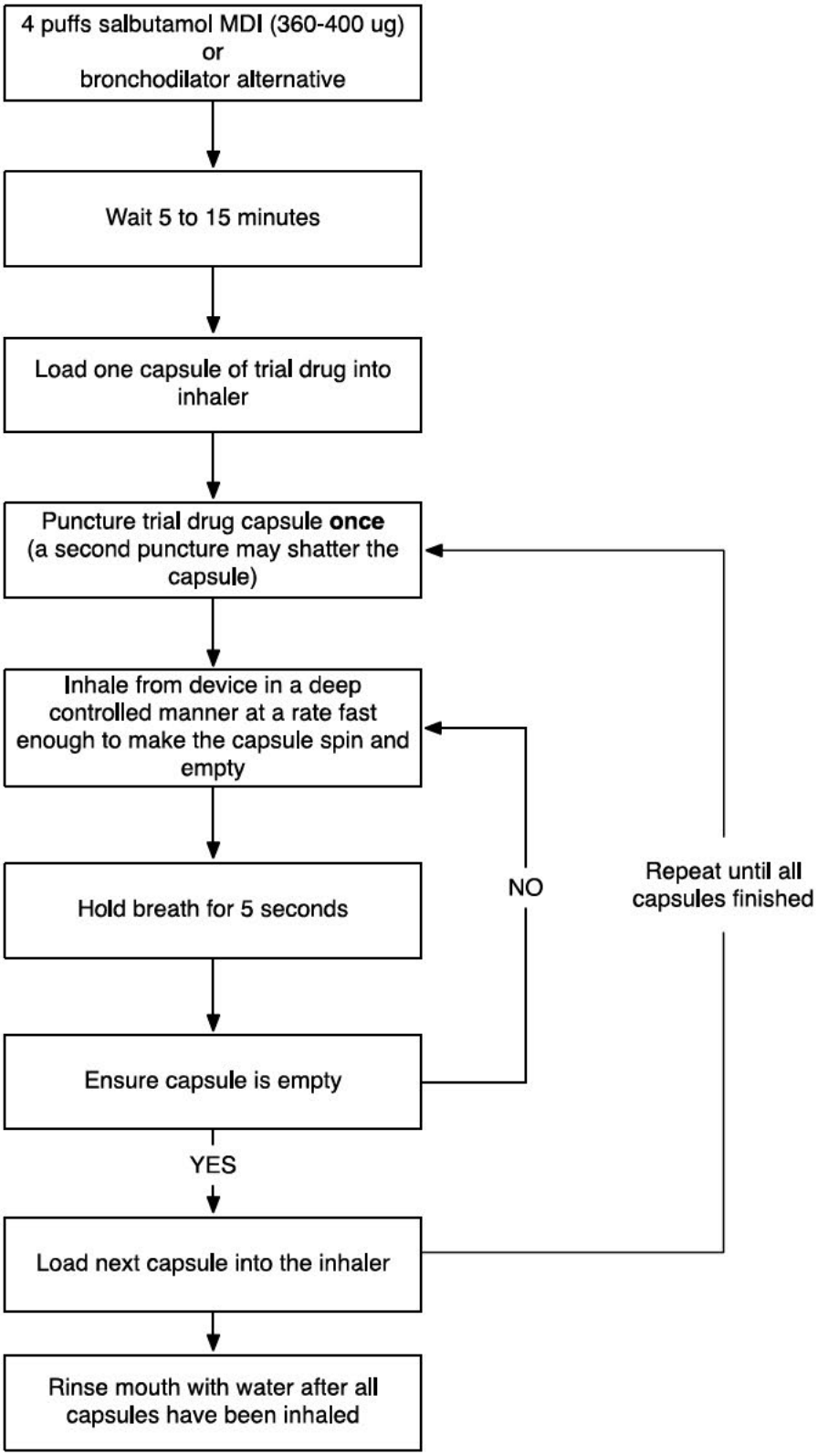
IMP is labeled according to local regulatory requirements. The mannitol dry powder is encapsulated in opaque capsules prior to blister packing in aluminum foil. Blisters are contained within cartons with a dry powder inhaler device(s). IMP blinding is maintained by the use of kit numbers enabling traceable labeling.

IMP is to be stored at room temperature (59°F to 77°F or 15°C to 25°C), in a secure area with restricted access. The site Investigator/Pharmacist is responsible for storage, temperature monitoring and maintaining accurate accountability records.

Once the IMP has been dispensed to subjects, it must not be refrigerated, nor kept in humid conditions, such as in the bathroom. IMP must be stored at room temperature, in a clean, dry, environment. Capsules should only be removed from the foil packaging immediately prior to use.

Important: Instruct your subjects the IMP can only be used with the inhaler provided.

Figure 2: IMP inhalation instructions



6.1.4 Investigational medicinal product supply and accountability

Pharmaxis will provide the participating sites with sufficient amount of IMP.

The site Investigator must ensure that deliveries of investigational product from the Sponsor are correctly received by a responsible person (e.g., Pharmacist), that all receipts are recorded in writing, that the product is stored in a secure area under recommended and proper storage conditions, and that they are used in accordance with the protocol. It is the responsibility of the Investigator to ensure that the integrity of the investigational medication is not jeopardized prior to dispensing. The investigational medication must be dispensed as provided by the Sponsor with no further repackaging done at site.

The site Pharmacist / Investigator(s) / legally qualified designee, as documented on the Site Signature / Delegation of Authority Log, will dispense/administer the investigational medication only to subjects enrolled in this trial following the procedures set out in Section 6. The site Pharmacist / Investigator(s) is responsible for assuring the retrieval of all trial supplies from the subjects. Unused investigational medication(s) (opened, unopened, or empty capsules) must be returned to the Sponsor or destroyed on site (if appropriate) after the investigational medication accountability has been completed and destruction has been authorized by the Sponsor.

Trial drug accountability should be performed by counting the used (empty blisters) and unused trial drug remaining in blisters.

The Investigator(s) must maintain accurate and adequate records including dates of receipt and return of IMP shipments, and batch numbers and quantities received/returned from/to Pharmaxis, as well as dates and amounts dispensed to/returned by trial subjects. Destruction of trial supplies should not be done without prior approval from the Sponsor. Full accountability documentation (with witnessing) is required for all destroyed IMP at an investigator site.

The Investigator / site Pharmacist will be required to provide the Monitor with access to the investigational medication and records for periodic review.

Copies of the accountability records will be provided to the pharmacy monitor for inclusion in the trial master file.

It is forbidden to use investigational medication for purposes other than defined in this protocol.

6.2 Pre-trial and concomitant medications

Any treatment that is considered necessary for the subject's welfare may be given at the discretion of the Investigator. Administration of all concomitant medications must be reported in the appropriate section of the CRF as noted below. Generic names are to be recorded, along with the start and stop dates, dose, frequency, route and indication. Trade names are acceptable for combination medications.

Concomitant rhDNase and maintenance antibiotics are permitted in this trial but treatment must have been established at least 1 month prior to screening (Visit 0). However, the subject should remain on the treatment for the duration of the trial, and should not commence treatment with rhDNase or maintenance antibiotics during the trial.

Subjects on regular antibiotics should maintain on/off cycles where appropriate.

All start and stop dates of antibiotic therapy must be captured on the Concomitant Medications *page of the CRF, even if the prescribed antibiotic therapy is cyclical..*

Rescue antibiotics and any other medication used for the treatment of acute exacerbations are permitted.

6.2.1 Pre-trial medication

Pre-trial therapies administered up to 3 months prior to the first dose of trial medication must be recorded following trial consent in the source records and on the *Concomitant Medications* page of the CRF.

6.2.2 Concomitant medication

Any medication (other than the investigational medication taken as specified in the protocol), including herbal and over-the-counter preparations, that the subject takes during the trial protocol period is considered concomitant medication and will be captured on the *Concomitant Medications* page of the CRF.

6.2.3 Concomitant medications that must be withheld prior to the trial visits

Concomitant medication will be taken as prescribed during the trial but must be withheld prior to each visit as per Table 4 below.

Table 4: Medication-withholding periods

Medication	**Time interval from last dose until spirometry**
Inhaled mannitol/control (Visit 1 onwards)	6 to 24 hours
Short-acting bronchodilators (isoproterenol, isoetharine, metaproterenol, salbutamol, albuterol, levalbuterol, terbutaline) (e.g., [redacted]®) Short-acting anticholinergics including combination therapies e.g., ipratropium (e.g., [redacted]®)	6 hours (minimum)
Long acting β2 agonists including combination therapies e.g. salmeterol; eformoterol (e.g. [redacted]) Long acting anticholinergics e.g. tiotropium (e.g., [redacted])	12 hours (minimum)
Oral bronchodilators e.g.. Theophylline and β-agonist tablets	12 hours (minimum)

6.2.3.1 Rescue medications

If a subject develops chest tightness or shortness of breath as a consequence of withholding bronchodilators or other respiratory medications on the morning of the due visit, they should take their usual bronchodilator and phone or attend the clinic for advice. Where the health of the subject is in question, the subject should attend the clinic for assessment. Note should be made of the event precipitating the use of rescue medication and the subsequent management. If necessary the due visit should be rescheduled.

6.2.4 Prohibited medications and non-pharmacological treatments

The following medications are prohibited from Screening (Visit 0) and throughout the trial protocol period:

- Maintenance treatment with nebulized hypertonic saline
- Non-selective oral beta-blockers

7 INVESTIGATIONAL MEDICATION TREATMENT ALLOCATION

Subjects will be randomized following informed consent and satisfying all the inclusion and none of the exclusion criteria. Subjects are randomized in a 1:1 ratio to receive either inhaled mannitol 400 mg b.i.d. or control (inhaled mannitol 50 mg b.i.d.) for a period of 26-weeks. Randomization will be stratified by rhDNase use and country.

The Pharmacist, Investigator or legally responsible designee will dispense the treatment allocated to the subject and record the randomized kit numbers in the dispensing log.

The subject, the investigative staff and Sponsor will be blinded to the treatment allocation. Appropriate site staff will be provided with access to unblinding information. In the event of a medical emergency, and when management of a subject's condition requires knowledge of the IMP, his/her randomization information may be obtained. Such emergencies should be discussed with the Sponsor in advance whenever possible prior to disclosure of the subject's treatment allocation. An automatic email is sent to the designated trial team member as soon as the subject is unblinded.

Any subject giving consent, but not fulfilling the criteria to participate in the trial will not be entered into the Case Report Form (CRF) with the exception of mannitol tolerance test (MTT) status. Subjects giving consent but not entered into the CRF will be documented as *screen failures.* Subjects who are administered the MTT should be entered onto the CRF irrespective of the MTT result; however, the subject's trial identifier and date of birth will be detailed in the IWRS.

8 TREATMENT EVALUATIONS

8.1 Visit procedures

This trial consists five trial visits and 9 telephone calls over a period of 28-weeks (see TIME AND EVENTS SCHEDULE).

8.1.1 Screening (Visit 0)

Subjects will be screened for eligibility during the Screening phase. Refer to the TIME AND EVENTS SCHEDULE for a complete list of procedures to be performed during the Screening visit.

- Obtain written informed consent - this must be obtained before any trial related procedures, including medication-withholding requests, are undertaken. This may be done prior to V0;
- Review inclusion / exclusion criteria;
- Obtain demographic information:
  - date of birth;
  - height and weight (no shoes using a stadiometer);
  - race and gender;
  - age at diagnosis;
  - CFTR mutation (if known)*; *and*
  - previous diagnosis of CF related bronchiectasis (yes, no, unknown)*.
- Review medical history;
- Review and record concomitant medications for the previous 3 months;
- Perform physical examination, including but not limited to:
  - pulmonary function tests (FEV_1, FVC, FEV_1 % predicted PEF, FEF_{25-75});
  - urine pregnancy test (for females of child-bearing potential); *and*
  - physical examination / vital signs (including chest sounds, weight, BP, HR, O_2 saturation, temperature, respiratory rate).
- Premedicate with four puffs of albuterol/salbutamol from a metered dose inhaler (MDI) (360-400µg)**;
- Conduct mannitol tolerance test (MTT) (see section 8.1.1.1);
- Collect 1 x sputum sample for qualitative microbiology (sent to local lab); alternatively, an oropharyngeal or cough swab is permitted.
- Collect blood sample for hematology, electrolytes, urea, creatinine and liver function testing (collected following local laboratory procedures and sent to local lab); and
- Issue subject diary and instruct the subject on how to complete it.

** Enter this data only if known and confirmed evidence is available.*

*** Bronchodilator is to be used approximately 5 -15 minutes prior to MTT. The default bronchodilator for this trial is 4 x puffs albuterol/salbutamol (360-400µg). However, the subject's preferred alternative bronchodilator may be used as an alternative premedication. Please record premedication details.*

Re-screening of subjects may be considered upon approval by the Sponsor or delegate. Re-screening will not be considered for subjects who have had a 'failed' or 'incomplete' mannitol tolerance test.

8.1.1.1 Mannitol tolerance test (MTT)

The MTT identifies subjects who have airway hyperresponsiveness in response to inhaled mannitol. Airway hyperresponsiveness is determined by measuring the degree of bronchoconstriction that occurs following sequential administrations of mannitol.

As is standard in administering agents that have the potential to cause bronchoconstriction, a physician, appropriately trained, to treat acute bronchospasm, including the use of resuscitation equipment, **must** be available to respond to an emergency.

As a minimum, the person conducting the MTT procedure must:

- Be competent/trained, qualified and experienced in performing bronchial provocation tests;
- Be familiar with safety and emergency procedures;
- Know when to stop further testing;
- Be proficient in the administration of inhaled bronchodilators and other rescue medications and equipment; *and*
- Not be the same person who administers IMP in the clinic

The following equipment must be available during the MTT procedure:

- MTT kit with a dry powder inhaler;
- Bronchodilator metered dose inhalers and spacers;
- A spirometry system that meets ATS/ERS requirements;
- Calculator and 60 second timer;
- Pulse oximeter;
- Adrenaline, atropine and oxygen; *and*
- Resuscitation equipment.

Technical considerations

The gelatin capsules may fragment if pierced more than once. Subjects must inhale from the device in a deep controlled manner at a rate fast enough to make the capsule spin and empty, followed by a 5 second breath hold. A second inhalation may be required if the capsule appears not to have emptied. Static charge can be problematic with dry powders, particularly in low humidity environments. If the capsule fails to spin in the device on inhalation, tilt the device mouthpiece down and firmly tap the base to drop the capsule into the spinning chamber. The subject should never exhale into the device.

To facilitate the development of an osmotic gradient within the airway, delays between sequential doses should be minimized.

Cough may be controlled by slowing the rate of inhalation, while still ensuring that the flow rate is enough to facilitate emptying the capsule. Water can be given following the procedure to clear residual powder from the oropharynx.

Safety considerations

During the MTT procedure, monitor the subject according to the schedule in Table 5. Measure post-test FEV_1 and FVC after 15 minutes for all subjects including those with passed tests, failed tests and incomplete tests and monitor the subject until adequately recovered to leave the clinic. If the subject shows clinical signs and symptoms of bronchoconstriction e.g. wheeze, dyspnea, shortness of breath treat accordingly.

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Table 5: Safety monitoring during MTT procedure

Parameter	Baseline (pre-bronchodilator)	At each dose step	After cumulative doses of 120 mg, 240 mg and 400 mg	Repeat pulmonary function test (PFT) 15 min post 400 mg dose (when FEV_1 falls between 20 – 50%)
SpO_2	X	X		X
FVC	X			
FEV_1	X		X	X
Clinical signs & symptoms		X		X

Stopping the MTT procedure

The MTT procedure must be stopped if:

- The subject's oxygen saturation is < 89% (record as an adverse event);
- Cough is highly distressing or vomiting occurs (record as an adverse event);
- Subject's FEV_1 has fallen ≥ 20% (from baseline) following cumulative doses of 120 mg or 240 mg of the procedure; *or*
- Clinical signs and symptoms are causing concern.

The MTT procedure

The flowchart for the MTT procedure is depicted in Figure 3

1. Perform baseline spirometry and measure SpO_2. Multiply the patient's best FEV_1 by 0.80 and 0.50 to obtain the 20% and 50% fall in FEV_1 values respectively.
2. Pre-medicate patient with 4 x puffs albuterol/salbutamol* and wait 5–15 min
3. Following the instructions for using the inhaler device, inhale contents of 1x 40mg capsule in a controlled, deep inhalation; breath hold for 5 seconds, then exhale, wait 60 seconds, then measure $Sp0_2$

   If $Sp0_2$ <89%, failed MTT, discontinue test and treat as required and go to step 7, otherwise go to step 4
4. Administer an additional 80mg mannitol (2 x 40mg) as above; wait 60 seconds, then measure FEV_1** & $Sp0_2$

   If FEV_1 fall is ≥ 20% (from baseline) or SpO_2 <89%, failed MTT discontinue test and treat as required and go to step 7, otherwise go to step 5
5. Administer an additional 120mg mannitol (3 x 40mg) as above. Wait 60 seconds, measure FEV_1** & $Sp0_2$

   If FEV_1 fall is ≥ 20% (from baseline) or SpO_2 <89%, failed MTT discontinue test and treat as required and go to step 7, otherwise go to step 6
6. Administer an additional 160mg mannitol (4 x 40mg) as above. Wait 60 seconds, measure FEV_1** & $Sp0_2$

   If FEV_1 fall is ≥ 50% (from baseline) or SpO_2 <89%, Failed MTT, treat as required and go to Step 7

   If FEV_1 fall is ≥ 20%, wait 15 minutes and Repeat PFT & $Sp0_2$

   ↓

   If FEV_1 fall is still ≥ 20% Failed MTT: Treat any symptoms as clinically appropriate and go to step 7
7. 15 minutes post test, measure recovery spirometry (including FVC) and Sp02 for all subjects including those who passed, failed or had an incomplete MTT
8. Monitor clinical signs and symptoms until stable for discharge

* alternative bronchodilator may be used

** only one acceptable effort required for this measurement

MTT Assessment

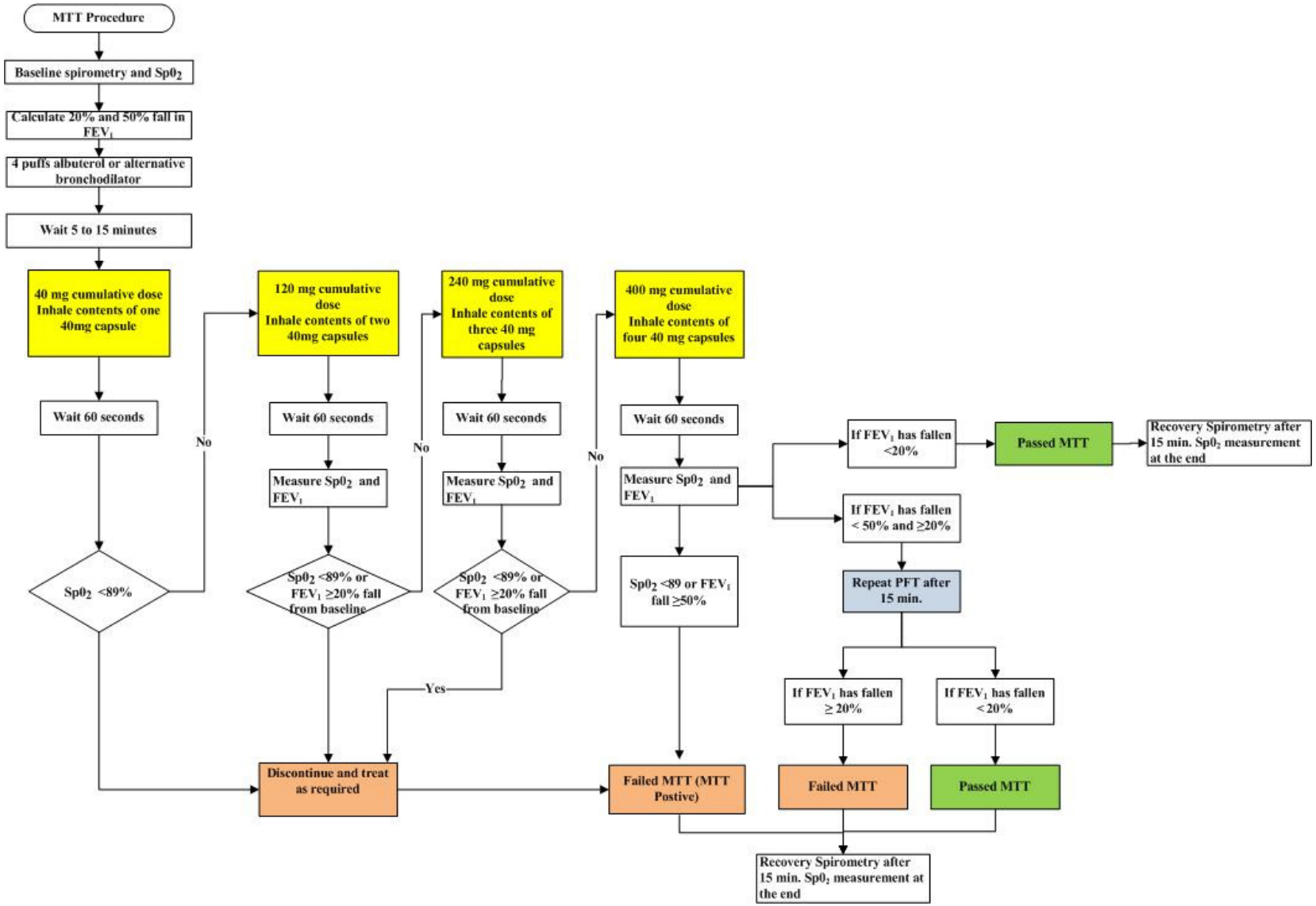
Failed MTT (i.e. "positive" MTT) when the subject's:	1. oxygen saturation falls to < 89 %. Record this as an adverse event; 2. FEV_1 falls ≥ 20% from baseline following cumulative doses of 120 mg or 240 mg of mannitol; 3. FEV_1 falls ≥ 20% from baseline following a cumulative dose of 400 mg of mannitol, and does not return to within 20% of baseline FEV_1 within 15 minutes; *or* 4. FEV_1 falls ≥ 50% from baseline following a cumulative dose of 400 mg of mannitol.
Passed MTT (i.e. "negative" MTT) occurs when:	1. a cumulative dose of 400 mg mannitol has been administered and no positive criteria have been met (see above); *or* 2. a cumulative dose of 400 mg mannitol has been administered and FEV_1 falls ≥ 20% but <50% from baseline but returns to within 20% of baseline FEV_1 within 15 minutes.
An **Incomplete (failed)** test occurs when:	1. Cough is highly distressing or vomiting occurs during the procedure. Record this as an adverse event; *or* 2. Any other medical event not listed above that leads to the test not being completed. Record this as an adverse event.

Record times of all spirometry during MTT procedure.

Bronchoconstriction may occur during the MTT. If the subject shows clinical signs and symptoms of bronchoconstriction measure FEV_1 and treat

accordingly. If bronchoconstriction is severe or distressing, record as an adverse event.

Figure 3: The MTT procedure



8.1.1.2 ☎ Phone call 1 week after visit 0 (Week -1 ± 1 day)

One week after the screening (Visit 0) visit of the trial, the Investigator or designate, will call the subject to:

- collect information on any adverse events experienced (including pulmonary exacerbations or hemoptysis);
- collect information on current concomitant medications;
- confirm attendance to next clinic visit;
- remind the subject to adhere to withholding periods; *and*
- remind the subject to complete their subject diary.

8.1.2 Double-blind treatment visits

Refer to the TIME AND EVENTS SCHEDULE for a complete list of procedures to be performed during the double-blind treatment visits.

8.1.2.1 Baseline (Visit 1)

Visit 1 will occur 2 to 5 weeks post screening visit

Prior to the visit, confirm medication-withholding periods have been adhered to (including 2-weeks wash out from hypertonic saline). If not, the subject's visit should be postponed.

*Confirm that the subject is **not** currently having a pulmonary exacerbation. In the event of a pulmonary exacerbation, visit 1 should occur 2 to 5 weeks from the end of the treatment for the exacerbation or of the adverse event, whichever is later.*

The following procedures will be performed:

- Review subject diary.
- Randomize eligible subjects if compliance with maintenance therapies (antibiotic & rhDNase) is at least 80% in the two weeks prior to visit 1 using the *Subject Diary*.;
- Review concomitant medications, and adverse events; (including pulmonary exacerbations or hemoptysis);
- Complete ease of expectoration VAS and respiratory domain of the CFQ-R questionnaire;
- Conduct physical examination (chest sounds, weight, BP, HR, O_2 saturation, temperature, respiratory rate);
- Perform pre-mannitol pulmonary function testing (FEV_1, FVC, PEF, FEF_{25-75});
- Assign IMP using IWRS;
- Dispense IMP and bronchodilator according to randomization schedule;
- Premedicate with four puffs of albuterol/salbutamol from a metered dose inhaler (MDI) (360-400µg), 5 to 15 minutes prior to each dose of IMP. The subject's preferred bronchodilator may be used as an alternative premedication. Care must be taken to ensure that a bronchodilator is used prior to ALL doses of IMP;

- Train the subjects on correct administration and inhalation of IMP, then administer initial dose of IMP (mannitol/control) according to 'IMP Administration Procedure' (see Section 6.1 figure 2) and randomization number; IMP administration should not be performed by the same person who administers the MTT.

- Perform pulmonary function test 30 minutes post dose. If the subject has a measured fall in FEV1 >20% at 30 minutes post dose completion, no further study medication should be administered. The subject should be continued in the study on an intention to treat basis. Symptomatic bronchoconstriction should be managed as medically appropriate. The respiratory technician performing spirometry could be the same person who administers the IMP as long as they have not performed the MTT.
- Access IWRS to complete the required visit information.

8.1.2.2 ☎ Phone call 2 weeks after visit 1 (Week 2 ±3 days)

Two weeks <u>after</u> visit 1, the Investigator or designate, will telephone the subject to:

- Collect information on any adverse events experienced (including pulmonary exacerbations or hemoptysis);
- Collect information on current concomitant medications;
- Remind the subject to adhere to withholding periods;
- Discuss adherence to trial treatment; Is this subject continuing with the IMP treatment? If the answer is NO but the subject is continuing their participation in the trial, please schedule an IMP discontinuation visit (within 2 weeks after IMP discontinuation);
- Remind the subject to return all trial drug (whether used or unused) at the next clinic visit*; and*
- Remind the subject to complete all relevant sections of the subject diary and to bring the diary to each visit.

8.1.2.3 ☎ Phone call 2 weeks before visit 2 (Week 4 ±3 days)

Two weeks <u>before</u> visit 2, the Investigator or designate, will call the subject to:

- Collect information on any adverse events experienced (including pulmonary exacerbations or hemoptysis);
- Collect information on current concomitant medications;
- Confirm attendance to next clinic visit;
- Remind the subject to adhere to withholding periods;
- Discuss adherence to trial treatment; Is this subject continuing with the IMP treatment? If the answer is NO but the subject is continuing their participation in the trial, please schedule an IMP discontinuation visit (within 2 weeks after IMP discontinuation);
- Remind the subject to return all trial drug (whether used or unused) at the next clinic visit; *and*

- Remind the subject to complete all sections of the subject diary and to bring the diary to each visit.

8.1.2.4 Visit 2

Visit 2 should occur on completion of 6 weeks trial treatment (±7 days)

Prior to the visit, confirm medication-withholding periods have been adhered to. If not, the subject's visit should be postponed.

The following procedures will be performed:

- Review subject diary.
- Review concomitant medications and adverse events (including pulmonary exacerbations or hemoptysis);
- Complete ease of expectoration VAS and respiratory domain of the CFQ-R questionnaire;
- Conduct physical examination (chest sounds, weight, BP, HR, O_2 saturation, temperature, respiratory rate);
- Perform pre-mannitol pulmonary function testing (FEV_1, FVC, PEF, FEF_{25-75});
- Dispense IMP and bronchodilator according to randomization schedule;
- Premedicate with four puffs of albuterol/salbutamol from a metered dose inhaler (MDI) (360-400µg), 5 to 15 minutes prior to each dose of IMP. The subject's preferred bronchodilator may be used as an alternative premedication. Care must be taken to ensure that a bronchodilator is used prior to ALL doses of IMP;
- Administer dose of IMP (mannitol/control) according to 'IMP Administration Procedure' (see Section 6.1 figure 2) and randomization number. Check the subject's inhalation technique and re-train if required;
- Perform pulmonary function test 30 minutes post dose. If the subject has a measured fall in FEV1 >20% at 30 minutes post dose completion, no further study medication should be administered. The subject should be continued in the study on an intention to treat basis. Symptomatic bronchoconstriction should be managed as medically appropriate);
- Conduct drug accountability; *and*
- Access IWRS to complete the required visit information.

8.1.2.5 ☎ Phone call 2 weeks after visit 2 (Week 8 ±3 days)

Two weeks after visit 2, the Investigator or designate, will telephone the subject, and complete the procedures as per Section 8.1.2.2.

8.1.2.6 ☎ Phone call 2 weeks before visit 3 (Week 12 ±3 days)

Two weeks before visit 3, the Investigator or designate will telephone the subject. Information collected will be as per Section 8.1.2.3.

8.1.2.7 Visit 3

Visit 3 should occur at the end of 14 weeks trial treatment (± 7 days)

Prior to the visit, confirm medication-withholding periods have been adhered to. If not, the subject's visit should be postponed.

The following procedures will be performed:

- Review subject diary.
- Review concomitant medications and adverse events (including pulmonary exacerbations or hemoptysis);
- Complete ease of expectoration VAS and respiratory domain of the CFQ-R questionnaire;
- Conduct physical examination (chest sounds, weight, BP, HR, O_2 saturation, temperature, respiratory rate);
- Perform pre-mannitol pulmonary function testing (FEV_1, FVC, PEF, FEF_{25-75});
- Dispense IMP and bronchodilator according to randomization schedule;
- Premedicate with four puffs of albuterol/salbutamol from a metered dose inhaler (MDI) (360-400µg), 5 to 15 minutes prior to each dose of IMP. The subject's preferred bronchodilator may be used as an alternative premedication. Care must be taken to ensure that a bronchodilator is used prior to ALL doses of IMP;
- Administer dose of IMP (mannitol/control) according to 'IMP Administration Procedure' (see Section 6.1 figure 2) and randomization number. Check the subject's inhalation technique and re-train if required;
- Perform pulmonary function test 30 minutes post dose. If the subject has a measured fall in FEV1 >20% at 30 minutes post dose completion, no further study medication should be administered. The subject should be continued in the study on an intention to treat basis. Symptomatic bronchoconstriction should be managed as medically appropriate.
- Conduct drug accountability; *and*
- Access IWRS to complete the required visit information.

8.1.2.8 ☎ Phone call 2 weeks after visit 3 (Week 16 ±3 days)

Two weeks after visit 3, the Investigator or designate will telephone the subject. Information collected will be as per Section 8.1.2.2.

8.1.2.9 ☎ Phone call 6 weeks before visit 4 (Week 20 ±3 days)

Six weeks before visit 4, the Investigator or designate will telephone the subject. Information collected will be as per Section 8.1.2.3.

8.1.2.10 ☎ Phone call 2 weeks before visit 4 (Week 24 ±3 days)

Two weeks before visit 4, the Investigator or designate will telephone the subject. Information collected will be as per Section 8.1.2.3.

8.1.2.11 Visit 4

Visit 4 should occur at the end of 26 weeks of trial treatment (-7 to +28 days)

Prior to the visit, confirm medication-withholding periods have been adhered to. If not, the subject's visit should be postponed.

The following procedures will be performed:

- Review and collect subject diary.
- Review concomitant medications and adverse events (including pulmonary exacerbations or hemoptysis);
- Complete ease of expectoration VAS and respiratory domain of the CFQ-R questionnaire;
- Conduct physical examination (chest sounds, weight, BP, HR, O_2 saturation, temperature, respiratory rate);
- Perform pre-mannitol pulmonary function testing (FEV_1, FVC, , PEF, FEF_{25-75});
- Premedicate with four puffs of albuterol/salbutamol from a metered dose inhaler (MDI) (360-400µg), 5 to 15 minutes prior to each dose of IMP. The subject's preferred bronchodilator may be used as an alternative premedication. Care must be taken to ensure that a bronchodilator is used prior to ALL doses of IMP;
- Administer dose of IMP (mannitol/control) according to 'IMP Administration Procedure' (see Section 6.1 figure 2) and randomization group;
- Perform pulmonary function test 30 minutes post dose. Symptomatic bronchoconstriction should be managed as medically appropriate;
- Conduct drug accountability; *and*
- Access IWRS to complete the required visit information.

8.1.2.12 ☎ Final phone call 1 week after visit 4 (Week 27 ±1 day)

One week after the final visit of the trial, the Investigator or designate, will call the subject to:

- Collect information on any adverse events experienced (including pulmonary exacerbations or hemoptysis); and
- Collect information on current concomitant medications; *and*
- Discharge subject from trial.

8.1.2.13 IMP discontinuation visit (within 2 weeks after IMP discontinuation)

Within two weeks of discontinuation of IMP, subjects should undergo an IMP discontinuation visit. At this visit, the following procedures will be performed:

- Review subject diary;
- Review concomitant medications and adverse events (including pulmonary exacerbations or hemoptysis);
- Complete ease of expectoration VAS and respiratory domain of the CFQ-R questionnaire;
- Conduct physical examination (chest sounds, weight, BP, HR, O_2 saturation, temperature, respiratory rate);
- Perform pulmonary function testing (FEV_1, FVC, PEF, FEF_{25-75});
- Access IWRS to complete the required visit information.

8.2 Efficacy

8.2.1 Efficacy evaluations

Efficacy evaluations are highlighted in the TIME AND EVENTS SCHEDULE. Briefly, these include:

8.2.1.1 Pulmonary function tests (Spirometry)

Perform all trial spirometry according to the American Thoracic Society / European Respiratory Society (ATS/ERS) criteria [12], using a standardized spirometer that meets ATS/ERS requirements and using a trained, experienced, qualified technician. **Pharmaxis or designee must approve all trial staff responsible for performing spirometry *prior* to the staff being delegated this task**.

Perform all lung function measures using the same spirometer throughout the trial. Perform spirometry at the same time of day for each visit (± 1 to 2 hours where possible). All visits should coincide with the trough treatment effect – that is, approximately 6 to 24 hours following the previous inhaled mannitol dose. Subjects must refrain from smoking within 1 hour of testing, consuming alcohol within 4 hours of testing, and performing vigorous exercise within 30 minutes of testing. Concomitant medications must be withheld prior to spirometry according to Table 4.

Complete spirometry after the physical exam, but prior to administration of any bronchodilator.

Seat the subject for the procedure unless they are obese, in which case testing in the standing position is preferred.

It is important that the subject is carefully instructed. Trial staff should demonstrate the appropriate technique and perform the procedure as described in **Figure 4**.

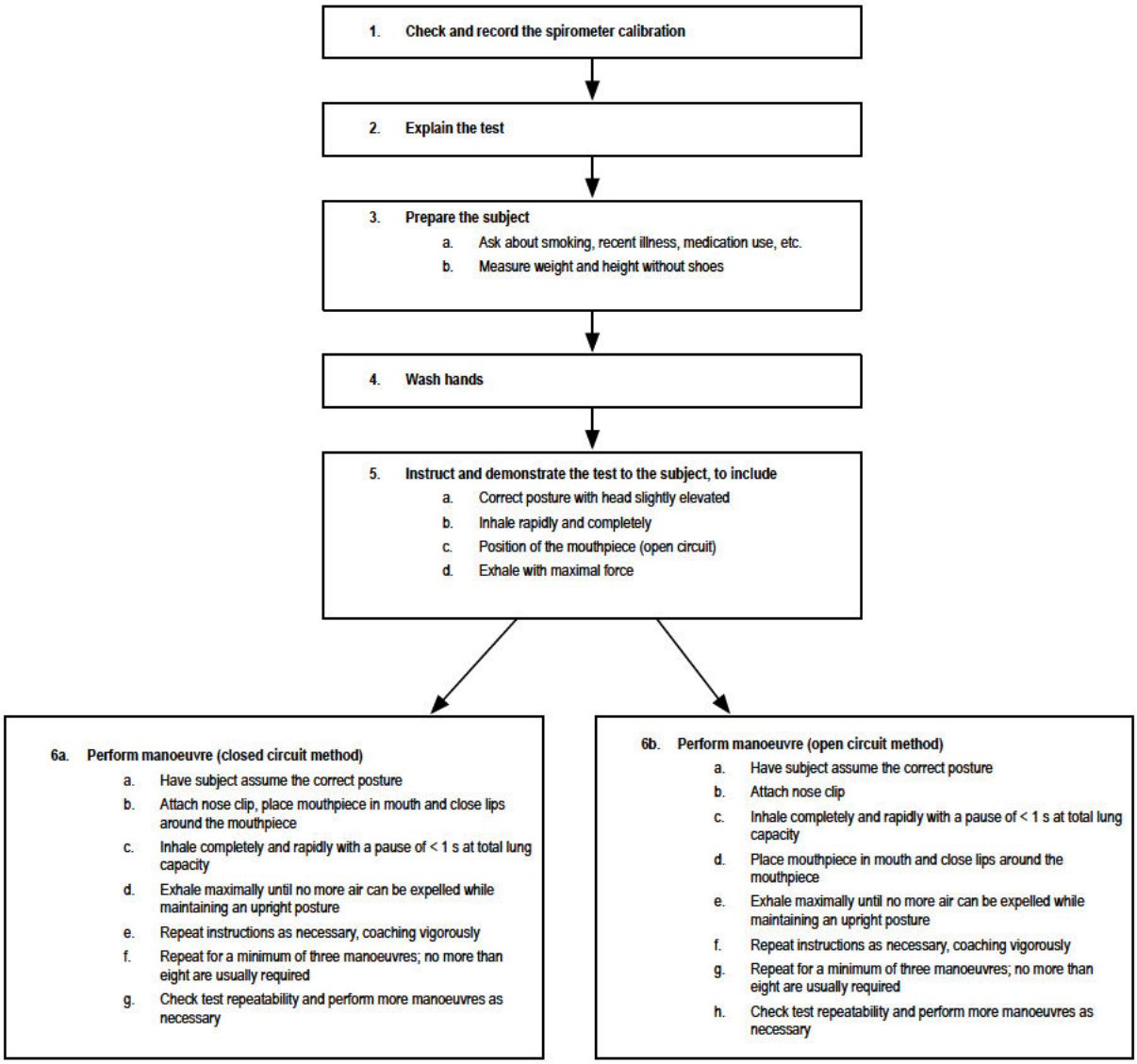


Figure 4: Procedures for recording forced vital capacity. Reproduced from [12]

Results of testing must meet the ATS/ERS criteria for the number of trials, acceptability and repeatability (see Table 6). The acceptability criteria should be applied before the repeatability criterion is checked.

Table 6: ATS/ERS Criteria for acceptable spirometry [12]

ATS/ERS criterion	
Number of trials	> 3 acceptable FVC maneuvers *If more than eight attempts are made with the subject unable to perform a single acceptable maneuver, then discontinue testing.* *In some cases, testing can be continued after additional instruction and demonstration, where the subject's clinical condition and tolerance allow.*
Acceptability	1. Satisfactory "start of test" • No excessive hesitation or false start extrapolated volume; • Extrapolated volume < 5% of FVC or 0.150 L (whichever is greater); 2. No coughing during the first second of the maneuver, or any other cough that the Trial Staff believe interferes with the measurement of accurate results; 3. No early termination of expiration; 4. No Valsalva maneuver (glottis closure) or hesitation during the maneuver that causes a cessation of airflow; 5. No leak at the mouth; 6. No obstructed mouthpiece*; and* 7. No evidence of an extra breath being taken during the maneuver. *A minimum exhalation time of ≥ 6 seconds or ≥ 3 seconds for children*
Repeatability	After three acceptable spirograms have been obtained, apply the following tests: 1. The two largest FVC must be within *0.150 L of each other*; and* 2. The two largest FEV_1 must be within *0.150 L of each other. *If both are met, testing can be concluded, otherwise continue testing until both criteria are met or a total of eight tests have been performed or the subject cannot or should not continue.* **For those with FVC of ≤1.0L, both these values are 0.100L.*

Reporting spirometry
The largest FEV_1 and FVC should be reported from the three acceptable efforts, regardless of whether they come from the same curve. Other measures should be reported from a single acceptable "best-curve" (defined as the curve with the largest sum of FEV_1 and FVC). Results should be reported at body temperature and pressure, saturated (BTPS). Report race-adjusted results. [redacted]

Print flow volume curves and annotate each spirometry report with the time of day, equipment serial number or instrumentation used, start and finish time of tests and the name of the technician administering the test. All values should be recorded and retained. Retention is to enable the quick identification of spirometry induced bronchospasm.

8.2.1.2 Pulmonary exacerbations

A protocol defined pulmonary exacerbation occurs when subjects are treated with **intravenous** antibiotics for **four or more** of the following twelve signs or symptoms [13]:

1. change in sputum production (volume, color, consistency);
2. increased dyspnea;
3. new or increased hemoptysis;
4. malaise, fatigue or lethargy;
5. fever ($\geq 38^{o}C$);
6. anorexia or weight loss;
7. sinus pain or tenderness;
8. change in sinus discharge;
9. FVC or FEV_1 decreased by >10% from previous recorded value;
10. radiographic signs indicative of pulmonary infection;
11. increased cough; *or*
12. changes in physical examination of the chest.

During regular clinic visits, subjects will be asked about their respiratory symptoms for an exacerbation update and history. Details will be collected on symptoms since the last visit and verbal information will be cross-referenced to diary entries and antibiotic use.

8.2.1.3 Antibiotic use

The number of days of antibiotic use is an outcome measure and as such antibiotic use must be carefully monitored and documented. This will include all nebulized antibiotics, oral and intravenous use. Subjects will be asked to keep a diary record of all antibiotic use while in the trial. This will include the antibiotic name, indication for use, the dose and the start and stop dates. Trial coordinators will track all antibiotic use, noting the diary records as well as outpatient clinic and hospital records. Routine maintenance antibiotic use will be distinguished from rescue antibiotics for exacerbations.

8.2.1.4 Hospitalizations

If a subject is formally admitted to hospital the event for which the subject is admitted is considered to constitute a serious adverse event and as such a 'serious adverse event' form must be completed. The only exceptions to this are hospital admissions that are pre-planned for elective procedures e.g. laser eye surgery, PEG replacement, cosmetic procedures, or are conducted on a regular and routine basis e.g. CF "tune up" that have been recorded at baseline in the subject's medical records.

"Hospital in the home" or an equivalent program; or attendance / observation in the emergency department is not considered to be a hospital admission. Medications administered as part of any elective procedure or hospital in the home process, are to be recorded as concomitant medications.

8.2.1.5 Hospital admission

At the time of hospital admission, the following information will be recorded in the CRF and on the SAE Form, if available:

- physical examination and medical history;
- signs and symptoms checklist;
- sputum microbiology results;
- spirometry;
- number of days since last hospital admission;
- results of laboratory tests; *and*
- chest X ray reports.

8.2.1.6 Hospital discharge

In addition to information recorded as per hospital admission, at the time of hospital discharge for an SAE the following information must be obtained as close as possible to the discharge date, and recorded in the CRF and on a follow-up SAE Form:

- medications during admission*; and*
- Start and end dates of hospitalization.

8.2.1.7 Ease of expectoration

A visual analogue scale will be used to assess the subjects' perceptions on ease of expectoration [14] while using IMP. Subjects will be asked to record the ease of expectoration over the previous week using a 10-cm visual analogue scale (0 = very difficult; 10 = very easy).

8.3 Safety

Safety assessments are highlighted in the TIME AND EVENTS SCHEDULE.

8.3.1 Safety evaluations

Any clinically significant change in safety evaluation will be recorded as an adverse event (AE) as detailed in Section 8.3.1.1.

8.3.1.1 Adverse events (AEs)

All observed or volunteered AEs regardless of treatment group or suspected causal relationship to the investigational medicinal product(s) will be recorded on the *Adverse Event* page of the CRF. Subjects will be questioned for the occurrence of any new or worsening signs or symptoms at each visit by the following methods:

- Information volunteered by the subject;
- Open ended and non-leading questions such as: Have you had any health problems since your last visit?;
- Observation by the investigational team, other care providers or relatives; *or*
- Medical Record and *Subject Diary* review.

The appropriate clinical individual at the trial site provides assessment and treatment of any trial related medical emergencies involving trial subjects.

Please refer to Section 8.3.3 for further details on AE reporting procedures.

8.3.1.2 Clinical laboratory tests

Sputum microbiology, and screening bloods (hematology, electrolytes, urea, creatinine and liver function tests) are the only laboratory tests required for this trial protocol. Laboratory tests are performed and analyzed following the local laboratory's standard procedures. If any other routine clinical laboratory testing is performed and a clinically significant result is obtained, then this must be recorded as an AE (see Section 8.3.3).

8.3.1.3 Vital signs

Temperature, pulse, respiratory rate and blood pressure will be assessed according to the TIME AND EVENTS SCHEDULE.

Temperature should be taken at the same site each visit (e.g. oral, tympanic, *or* axillary).

Blood pressure will be assessed seated.

8.3.1.4 Physical examination

Physical examinations will be as per the TIME AND EVENTS SCHEDULE. A physician should preferably conduct the physical examination, but where timing prohibits this, a suitably trained nurse may undertake this task. The physician should be consulted if any abnormality in the examination is detected.

8.3.1.5 Pregnancy testing

Perform urine pregnancy tests at screening on all females of child-bearing potential. Subjects who are pregnant are excluded from the trial. Female subjects who are at risk of becoming pregnant are expected to prevent pregnancy occurring while on treatment by using effective birth control methods e.g. oral contraceptive, condom, IUD, injectable contraceptive, or diaphragm. Male subjects have no contraceptive restrictions.

The Investigator will inform the Sponsor immediately of any case of pregnancy and collect information on any subject who becomes pregnant during the trial using the *Pregnancy Report Form*. Discontinue pregnant subjects from treatment but continue to follow-up for

scheduled safety assessments for the duration of the trial. Subjects will also be followed to determine the outcome of pregnancy. See also section 8.3.4.3.

8.3.2 Criteria

An assessment of safety will be made on the basis of reviewing changes in physical examination, spirometry and using adverse event data.

8.3.3 Adverse event reporting

8.3.3.1 Definitions

Adverse event

An AE is any untoward medical occurrence in a patient or clinical investigation subject administered a medicinal product and which does not necessarily have to have a causal relationship with this product.

An AE can therefore be any unfavorable or unintended sign (for example an abnormal laboratory finding), a symptom or disease temporally associated with the use of a medicinal (investigational) product, whether or not considered related to that product.

Any worsening (e.g. clinically significant adverse change in frequency and/or intensity) of a pre-existing condition, which is temporally associated with the use of a medicinal product, is also considered an AE. Recurring symptoms associated with pre-existing conditions **will not** be considered AEs during the trial, unless they have a clinically significant increase in severity and/or frequency.

Hemoptysis *is an event of special interest in this trial and should be reported as a separate Adverse Event every time it occurs, even if it is considered to be a component of a pulmonary exacerbation and/or if it is an ongoing / intermittent medical history condition.*

Serious adverse event (SAEs)

A serious adverse event (SAE) for this trial is defined as any unfavorable medical occurrence that at any dose:

1) Results in **death**

2) Is **life-threatening**

   NOTE: the term "life-threatening" refers to an event in which the subject was at risk of death at the time of the event; it does not refer to an event, which hypothetically might have caused death if it were more severe.

3) Requires inpatient **hospitalization** or **prolongation of existing hospitalization**

   NOTE: The following do not meet the definition of hospitalizations for SAE reporting purposes:

   - An Emergency Department visit that did not result in an admission to the hospital
   - Hospitalizations for social reasons
   - Planned hospitalizations for surgery or procedure planned prior to the subject's entry into the trial, unless the subject experiences an additional AE that prolongs the pre-planned hospitalization. This pre-planned hospitalization must be documented in the subject's CRF medical record page at screening (Visit 0).

4) Results in **persistent** or **significant disability** or **incapacity**

5) Is a **congenital anomaly** or **birth defect**

6) Other **important medical event**

   NOTE: Appropriate medical judgment should be exercised for important medical events that may not be immediately life-threatening or result in death or hospitalization but may jeopardize the subject or may require intervention to prevent one of the other outcomes listed in the definitions above. **These should usually be considered serious**.

8.3.3.2 Causality assessment

The Investigator will make a causality assessment according to his/her best judgment. The following criteria can be used as a guidance to assess the causal relationship. These are not definitive, but presented as examples.

Definitely related:

- There is evidence of exposure to the investigational medication or procedure
- The temporal sequence of the AE onset relative to administration of the investigational medication or procedure is reasonable
- The SAE is more likely explained by the investigational medication or procedure than by another cause
- De-challenge (if performed) is positive
- Re-challenge (if feasible and if performed) is positive
- The AE shows a pattern consistent with previous knowledge of the investigational medication or investigational medication class

Probably related:

- There is evidence of exposure to the investigational medication or procedure
- The temporal sequence of the AE onset relative to administration of the investigational medication or procedure is reasonable
- The AE is more likely explained by the investigational medication or procedure rather than by another cause
- De-challenge (if performed) is positive
- Re-challenge (if performed) is positive or ambiguous

Possibly related:

- There is evidence of exposure to the investigational medication or a protocol procedure
- The temporal sequence of the AE onset relative to administration of the investigational medication or procedure is reasonable
- The AE could have been due to another equally likely cause
- De-challenge (if performed) is negative or ambiguous
- Re-challenge (if performed) is negative or ambiguous

Probably not related:

- There is evidence of exposure to the investigational medication or protocol procedure
- There is another more likely cause of the AE
- De-challenge (if performed) is negative or ambiguous
- Re-challenge (if performed) is negative or ambiguous

Definitely not related:

- The subject did not receive the investigational medication or undergone any protocol procedure
- Temporal sequence of the AE onset relative to administration of the investigational medication or procedure is not reasonable
- There is another obvious cause of the AE
- De-challenge (if performed) is negative
- Re-challenge (if performed) is negative

8.3.3.3 Severity assessment

Mild: the subject has an awareness of a sign or symptom, but it is easily tolerated and does not alter normal activity

Moderate: the sign or symptom causes discomfort and/or interference with the subject's usual activity

Severe: the sign or symptom causes significant impairment of function or incapacitation, and/or the subject is unable to perform usual activities

8.3.4 Reporting procedures

8.3.4.1 All adverse events

The Investigator will assess all AEs and record details of seriousness (see Section 8.3.3.1), severity (see Section 8.3.3.3), duration, action taken with the investigational medication, and relationship to the investigational medication (causality, see Section 8.3.3.2). The Investigator will also record details of de-challenge and re-challenge with the investigational medication.

All adverse events that meet any of the serious criteria defined in Section 8.3.3.1 will be reported as SAEs.

All AEs, whether serious or not, will be reported from signing of the informed consent form until 7 days after the last trial visit.

All SAEs, including those spontaneously reported to the Investigator by the subject, must be reported using the *SAE Form*.

If a subject experiences an SAE any time after the 30 day post final trial visit follow-up period and the SAE is considered to be related to the investigational medication, the Investigator should report this to Pharmaxis immediately.

All AEs, regardless of seriousness, severity or presumed causality must be recorded in the subject's medical records and in the CRF. AEs that occur in subjects who have screen failed and are not entered into the CRF will be captured on the AE Reporting Log in the Investigator Site File. All SAEs that occur in subjects who have screen failed will be reported from informed consent.

The Sponsor is responsible for reporting AEs to the relevant regulatory authorities according to international and local regulations applicable to this trial.

The Investigator is responsible for reporting AEs to the appropriate IEC that approved the protocol, as required and documented by the IEC.

Pulmonary exacerbations are expected to occur as part of the disease but will be recorded as adverse events. Expedited reporting of serious exacerbations to Regulators and Ethics Committees will only be carried out if the exacerbation is considered to be related to trial drug and is clinically different to that normally expected in a particular subject (e.g. more severe). In such cases, the difference should be noted on the AE form.

8.3.4.2 Serious adverse events

The Principal Investigator or designee will report all SAEs immediately and in any situation no later than 24 hours after any member of the investigational team becomes aware of the event.

The Investigator must make an assessment of causality (see Section 8.3.3.2) and severity (see Section 8.3.3.3) of all SAEs. All actions taken and outcomes must also be recorded.

The *SAE Report Form* should be completed and forwarded to:

[redacted]

SAEs will be followed up until the event has resolved and the report is assessed as complete; or the subject is lost to follow-up. In addition, an Investigator may be requested by Pharmaxis to provide or obtain specific additional follow-up information in an expedited fashion for the purposes of full safety assessment.

All follow-up information should be reported as described above.

8.3.4.3 Pregnancy

Pregnancy is not usually considered an SAE or an AE.
If an Investigator becomes aware that a subject, or the sexual partner of a male subject, becomes pregnant while they are participating in the trial or within the 7-day post trial follow-up period they will notify Pharmaxis immediately.

A *Pregnancy Report Form* should be completed and be forwarded to;

Female subjects who become pregnant will be withdrawn from treatment but will continue to be followed up for scheduled safety assessments for the duration of the trial.

All pregnancies should be followed to term and the outcome reported to Pharmaxis. In the case of a pregnancy in the sexual partner of a male subject consent will be sought from the partner to obtain follow-up information.

If a subject or baby experience any adverse event during the pregnancy or after birth this will be reported as described throughout Section 8.3.

9 SUBJECT COMPLETION / DISCONTINUATION

9.1 Completion
A subject will be considered to have completed the trial if he or she has completed assessments up to visit 4. Subjects who prematurely discontinue investigational drug treatment during the trial and fail to present for the scheduled trial visits for any reason will not be considered to have completed the trial. Subjects who discontinue from the IMP but continue to participate in the trial to the final assessment will be considered to have completed the trial.

9.2 Trial treatment discontinuation
All subjects who discontinue IMP treatment and remain in the trial will be requested to attend an IMP discontinuation visit within 2 weeks of the last dose. A complete evaluation should be made at this visit.

All efforts should be made to ensure that subjects who discontinue IMP treatment remain in the trial, and continue all other scheduled visits as per protocol.

A subject **will be** discontinued from treatment if she becomes pregnant.

9.3 Trial withdrawal
A subject will be withdrawn from the trial for any of the following reasons:

- Loss to follow-up;
- Withdrawal of consent;
- The subject's attending physician requests that the subject be withdrawn from the trial; *or*

- The Investigator or the Sponsor, for any reason, stops the trial or stops the subject participating in the trial.

Protocol deviations will not lead to automatic withdrawal unless they indicate a significant risk to the subject's safety. All protocol deviations must be discussed promptly with the Sponsor.

All subjects who are withdrawing from the trial will attend their next scheduled visit. This should be within two weeks of administration of the last dose of IMP. A complete final evaluation at the time of the subject's withdrawal should be made and an explanation be given as to why the subject is withdrawing or being withdrawn from the trial. The reason and date of withdrawal must be noted on the CRF. If the reason for withdrawal is an AE or a clinically significant laboratory test result, monitoring will continue until resolution or until an appropriate medical judgment concerning the cause or importance has been made. The specific event or test result(s) must be recorded on the CRF.

10 STATISTICAL METHODS

10.1 General considerations

A full description of the statistical methods planned for this trial can be found in the DPM-CF-303 Statistical Analysis Plan (SAP).

Efficacy analyses will be conducted using the intention to treat (ITT) analysis set (refer **Section 10.1.2.1**) as well as the per protocol (PP) analysis set (refer **Section 10.1.2.2**). Safety analyses will be conducted on the safety analysis set (refer **Section 10.1.2.3**).

Significance tests will be performed assuming a two-sided 0.05 significance level; two-sided 95% confidence intervals will also be computed.

[redacted]

10.1.1 Sample size justification

Enrolment will continue until at least 350 subjects have been randomized to treatment in a 1:1 ratio.

Assuming that the change from baseline in FEV_1 on control is 0 mL and the change from baseline in FEV_1 on mannitol is 80 mL, then a sample size of 350 (175 Mannitol : 175 Control) subjects will yield 90 % power at the 0.05 significance level to detect this difference between the treatment groups assuming a pooled standard deviation of 230 mL. The sample size calculation is based on a t-test of the treatment contrast at week 26. While this approach is not completely concordant with the primary analysis which computes the mean change in FEV_1 over all three post-baseline visits it can be considered a suitable method for estimating sample sizes for longitudinal analyses and is likely to be conservative.

The estimated pooled standard deviation of 230 mL is based on the observed pooled standard deviation in the pooled adult DPM-CF-301 and DPM-CF-302 data from subjects who completed the studies.

This minimum sample size is based upon the observed pooled standard deviation from both DPM-CF-301 and DPM-CF-302 studies in subjects with baseline FEV_1 of more than 40% but less than 90%. However the observed standard deviations were different in these two

previous studies, and were highly influenced by a few outlying values. In addition, the present trial (DPM-CF-303) will be conducted at different sites and in different countries to the previous studies. Thus there is some uncertainty as to what the observed standard deviation is likely to be in this trial (DPM-CF-303). For these reasons a sample size re-estimation is planned to be completed after at least 300 subjects have been recruited and will be based on all available 26-week data. This blinded sample size re-estimation will be based on the method of Kieser and Friede [15] which preserves the overall type 1 error rate. Therefore no adjustments to the overall significance level for the trial is required. See Section 10.1.5 for more information.

[redacted]

10.1.2 Analysis sets

10.1.2.1 Intention to treat (ITT) analysis set

The ITT analysis set (also known as the Full Analysis Set) will include all subjects who were randomized to treatment. Subjects will be analyzed according to the treatment group to which they were randomized.

10.1.2.2 Per protocol (PP) analysis set

The per protocol analysis set consists of all randomized subjects, who have:

- Strictly met all the inclusion and exclusion criteria (i.e. will exclude those subjects who have been granted an exception to be included in the trial);
- No major protocol deviations which would affect efficacy evaluations;
- Taken trial drug for at least 60% of the 26-week treatment period;
- A valid baseline FEV_1 measure; *and*
- At least two valid assessments of FEV_1 following the baseline visit (Visit 0).

The decision to include subjects in the per protocol dataset will be made prior to unblinding of the randomization schedule. For the per protocol analysis any non-valid assessments of FEV_1 will be excluded from the analysis. The validity of the FEV_1 measurements will be assessed prior to unblinding. The subject must meet the requirement for a valid baseline and two valid post-baseline FEV_1 assessments.

10.1.2.3 Safety analysis set

The safety analysis set will include all subjects who received at least one dose (or part thereof) of randomized trial treatment. Subjects will be analyzed in the treatment group based on the treatment they received.

10.1.2.4 Mannitol Tolerance Test (MTT) analysis set

The MTT set will include all subjects participating in the mannitol tolerance test prior to randomisation regardless of their subsequent randomisation status. It includes subjects who fail the MTT (MTT positive) or who have an incomplete procedure, along with those who pass the MTT (MTT negative).

10.1.3 Missing data

Where a subject has missing baseline data, where possible, the screening (Visit 0) value will be used.

Handling of missing post-baseline data for the primary efficacy variable (FEV_1) and secondary variables is discussed further in Sections 10.2.4 and 10.2.9.

10.1.4 Multiple comparisons

No adjustments for multiple comparisons will be performed because a hierarchical testing approach will be used for the key secondary comparisons using a sequentially rejective method (refer to Section 10.2.8).

10.1.5 Sample size re-estimation

A formal sample size re-estimation will be performed after at least 300 subjects have been recruited and will be based on all available 26-week data.

This blinded sample size re-estimation will be based on the methods of Kieser and Friede [15] which preserve the overall type 1 error rate. Thus no adjustments to the overall significance level for the trial is required.

The sample size calculation will be performed such that the trial achieves at least 90% power at the two-sided significance level of 0.05 to detect an 80 mL difference in FEV_1 change from baseline between the treatment arms, using the observed pooled standard deviation observed in the cohort of subjects with available week 26 data. [redacted]

Table 7: Required sample size based upon re-estimation of standard deviation

Observed standard deviation	Re-estimated required sample size
≤ 230	N=350 (as originally planned)
>230 and ≤ 239	N=380
>239 and ≤ 249	N=410
>249	N=440 (maximum sample size)

10.1.6 Interim analysis

No interim analysis is planned.

10.1.7 Subject disposition

The number and percentage of subjects enrolled (meet inclusion and exclusion criteria), randomized and treated in the trial will be presented, together with the number and percentage of subjects included in each of the analysis populations. The number and percentage of subjects randomized at each center will also be presented.

A tabulation of the number and percentage of subjects who discontinued from treatment, or withdrew from the trial prematurely, including a breakdown of the corresponding reasons for discontinuation/withdrawal, will be presented.

10.1.8 Baseline demographic and clinical characteristics

Baseline characteristics will be described using the safety population. If required, these may also be produced for the intent to treat analysis set (ITT). Demographics, medical history and other baseline variables will be summarized as appropriate to the type of data.

10.2 Efficacy analysis

10.2.1 Primary objective

To determine whether inhaled mannitol (400mg b.i.d.) is superior to control for improving lung function as measured by mean change from baseline FEV_1 (mL) over the 26-week treatment period in adult subjects (18 years or older) with CF.

10.2.2 Primary endpoint

The primary endpoint is the mean change from baseline FEV_1 (mL) measured at weeks 6, 14 and 26.

10.2.3 Primary analysis

The mean absolute change from baseline FEV_1 (mL) over weeks 6, 14 and 26 will be compared between the two treatment groups with a REML based repeated measures approach.

Analyses will include the fixed, categorical effects of treatment, rhDNase use, country, visit, and treatment-by-visit interaction, as well as the continuous, fixed covariate of baseline percent predicted FEV_1. An unstructured (co)variance structure will be used to model the within-subject errors.

The planned primary efficacy model will take the form:

$$\text{Change from baseline } FEV_1 = \text{Baseline \% predicted } FEV_1 + \text{Treatment} + \text{rhDNase} + \text{Visit} + \text{Country} + (\text{Treatment} \times \text{Visit})$$

Significance tests will be based on least-squares means using a two-sided 0.05 significance level. Two-sided 95% confidence intervals will be computed. [redacted]

Change in % predicted FEV_1, relative change in FEV_1 (% change) and relative change in % predicted FEV_1 will also be analyzed using the primary model.

10.2.4 Primary analysis – missing data

Subjects who discontinue drug treatment will remain in the trial and continue to provide all scheduled FEV_1 measurements. While all efforts will be made to ensure subjects remain in the trial, the following planned imputation strategy will allow for an appropriate primary analysis in the presence of missing FEV_1 data.

Missing baseline (Visit 1) measurements for the primary efficacy variable (FEV_1) will be imputed using screening (Visit 0) values, if available.

Missing post-baseline measurements for the primary efficacy variable (FEV_1) will be imputed utilizing an Imputation Using Dropout Reason *(IUDR)* methodology. For subjects who discontinue due to safety, tolerability, lack of efficacy or were lost to follow-up, missing post-baseline FEV_1 measures will be imputed using the baseline (Visit 1) FEV_1 measurement (baseline observation carried forward [BOCF] procedure). Missing FEV_1 measurements as a

result of other causes of trial discontinuation (e.g. relocation, pregnancy) will not be explicitly imputed.

Imputation of missing FEV_1 measurements by means of BOCF is a reasonable assumption in the studies of adult subjects with cystic fibrosis, as these subjects who discontinue will no longer receive treatment and are thus likely to promptly return to their baseline lung function. Imputation by BOCF specifically inhibits any possible prolongation of an observed early treatment benefit implicitly conferred by a mixed model.

A framework of imputation by BOCF in combination with the IUDR strategy seeks to specifically address the primary regulatory question of interest, that is to provide a reliable estimation of the treatment effect over 26 weeks as observed in all randomized subjects.

10.2.5 Primary sensitivity analyses

The following planned sensitivity analyses are intended to gain insights into how different analytical assumptions influence the results of the planned primary analysis. Depending on the extent and pattern of withdrawals and "missingness" further sensitivity analyses in additional to those given below may be performed. If necessary, further details will be provided in the SAP.

10.2.5.1 Primary sensitivity analysis #1:

To elicit insights into the effect of imputation via the planned BOCF + IUDR framework the primary analyses will be repeated with no explicit imputation of missing post-baseline FEV_1.

10.2.5.2 Primary sensitivity analysis #2:

To elicit insights into the effects of including data from subjects after cessation of trial treatment (by including these data, the effects of subsequent therapy are attributed to trial treatment), the primary analyses will be repeated excluding these data both with imputation of missing (and excluded) post-baseline FEV_1 assessments and without explicit imputation. Data from IMP discontinuation visit or early withdrawal from the study that has been collected will be examined here also.

10.2.5.3 Primary sensitivity analysis #3:

Sensitivity of the primary result to choice of "across weeks 6 to 26" versus "at week 26" estimates of the treatment effect will be gauged by computing the least square mean difference at week 26 for the purposes of comparison.

10.2.5.4 Primary sensitivity analysis #4:

A potential disadvantage of employing a single imputation method such as BOCF is the risk of artificially attenuating the natural variability in the longitudinal data. To gauge the possible impact of this effect, a two-sample t-test at week 26 will be undertaken computing point estimates from the data with BOCF *but computing variation only from subjects who completed the trial*. Furthermore a non-parametric equivalent (e.g. Wilcoxon rank sum test) will be undertaken on the data with BOCF imputation. These results will be compared to the week 26 contrast computed by the primary efficacy model (Primary sensitivity analysis #2).

10.2.5.5 Primary sensitivity analysis #5:

The primary analysis will be repeated using the mean FEV_1 measurement from screening (Visit 0) and baseline (instead of the baseline FEV_1 measurement) in order to determine if the primary results are sensitive to any observed changes between screening and baseline in FEV_1.

10.2.5.6 Primary sensitivity analysis #6

Two responder analyses will be undertaken:

1. A responder will be defined as those who have an increase from baseline FEV_1 of greater than 100 mL (subjects who discontinued from the trial will be classified as non-responders).
2. A non-responder will be defined as those who have a reduction from baseline FEV_1 of more than 100 mL (subjects who discontinued from the trial will be classified as non-responders).

Proportions in each treatment group will be assessed for each definition and compared between treatment groups using logistic regression.

10.2.5.7 Primary sensitivity analysis #7

In addition to the responder analysis described above, a responder analysis will be undertaken where the cut-off for classification as a responder will be change from baseline FEV_1 greater than: -300, -250, -200, -150, -100, -50, 0, 50, 100, 150, 200, 250 & 300mL respectively. These results will be plotted in a graphical summary for both treatment groups. These results can be scrutinized to ensure a consistent trend of treatment effect that is insensitive to any particular definition of response.

10.2.5.8 Primary sensitivity analysis #8

To explore the impact of patterns of "missingness" in the data, multiple imputation (MI) with penalties will be used. Missing data across the treatment period, namely week 6 to week 26, will be first imputed by MI (i.e. multiple times). Then the impact of various penalties will be explored including linking penalties to reason for "missingness" and increasing penalties for subjects with multiple missing data (as these subjects could be less likely benefit from the treatment). The imputed longitudinal data will be analyzed by mixed-effect model repeated measures (MMRM) model as above.

10.2.5.9 Primary sensitivity analysis #9

To explore the effect of different imputation methods, the primary analysis will be repeated using a worst observation carried forward (WOCF) + IUDR method rather than the BOCF + IUDR framework.

10.2.6 Primary per protocol analysis

The primary analysis is planned to estimate the treatment effect of being *randomized to mannitol*. In order to estimate the treatment effect of *taking mannitol* a per protocol analysis is planned. This entails re-fitting the primary efficacy model to the per-protocol analysis set.

The results of this per protocol analysis will provide an extra robustness check on the main results from the ITT population.

10.2.7 Secondary analyses

Three key secondary objectives are ordered as follows and will be subjected to a hierarchical testing procedure outlined in **Section 10.2.8**

10.2.7.1 Hierarchical secondary objectives

Objective #1: To determine whether inhaled mannitol (400 mg b.i.d.) is superior to control for improving lung function as measured by mean change from baseline FVC (mL) over the 26-week treatment period in adult subjects with CF.

Analysis: The absolute change in FVC will be analyzed as per the primary analysis (i.e. REML based repeated measures approach using a model including the fixed, categorical effects of treatment, rhDNase use, country, visit, and treatment-by-visit interaction, as well as, the continuous, fixed covariate of baseline percent predicted FEV_1).

Objective #2: To determine whether inhaled mannitol (400 mg b.i.d.) is superior to control in increasing the time to first pulmonary exacerbation over the 26-week treatment period in adult subjects with CF.
Analysis: The time (in days) from the date of first treatment to the date of the first pulmonary exacerbation (any grade) will be analyzed using the Cox's Proportional Hazards model with time to first exacerbation as the outcome, and treatment, country, rhDNase use and historical exacerbation rate as factors. The hazard ratio will be presented with 95% confidence limits. When a subject does not have an exacerbation during the trial period the subject will be censored at the date of their last participation in the trial.

Objective #3: To determine whether in adult subjects with CF, inhaled mannitol (400 mg b.i.d.) is superior to control for reducing the number of days on antibiotics (oral, inhaled or IV) due to pulmonary exacerbation.
Analysis: The number of days on antibiotics will be compared between treatment groups using a negative binomial model with the number of days on antibiotics recorded by each subject over the trial period as the outcome variable, and treatment group, country, rhDNase use and historical exacerbation rate as predictors. An offset variable of the natural log of number of days in the trial will be used in the model to adjust for different lengths of follow-up. The rate ratio will be presented with 95% confidence limits.

Objective #4: To determine whether in adult subjects with CF, inhaled mannitol (400 mg b.i.d.) is superior to control for decreasing the number of days in hospital due to pulmonary exacerbation.
Analysis: The number of days in hospital (admissions only) due to pulmonary exacerbation will be compared between treatment groups using a negative binomial model with the number of days in hospital due to pulmonary exacerbation experienced by each subject over the trial period as the outcome variable, and treatment group, country, rhDNase use and historical exacerbation rate as predictors. An offset variable of the natural log of number of days in the trial will be used in the model to adjust for different lengths of follow-up. The rate ratio will be presented with 95% confidence limits.

Objective #5: To determine whether inhaled mannitol (400 mg b.i.d.) decreases the rate of pulmonary exacerbations over the 26-week treatment period compared to control in adult subjects with CF.
Analysis: The rate of pulmonary exacerbations will be compared between treatment groups using a negative binomial model with the number of pulmonary exacerbations experienced by each individual over the trial period as the outcome variable, and treatment group, country and rhDNase use as predictors. Historical exacerbation rate will be used as a covariate. An offset variable of the natural log of number of days in the trial will be used in the model to adjust for different lengths of follow-up. The rate ratio will be presented with 95% confidence limits.

10.2.7.2 Non-hierarchical secondary objectives

Objective #1: To determine whether in adult subjects with CF, inhaled mannitol (400 mg b.i.d.) is superior to control for decreasing incidence of pulmonary exacerbations.
Logistic regression will be used to estimate the treatment effect (odds ratio) on the proportion

of subjects with one or more exacerbations. Covariates of country, rhDNase use and historical exacerbation rate will be included in the logistic regression model.

Objective #2: To determine whether in adult subjects with CF, inhaled mannitol (400 mg b.i.d.) is superior to control for improving ease of expectoration.
Analysis: The change in VAS score from baseline will be analyzed in a similar manner to the primary endpoint. The mean absolute change from baseline VAS score over weeks 6, 14 and 26 will be compared between the two treatment groups with a REML based repeated measures approach. Analyses will include the fixed, categorical effects of treatment, rhDNase use, country, visit, and treatment-by-visit interaction, as well as the continuous, fixed covariates of baseline VAS score and baseline % predicted FEV_1. An unstructured (co)variance structure will be used to model the within-subject errors.

Objective #3: To determine whether in adult subjects with CF, inhaled mannitol (400 mg b.i.d.) is superior to control for improving respiratory symptoms measured by CFQ-R respiratory domain.
Analysis: Component questions in the questionnaire will be appropriately transformed according to the scoring manual, so that the best response will be uniformly the highest score and the worst response will be uniformly the lowest score. The mean absolute change from baseline in the CFQ-R respiratory domain score over weeks 6, 14 and 26 will be compared between the two treatment groups with a REML based repeated measures approach. Analyses will include the fixed, categorical effects of treatment, rhDNase use, country, visit, and treatment-by-visit interaction, as well as the continuous, fixed covariates of baseline CFQ-R respiratory domain score and baseline % predicted FEV_1. An unstructured (co)variance structure will be used to model the within-subject errors.

The CFQ-R respiratory domain includes the question "Have you had to cough up mucus?" The scoring algorithm scores an affirmative answer to this question as a negative outcome for the subject, however, due to the mechanism of action of mannitol (i.e. inducing coughing up mucus) an affirmative answer here may not be a negative outcome and hence this question is inappropriate here. Therefore, the above analyses will be repeated on the CFQ-R respiratory domain score omitting this question.

10.2.8 Hierarchical testing of key secondary objectives

The hypotheses for the primary efficacy endpoint and the three key secondary efficacy endpoints will be formally tested utilizing a hierarchical testing approach to preserve the type-I error rate. Objectives will be tested in the order in which they are ranked in Section 2.

10.2.9 Secondary analyses – missing data

Change from baseline FVC:
Missing post-baseline measurements will be handled as per the primary efficacy endpoint (FEV_1).

Time to first pulmonary exacerbation:
Subjects who withdraw from the trial prior to experiencing an exacerbation will be censored at the date of the last participation in the trial.

Pulmonary exacerbation rate:
In the event a subject discontinues from the trial before week 14 with no observed instances of a pulmonary exacerbations the number of pulmonary exacerbations will be imputed using half the subject's historical (previous 12 months) pulmonary exacerbation count (rounded upwards) and their treatment exposure time will be imputed as 26 weeks.

In the event a subject discontinues from the trial after week 14 with no observed instances of a pulmonary exacerbations the number of pulmonary exacerbations will be imputed using one quarter the subject's historical (previous 12 months) pulmonary exacerbation count (rounded upwards) and their treatment exposure time will be imputed as 26 weeks.

Number of days in hospital and number of days on antibiotics:
Primary negative binominal analysis allows for different lengths of follow-up via offset variable. No further allowance for missing data will be made in these analyses.

Pulmonary exacerbation incidence:
No allowance for missing data will be made in this analysis.

Ease of expectoration:
Missing post-baseline measurements will be handled as per the primary efficacy endpoint (FEV_1).

CFQ-R respiratory domain score:
Missing post-baseline measurements will be handled as per the primary efficacy endpoint (FEV_1).

10.2.10 Key secondary sensitivity analyses

Change in absolute FVC from baseline:
* Analysis will be repeated without imputation of missing data.

Pulmonary exacerbation rate:
* Analysis will be repeated without imputation of missing data.
* A zero inflated negative binomial model will be fitted and compared to the specified negative binomial model.

10.2.11 Subgroup analyses

Limited subgroup analyses of relevant primary and secondary will be performed to assess consistency of response between those:

- with and without rhDNase use;
- with and without *Pseudomonas aeruginosa* infection;
- with differing baseline percentage of predicted FEV_1.

11 ETHICAL ASPECTS

The trial will be conducted in accordance with the International Conference on Harmonization (ICH) and Good Clinical Practice (GCP) guidelines. In addition, the trial will be conducted in compliance with all applicable laws and regulatory/health authority(ies) requirements relevant to the use of new therapeutic agents in each participating country.

11.1 Informed consent

This trial will be conducted in compliance with the US federal FDA 21 CFR Part 50-Protection of Human Subjects requirements. Written informed consent will be obtained from each subject before any trial related procedures or assessments are performed and after the aims, methods, anticipated benefits, and potential hazards are explained. Explanation will also be provided to the subjects that they are free to refuse participation in the trial and free to withdraw from the trial at any time without prejudice to future treatment.

The subject's willingness to participate in the trial will be documented in writing on an informed consent form, which will be signed by the subject and the person obtaining the consent with the date and time of that signature indicated. The Investigator(s) will keep the original informed consent forms and copies will be given to the subjects. In addition, the person conducting the informed consent discussion will document the process of obtaining consent in the subject's medical record.

Written and/or oral information about the trial in a language understandable by the subject will be given to all subjects. The information provided must include a statement that this is a clinical research study/trial, the duration and an adequate explanation of the aims, methods, anticipated benefits, potential hazards, and insurance arrangements in force.

11.2 Heath authorities, independent ethics committees / institutional review boards

The Investigator(s) will ensure that the conduct of the trial conforms to ICH/GCP Guidelines and with national laws and regulations for clinical research.

Before starting this trial, the protocol will be submitted to the national/local health authorities and to the IEC/IRB for evaluation. As required, the trial will not start before the IEC/IRB and health authorities give approval or a favorable opinion.

11.3 Confidentiality regarding trial subjects

The Investigator(s) must assure that the privacy of the subjects, including their personal identity and all personal medical information, will be maintained at all times. In CRFs and other documents submitted to the Sponsor, subjects will not be identified by their names, but by an identification code (e.g., subject or screen number).

The Monitor, properly authorized persons on behalf of the Sponsor's quality assurance unit, or competent authorities may scrutinize personal medical information for the purpose of verifying data recorded on the CRF. Personal medical information will always be treated as confidential, according to local privacy regulations.

11.4 Data safety monitoring board (DSMB)

A DSMB will be established. The constitution and duties of the DSMB will be documented in a DSMB charter, but the primary function will be to provide ongoing review of safety data.

12 TRIAL MANAGEMENT & ADMINISTRATIVE REQUIREMENTS

12.1 Protocol amendments

[redacted]

12.2 Direct access to source data / documents

The Monitor(s), Auditor(s), and Regulatory Inspector(s) will be given direct access to source data and documentation (e.g., medical records, laboratory reports, diagnostic imaging) for source data verification, provided that subject confidentiality is maintained in accordance with local requirements and privacy regulations.

12.3 Monitoring and quality assurance

12.3.1 Information to trial personnel

The Investigator(s) is responsible for ensuring that all trial personnel are qualified and trained for their designated roles and responsibilities. He/she shall provide information about the trial to all staff members directly involved in the trial or in any element of subject management, both before starting the practical performance of the trial and during the course of the trial (e.g., when new staff become involved).

The Monitor(s) is responsible for initiating the site, for ensuring site compliance with the protocol and for closing out the site at the end of the trial. Additional information available during the trial should be provided to the Investigator by the Sponsor on an ongoing basis. The Investigator is responsible for ensuring that the site staff are fully informed and appropriately trained. Specific training in relation to additional information should be supported and documented by the Monitor as required. (The Investigator must ensure and document that new staff members involved in the trial are fully informed and trained prior to completing any trial related tasks. The Monitor is responsible for ensuring that training has been completed to ensure trial compliance).

12.3.2 Trial monitoring

For this trial, the average monitoring frequency is expected to be once every five to ten weeks, but is subject to recruitment rates. The Monitor(s) or Sponsor representative is responsible for ensuring that the trial is conducted according to the protocol, Standard Operating Procedures (SOPs) and in compliance with GCP.

The Monitor(s) is the primary link between the Sponsor and the Investigator. The main responsibilities of the Monitor(s) is to visit the Investigator before, during, and after the trial to ensure adherence to the protocol, and to assure that all data are correctly and completely recorded and reported and that informed consent is obtained and recorded for all subjects before their participation in the trial.

The Monitor(s) will contact and visit the Investigator at regular intervals throughout the trial. The Monitor(s) will be allowed access to check and verify the various records (CRFs and other pertinent data records) relating to the trial to verify adherence to the protocol and to ensure the completeness, consistency, and accuracy of the data being recorded.

As part of the supervision of the trial progress other Sponsor personnel may, on request, accompany the Monitor(s) on visits to the trial center. The Investigator and assisting staff must agree to cooperate with the Monitor(s) to resolve any problems, errors, or possible misunderstandings concerning any data discrepancies detected in the course of these monitoring visits.

12.3.3 Audit and inspection

According to ICH / GCP Guidelines, the Sponsor may audit the investigational site to compare raw data, source data, and associated records with the interim (if applicable) or final report of the trial to assure that data have been accurately reported and the trial was conducted in compliance with the protocol, SOPs, GCPs and applicable regulatory requirements. The Sponsor's worldwide Clinical Quality Assurance department is responsible for the auditing of the trial. Auditing may be contracted to an external body.

The Investigator(s) must accept that regulatory authorities may conduct an inspection to verify compliance of the trial with the protocol, GCPs and applicable regulatory requirements.

12.4 Protocol deviations

The protocol must be read thoroughly and the instructions must be followed. However, exceptions will be made in emergency situations when the protection, safety, and well-being of the subject requires immediate intervention based on the judgment of the Investigator or a responsible, appropriately trained, and credentialed professional(s) designated by the Investigator as a sub-Investigator.

In the event of a significant protocol deviation due to an emergency, accident, or error, the Investigator or designee must contact the Medical Monitor at the earliest possible time by telephone. This allows for an early joint decision to be made as to whether or not the subject should continue in the trial. The Investigator, the Sponsor, and the Medical Monitor will document this decision.

13 DATA HANDLING AND RECORD KEEPING

13.1 Source data

The Investigator is required to prepare and maintain adequate and accurate case histories (i.e., medical records) designed to record all observations and other data pertinent to the trial for each trial subject. The medical records must contain adequate information to allow for verification of subject identity throughout the trial.

Any data recorded directly on the CRF, as agreed to by the Sponsor for which no other written or electronic record will be maintained in the subject's medical record, will be considered source data (e.g., results of physical examinations, vital signs testing, or the drug administration procedure). The agreement between the Investigator and the Sponsor, including the type of parameters allowed to be entered directly on the CRFs, must be documented in the subject's source documentation.

The CRF and the subject's medical records pertinent to the trial will be reviewed by the Pharmaxis' designated Monitor, auditors and possibly by representatives from the IRB/IEC and regulatory bodies such as the US Food and Drug Administration (FDA) to the extent permitted by regulations.

The Investigator is required to retain a subject identification code list to allow unambiguous identification of each subject included in the trial. This list should contain the subject's full name, date of birth, dates of participation and identification number as per country regulations. The Investigator must agree to supply all details to the Sponsor auditor(s) and/or regulatory authorities, ensuring the data are held confidentially at the Investigator's center after completion of the trial. A note will be made in the hospital or clinical medical records, if appropriate, that the subject is participating in a clinical trial.

13.2 Case report form (CRF)

In this trial, the CRF will be an electronic CRF (eCRF). The Investigator or the designated site person must complete the CRF and supporting documentation for each subject within a timely manner of the visit occurring.

The Monitor(s) and Data Manager(s) will review the completed data for accuracy, completeness and consistency with source documentation. The Monitor or Data Manager will submit requests for correction/clarification of data (e.g., queries) to the Investigator or designee when inconsistencies are identified during monitoring and source data verification or during the edit check process.

All corrections and alterations to CRF data must be made by the Investigator or by the designated site personnel in a timely manner and according to the instructions provided. Completed CRFs should then be reviewed and signed by the Principal Investigator or designated site personnel. All persons, their roles and responsibilities delegated by the Investigator participating in the trial must be indicated on the delegation of authority log.

A full audit trail detailing corrections and alternations made to the CRF will be maintained.

13.3 Archiving of trial documentation

Trial data and other essential documents must be retained for a period of at least 15 years for adult trials; at least 25 years for trials involving children; or at least 2 years after the last approval of a marketing application, and until there are no pending or contemplated marketing applications in an ICH region, or at least 2 years have elapsed since the formal discontinuation of clinical development of the investigational product. It is the responsibility of the Sponsor to inform the Investigator(s)/institution(s) when these documents need no longer be retained. The original record of the CRFs will be archived by the Sponsor for the lifetime of the product. No trial document or image will be destroyed without prior written agreement between the Sponsor and the Investigator(s). Should the Investigator(s) wish to assign the trial records to another party or move them to another location, advance written notice will be given to the Sponsor.

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15 REPORTING AND PUBLICATION OF RESULTS

The Sponsor is responsible for publically registering this trial on the National Library of Medicine's website (http://clinicaltrials.gov) within 21 days of initiating enrolment.

All information, including but not limited to information regarding dry powder mannitol or the Sponsor's operations supplied by the Sponsor to the Investigator and not previously published, along with any data generated as a result of this trial, are considered confidential and remain the sole property of the Sponsor. The Investigator agrees to maintain this information in confidence, and will use the information only to perform the trial.

The Sponsor is responsible for preparing a clinical trial report, in cooperation with the Investigator(s). The Sponsor and the Principal/Coordinating Investigator will sign the final report.

The Sponsor shall have the right to publish data and information without approval from the Investigator. If an Investigator wishes to publish information from the trial, a copy of the manuscript must be provided to the Sponsor for review at least 60 days before submission for publication or presentation. Expedited reviews will be arranged for abstracts, poster presentations, or other materials. If requested by the Sponsor in writing, the Investigator will withhold such publication for up to an additional 60 days to allow for filing of a patent application. ***The Sponsor will not mandate modifications to scientific content and does not have the right to suppress information.*** The Investigator will recognize the integrity of a multicenter trial by not publishing data derived from the individual site until the combined results from the completed trial have been published in full, within 12 months after conclusion, abandonment, or termination of the trial at all sites, or the Sponsor confirms there will be no multicenter trial publication. Authorship of publications resulting from this trial will be based on the guidelines on authorship, such as those described in the Uniform Requirements for Manuscripts Submitted to Biomedical Journals, which state that the named authors must have made a significant contribution to the design of the trial or analysis and interpretation of the data, provided critical review of the paper, and given final approval of the final version.

15.1 Authorship

The publications should acknowledge the contribution of investigators jointly as the *CF-303 Trial Investigators* and, where journal and space permit, the names of all investigators will be listed in the acknowledgement section.

For the primary publication and any secondary analyses of the data set, the Principal / Coordinating Investigator plus the three highest recruiters who also fulfill the authorship criteria as outlined in the international committee of medical journal editors (ICMJE) guidelines will be named as authors on behalf of the *CF-303 Trial Investigators*. Sponsor personnel who also fulfill the ICMJE's criteria for authorship will also be offered authorship.

16 REFERENCES

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17 SIGNATURE PAGE

Protocol

I confirm that I have read this protocol, I understand it, and I will work according to this protocol and in accordance with the International Conference on Harmonization (ICH) and Good Clinical Practice (GCP). I agree to report all information or data in accordance with the protocol, and in particular, I agree to report any serious adverse events. I will accept the monitors', auditors', and regulatory inspectors' oversight of the trial. I will abide by the publication plan set forth in the Investigator Agreement with Pharmaxis. I will promptly submit the protocol to the applicable ethical review board.

Clinical Site:

Site Number:

Site Principal Investigator:

_____ _____

Print Name **Title**

_____ _____

Signature **Date**

Accepted for the Sponsor – Pharmaxis Ltd

_____ _____

Print Name **Title**

_____ _____

Signature **Date**

Accepted for the Sponsor's Representative (if applicable_____)

(Organization name)

_____ _____

Print Name **Title**

_____ _____

Signature **Date**

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