ERJ Express. Published on April 8, 2011 as doi: 10.1183/09031936.00187510

Title: Inhaled dry powder mannitol in cystic fibrosis: an efficacy and safety study

Running Head: Inhaled mannitol in CF: an efficacy study

Key Words: cystic fibrosis, mannitol dry powder, FEV_1, airway mucociliary clearance, clinical study, dry powder inhalers

Authors: Bilton D[1], Robinson P[2], Cooper P[3], Gallagher CG[4], Kolbe J[5], Fox H[6], Jaques A[6], Charlton B[6] for the CF301 Study Investigators*

Author affiliations: [1]Royal Brompton Hospital, London, United Kingdom; [2]Royal Children's Hospital, Melbourne, Australia; [3]Children's Hospital, Westmead, Sydney, Australia, [4]St. Vincent's University Hospital, Dublin, Ireland, [5]University of Auckland, Auckland, New Zealand, [6]Pharmaxis Ltd, Frenchs Forest, Sydney, Australia

***CF301 Study Investigators**

AUSTRALIA: Scott Bell, Anne Chang, Peter Cooper, Iain Feather, Hugh Greville, Adam Jaffe, David Serisier, Martin Phillips, Phil Robinson, Alan Young, IRELAND: Gerry Canny, Charles Gallagher, Peter Greally, Noel G McElvaney, NEW ZEALAND: John Kolbe, Robin Taylor, UNITED KINGDOM: Diana Bilton, Alan Cade, Siobhan Carr, Mary Carroll, Meya Desai, Stuart Elborn, Iolo Doull, Charles Haworth, Ian Ketchell, Alan Knox, Leiske Kuitert, Simon Langton-Hewer, Julian Legg, Claire Murray, Nabil Jarad, Daniel Peckham, Simon Range, Simon Robertson, Alan Smyth, Kevin Southern, David Spencer, Chris Taylor, Chris Upton, Martin Walshaw, Jo Whitehouse

This work was supported by the NIHR Respiratory Disease Biomedical Research Unit at the Royal Brompton and Harefield NHS Foundation Trust and Imperial College London**.**

Funding: Pharmaxis Limited

Role of the funding source: The study sponsor participated in the study design, data collection, data analysis, data interpretation and writing of the report. Following completion of the trial, the data were held and analysed by the sponsor. The corresponding author had full access to all of the data, and had final responsibility for publication.

This trial is registered at ClinicalTrials.gov, number NCT00446680.

Abstract

This international phase III study of inhaled dry powder mannitol was a randomised double blind 26 week study, followed by a further 26 week open-label extension. 324 subjects were randomised 3:2 to mannitol (400 mg bid) or control. The primary efficacy endpoint was to determine the change in FEV_1 over the double-blind phase. Secondary endpoints included changes in FVC and, pulmonary exacerbations.

A significant improvement in FEV_1 was seen over 26 weeks ($p<0.001$) and was apparent by 6 weeks irrespective of concomitant rhDNase use. At 26 weeks, there was a significant improvement of 92.9 mL in FEV_1 for subjects receiving mannitol compared with control (change from baseline 118.9 mL [6.5%] vs. 26.0 mL [2.4%]; $p <0.001$). Improvements in FEV_1 were maintained up to 52 weeks in the open-label part of the study. There was a 35.4% reduction in the incidence of having an exacerbation on mannitol ($p=0.045$).

The incidence of adverse events (AE) was similar in both groups, though treatment related AEs were higher in the mannitol compared to the control group. The most common mannitol related AEs were cough, haemoptysis and pharyngolaryngeal pain.

Mannitol shows sustained, clinically meaningful benefit in airway function in CF, irrespective of concomitant rhDNase use. Mannitol appears to have an acceptable safety profile for patients with CF.

Introduction

Cystic Fibrosis (CF) is characterised by a failure to control ion transport across the epithelial cell membrane [1, 2, 3]. There are several hypotheses postulated about CF pathogenesis and the underlying mechanism for CF lung disease. The most recognised of these is that in the lungs there is a relative dehydration and a reduction in volume of airway surface liquid, which is associated with increased mucus viscosity and impaired mucociliary clearance [4-6]. This leads to retention of bacteria and inhaled particles, resulting in chronic airway infection and inflammation, airway damage and respiratory failure [1, 3, 7]. Pulmonary disease is the major cause of morbidity and mortality in CF, with over 90% of deaths due to respiratory failure [7]. Pulmonary exacerbations are an important clinical feature of CF, as they have both acute and chronic consequences and are the most common reason for hospitalisation and thus have major influence on health care costs [8].

Because CF cannot yet be cured, the goal of therapy is to slow disease progression, alleviate symptoms and improve quality of life. Improvement of airway hydration and mucus clearance from the lung is a major therapeutic goal, with the aim of maintaining/restoring respiratory function [9, 10]. Mannitol is a sugar alcohol that is currently used in medicine as an osmotic agent [11]. When inhaled, it creates an osmotic gradient that is thought to facilitate movement of water into the lumen of the airways thereby increasing volume of airway surface liquid and improving clearance of mucus [12, 13]. It is currently used as a bronchial provocation agent in measuring airway hyperresponsiveness [14, 15]. Inhaled mannitol has been shown to benefit-patients with bronchiectasis and CF [16-19]. A Phase II study demonstrated that inhaled mannitol administered over 2 weeks improved lung function in patients with CF [18]. A dose

finding phase II study further demonstrated that 400mg bid of inhaled mannitol provided an optimal balance between efficacy, safety and ease of administration [19]. The primary aim of this Phase III study was to determine the efficacy and safety of inhaled dry-powder mannitol over 26 weeks with further assessments at week 52 in the open label phase of the study.

Methods

Subjects

A total of 389 subjects with confirmed cystic fibrosis were enrolled from sites in Australia, Ireland, New Zealand and the UK. Eligible subjects were aged 6 years and above with baseline FEV_1 of ≥ 30 and $< 90\%$ predicted. Exclusion criteria included failing a mannitol tolerance test (MTT) at screening, concurrent use of hypertonic saline or beta-blockers, pregnancy or breastfeeding, and intolerance of beta-agonists. Subjects could continue with rhDNase and other standard therapies.

Informed written consent was obtained from all participants (or their parent/guardian). The study was conducted in accordance with the Declaration of Helsinki and the protocol was approved by the institutional review board or research ethics committee at each study centre.

Study design

The first phase of the study was a multi-centre, randomised, controlled, parallel-arm, double-blind phase III clinical trial to determine the efficacy and safety of inhaled dry powder mannitol administered over 26 weeks to subjects with cystic fibrosis. This was

followed by an optional 26 weeks, open-label (OL) extension of the study during which all subjects received inhaled dry powder mannitol.

Prior to randomisation, all subjects were screened for significant airway hyper-responsiveness to mannitol. Following pre-medication with a bronchodilator, the degree of bronchoconstriction after sequential administrations of incremental doses of mannitol up to a maximum dose of 395 mg was measured. A negative MTT to allow inclusion was defined as < 20% fall in FEV_1 from baseline (or a ≥ 20% fall in FEV_1 at the end of the test that had returned to < 20% within 15 min) and SpO_2 ≥ 90% with mannitol at a total dose of 395 mg.

Three hundred and twenty-four eligible subjects were randomised using a 3:2 ratio to 400 mg inhaled dry powder mannitol bid or matched control. Unlike the first phase II efficacy study where a non-respirable dose of mannitol was given, this study used a sub-therapeutic (50mg) dose of mannitol as the control [18, 19]. The choice of control for this study was based on the need to maintain the blinding, provide an appropriate comparator and comply with scientific advice from regulatory agencies. Randomisation was stratified according to concurrent rhDNase use. Subjects took a dose of the short acting bronchodilator salbutamol (400 µg) 5–15 minutes before each dose of study medication.

Inhaled mannitol was supplied as ten 40mg capsules together with an inhaler device (RS01, Mondose Inhaler Model 7, Plastiape, Italy). Capsules were loaded into the inhaler device, punctured, then mannitol inhaled in a deep controlled manner followed by a five second breath hold. The process was repeated until the content of ten capsules were inhaled. The time taken to inhale from ten capsules was usually between 2-5

minutes. The inhaler device was replaced after one week of use to minimise the need for cleaning or disinfection.

As FEV_1 is a recommended variable for assessing efficacy of CF therapies [9], change in FEV_1 over 26 weeks was the primary endpoint selected for this trial. Secondary endpoints included: the percentage of responders (based on a 100 mL or ≥ 5% relative change in FEV_1 or ≥ 5% absolute change in % predicted FEV_1) at 26 weeks; change in other lung function parameters (forced vital capacity [FVC] and forced expiratory flow in the middle half of expiration [FEF_{25-75}]); protocol defined pulmonary exacerbations (PDPE), clinically defined pulmonary exacerbations (including exacerbations not meeting the protocol-defined criteria [PE]), rescue antibiotic use (number of agents, course, and days of use); and quality of life (QoL) scores (using age-appropriate Cystic Fibrosis Questionnaire) [20]. The protocol PDPEs used Fuchs' criteria: exacerbations in which subjects are treated with intravenous antibiotics for four or more of the following signs or symptoms: change in sputum production, dyspnoea, new or increased haemoptysis, malaise, fatigue or lethargy, fever [≥38°C], anorexia or weight loss, sinus pain or tenderness, change in sinus discharge, FVC or FEV_1 decreased by ≥10% from previous recorded value, radiographic signs indicative of pulmonary infection, increased cough, changes in physical examination of the chest [21].

Spirometry measurements were taken to coincide with trough treatment effect of inhaled dry powder mannitol; subjects were required to withhold study medication, long-acting β_2-agonists and combination inhaled corticosteroid/long acting β_2-agonists for 12 hours, and short-acting β_2-agonists for 6 hours, prior to spirometry assessment. The OL phase included patient follow up visits at 38 and 52 wks to assess lung function, sputum

microbiology and review pulmonary exacerbations and safety. Safety was assessed by tracking the number and percentage of adverse events as well as monitoring for changes in haematology, liver and renal function, and sputum pathogens. Safety and efficacy endpoints were examined in cohorts according to rhDNase use as well as within the total cohort.

Treatment compliance was determined by counting returned unused medication and empty blister packaging. The Cystic Fibrosis Questionnaire – Revised (CFQ-R) includes several domains to assess quality of life, which were analysed separately. An increased score is associated with an improvement in that domain. A difference of 4 or more points in the respiratory domain is considered to be clinically meaningful [20, 22]. The minimal clinically important differences of other domains have yet to be established.

The study was 80% powered (two tailed significance level of 4.98%, primary endpoint), to detect a change in FEV_1 from baseline at week 26 of 70 mL in the total intention to treat (ITT) cohort (consisting of all subjects who were randomised and received at least one dose of study medication) and 85 mL (5% significance, secondary endpoint) in the subgroup of subjects taking concomitant rhDNase. The study was not powered to detect a difference in exacerbation rate of less than 50%.

Statistical analysis

For the double-blind phase analyses relating to change from baseline in spirometry values, mixed-effect model repeated measure (MMRM) models were used with absolute change from baseline in spirometry value as the outcome variable; fixed effect terms for

treatment, rhDNase use, region, gender and time point; disease severity as characterised by FEV_1 percent predicted at screening, age and baseline spirometry value as covariates and subject as a random effect. Treatment-by-time, treatment-by-rhDNase use and treatment-by-time-by-rhDNase use were also included in models to estimate changes by time, by rhDNase use and week 6-26 week data. Subject was fitted as a random effect with a first order autoregression covariance structure in the MMRM analysis overall (week 0-26) and (6-26) week data. For rate-based analyses (pulmonary exacerbations, hospitalisation, antibiotic use), negative binomial regression models were used with treatment, age, rhDNase use and region included as covariates and the time on study during the blinded phase used as an offset.

Pulmonary exacerbations were analysed using Cox proportional hazards regression modelling, with treatment, age, rhDNase use and region included as covariates from which hazard ratios were obtained. In addition Kaplan-Meier estimates of time to first event were provided.

For responder-based analysis (improvement in FEV_1, improvement in QoL respiratory score), subjects were classified as a responder or non-responder at week 26. A logistic regression model was fitted to compare the odds of responding to mannitol vs. control. Terms used in the model were as per the primary analysis model with response as the outcome and treatment, age, sex, rhDNase use, region, disease severity as characterised by percent predicted FEV_1 at screening, and baseline FEV_1/QoL respiratory score included as covariates.

For the open-label analysis, long term safety was the primary focus with FEV_1 data summarised descriptively at week 52. No between treatment group analysis was undertaken.

Results

Patient disposition

The ITT study population consisted of subjects (mannitol: n=177; control: n=118) who tolerated MTT (see Figure 1), were randomised to treatment, provided a baseline FEV_1, and received at least one dose of study medication. The per protocol population (mannitol: n=111; control: n=89) was defined as all subjects in the ITT population with ≥ 60% treatment compliance and no major protocol violations who provided baseline and at least one FEV_1 value after commencement of study treatment. The completers population were those subjects who completed the blinded phase of the study (visit 4) with FEV_1 data. Patient disposition is shown in Figure 1 and demographics and characteristics at baseline are given in Table 1. The median compliance was 89% and 91% for mannitol and control groups respectively.

Table 1: Patient demographic data and baseline characteristics

Characteristic		**Mannitol n=177**	**Control n=118**	**Total n=295**
Age (years): mean (± SD)		23·1 (± 11·66)	22·8 (± 10·75)	23·0 (±11·29)
Age range	6–11 years: n (%)	31 (17·5%)	17 (14·4%)	48 (16·3%)
	12–17 years: n (%)	32 (18·1%)	25 (21·1%)	57 (19·3%)
	≥18: n (%)	114 (64·4%)	76 (64·4%)	190 (64·4%)
Number and proportion of subjects with diagnosis at		130 (73·4%)	94 (80·0%)	224 (75·9%)

or before age 1 year:			
Caucasian race	169 (95·5%)	115 (97·5%)	284 (96·3%)
BMI mean (± SD) kg/m^2	21·07(± 3·99)	20·38 (± 3·59)	20·80 (± 3·84)
Female (n, %)	71 (40·1%)	61 (51·7%)	132 (44·7%)
FEV_1 (L): mean (± SD)	2·07 (±0·82)*	1·95 (±0·69)	2·02 (± 0·77)**
FEV_1 (% predicted): mean (± SD)	62·4 (± 16·45)*	61·4 (± 16·13)	62·0 (± 16·30)**
Microbiology			
Pseudomonas aeruginosa (mucoid)	58 (32·8)	48 (40·7%)	106 (35·9)
Pseudomonas aeruginosa (non-mucoid)	42 (23·7)	32 (27·1%)	74 (25·1)
Staphylococcus aureus	32 (18·1)	25 (21·2)	57 (19·3)
Aspergillus spp	28 (15·8)	11 (9·3%)	39 (13·2)
Chronic antibiotic use >10% at baseline: n (%)			
azithromycin	98 (55·4%)	60 (50·8%)	158 (53·6%)
colistin+	73 (41.2%)	45 (38.1%)	118 (40.0%)
tobramycin$^+$	43 (24.3%)	35 (29.7%)	78 (26.4%)
flucloxacillin	45 (25.4%)	23 (19.5%)	68 (23.1%)
Drugs for obstructive airway diseases ^	148 (83·6%)	101 (85·6%)	249 (84·4%)
ICS (including combinations and single agents)	103 (58.2%)	73 (61.9%)	176 (59.7%)
SABA (salbutamol, terbutaline)	137 (77.4%)	96 (81.4%)	233 (79.0%)
LABA (salmeterol, formoterol or combinations)	98 (55.4%)	64 (54.2%)	162 (54.9%)
Other^	38 (21.5%)	21 (17.8%)	59 (20.7%)
rhDNase users (n, %)	96 (54·2%)	67 (56·8%)	163 (55·3%)
FEV_1 (L): mean (SD)	1·96 (±0·78)†	1·87 (±0·64)	1·92 (±0·72)
FEV_1 (% predicted): mean (± SD)	59·2 (±17·26)†	57·9 (±16·38)	58·7 (±16·86)
rhDNase non-users (n, %)	81 (45·8%)	51 (43·2%)	132 (44·7%)
FEV_1 (L): mean (SD)	2·20 (±0·85)	2·06 (±0·75)	2·14 (0·81)
FEV_1 (% predicted): mean (± SD)	66·2 (±14·68)	66·1 (±14·69)	66·1 (±14·63)

*n=176, **n=294, †n=95, ICS: inhaled corticosteroids ^ Other medications included: LTA, anticholinergic bronchodilators, theophylline, aminophylline, nedocromil. $^+$ 98.3% of colistin and 92.3% of tobramycin was nebulised at baseline.

85·9% of the 198 subjects (112 mannitol, and 86 control) who completed the double-blind phase elected to enter the optional 26 week OL phase.

Efficacy

A statistically significant improvement in FEV_1 was apparent within 6 weeks of treatment with mannitol, and was maintained throughout the double blind phase of the study (Figure 2). Overall, the treatment effect of mannitol across the study was statistically superior compared to control (p<0·001). The absolute difference in FEV_1 averaged across all post randomisation visits (week 6, 14 and 26) in the double blind phase of the study for mannitol-treated subjects compared to control was 85.03 mL [95% CI=(53.5 mL, 116.6 mL)] (p <0·001); this treatment effect was similar and statistically significant for both the rhDNase subgroup; 85.4 mL [95% CI = (42.9 mL, 127.9 mL)], and non-rhDNase sub-group; 84.6 mL [95% CI=(38.2 mL, 131.1 mL)].

At 26 weeks, a 118.9mL (6·5%) increase from baseline in the mannitol group resulted in a statistically significant improvement of 92·9 mL (p <0·001) in FEV_1 compared with control.

Significantly more mannitol-treated subjects achieved clinically meaningful responses compared with control (odds ratio of 1.97 [95% CI: 1.08, 3.58] based on a change in FEV_1 of at least 100 mL, p= 0.026) in the completer population. Similar odds of being a responder were seen regardless of the definition of responder used (respective odds ratios of 2.00 [95% CI: 1.09, 3.66] and 2.30 [95% CI: 1.20, 4.38] achieve either a FEV_1 increase of at least 5% or a % predicted FEV_1 increase of at least 5%, p < 0.05 for both).

At the end of the double blind phase, changes in FVC (Figure 3), were consistent with an improvement in FEV_1 (at week 26, FVC increase from baseline was 128.9 mL vs. 15.9 mL for the mannitol and control groups respectively, p=0.002). $FEF_{25–75}$ also improved

with mannitol, but compared to control, differences in $FEF_{25–75}$ were not statistically significant.

During the uncontrolled open label phase of the study, subjects who were switched to mannitol after initial randomisation to control in the double blind phase showed a clinically and statistically significant improvement in FEV_1 that was comparable to the improvements seen in the mannitol group during the double blind phase of the study. Subjects initially randomised to mannitol maintained the increase in FEV_1 seen during the double-blind phase over the second 26 weeks (Figure 4). The change in FEV_1 in the control group who moved to the open label phase, subsequently received mannitol for six months and completed the study showed an absolute improvement from baseline (148.5mL), which was similar to that seen with the completers from the original mannitol group (155.7mL).

The protocol defined pulmonary exacerbation (PDPE) results are presented in Table 2. There was a significant 35.4% (p=0.045) reduction in the incidence of having a PDPE during the double-blind phase of the study, though there was no statistically significant reduction in the rate of exacerbations. For the per protocol patient population, there was a significant increase in the time to first exacerbation (hazard ratio 0·47, p=0·024, Figure 5), which did not reach statistical significance in the ITT population (hazard ratio 0·68, p=0·119).

The trend to improvement in exacerbation related endpoints was consistent with lung function improvement and there was a correlation between PDPE incidence and improvement in FEV_1 over the 26 weeks of the study (p>0.0001).

The result from the CFQ-R treatment burden domain at baseline was similar to the results at the end of the study, demonstrating that there was no meaningful increase in treatment burden resulting from additional therapy with 10 capsules in a dry powder inhaler twice a day.

There was a 3.8 point difference in mean change from baseline in the Cystic Fibrosis Questionnaire respiratory score in favour of the mannitol group (p=0.096). Differences in the vitality and physical domains were 7.2 and 4.2 points respectively, again trending in favour of mannitol.

Table 2 CF301 Protocol Defined Pulmonary Exacerbation (PDPE) Data

Exacerbation Measure	**Reduction in incidence (Bronchitol vs. Control %)***	
Incidence of PDPE (ITT)	35.4%	p = 0.045
Rescue Antibiotic Use (ITT)	35.4%	p= 0.045
PDPE related Hospitalisations (ITT)	20.6%	p = 0.395
	Rate Ratio [95%CI] (Bronchitol vs. Control)#	
Rate of PDPE (ITT)	0.74 [0.47, 1.18]	p = 0.205
Rate of PDPE (PP)	0.65 [0.34, 1.21]	p = 0.174
	Hazard Ratio [95% CI] (Bronchitol vs. Control)^	
Time to First PDPE [Hazard Ratio] (ITT)	0.68 [0.42, 1.11]	p = 0.119
Time to First PDPE (Hazard Ratio) (PP)	0.47 [0.25, 0.91]	p = 0.024

*Posthoc analysis chi squared test, # Rate ratio analysed using a negative binomial regression of the rate.^ Time to first event analysed using Hazard Ratio.

Safety

Overall, the proportion of subjects reporting at least one adverse event during the blinded study period was similar for the mannitol and control groups (any adverse event: 87.0% vs. 92.4%, respectively; serious adverse event: 26.0% vs. 29.7%, respectively).

Pulmonary exacerbations are an expected feature of the underlying disease in CF and were carefully documented as an efficacy endpoint as well as being recorded as AEs (condition aggravated) and this was the the most commonly reported adverse event in both treatment groups. Treatment-related adverse events were reported in 40.7% of mannitol-treated subjects and 22% of the control group. Adverse events that were more common in the mannitol group included cough, (haemoptysis – see below) and pharyngolaryngeal pain. Lower respiratory tract infection and exacerbation was less common in the mannitol group than in the control group (Table 3).

Haemoptysis was reported either as an adverse event (11.9% vs. 8.5% in mannitol and control groups respectively), or as a sign in association with an exacerbation; overall, haemoptysis, including that associated with exacerbations of CF, was reported by 15.8% of mannitol-treated subjects and 15.3% of subjects receiving control. The majority of the cases of haemoptysis were considered to be not related to study drug, transient and mild to moderate in intensity.

A total of 28 (15.8%) subjects in the mannitol group and 10 (8.5%) in the control group discontinued the study due to adverse events; of these, 24 (13.6%) and 6 (5.1%) subjects, respectively, withdrew due to treatment-related adverse events (Table 3). All subjects who withdrew due to a serious adverse event were in the mannitol group (moderate haemoptysis, n=2, moderate severity pulmonary exacerbation, n=1; severe, asymptomatic bronchoconstriction, n=1).

Table 3. Summary of adverse events, Treatment-emergent adverse events (TEAEs) and Treatment-related adverse events leading to withdrawal

Subjects (n, %) with:	Mannitol (n=177)	Control (n=118)	Total (n=295)
At least one adverse event (AE)	154 (87·0%)	109 (92·4%)	263 (89·2%)
At least one treatment-related AE	72 (40·7%)	26 (22·0%)	98 (33·2%)
At least one serious adverse event (SAE)	46 (26·0%)	35 (29·7%)	81 (27·5%)
At least one treatment-related SAE	6 (3·4%)	1 (0·8%)	7 (2·4%)
TEAEs by MedDRA preferred term (occurring in ≥10% subjects):			
condition aggravated	57 (32·2%)	42 (35·6%)	99 (33·6%)
cough	45 (25·4%)	24 (20·3%)	69 (23·4%)
headache	38 (21·5%)	28 (23·7%)	66 (22·4%)
bacteria sputum identified	33 (18·6%)	22 (18·6%)	55 (18·6%)
nasopharyngitis	25 (14·1%)	17 (14·4%)	42 (14·2%)
lower respiratory tract infection	15 (8·5%)	20 (16·9%)	35 (11·9%)
haemoptysis	21 (11·9%)	10 (8·5%)	31 (10·5%)
pharyngolaryngeal pain	24 (13·6%)	5 (4·2%)	29 (9·8%)
AE leading to withdrawal from study	28 (15·8%)	10 (8·5%)	38 (12·9%)
Treatment-related AEs leading to withdrawal in ≥ 2 subjects:			
cough	11 (6·2%)	4 (3·4%)	15 (5·1%)
haemoptysis	5 (2·8%)	0 (0·0%)	5 (1·7%)
condition aggravated	3 (1·7%)	1 (0·8%)	4 (1·4%)
chest discomfort	3 (1·7%)	0 (0·0%)	3 (1·0%)
bronchospasm	2 (1·1%)	0 (0·0%)	2 (0·7%)
pharyngolaryngeal pain	2 (1·1%)	0 (0·0%)	2 (0·7%)
wheezing	0 (0·0%)	2 (1·7%)	2 (0·7%)

The incidence of adverse events and serious adverse events among rhDNase users and non-users were similar to the overall results in both treatment groups. More rhDNase users in both treatment groups (32·3% and 32·8% subjects in the mannitol and control groups respectively) experienced serious adverse events than rhDNase non-users (18·5% and 25·5% subjects respectively).

The most common organisms in both treatment groups at week 0 were *Pseudomonas aeruginosa* (mucoid and non-mucoid), *Staphylococcus aureus* and *Aspergillus* species. At week 26 the proportion of subjects in both treatment groups with abnormal flora was similar to week 0 for all grades of growth. Sputum microbiology was similar for rhDNase users and non-users, and for paediatric and adolescent subjects. There were no apparent differences in laboratory results for mean haematology, liver function, urea and electrolyte parameters between treatment groups or over time.

Discussion

Treatment with inhaled dry powder mannitol (400 mg bid) provided early and sustained, statistically and clinically significant increases in FEV_1 over a 26-week treatment period ($p<0.001$) in subjects with cystic fibrosis receiving high standard of care. This improvement was maintained to 52 weeks in the open label phase of the study.

The improvement in lung function compared to control was seen irrespective of concomitant rhDNase use. The additional benefit of mannitol over the study period was very similar in rhDNase users and non-users when compared to control; this is in contrast with a recent small phase II study [23] which suggested that the combined use of

rhDNase and mannitol may not result in greater improvement than rhDNase alone. This larger study demonstrated that mannitol offered an additional lung function benefit on top of rhDNase as part of standard therapy and confirms its applicability to a wide range of CF patients.

This study also evaluated the proportion of subjects reaching a clinically meaningful threshold change in FEV_1. There are no guidelines on this threshold in CF, and the size of effect required to be meaningful varies according to individual perception, pre-existing lung impairment, size and age. For this study, a conservative threshold for a clinically significant improvement from baseline in FEV_1 was evaluated by absolute changes of both 100 mL and 5% FEV_1 % predicted as well as a 5% change in FEV_1. More subjects treated with mannitol reached these clinically meaningful lung function thresholds. Improvements with mannitol using other lung function parameters (such as the FVC and PEF) reflected the positive improvements in FEV_1 and support the consistent effect of mannitol on lung function. While FEF_{25-75} was not statistically different between the Mannitol and control groups, the variability of this measure across the study was high, and the mean change from baseline with Mannitol at week 26 was 86.2 mL/s [95%CI: 29.33, 143.11] .

The frequency of pulmonary exacerbations is an important outcome measure in clinical trials, as exacerbations contribute to the burden of disease and are associated with impaired quality of life, increased hospitalisation and associated health care costs and increased risk of mortality [8, 9, 24, 25]. In this study, many different measures of exacerbation including incidence and rate of PDPE or PE, time to first PDPE or PE, and rescue antibiotic use were assessed in a range of patient populations (ITT population, per-

protocol population, completers). There was a significant reduction in the incidence of having a PDPE in the ITT group and significant improvement in time to first exacerbation in the per-protocol population with mannitol (p=0.02). While other exacerbation endpoints, did not reach statistical significance. Nevertheless, the trial was not powered to detect differences in exacerbation rates that would still be considered clinically meaningful.

Currently only two nebulised agents are used in clinical practice to help airway clearance in CF subjects. Inhaled mannitol demonstrated a similar benefit in terms of relative percent improvement in lung function to rhDNase (a mucolytic), which has had regulatory approval for well over 10 years in most countries [21, 26]. This similar improvement with mannitol is considered significant given the differing standards of care available at the time of the original rhDNase studies and in a population who are now slightly older and on more concomitant medications (including beta agonists and antibiotics). Hypertonic saline is frequently used as an osmotic agent at various dosing frequencies, doses and concentrations largely driven by tolerability, though the primary evidence base for hypertonic saline comes from one study in a milder population of 164 patients where 4 mL of 7% saline was used twice a day to improve lung function and exacerbation rates [27]. The primary outcome measure of rate of change in lung function for hypertonic saline was not significant between groups over the duration of the study. By comparison, mannitol improved FEV_1 early (at 6 weeks) in this study, and the significant effect size with mannitol was maintained out to 26 weeks, and further to 52 weeks in patients extending the treatment period. Compared with this study using mannitol, Elkins hypertonic saline study, with its notably lower rates of regular

concomitant antibiotic use demonstrated a statistically significant reduction in exacerbation rate, though a similar magnitude of reduction in incidence of exacerbations was reported (35% for mannitol vs. 37% for hypertonic saline).[27]

Osmotic agents offer a rational approach to the treatment of CF, and non-ionic agents like mannitol would hypothetically have a more sustained duration of action in the airway, as epithelia may remove salts ions more rapidly [5]. Mannitol has properties which make it a good osmotic agent for the treatment of CF; it has sufficient osmotic load per unit weight, can be formulated as a powder for inhalation, is stable, and is not subject to ionic movement.

Non-compliance and adherence with therapy is an important issue in cystic fibrosis and can influence the therapeutic value of treatment. Therapies are often time consuming and challenging to deliver, so adherence may be poor – especially among certain groups such as adolescents [28, 29]. Dry powder mannitol offers the convenience of delivery to the lung via a simple, hand-held inhaler, rather than via a nebuliser, and it can be administered within 5 minutes [30]. The shorter delivery time, and lack of need for sophisticated equipment with its associated need for maintenance and cleaning may lead to improved adherence, although this would need to be confirmed in future studies. The withdrawal rate at the start of this study was higher than expected, however the need to provide education about the device and expectations for patients and healthcare professionals may have been underestimated. The impact of additional education will be able to be evaluated from the second phase III study CF302 [NCT00630812].

Improvements in QoL scores for vitality, respiratory and physical domains are all considered to be relevant in the assessment of a pulmonary-directed therapy; differences in these scores all approached or exceeded 4 points, but did not reach statistical significance. While the CFQ-R is a widely accepted and validated tool for use in patients with CF, questions such as “Have you had

to cough up mucus" and "Have you been coughing during the day" may favour the control group as sputum production with mannitol increased during the study compared to control, and thus the tool may be inappropriate to reflect improvements associated with mucoactive therapies.

Mannitol was well tolerated by most study subjects with an acceptable safety profile. Cough and haemoptysis were the most commonly reported treatment-related adverse events. Haemoptysis, similar to exacerbations, is an underlying feature of cystic fibrosis and therefore expected in CF studies [31]. The total incidence of haemoptysis when considered either occurring during a pulmonary exacerbation or as a treatment-related adverse event was, however, similar between the two treatment groups. Cough was more common in the mannitol group but as it may be considered to be a component of the therapeutic effect it is difficult to interpret the potential benefit /risk ratio of this finding. Importantly, cough was only sufficiently troublesome in a small proportion of subjects to lead to treatment discontinuation. Overall most adverse events were mild or moderate in intensity.

As mannitol may potentially cause bronchoconstriction in those with airway hyperresponsiveness, the trial excluded such subjects at screening with an MTT. However, 87.8% of screened subjects passed the MTT.

Exploratory analyses of bronchoconstrictor response to study drug at each double blind visit and occurrence of bronchoconstriction related AEs was performed. There was no suggestion of an increased risk of airway hyperresponsiveness in either the measured response to mannitol or when reviewing the cluster of associated side effects of wheeze, asthma and bronchospasm. Although there was a greater proportion of subjects in the mannitol group compared to the control group who had a medical history of asthma (34.5% vs. 19.5%); the proportion of subjects experiencing AEs pertaining to bronchospasm was low and comparable between the two treatment groups (bronchospasm: 1.1% vs. 0.0%; asthma: 1.1% vs. 2.5%; dyspnoea: 1.1% vs. 0.8%; wheezing: 2.3% vs. 3.4%).

The study results suggest there is no added risk involved in treating subjects with mannitol with respect to sputum growth of microorganisms. Mannitol is a growth substrate for certain bacteria in vitro, particularly *Staphylococcus aureus*, *Burkholderia cepacia* and *Pseudomonas aeruginosa* [32]. It was therefore important to assess if this impacted on sputum microbiology over time in subjects. While most subjects grew abnormal flora in their sputum at randomisation, there was no evidence for either an increase or decrease in microorganism growth after 26 weeks of treatment.

In those who completed the double-blind period, the study drug appears to have been well tolerated as the majority (85%) of subjects elected to continue into the open label study.

Consistent with regulatory agency guidance, the control used was an anticipated sub-therapeutic 50mg dose of mannitol, meaning that the size of effect is possibly conservative, but safety (essentially that related to the inhaled route) when assessed relative to control may potentially be under estimated (although incidence of adverse event rates did not exceed the expected based on incidence in published studies.[21,33] Nevertheless, while this could be considered a possible limitation to the study, the use of a sub-therapeutic mannitol dose was supported by the dose response study (DPM-CF-202) [26] in which a single 40 mg capsule b.i.d. did not demonstrate any efficacy over a two week treatment period while the 400 mg therapeutic dose was efficacious.

In conclusion, inhaled mannitol appears to have an acceptable safety profile for patients with CF, and demonstrates that mannitol treatment, over and above existing standard care provides both early and sustained clinically meaningful improvements in airway function,

irrespective of concomitant rhDNase use, in a representative population of cystic fibrosis subjects.

Authors' contributions

All authors helped to interpret data, write the manuscript and have seen and approved the final version.

Diana Bilton was the Global Principle Investigator for CF301 and had full access to all the data in the study and had final responsibility to submit for publication. Phil Robinson was Australian Lead Regional Investigator for CF301. Peter Cooper was an Investigator for CF301. Charles G Gallagher was Ireland was Ireland Lead Regional Investigator for CF301. John Kolbe was New Zealand Lead Regional Investigator for CF301. Howard Fox approved the statistical plans and interpreted the data. Anna Jaques was the CF301 protocol author and clinical study report author. Brett Charlton designed the CF301 study, approved the statistical plans, interpreted the data and was the Sponsor's Responsible Medical Officer.

Conflicts of interest

DB and PR have received fees for chairing an advisory board for Pharmaxis. DB, PR, PC, JK, CG were all investigators during the study and their institutions received standard clinical trial support from Pharmaxis. No Investigator received any personal funding to participate in the study. BC is the Medical Director of and holds stock options in Pharmaxis Ltd. HF is Chief Medical Officer of and holds stock options in Pharmaxis Ltd. AJ is the clinical development manager of and holds shares and stock options in Pharmaxis Ltd.

Acknowledgement

Annie Solterbeck from Statistical Revelations for her statistical analysis and expertise.

References

1. Davies JC, Alton EWFW, Bush A. Cystic Fibrosis. *BMJ* 2007; **335**: 1255–59.

2. Riordan JR, Rommens JM, Kerem B, *et al.* Identification of the cystic fibrosis gene: cloning and characterization of complementary DNA. *Science* 1989; **245**: 1066–73.

3. Ratjen FA. Cystic fibrosis: pathogenesis and future treatment strategies. *Respiratory Care* 2009; **54**: 595–605.

4. Puchell E, Bajolet O, Abely M. Airway mucus in cystic fibrosis. *Paed Resp Reviews.* 2002: **3**: 115-19.

5. Tarran R, Grubb BR, Parsons D, *et al.* The CF Salt Controversy: *in vivo* observations and therapeutic approaches. *Mol Cell* 2001; 8:149-58.

6. Matsui H, Grubb BR, Tarran R, *et al.* Evidence for periciliary liquid layer depletion, not abnormal ion composition in the pathogenesis of cystic fibrosis airways disease. *Cell* 1998; 95:1005-15.

7. Ramsey BW. Management of pulmonary disease in patients with cystic fibrosis. *N Engl J Med* 1996; **335**: 179–88.

8. Lieu TA, Ray GT, Farmer G, Shay GF. The cost of medical care for patients with cystic fibrosis in a Health Maintenance Organization. Pediatrics 1999; **103:** 6: e72 http://www.pedatrics.org/cgi/content/full/103/6/e72.

9. Zemanick ET, Harris JK, Conway S, et al. Measuring and improving respiratory outcomes in cystic fibrosis lung disease: Opportunities and challenges to therapy. *J Cyst Fibrosis* 2010; **9**: 1-16.

10. Flume PA, O'Sullivan BP, Robinson KA, et al. Cystic Fibrosis Pulmonary Guidelines. Chronic Medications for Maintenance of Lung Health. *Am J Respir Crit Care Med* 2007; **176**: 957-69.

11. Rowe RC, Sheskey PJ, Weller PJ, editors. Handbook of Pharmaceutical Excipients. London and Chicago: Pharmaceutical Press and American Pharmaceutical Association; 2003.

12. Daviskas E, Anderson SD, Brannan JD, Chan HK, Eberl S, Bautovich G. Inhalation of dry-powder mannitol increases mucociliary clearance. *Eur Respir J* 1997; **10**: 2449-54.

13. Robinson M, Daviskas E, Eberl S, et al. The effect of inhaled mannitol on bronchial mucus clearance in cystic fibrosis patients: a pilot study. *Eur Respir J.* 1999; **14**:678-85.

14. Anderson SD, Brannan J, Spring J, *et al.* A new method for bronchial-provocation testing in asthmatic subjects using a dry powder of mannitol. *Am J Respir Crit Care Med* 1997; **156**: 758–65.

15. Brannan JD, Anderson SD, Perry CP, Freed-Martens R, Lassig AR, Charlton B. The safety and efficacy of inhaled dry powder mannitol as a bronchial provocation test for airway hyperresponsiveness: a phase 3 comparison study with hypertonic (4.5%) saline. *Respir Res* 2005; **6**: 144.

16. Daviskas E, Anderson SD, Eberl S, Chan HK, Bautovich G. Inhalation of dry powder mannitol improves clearance of mucus in patients with bronchiectasis. *Am J Respir Crit Care Med* 1999; **159**: 1843–48.

17. Daviskas E, Anderson SD, Eberl S, Chan HK, Young IH. The 24-h effect of mannitol on the clearance of mucus in patients with bronchiectasis. *Chest* 2001; **119**: 414–21.

18. Jaques AJ, Daviskas E, Turton JA, *et al.* Inhaled mannitol improves lung function in cystic fibrosis. *Chest* 2008; **133**: 1388–96.

19. Teper A, Jaques A, Charlton B. Inhaled mannitol in patients with cystic fibrosis: A randomised open-label dose response trial. J Cystic Fibrosis 2010; doi:10.1016/j.jcf.2010.08.020.

20. Quittner AL, Buu A, Messer MA, Modi AC, Watrous M. Development and validation of The Cystic Fibrosis Questionnaire in the United States: a health-related quality-of-life measure for cystic fibrosis. *Chest* 2005; **128**: 2347–54.

21. Fuchs HJ, Borowitz DS, Christiansen DH, et al. Effect of aerosolized recombinant human DNase on exacerbations of respiratory symptoms and on pulmonary function in patients with cystic fibrosis. *N Engl J Med*. 1994; **331**: 637-42.

22. Quittner AL, Modi AC, Wainwright C, Otto K, Kirihara J, Montgomery AB. Determination of the minimal clinically important difference scores for the Cystic Fibrosis Questionnaire-Revised respiratory symptom scale in two populations of patients with cystic fibrosis and chronic Pseudomonas aeruginosa airway infection. *Chest* 2009; **135**: 1610–18.

23. Minasian CC, Wallis C, Metcalfe C and Bush A. Comparison of inhaled mannitol, daily rhDNase, and a combination of both in children with cystic fibrosis: a randomised trial. *Thorax* 2010; **65**: 51-56.

24. Amadori A, Antonelli A, Balteri I, Schreiber A, Bugiani M, De Rose V. Recurrent exacerbations affect FEV_1 decline in adult patients with cystic fibrosis. *Respir Med.* 2009; **103**: 407–13.

25. Ziaian T, Sawyer MG, Reynolds KE, et al. Treatment burden and health-related quality of life of children with diabetes, cystic fibrosis and asthma. *J Paediatr Child Health* 2006; **42**: 596-600.

26. Jones AP, Wallis C, Kearney CE. Dornase alfa for cystic fibrosis. *Cochrane Database of Systematic Reviews* 2003, Issue 3. Art. No.:CD001127. DOI: 10.1002/14651858.CD001127.

27. Elkins MR, Robinson M, Rose BR, Harbour C, Moriarty CP, *et al*. A controlled trial of long-term inhaled hypertonic saline in patients with cystic fibrosis. *N Engl J Med* 2006; 354:229-40.

28. Sawicki GS, Sellers DE, Robinson WM. High treatment burden in adults with cystic fibrosis: challenges to disease self-management. *J Cyst Fibros* 2009; **8**: 91–96.

29. Modi AC, Marciel KK, Slater SK et al. The influence of parental supervision on medical adherence in adolescents with cystic fibrosis: developmental shifts from pre to late adolescence. *Child Health Care* 2009; **37**: 78-92.

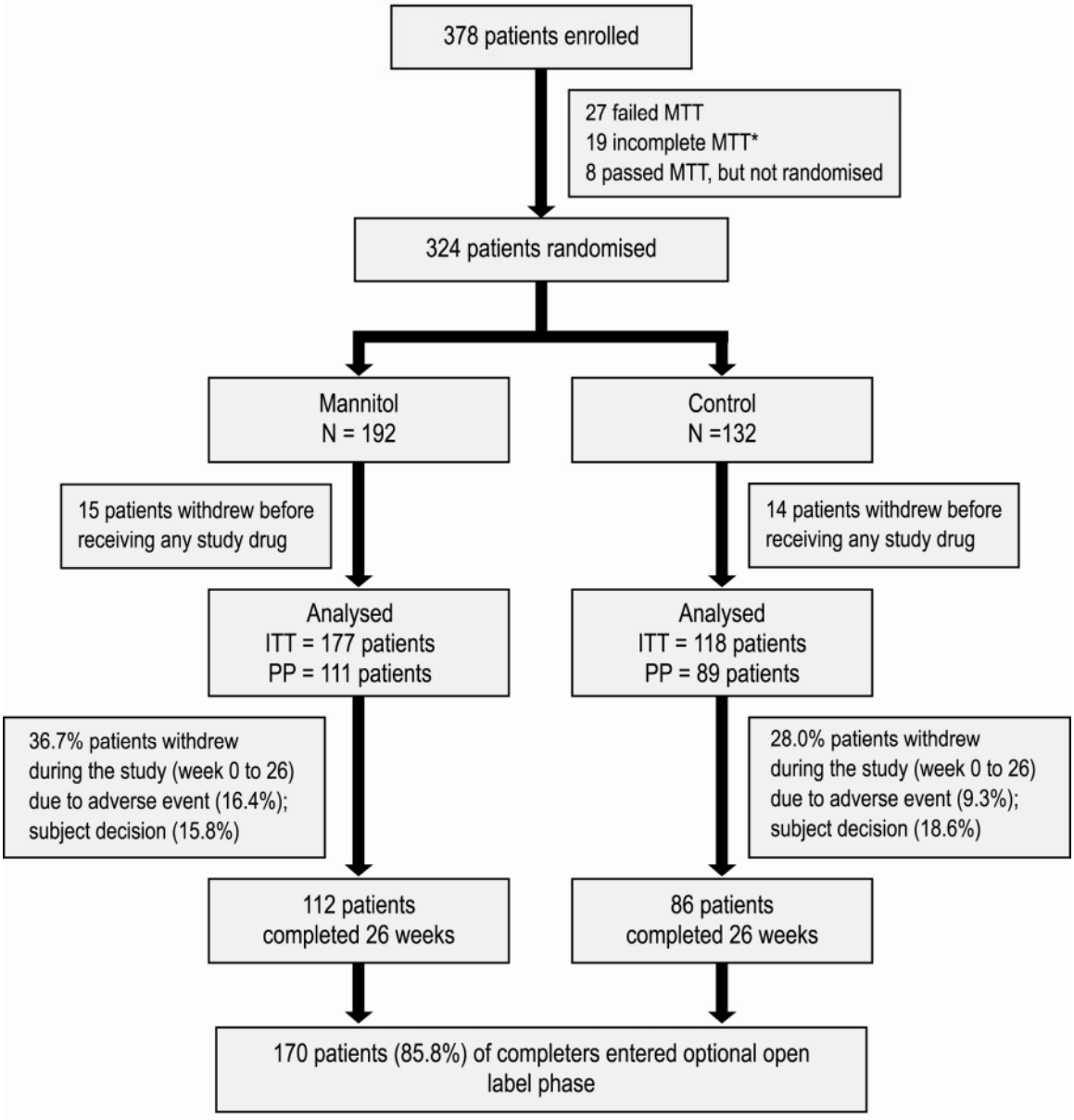
30. Kesser KC, Geller DE. New Aerosol Delivery Devices for Cystic Fibrosis. *Respir Care* 2009; **54**: 754-67.

31. Flume PA, Mogayzel PJ, Robinson KA, Rosenblatt RL, Quittell L, *et al*. Cystic Fibrosis Pulmonary Guidelines, Pulmonary Complications: Hemoptysis and Pneumothorax. *Am J Respir Crit Care Med* 2010; 182:298-306.

32. Bartholdson SJ, Brown AR, Mewburn BR, Clarke DJ, Fry SC, Campopiano DJ, Govan JR. Plant host and sugar alcohol induced exopolysaccharide biosynthesis in the Burkholderia cepacia complex. *Microbiology* 2008; **154**: 2513-21.

33. Konstan MW, Flume PA, Kappler M, et al. Safety, Efficacy and convenience of tobramycin inhalation powder in cystic fibrosis patients: The EAGER trial. *J Cystic Fibrosis* 2010, doi:10.1016/j.jcf.2010.10.003.

Figure 1. CF301 Trial Profile



MTT = Mannitol Tolerance Test

* An incomplete MTT was defined as a test that was stopped prior to either a test failure criteria being reached or before a cumulative dose of 395mg of mannitol was reached

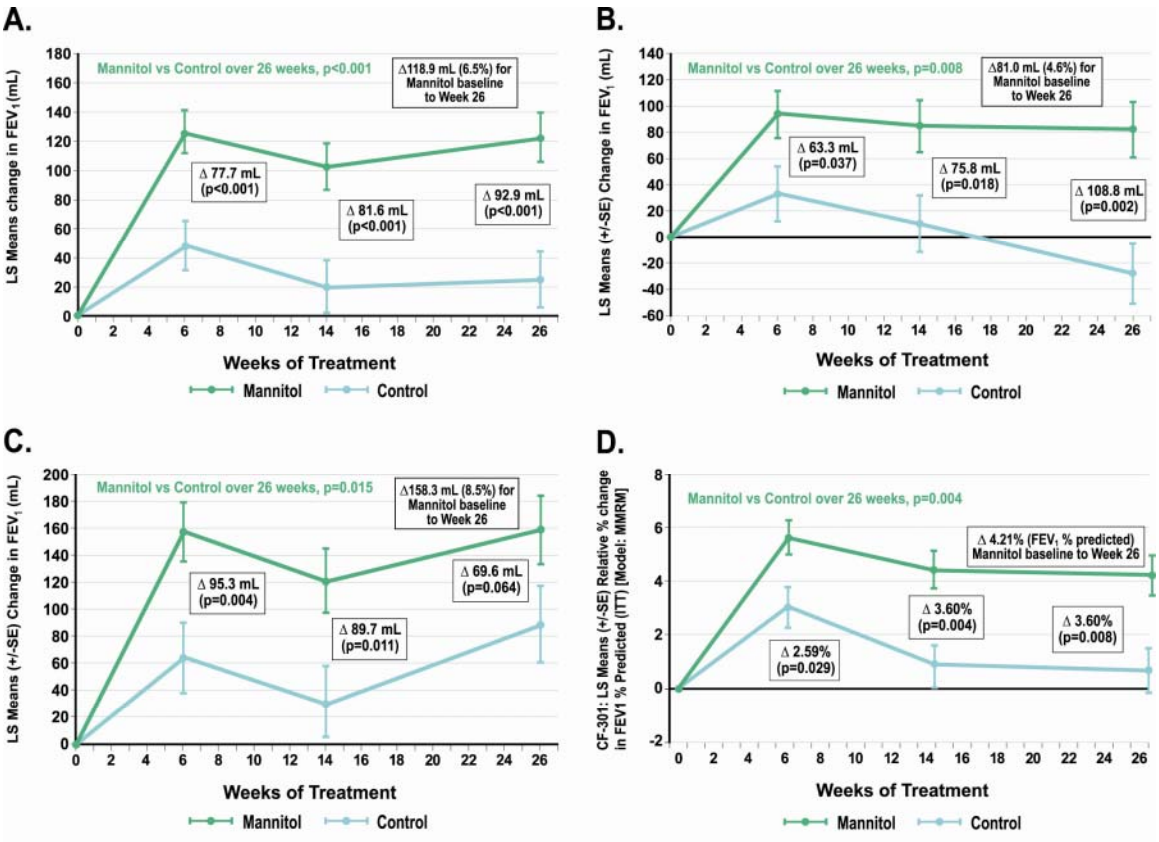


Figure 2: Change in Forced Expiratory Volume (FEV_1) over the 26 week blinded phase in patients with mannitol and control. FEV_1 mL in the ITT group (A), the subgroup of rhDnase patients (B), the non-rhDnase group (C) and the relative % change in FEV_1 % predicted (D).

The mean changes are based on a repeated-measures analysis. Error bars are SE. A mixed effects model with change in lung function as the outcome and treatment, time-point, rhDNase use, region and gender as fixed effects and baseline FEV_1, age and disease severity (FEV_1 as % predicted at baseline) as covariates in the model are used to determine the effects across the 26 week period [week 0-26 for (A),(B),(C) and week 6-26 for (D)]. A treatment effect by time-point was obtained by adding a time by interaction term to the model.

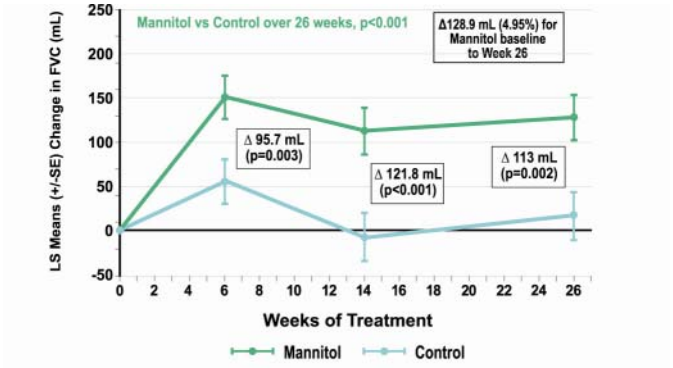
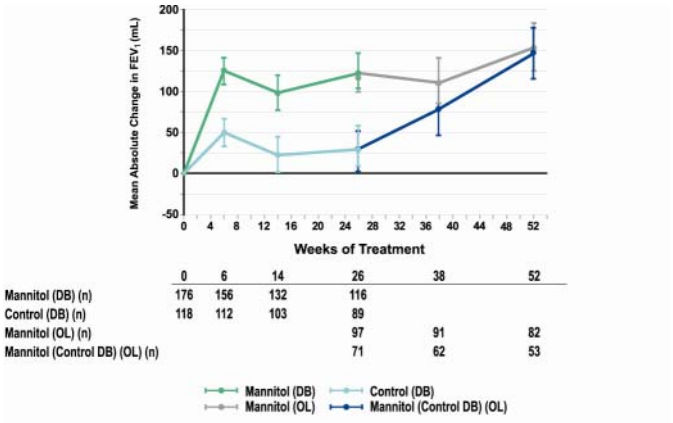


Figure 3: Change in Forced Vital Capacity (FVC) over the 26 week blinded phase in patients with mannitol and control in the ITT group.

The mean changes are based on a repeated-measures analysis. Error bars are SE. A mixed effects model with change in lung function as the outcome and treatment, time-point, rhDNase use, region and gender as fixed effects and baseline FEV_1, age and disease severity (FEV_1 as % predicted at baseline) as covariates in the model are used to determine the effects across the 26 week period (week 0-26). A treatment effect by time-point was obtained by adding a time by interaction term to the model.



	0	6	14	26	38	52
Mannitol (DB) (n)	176	156	132	116		
Control (DB) (n)	118	112	103	89		
Mannitol (OL) (n)				97	91	82
Mannitol (Control DB) (OL) (n)				71	62	53

Figure 4: Mean Absolute Change in FEV_1 from Baseline over 2 Phases of the Study (Week 0-26: Double-blind phase and Week 26-52: Open-label mannitol phase)

(Control patients went on to receive Mannitol in the open-label phase of the study, Numbers contributing to each mean FEV_1 at timepoints are listed below the graph.)

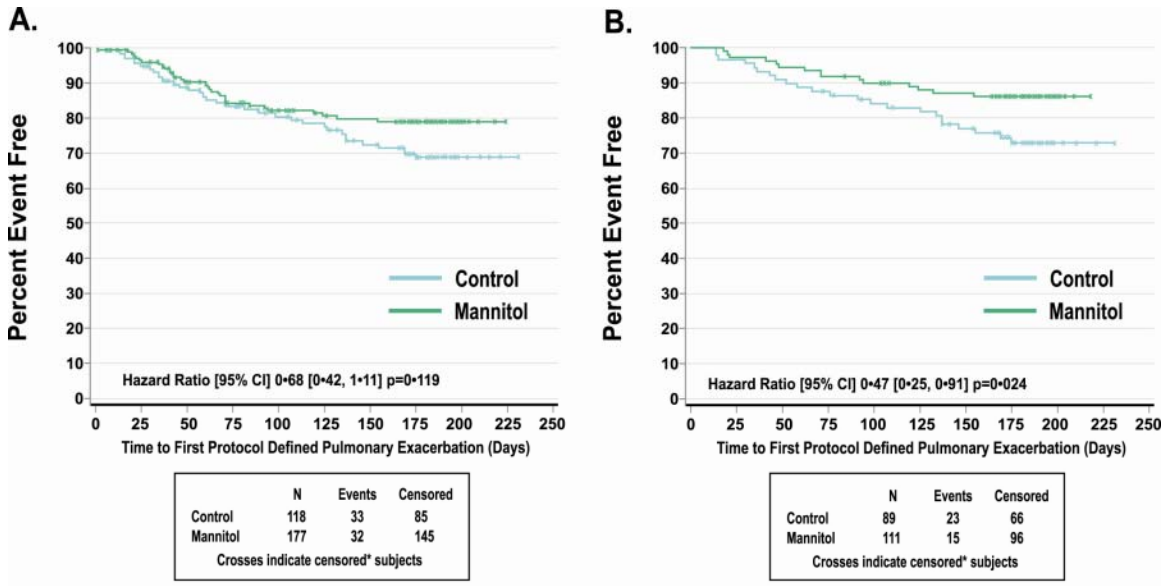


Figure 5: Protocol Defined Pulmonary Exacerbation (PDPE) Event Free Survival in the Intention to Treat Population (A) and the Per Protocol Population (B)

*Censored subjects are those with no pulmonary exacerbations at the time of last contact in the blinded study period (either week 26 or early withdrawal). The Study had 2 week study windows for each visit, that allowed a number of patient in both arms to continue in the double-blind phase of the study for up to 8 months.