# Long-term inhaled dry powder mannitol in cystic fibrosis: an international randomized study

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| Keywords: | cystic fibrosis, mannitol, clinical trial, mucociliary clearance, dry powder inhaler |
Title

Long-term inhaled dry powder mannitol in cystic fibrosis: an international randomized study

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Contributions made by each author

All authors helped to interpret data, write the manuscript and have seen and approved the final version. Moira L Aitken was the Global Principal Investigator for CF302. Moira L Aitken, Patrick A Flume, David E Geller, Allen Lapey and Jonathan B Zuckerman were on the CF302 Steering Committee. Gabriel Bellon was Lead Regional Investigator for France. Kris De Boeck was Lead Regional Investigator for Belgium. Eric G Haarman was Lead Regional
Investigator for The Netherlands. Helge U Hebestreit was Lead Regional Investigator for Germany. Howard G Fox approved the statistical plans and assisted the design of CF302. Brett Charlton designed the CF302 study, approved the statistical plans, and was the Sponsor’s Responsible Medical Officer. Manjula Schou wrote the statistical plans and was responsible for the data analyses.

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**Descriptor** that best classifies the subject of manuscript [probably 9.17, i.e. Cystic Fibrosis: Translational and clinical studies; or possibly 9.1, i.e. Adult CF, given median age of subjects]

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**Running Head:** Inhaled mannitol study in CF
Keywords: cystic fibrosis, mannitol, dry powder inhaler, airway mucociliary clearance, mucoactive agents, lung function, clinical trial

AT A GLANCE COMMENTARY

Scientific Knowledge on the Subject

Despite recent advances in treatment, patients with cystic fibrosis continue to experience a progressive decline in lung function and premature mortality. Mannitol is a dry powder agent with osmotic properties that hydrates the airway surface and changes mucus rheology, thus aiding mucociliary clearance from the lungs.

What This Study Adds to the Field

This double-blind randomized Phase III trial demonstrates that adding mannitol to standard therapy produces sustained improvement in lung function for 26 to 52 weeks. Quantitative microbiology showed added safety.
CF 302 Abstracts submitted/accepted at International conferences


Aitken ML, Bilton D, Pinero R, Fox H, Charlton B. Mannitol inhaler device culture study: no evidence of an increased microbiological contamination. Amer J Crit Care Med 2011; 185 A1125 (ATS 2011)


Aitken ML, D Bilton, P Robinson, P Cooper, J Kolbe, CG Gallagher, plus the European Union Investigators/ US steering committee, H Fox, B Charlton. Efficacy and safety by age group from the phase III studies of Bronchitol (inhaled mannitol) in patients with CF. [Accepted for NACF 2011 November (Abstract ID 236)]

ABSTRACT

Rationale: New treatment strategies are needed to improve airway clearance and reduce the morbidity and the time burden associated with cystic fibrosis (CF).

Objective: To determine whether long-term treatment with inhaled mannitol an osmotic agent, improves lung function and morbidity.

Methods: Double-blind, randomized, controlled trial of inhaled mannitol 400 mg bid (n=192, “treated” group) or mannitol 50 mg bid (n=126, “control” group) for 26-weeks, followed by 26 weeks of open-label treatment.

Measurements: The primary endpoint was absolute change in forced expiratory volume in one second (FEV₁) from baseline in treated versus control groups, averaged over the study period. Secondary endpoints included other spirometric measurements, pulmonary exacerbations, and hospitalization. Clinical, microbiological and laboratory safety were assessed.

Main results: The treated group had a mean improvement in FEV₁ of 105 mL (8.2% above baseline). The treated group had a relative improvement in FEV₁ of 3.75% (p=0.029) versus the control group. Adverse events and sputum microbiology were similar in both treatment groups. Exacerbation rates were low, but there were fewer in the treated group (HR [95% CI] 0.74 [0.42, 1.32], p=0.31) though this was not statistically significant. In the 26-week open-label extension study, FEV₁ was maintained in the original treated group, and improved in the original control group to the same degree.
Conclusions: Inhaled mannitol 400 mg bid resulted in improved lung function over 26 weeks, that was sustained after an additional 26 weeks of treatment. The safety profile was also acceptable, demonstrating the potential role for this chronic therapy for CF.

Clinical trial registered with www.clinicaltrials.gov (NCT00630812).

Word count: 250
INTRODUCTION

Cystic fibrosis (CF) lung disease is characterized by reduced hydration of the airway surface liquid and impaired mucociliary clearance. The difficulty in clearing airway secretions and pathogens leads to chronic airway obstruction, inflammation, and infection, with intermittent pulmonary exacerbations and ultimately respiratory failure (1,2).

There are numerous therapies directed toward the treatment of the lungs to slow the progression of the disease. These treatments include antibiotics, anti-inflammatory agents, mucolytics and agents and maneuvers that enhance airway clearance (3,4,5). Current treatment guidelines recommend inhaled dornase alfa (rhDNase, Pulmozyme, Genentech, San Francisco, CA) and inhaled hypertonic saline (an osmotic agent that may help to restore the airway surface liquid) to improve lung function and reduce exacerbations (6).

However, the number of treatments prescribed for CF patients and the amount of time it takes to accomplish them imposes a significant burden on the patients. There remains a need for effective therapies that can improve mucociliary clearance and decrease the time burden of disease associated with CF. When inhaled, mannitol (a sugar alcohol) is believed to increase surface liquid in the airways by creating an osmotic gradient that encourages movement of water into the lumen, and thereby enhancing mucociliary clearance (7). Mannitol is a dry powder and is inhaled from a simple, disposable, capsule-based dry-powder inhaler (DPI). As such, it has the potential to be a more convenient alternative as it does not require refrigeration, nebulization, equipment cleaning or sterilization.

In a small, double-blind crossover study in which 39 patients with mild-moderately severe CF inhaled 420 mg mannitol bid and placebo (non-respirable mannitol; each for 2 weeks), the active treatment produced a significantly greater improvement in forced expiratory volume in
one second (FEV\textsubscript{1}) from baseline (8). Sputum samples from the treated group showed improved hydration and surface properties, and that these changes correlated with improvements in airway function (9). A phase III trial of mannitol in 295 subjects from Europe, Australia and New Zealand demonstrated that mannitol was effective in improving lung function (10).

The purpose of this second international phase III study was to determine whether long-term (26-week) administration of mannitol improves FEV\textsubscript{1}. FEV\textsubscript{1} was used as the primary outcome variable, as it is the strongest clinical predictor of survival in patients with CF (11,12,13).

In addition to extending our understanding of potential benefits for lung function, the study explored the effect of mannitol treatment on the frequency of pulmonary exacerbations, which is known to associate with accelerated loss of lung function and decreased survival (14,15,16). Given a possible influence of mannitol on lung microbiology, quantitative microbiology was explored.

**METHODS**

This was a 26-week double-blind, randomized, controlled trial of inhaled mannitol (Pharmaxis, Sydney NSW, Australia) 400 mg bid versus a control of mannitol 50 mg bid followed by a 26-week open-label extension during which all subjects received active treatment. Low dose mannitol was chosen as the placebo following discussion with regulatory agencies and in accordance with the results of the dose-finding study (17).

**Selection of Patients**

The study was conducted in full accordance with the current revision of the Declaration of Helsinki and the *Good Clinical Practice: Consolidated Guideline* approved by the
International Conference on Harmonization. The study was approved by the institutional review board or ethical committee of each participating hospital and written consent was obtained from each patient or their legally authorized representative.

The study was conducted at 53 sites in North America [31], South America [8] and Europe [14]. In order to be eligible, patients had to have a confirmed diagnosis of CF, be at least 6 years of age, and have an FEV\textsubscript{1} between 40-89% of predicted values (18,19). Eligible patients were given a mannitol tolerance test (MTT) to exclude those with mannitol-induced bronchospasm. Use of nebulized hypertonic saline during the study was prohibited but all other therapies were continued. (Full details of the inclusion/exclusion criteria and the MTT can be found in the online supplement).

Randomization and Blinding

Patients were randomized to active treatment or the control arm in a 3:2 ratio. Randomization was stratified by country and use of dornase alfa.

Study drug

Patients were assigned to receive ten capsules of inhaled mannitol 40 mg (mannitol group) or sub-therapeutic 5 mg per capsule (control group) bid for 26 weeks. Drug was administered via a single-dose dry powder RS01 inhaler Model 7 (Plastiape, Italy). Patients had five visits over 26 weeks and a further two visits during the open-label phase to 52 weeks.
Outcome Measures

The primary efficacy endpoint was the between-group difference in the absolute value of FEV₁ averaged over the double-blind phase of the study.

Secondary endpoints indicative of pulmonary function included group differences in percent predicted FEV₁ at 26 weeks, FVC and FEF₂₅₋₇₅. Mannitol had to be withheld for at least 6 hours prior to spirometry testing as were short acting bronchodilators. Long acting bronchodilators were withheld for at least 12 hours. Other secondary endpoints included the number of pulmonary exacerbations meeting the pre-specified protocol definition (PDPEs), which included treatment with intravenous antibiotics and presence of specified signs and symptoms (20), hospitalizations and use of antibiotics for a pulmonary exacerbation. During and for the first 30 minutes following the initial administration of mannitol/control at Visit 1 (week 0) and Visit 3 (week 14), sputum weights were measured. The quality of spirometry met the 2005 American Thoracic Society (ATS) / European Respiratory Society (ERS) criteria (21) thus the FEV₁ and FVC used were the best values even if they were from different attempts. Quality of life was assessed using the Cystic Fibrosis Questionnaire-R (22).

Adverse events

Assessment of adverse events (AEs) included complete blood count, liver and renal function tests and physical examinations including vital signs, and patients were also asked to record a study diary.

Microbiology
Given a possible influence of mannitol on lung microbiology, sputum samples were collected at the baseline visit and at each of the four visits during the study’s double-blind phase. Qualitative sputum microbiology was performed, for *Staphylococcus aureus* and *Pseudomonas aeruginosa* at baseline, week 6 and week 26. Central laboratory facilities for the USA and Canada were provided by Quest Diagnostics, Valencia, California; for Europe by Quest Diagnostics, Middlesex; and for Argentina by Centralab, Buenos Aires.

**Statistics and Statistical Methods**

The study was designed to have 80% power to detect a change from baseline of 79.3 mL with active treatment. Calculation of total sample size assumed a drop out rate of 30% in the mannitol 400mg bid arm. (More details can be found in the online supplement.)

Spirometry endpoints were analyzed using mixed model repeated measures (MMRM) methodology and an unstructured variance-covariance structure. MMRM produced an estimate of the difference between mannitol and control patients based on measures repeated at Weeks 6, 14 and 26 (overall treatment effect). Change in percent predicted FEV₁ was assessed by analysis of covariance (ANCOVA) at week 26. Data on pulmonary exacerbations, hospitalization and antibiotic use were analyzed by Poisson regression and the rate ratio estimated. Covariates included treatment group, baseline response (for spirometry measures only), disease severity at baseline, age, gender, rhDNase use, country of participation, visit and visit by treatment group interaction term for the MMRM models only, and, for pulmonary exacerbations, PDPEs and hospitalization, prior history of these events.

A single pre-planned interim analysis was conducted during the study and the Haybittle-Peto stopping rule was to be employed to determine whether the study was to terminate early. As a
result of this interim analysis, the primary efficacy endpoint was tested at a 4.98% significance level.

RESULTS

Patient Characteristics

The study was conducted between September 2008 and April 2010. Figure 1 shows that of the 342 potentially eligible patients, 318 (93%) completed and passed the MTT and were randomized, of whom 305 patients began study treatment. Patient demographics are shown in Table 1, and reveal that patients in the two arms of the study were well matched.

One important difference between treatment groups was the mean change in FEV$_1$ measures from the screening visit to the baseline visit (Visit 1), which dropped in the control group but remained stable in the mannitol group (Table 1).

Compliance and Completion

At each visit, patients returned all used and unused blister packs of active drug or control. These were reconciled against numbers dispensed. Compliance with protocol treatment was defined as use of 60% or more of drug dispensed. By this criterion, compliance was good in both arms: mean 85.2% (standard deviation [SD] 23.81%) of mannitol patients and 88.7% (SD 17.66%) of controls.

Among the safety/ITT population of 305 in total (mannitol 184; controls 121), 260 (mannitol 153; controls 107) completed the 26 weeks of double-blind treatment. The main reason for discontinuation was withdrawal of consent (13 mannitol patients and 7 controls). Lack of time was the most common reason cited. Thirteen subjects in the mannitol group and 5 in the
control group discontinued the study due to AEs, with increased cough being the most frequently cited event.

**Efficacy**

**Change in FEV\(_1\) (mL) and FEV\(_1\) (% predicted)**

The mean improvement in FEV\(_1\) was greater in the mannitol group than the control group (106.5 vs. 52.4 mL, \(p=0.059\)) see Figure 2a. The relative change from baseline FEV\(_1\) (mL) in the mannitol group was 8.22% while that in the control group was 4.47% (effect between groups 3.75%, \(p=0.029\)). There was a difference in the secondary endpoint of absolute FEV\(_1\)% predicted (2.42% FEV\(_1\), \(p=0.024\)) at 26 weeks. Relative % change in FEV\(_1\) (% predicted) over the study was greater in the mannitol group than in the control group (3.59%, \(p = 0.033\)) see Figure 2b.

Prior clinical intervention studies have used an average FEV\(_1\) over 2 or more visits to establish a baseline from which to measure change, as opposed to a single visit (20). Therefore, in a post-hoc sensitivity analysis, baseline FEV\(_1\) was calculated as the mean of the values at screening and at Visit 1 (rather than taking the Visit 1 value alone). Using these baseline-corrected values, the overall increase in absolute FEV\(_1\) seen in the mannitol group was significantly greater than that in the control group (difference 71.1 mL, \(p=0.008\)). A post hoc examination of the relative change in FEV\(_1\) (%) using the mean of the screening and baseline FEV\(_1\) (mL) value showed an overall treatment effect that favored the mannitol group (difference 3.97%, \(p=0.008\)).
FVC

In the mannitol group, FVC increased 136.3 mL compared to a 65.0 mL increase in the control group. There was an overall increase of 71.4 mL in FVC in the mannitol group compared to the control group (p=0.022) (Figure 2c).

Pulmonary Exacerbations, Hospitalization and Other Outcomes

Over the 26-week period of double-blind treatment, patients in the mannitol group experienced fewer pulmonary exacerbations whether protocol-defined (PDPE) or from any cause (Table 3), but the difference was not statistically significant. Similar numbers of patients were treated with IV antibiotics for a PDPE in both the mannitol (15.2%) and control (19.0%) groups. Of these acute exacerbations, most were hospitalized (12% and 15.7% respectively). The duration of hospital stay was three days shorter in patients in the mannitol group. There was no significant difference in quality of life from baseline for either treatment group or between treatment groups for any of the quality of life domains. At Visit 1, patients in the Bronchitol group cleared more sputum during and for 30 minutes post Bronchitol administration than patients in the control group. The median sputum weights were 2.7 vs. 1.7 grams respectively (p=0.04). At Week 14, patients in the Bronchitol group cleared less sputum than at Visit 1, but still more than the control group. Median weights were 2.0 vs. 1.5 grams respectively (p=0.26).

Adverse Events

The proportion of patients reporting AEs was similar in the mannitol and control groups (89.7% and 87.6% of patients, respectively). The incidence of severe AEs was similar in the two arms of the study, occurring in 29 (15.8%) of the mannitol group and in 19 (15.7%) of the control group. Serious treatment-related AEs occurred in 2.2% and 2.5%, respectively. The
proportion of patients with treatment-related AEs leading to withdrawal of treatment was 6.5% compared to 1.7%, respectively (Table 4). Condition aggravated (i.e. acute pulmonary exacerbation) was the most frequently reported AE (mannitol 41.3%, control 44.6%). Headache was reported by 14.1% of mannitol subjects and 18.2% of control subjects, while 15.2% of mannitol subjects experienced cough (control 13.2%). Hemoptysis reported as either an AE or as part of an exacerbation was similar in the treatment arms at 11.4% vs 10.7% in the mannitol and control groups, respectively).

There was one death in the study population: a patient in the control group died approximately 3 months after discontinuing study treatment because of a serious AE (pneumothorax). The death was considered unrelated to treatment.

Clinically significant findings regarding vital signs, the physical exam, hematology and biochemistry were generally similar in the two treatment groups and related to the underlying disease. Abnormal white cell count was the most common hematological abnormality, and seen in 5 patients from each treatment group. There were no significant findings for liver function enzymes or diabetes control.

**Microbiology**

There were no qualitative changes in microbiology results from baseline in either group. Mannitol and control groups showed no change from baseline to Week 26 in the frequency of sputum colonization by *S. aureus* or *P. aeruginosa*. There was no change in the number of colony forming units per gram sputum. Data are given in Table 5a and b and c.
Open-label Phase

260 patients completed the double-blind phase (153 mannitol and 107 control) and all entered the open-label phase of the study. Of these, 242 patients completed the open-label phase including 143 mannitol (93.4% who started) and 99 controls (92.5% who started). 10 patients (5.4%) from the mannitol group and 8 patients (6.6%) from the control group withdrew during the open-label phase. Withdrawals due to treatment related adverse events were low (one patient from the original mannitol group and 3 patients from the original control group), while overall adverse events in the open-label phase were similar between the groups. Eight patients experienced serious treatment related adverse events (mannitol 2.6% and control 3.7%).

For those patients originally randomized to the mannitol group, the increase in FEV₁ was maintained for the 12 months of the study, with a mean increase in FEV₁ of 87.2 mL or an 8.2% relative change compared to baseline (p=0.001). Those patients who were initially randomized to the control group during the double-blind phase and then went on to receive mannitol 400 mg twice daily in the six-month open-label phase had a mean FEV₁ improvement from baseline of 84.0 mL (6.3%; p=0.031) at the end of the open-label phase.

DISCUSSION

We demonstrated a 105 mL mean improvement in the FEV₁ of the mannitol-treatment group, an 8.2% improvement from baseline. However, for the primary end point for the study, i.e. the difference in absolute FEV₁ between the treated and control groups over 26 weeks of the study, statistical significance was narrowly missed (although was significant by relative difference).
We believe that this end point was not reached for several reasons. Importantly, analysis of the baseline FEV\textsubscript{1} was taken from only one data point (baseline visit) and was not \textit{a priori} averaged with the screening visit value. Other Phase 3 CF studies (20) have averaged more than one FEV\textsubscript{1} to establish a stable baseline for comparison. The absolute difference in mL was significant when a post-hoc analysis accounted for the observed baseline variability that was limited to control patients.

A second point is that the control arm received a lower dose of the same drug. It was felt that 50 mg would not be clinically effective because of results from the dose escalation study (17) where 40 mg dose seemed to have no effect on FEV\textsubscript{1}. However, it is possible that the lower dose of mannitol may have some benefit, but not as much efficacy as the higher dose, and this finding emerged because of the larger number of patients in this study. The improvement in FEV\textsubscript{1} at the lower dose of mannitol (50 mg) does appear to contribute to the lack of statistically significant absolute (mL) difference between the two doses of mannitol.

As for the other spirometric measures (% predicted FEV\textsubscript{1} and FVC) the 400 mg inhaled mannitol dose resulted in a statistically and clinically significant average improvements in lung function over the treatment period compared to control.

There were fewer pulmonary exacerbations in the mannitol group, although this did not reach statistical significance. This study was not powered to observe an effect on acute pulmonary exacerbations as they can be relatively uncommon in the course of six months. Prior multi-center trials have sometimes detected a decrease in the number of acute pulmonary exacerbations or a delay to the next pulmonary exacerbation (20, 23). However, in this study all preventative medications against acute exacerbations were continued, and thus we believe that it would be difficult to detect change in acute exacerbations in this study over 6-months. Overall there was a low rate of acute exacerbations (<1 per year) in the study population and
this was a heavily treated group on many chronic medications. Moreover, we designed this study using a stringent definition of acute pulmonary exacerbation with the use of IV antibiotics (20), rather than other definitions that have been developed since the start of the trial (24). Interestingly, patients with a higher rate of pulmonary exacerbation in the year preceding the study were more likely to experience a pulmonary exacerbation during the trial, and the historical rate of pulmonary exacerbations in the year prior to treatment was 19.7% higher among mannitol than among control patients).

Mannitol was well tolerated, and the proportion of patients discontinuing from the study due to AEs was similar between the groups. The AEs reported were generally mild or moderate and consistent with CF and its treatment. Cough was more common in the mannitol than in the control arm and is a known side-effect of this agent. When it contributes to clearance of mucus, cough can be a positive event. However, a small proportion of patients are unable to tolerate it, and cough was the most common treatment-related AE leading to withdrawal. The incidence of severe AEs and serious treatment-related AEs were similar in the two arms of the study. No safety signals of concern were detected.

Hemoptysis is frequently associated with CF exacerbation and therefore many occurrences of hemoptysis were captured as symptoms of pulmonary exacerbation or PDPE but not necessarily as AEs. When hemoptysis reported as a symptom of a pulmonary exacerbation was included in the analysis of hemoptysis AEs, the frequency of hemoptysis was similar overall in both the mannitol and control groups. Inhaled mannitol like hypertonic saline is known to induce bronchospasm in other patient populations. To reduce any potential risk of bronchospasm, a test dose of mannitol was instituted at screening (MTT), and bronchodilator was routinely used prior to inhaled mannitol administration in the study. A small minority of the CF population (6.4%) did not proceed into the study on initial testing due to bronchial
hyper-responsive. There was no bronchospasm reported with inhaled mannitol during the study and surprisingly, perhaps, this compares favorably with existing therapies that do not mandate screening or pre-dose bronchodilator use, yet may cause bronchoconstriction. Furthermore, unlike antibiotics that carry a potential for anaphylaxis, bronchospasm following bronchial hyper-reactivity is short lived and responsive to bronchodilator.

Mannitol treatment was not associated with an increase in the isolation of *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Burkholderia cepacia*, *Stenotrophomonas maltophilia*, other gram negative aerobic organisms, Methicillin-resistant *Staphylococcus aureus* (MRSA), small colony variant or other pathogens commonly seen in patients with CF. This is a reassuring finding since many organisms are able to use mannitol as a carbon source.

Interestingly, a recent study exploring the aminoglycoside gentamicin in combination with mannitol demonstrated that mannitol and gentamicin could potentiate the eradication of gram negative bacterial persisters and biofilms suggesting mannitol might potentially be beneficial in clearing organisms (25).

Inhaled mannitol has other advantages that may reduce the treatment burden for patients. The simple DPI is small, portable, and fairly easy to use, especially in a population of patients who are experienced with inhaled therapies. Each dose takes an average of 5 minutes to administer (data not shown), and it requires no power source other than the patient’s inspiratory effort. Another favorable feature of the formulation is that the DPI (unlike a nebulizer) does not require thorough cleaning and disinfection after each use. We speculate that in patients with adequate inspiratory flow and lung volume to successfully actuate the DPI, patients will have more flexibility and may be more adherent with this therapy.
To summarize, in this Phase III study, 12 month use of inhaled mannitol 400 mg bid resulted in sustained improvement in lung function relative to control, as measured by FEV₁ and FVC and reduced exacerbations, and had a good safety profile with excellent treatment compliance over 26 weeks of treatment. While both this study and the earlier Phase III study (26) were statistically significant for change in FEV₁ by relative % predicted, this study did not reach statistical significance for absolute change in mL (p=0.059). The efficacy of inhaled mannitol was demonstrated on top of a background of typical concomitant therapy such as rhDNase and inhaled antibiotics, perhaps reflecting its different mechanism of action. These results support the use of mannitol as an osmotic, inhalation dry powder treatment for the daily management of CF patients to improve overall pulmonary function with a shortened time of treatment burden.

**CF302 Investigators**

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REFERENCES


TABLE 1. BASELINE CHARACTERISTICS OF PATIENTS (SAFETY POPULATION)

<table>
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<td>0.6 (1.11)</td>
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<td>L [Mean (SD)]</td>
<td>2.06 (0.71)</td>
<td>2.02 (0.72)</td>
</tr>
<tr>
<td>% predicted [Mean (SD)]</td>
<td>65.2 (13.9)</td>
<td>64.4 (15.3)</td>
</tr>
<tr>
<td><strong>FEV₁ at baseline</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L [Mean (SD)]</td>
<td>2.06 (0.77)</td>
<td>1.96 (0.74)</td>
</tr>
<tr>
<td>% predicted [Mean (SD)]</td>
<td>64.8 (15.7)</td>
<td>62.5 (16.0)</td>
</tr>
<tr>
<td><strong>BMI kg/m² [Mean (SD)]</strong></td>
<td>20.0 (4.1)</td>
<td>19.8 (3.7)</td>
</tr>
<tr>
<td><strong>Use of systemic antibacterials</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>75.5%</td>
<td>81.0%</td>
<td></td>
</tr>
<tr>
<td><strong>Azithromycin</strong></td>
<td>43.5%</td>
<td>43.8%</td>
</tr>
<tr>
<td><strong>Use of inhaled antibiotics</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>60.3%</td>
<td>57.9%</td>
<td></td>
</tr>
<tr>
<td><strong>colistin</strong></td>
<td>17.9%</td>
<td>21.5%</td>
</tr>
<tr>
<td>Drug</td>
<td>Mean</td>
<td>Median</td>
</tr>
<tr>
<td>-----------------------------</td>
<td>-------</td>
<td>--------</td>
</tr>
<tr>
<td>Tobramycin</td>
<td>48.4%</td>
<td>38.0%</td>
</tr>
</tbody>
</table>

**Drugs for Obstructive Airway disease**

<table>
<thead>
<tr>
<th>Drug</th>
<th>Mean</th>
<th>Median</th>
</tr>
</thead>
<tbody>
<tr>
<td>Salbutamol (albuterol)</td>
<td>98.9%</td>
<td>99.2%</td>
</tr>
<tr>
<td>Inhaled corticosteroids*</td>
<td>51.1%</td>
<td>50.4%</td>
</tr>
</tbody>
</table>

**KEY:** BMI = body mass index; CF = cystic fibrosis; FEV\textsubscript{1} = forced expiratory volume in one second; L = liter; max = maximum; min = minimum; rhDNase = recombinant human deoxyribonuclease; SD = standard deviation. *includes subjects who were on combination of an inhaled corticosteroid and a beta agonist.
### TABLE 2. CHANGES IN FEV\(_1\) ENDPOINTS IN ACTIVE TREATMENT AND CONTROL GROUPS

<table>
<thead>
<tr>
<th>Endpoint</th>
<th>Mannitol</th>
<th>Control</th>
<th>Treatment effect</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n = 184</td>
<td>n = 121</td>
<td></td>
</tr>
</tbody>
</table>

**Absolute increase in FEV\(_1\) (mL) from baseline** (Visit 1)
- **Mean**: 106.5, 52.4, 54.1
- 95% CI: 62.4, 150.6, 2.1, 102.7
- p vs. baseline: p < 0.001, p = 0.04
- p mannitol vs. Control: p = 0.059

**Absolute increase in FEV\(_1\) (mL) from adjusted baseline (taken as mean of screening and Visit 1)**
- **Mean**: 108.7, 37.6, 71.1
- 95% CI: 67.8, 149.5, -9.1, 84.2
- p vs. baseline: p < 0.001, p = 0.114
- p mannitol vs. Control: p = 0.008

**Relative % change in FEV\(_1\) (mL) from baseline**
- **Mean**: 8.22, 4.47, 3.75
- 95% CI: 5.57, 10.88, 1.44, 7.50
- p vs. baseline: p < 0.001, p = 0.004
- p mannitol vs. Control: p = 0.029

**Relative % change in FEV\(_1\) (% predicted) from baseline**
- **Mean**: 6.73, 3.13, 3.59
- 95% CI: 4.12, 9.33, 0.16, 6.10
- p vs. baseline: p < 0.001, p = 0.039
<table>
<thead>
<tr>
<th></th>
<th>Mannitol vs. Control</th>
<th>p = 0.033</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>At Week 26 absolute increase in % predicted FEV₁</strong></td>
<td><strong>Mean</strong></td>
<td><strong>95% CI</strong></td>
</tr>
<tr>
<td></td>
<td>3.14</td>
<td>0.72</td>
</tr>
</tbody>
</table>

KEY: CI=confidence interval; FEV₁=forced expiratory volume in one second; mL=milliliter.

* Post-hoc analysis

† ANCOVA analysis: prespecified secondary endpoint for % predicted FEV₁ at week 26
### TABLE 3. PULMONARY EXACERBATION RATES AND HOSPITALIZATION IN MANNITOL AND CONTROL GROUPS

<table>
<thead>
<tr>
<th></th>
<th>Mannitol</th>
<th>Control</th>
<th>Reduction</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>n</strong> = 184</td>
<td>15.2%</td>
<td>19.0%</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Annualized Rate</strong></td>
<td></td>
<td></td>
<td>15%</td>
<td>0.520</td>
</tr>
<tr>
<td>Proportion of patients with ≥1 PDPE during 26 weeks of double-blind treatment</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PDPE rate ratio (95% CI)</td>
<td>0.85 (0.51, 1.41)</td>
<td>15%</td>
<td>0.520</td>
<td></td>
</tr>
<tr>
<td>PDPE Hazard ratio (95% CI)</td>
<td>0.74 (0.42, 1.32)</td>
<td>0.308</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Patients hospitalized due to PDPE</td>
<td>12.0%</td>
<td>15.7%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PDPE hospitalization rate ratio (95% CI)</td>
<td>0.75 (0.42, 1.33)</td>
<td>25%</td>
<td>0.328</td>
<td></td>
</tr>
<tr>
<td>Mean duration of hospital stay for PDPE (days)†</td>
<td>12.09</td>
<td>15.42</td>
<td>SD 7.91</td>
<td>SD 10.16</td>
</tr>
</tbody>
</table>

PDPE=protocol-defined pulmonary exacerbation; SD=standard deviation. *adjusted for pre-specified covariates including pulmonary exacerbation history, † Only includes those subjects who were hospitalized ‡ Cox proportional hazards
## TABLE 4. PROPORTION OF PATIENTS WITH ADVERSE EVENTS DURING 26 WEEKS OF DOUBLE-BLIND TREATMENT WITH MANNITOL OR CONTROL

<table>
<thead>
<tr>
<th>Category of AE</th>
<th>Mannitol</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n = 184</td>
<td>n = 121</td>
</tr>
<tr>
<td>One or more AE</td>
<td>89.7%</td>
<td>87.6%</td>
</tr>
<tr>
<td>TEAEs by MedDRA preferred term (occurring in ≥ 5% patients overall):</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Condition aggravated</td>
<td>41.3%</td>
<td>44.6%</td>
</tr>
<tr>
<td>Headache</td>
<td>14.1%</td>
<td>18.2%</td>
</tr>
<tr>
<td>Cough</td>
<td>15.2%</td>
<td>13.2%</td>
</tr>
<tr>
<td>Pharyngolaryngeal pain</td>
<td>10.3%</td>
<td>10.7%</td>
</tr>
<tr>
<td>Pyrexia</td>
<td>9.2%</td>
<td>10.7%</td>
</tr>
<tr>
<td>Abdominal pain</td>
<td>7.6%</td>
<td>6.6%</td>
</tr>
<tr>
<td>Upper respiratory tract infection</td>
<td>5.4%</td>
<td>9.1%</td>
</tr>
<tr>
<td>Nasopharyngitis</td>
<td>6%</td>
<td>5%</td>
</tr>
<tr>
<td>Hemoptysis*</td>
<td>7.1%</td>
<td>2.5%</td>
</tr>
<tr>
<td>Severe AE</td>
<td>15.8%</td>
<td>15.7%</td>
</tr>
<tr>
<td>AE leading to discontinuation from study</td>
<td>7.1%</td>
<td>4.1%</td>
</tr>
<tr>
<td>Treatment-related AE leading to discontinuation from study</td>
<td>6.5%</td>
<td>1.7%</td>
</tr>
<tr>
<td>Treatment-related serious AE</td>
<td>2.2%</td>
<td>2.5%</td>
</tr>
</tbody>
</table>


*N.B. when using haemoptysis symptoms reported as part of a PDPE the incidence was 11.0% and 11.6%, respectively.*
TABLE 5. QUANTITATIVE MICROBIOLOGY FOR \textit{P. aeruginosa} (5A), \textit{S. aureus} (5B) AT BASELINE AND WEEK 26 BY TREATMENT GROUP AND QUALITATIVE MICROBIOLOGY (5c)

5a

<table>
<thead>
<tr>
<th>\textbf{P. aeruginosa}</th>
<th>\textbf{Mannitol}</th>
<th>\textbf{Control}</th>
</tr>
</thead>
<tbody>
<tr>
<td>\textbf{Baseline}</td>
<td>n = 184</td>
<td>n = 121</td>
</tr>
<tr>
<td>Percent of patients with pathogen present</td>
<td>48.1%</td>
<td>52.9%</td>
</tr>
<tr>
<td>Mean (SD) log CFU/g</td>
<td>6.9 (1.6)</td>
<td>6.1 (1.9)</td>
</tr>
<tr>
<td>\textbf{Week 26}</td>
<td>n=82</td>
<td>n= 67</td>
</tr>
<tr>
<td>Percent of patients with pathogen present</td>
<td>41.5%</td>
<td>58.2%</td>
</tr>
<tr>
<td>Mean (SD) log CFU/g</td>
<td>6.4 (1.7)</td>
<td>6.3 (2.0)</td>
</tr>
</tbody>
</table>

CFU=colony forming unit; \textit{P.}=\textit{Pseudomonas}; SD=standard deviation.

5b

<table>
<thead>
<tr>
<th>\textbf{S. aureus}</th>
<th>\textbf{Mannitol}</th>
<th>\textbf{Control}</th>
</tr>
</thead>
<tbody>
<tr>
<td>\textbf{Baseline}</td>
<td>n = 184</td>
<td>n = 121</td>
</tr>
<tr>
<td>Percent of patients with pathogen present</td>
<td>64.3%</td>
<td>73.7%</td>
</tr>
<tr>
<td>Mean (SD) log CFU/g</td>
<td>6.8 (1.6)</td>
<td>6.1 (1.7)</td>
</tr>
<tr>
<td>\textbf{Week 26}</td>
<td>n=93</td>
<td>n= 70</td>
</tr>
<tr>
<td>Percent of patients with pathogen present</td>
<td>62.4%</td>
<td>62.9%</td>
</tr>
<tr>
<td>Mean (SD) log CFU/g</td>
<td>6.7 (1.5)</td>
<td>6.7 (1.4)</td>
</tr>
</tbody>
</table>

CFU=colony forming unit; \textit{S.}=\textit{Staphylococcus}; SD=standard deviation.
<table>
<thead>
<tr>
<th>Visit 1 (baseline)</th>
<th>Mannitol (N=184)</th>
<th>Control (N=121)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Any organism</td>
<td>N=184</td>
<td>N=121</td>
</tr>
<tr>
<td>P. aeruginosa (mucoid)</td>
<td>165 (89.7)</td>
<td>105 (86.8)</td>
</tr>
<tr>
<td>P. aeruginosa (non mucoid)</td>
<td>52 (28.3)</td>
<td>33 (27.3)</td>
</tr>
<tr>
<td>Pseudomonas spp (other)</td>
<td>8 (4.3)</td>
<td>3 (2.5)</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>84 (45.7)</td>
<td>55 (45.5)</td>
</tr>
<tr>
<td>MRSA</td>
<td>19 (10.3)</td>
<td>12 (9.9)</td>
</tr>
<tr>
<td>Burkholderia cepacia (cenocepacia)</td>
<td>5 (2.7)</td>
<td>8 (6.6)</td>
</tr>
<tr>
<td>Aspergillus spp</td>
<td>21 (11.4)</td>
<td>13 (10.7)</td>
</tr>
<tr>
<td>Visit 4 (week 26)</td>
<td>N=153</td>
<td>N=110</td>
</tr>
<tr>
<td>Any organism</td>
<td>137 (89.5)</td>
<td>100 (90.9)</td>
</tr>
<tr>
<td>P. aeruginosa (mucoid)</td>
<td>44 (28.8)</td>
<td>36 (32.7)</td>
</tr>
<tr>
<td>P. aeruginosa (non mucoid)</td>
<td>26 (17.0)</td>
<td>30 (27.3)</td>
</tr>
<tr>
<td>Pseudomonas spp (other)</td>
<td>3 (2.0)</td>
<td>1 (0.9)</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>75 (49.0)</td>
<td>55 (50.0)</td>
</tr>
<tr>
<td>MRSA</td>
<td>18 (11.8)</td>
<td>11 (10.0)</td>
</tr>
<tr>
<td>Burkholderia cepacia (cenocepacia)</td>
<td>5 (3.3)</td>
<td>8 (7.3)</td>
</tr>
<tr>
<td>Aspergillus spp</td>
<td>19 (12.4)</td>
<td>22 (20.0)</td>
</tr>
</tbody>
</table>
FIGURE LEGENDS

FIGURE 1 SUBJECT DISPOSITION AND DISCONTINUATION

FIGURE 2 TREATMENT EFFECT OVER TIME - ABSOLUTE CHANGE IN FEV₁

(ML AND % PREDICTED)

A. ABSOLUTE CHANGE IN FEV₁ (ML)

♦ Mannitol
▲ Control
● Mannitol Overall Effect
■ Control Overall Effect

B. RELATIVE % CHANGE IN % PREDICTED FEV₁ (%)

♦ Mannitol
▲ Control
● Mannitol Overall Effect
■ Control Overall Effect

C. ABSOLUTE CHANGE IN FVC (ML)

♦ Mannitol
▲ Control
● Mannitol Overall Effect
■ Control Overall Effect

D. 12 MONTH CHANGE IN FEV₁ (ML) INCLUDING OPEN LABEL PHASE
**FIGURE 1**

- **Allocated to Mannitol (n=192)**
  - Received Mannitol (n=184)
  - Discontinued before treatment (n=8)
    - Adverse event (1)
    - Withdrew consent (2)
    - Lost to follow-up (1)
    - Ineligible (randomized in error; 2)
    - Other (2)

- **Allocated to control (n=126)**
  - Received control (n=121)
  - Discontinued before treatment (n=5)
    - Adverse event (1)
    - Withdrew consent (3)
    - Ineligible (randomized in error; 1)

- **Randomized (n=318)**
  - Not randomized (n=24)
    - Positive MTT (n=14)
    - Incomplete MTT (n=8)
    - Negative MTT (n=1)
    - Unknown MTT (n=1)

- **Completed DBP (n=153)**
  - Discontinued Study (n=31)
    - Adverse event (13)
    - Lost to follow-up (1)
    - Other (1)
    - Physician decision (2)
    - Protocol violation (1)
    - Withdrew consent (13)

- **Analysis populations**
  - Safety (ITT) (n=184)
  - Per protocol (n=152)

- **Entered OLP* (n=153)**
  - Discontinued Study (n=10)
    - Adverse event (1)
    - Lost to follow-up (1)
    - Other (1)
    - Physician decision (2)
    - Withdrew consent (5)

- **Completed OLP* (n=143)**
  - Discontinued Study (n=8)
    - Adverse event (4)
    - Lost to follow-up (1)
    - Other (1)
    - Physician decision (1)

- **Completed DBP (n=107)**
  - Discontinued Study (n=14)
    - Adverse event (5)
    - Lost to follow-up (0)
    - Other (1)
    - Physician decision (1)
    - Protocol violation (0)
    - Withdrew consent (7)

- **Analysis populations**
  - Safety (ITT) (n=121)
  - Per protocol (n=109)

- **Entered OLP* (n=107)**
  - Discontinued Study (n=8)
    - Adverse event (1)
    - Lost to follow-up (1)
    - Other (1)
    - Physician decision (1)
    - Protocol violation (1)
DBP=double-blind phase; ITT=intent-to-treat; MTT=mannitol tolerance test; OLP=open-label phase.

*All patients received 400 mg mannitol bid in the OLP
Legend  Statement explaining Figures 2a, 2b, 2c, 2d.

In comparing change in FEV\textsubscript{1} with 50 mg (control) and 400 mg of inhaled mannitol over 26 weeks, Figure 2A shows a 54.14 mL absolute difference, and Figure 2B shows a 3.59% difference in relative change in percent predicted FEV\textsubscript{1}. Figure 2C shows a 71.35 mL difference in absolute FVC. Figure 2D shows that the initial control group and the initial treatment group had 84 mL and 87 mL increase in FEV\textsubscript{1} from baseline respectively at the end of a six-month open label period.

FIGURE 2A.
FIGURE 2B

Relative % change of % predicted FEV1 (%)

Treatment Week

Overall Effect:
3.59%,
95% CI [0.29, 6.90]
p=0.033

Δ4.55%
95% CI [0.54, 8.55]
p=0.026

Δ3.89%
95% CI [0.33, 7.45]
p=0.032

Δ2.34%
95% CI [-1.67, 6.35]
p=0.251
FIGURE 2C.

Overall Effect:
71.35 mL
95% CI [10.57, 132.13]
p=0.022

Δ 78.17 mL
95% CI [9.61, 146.72]
p = 0.026

Δ 49.12 mL
95% CI [-23.91, 122.14]
p = 0.187

Δ 86.77 mL
95% CI [2.7, 170.85]
p = 0.043
FIGURE 2D.

![Graph showing mean change in FEV1 (mL) over treatment weeks for Double Blind Phase and Open Label Phase with different patient counts (n values).

- Double Blind Phase: n=153, n=184
- Open Label Phase: n=107, n=99

Mean change in FEV1 (mL) plotted against treatment week.
Title

Long-term inhaled dry powder mannitol in cystic fibrosis: an international randomized study

Authors

Moira L Aitken MD¹, Gabriel Bellon MD², Kris De Boeck MD PhD³, Patrick A Flume MD⁴, Howard G Fox MD⁵, David E Geller MD⁶, Eric G Haarman MD PhD ⁷, Helge U Habetreit MD⁸, Allen Lapey MD⁹, I. Manjula Schou (MSc)⁵, Jonathan B Zuckerman MD¹⁰, Brett Charlton MD⁵

Online Data Supplement
CF302 Study Inclusion and Exclusion Criteria

Subject Inclusion Criteria
Subjects may be included in the study if all of the following criteria are met. The subject must:
• Have given written informed consent to participate in this study in accordance with local Regulations
• 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
• Be aged > 6 years old
• Have FEV₁ >40 % and < 90% predicted (using Wang32 <8 years and NHanes III33 >8 years)
• Be able to perform all the techniques necessary to measure lung function

Subject Exclusion Criteria
Subjects are excluded from participating in this study if one or more of the following criteria are met. The subject must NOT:
1. Be investigators, site personnel directly affiliated with this study, 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 nebulized hypertonic saline in the 4 weeks prior to visit 1*
5. Have had a significant episode of hemoptysis (>60 mL) in the three months prior to enrolment
6. Have had a myocardial infarction in the three months prior to enrolment
7. Have had a cerebral vascular accident in the three months prior to enrolment
8. Have had major ocular surgery in the three months prior to enrolment
9. Have had major abdominal, chest or brain surgery in the three months prior to enrolment
10. Have a known cerebral, aortic or abdominal aneurysm
11. Be breast feeding or pregnant, or plan to become pregnant while in the study
12. Be using an unreliable form of contraception (female subjects at risk of pregnancy only)
13. Be participating in another investigative drug study, parallel to, or within 4 weeks of visit 0
14. Have a known allergy to mannitol
15. Be using beta blockers
16. Have uncontrolled hypertension – e.g. for adults: 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 patient’s participation in the study
18. Be ‘MTT positive or incomplete’.

* Subjects may be eligible providing a 4 week washout period occurs between cessation of hypertonic saline at visit 0 and visit 1.
Mannitol Tolerance Test (MTT)

The MTT procedure identified subjects who had airway hyperresponsiveness in response to inhaled mannitol. Airway hyperresponsiveness was determined by measuring the degree of bronchoconstriction that occurs following sequential administrations of inhaled mannitol.

Monitoring Subject Safety during the MTT Procedure

Subjects were monitored during the MTT procedure by following the schedule listed in Table E1.

Table E1: Safety Monitoring During the MTT Procedure

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Baseline (pre-bronchodilator)</th>
<th>At each dose step</th>
<th>At steps 4, 5 and 6</th>
<th>15 min post test *</th>
</tr>
</thead>
<tbody>
<tr>
<td>SpO₂</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>FVC</td>
<td>X</td>
<td></td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>FEV₁</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Clinical signs &amp; symptoms</td>
<td></td>
<td>X</td>
<td></td>
<td>X</td>
</tr>
</tbody>
</table>

* Post test FVC was measured for all patients including those with positive tests, negative tests and incomplete tests

When was the MTT Procedure stopped

The MTT procedure was stopped if:
- The subject’s oxygen saturation fell below 89% (recorded as an adverse event)
- Cough was highly distressing or vomiting occurred (recorded as an adverse event)
- Subject’s FEV₁ had fallen >20% (from baseline) at step 4 or 5 of the procedure
- A total of 395 mg inhaled mannitol had been administered
- Clinical signs and symptoms were causing concern

See Figure E1 for the MTT Procedure

MTT Assessment

The MTT procedure was assessed as follows:

MTT Positive Test
1. The subject’s oxygen saturation fell below 89% (record as adverse event).
2. Subject’s FEV₁ fell >20% (from baseline) at step 4 or 5.
3. Subject’s FEV₁ fell >20% (from baseline) at step 6 and does not return to <20% within 15 minutes
4. Subject’s FEV₁ fell >50% (from baseline) at step 6.

MTT Negative Test
1. A total of 395 mg MTT was administered (and no positive criteria were met).
2. Subject’s FEV₁ fell >20% (from baseline) at step 6 and returned to <20% within 15 minutes.

MTT Incomplete Test
1. Cough was highly distressing or vomiting occurred during the procedure (record as adverse event).
2. Any other reason not listed above where test was incomplete.

N.B. If the subject showed clinical signs and symptoms of bronchoconstriction e.g. wheeze, dyspnea, shortness of breath; FEV₁ was measured and treated accordingly.
Figure E1. MTT Procedure

1. Perform baseline spirometry and measure SpO\textsubscript{2}. Multiply the patient’s test FEV\textsubscript{1} by 0.80 and 0.50 to obtain the 20% and 50% fall in FEV\textsubscript{1} values respectively.
2. Premedicated patient with 4 x puffs albuterol* and wait 5-15 min
3. Administer the MTT as follows:
   - Inhale contents of 1 x 40 mg capsule in a controlled, deep inhalation; breath hold for 5 seconds then exhale
   - Wait 60 seconds, then measure SpO\textsubscript{2}

   \textit{If SpO\textsubscript{2} < 89%, discontinue test and treat as required, otherwise go to step 4}

4. Administer an additional 80 mg MTT (2 x 40 mg) as above. Wait 60 seconds, measure FEV\textsubscript{1} & SpO\textsubscript{2}

   \textit{If FEV\textsubscript{1} fall is \geq 20\% (from baseline) or SpO\textsubscript{2} < 89\%, discontinue test and treat as required, otherwise go to step 5}

5. Administer an additional 120 mg MTT (3 x 40 mg) as above. Wait 60 seconds, measure FEV\textsubscript{1} & SpO\textsubscript{2}

   \textit{If FEV\textsubscript{1} fall is \geq 20\% (from baseline) or SpO\textsubscript{2} < 89\%, discontinue test and treat as required, otherwise go to step 6}

6. Administer an additional 160 mg MTT (4 x 40 mg) as above. Wait 60 seconds, measure FEV\textsubscript{1} & SpO\textsubscript{2}.

   Assess step 6 (only) as follows:

   \begin{itemize}
   \item Is FEV\textsubscript{1} fall \geq 50\%?
   \item Is FEV\textsubscript{1} fall \geq 20\%?
   \item Is FEV\textsubscript{1} still \geq 20\%?
   \end{itemize}

   \textit{If FEV\textsubscript{1} fall \geq 50\%, subject has failed the mannitol tolerance test = MTT positive (treat as required)}

   \textit{If FEV\textsubscript{1} fall \geq 20\%, subject has passed the mannitol tolerance test = MTT negative}

   \textit{If FEV\textsubscript{1} still \geq 20\%, wait 15 minutes† and repeat FEV\textsubscript{1}}

   \textit{If FEV\textsubscript{1} still \geq 20\%, discontinue test and treat as required}

7. 15 minutes post test, measure recovery spirometry (irrespective of result or when the test was terminated).

*alternative short acting bronchodilator may be used
†consider using PEP mask/Acapella/cough clearance to facilitate airway opening
Sample size assumptions and calculations

A total sample size of 250 patients was planned for the CF302 study. 150 patients were to be randomized to receive inhaled mannitol, while 100 were to be randomized to receive control therapy. It was planned that 100 of the patients in the mannitol arm would also be receiving rhDNase, while 50 would not be taking rhDNase. With a dropout rate of 20%, 120 patients in the mannitol arm were expected to complete the study (80 taking rhDNase, 40 not taking rhDNase) and 80 patients in the control arm were expected to complete the study.

The primary hypothesis, that there was no difference in the change from baseline FEV$_1$ ($\Delta$FEV$_1$) between patients taking inhaled mannitol and patients in the control group, was to be analysed using a repeated measures analysis. Age and baseline % predicted FEV$_1$ were to be included in the model as covariates for each patient.

The CF201 phase II study findings were used as assumptions for determining the power for testing this hypothesis, given the sample size chosen. The $\Delta$FEV$_1$ for patients taking rhDNase and inhaled mannitol was expected to be 120 mL (SD =200), while the $\Delta$FEV$_1$ was expected to be 0 mL (SD=200). Given these assumptions, and a type one error rate of 5%, the power to detect a difference of 120 mL in $\Delta$FEV$_1$ was 96%. When both rhDNase and non-rhDNase patients were taken into consideration, the power to detect the same difference of 120 mL $\Delta$FEV$_1$ was 98%.

The power estimate provided was obtained from the following formula for the sample size:

$$N_{control} = \frac{2\sigma^2(z_{\alpha} + z_{\beta})^2}{\Delta^2}$$

In formula 1, $\Delta$ represented the expected difference in $\Delta$FEV$_1$ between patients in the mannitol arm and the control arm (120 mL). The value $\sigma$ represented the common standard
deviation (200 mL). The values of $z\alpha$ and $z\beta$ represented values of the standard normal
distribution that corresponded to a type I error rate of $\alpha=0.025$ and a type II error rate of $1-\beta$,
respectively.

The formula was reworked to solve for $z\beta$ and thereby determine the power of $\beta$:

$$z_{\beta} = \left( \frac{N_{control} \times \Delta^2}{2\sigma^2} - z_{\alpha^2} \right) \left( \frac{1}{1+2z_{\alpha}} \right)^{\frac{1}{2}}$$

Using formula 2, it was shown that by having 45 patients taking rhDNase in the mannitol arm
and 45 patients in the control group, a power of 80% would be achieved.