Randomized controlled trial of prednisone in cystic fibrosis (CF) pulmonary exacerbations (PIPE Study)

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A. Rationale

Cystic fibrosis (CF) is the most common fatal genetic disorder in the Caucasian population and respiratory failure secondary to chronic pulmonary bacterial infection remains the leading cause of death (1). Much of the lung damage that occurs in CF is associated with periods of pulmonary exacerbations (PEx), or flares of respiratory symptoms, which are typically treated with antibiotic therapy (2). Despite aggressive, frequently prolonged intravenous (IV) antibiotic courses, quarter to a third of CF patients do not regain >90% of baseline lung function and only approximately 40% recover full lung function following an exacerbation (3-5). In a large epidemiologic study using the Toronto CF database, we showed that over half of the decline in forced expiratory volume in 1 second (FEV_1) over the lifetime of an individual with CF is directly attributable to these episodes of exacerbation (6). Irrevocable loss of lung function ultimately leads to earlier death in persons with CF (7).

Despite the well-recognized fact that many CF patients will not recover their baseline FEV_1 following treatment for a PEx, it is not clear how to improve the outcomes in these non-responders. Although the exact cause of PExs is not known, there are likely many factors at play in the response to antibiotic treatment. Antibiotics have several different mechanisms of action; therapy during PExs is associated with a decrease in bacterial load in the lungs as well as a decrease in both systemic and pulmonary inflammation (8). There is a correlation between reduction in these variables and increases in lung function (9) but it has not been previously shown which factor is most important in predicting response to therapy. We demonstrated using a multivariable analysis that failure to effectively reduce pulmonary inflammation as measured by sputum neutrophil elastase was independently associated with lack of lung function recovery after 14 days of antibiotic treatment for a pulmonary exacerbation in individuals with CF (10). In addition, elevated markers of pulmonary and systemic inflammation were associated with earlier time to subsequent exacerbation. This suggests that to improve our success in recovering lung function following exacerbations, we may need to target the underlying inflammatory process in the CF lung with adjuvant therapies in individuals not responding to antimicrobial treatment.

Several anti-inflammatory therapies have been used in patients with CF including corticosteroids. Corticosteroids are potent anti-inflammatory drugs that are known to inhibit neutrophilic accumulation at sites of inflammation (11). They have been studied mostly as chronic suppressive medications in CF, however, long-term use has been limited by concerns of side effects (12). A randomized controlled trial examining the short-term use of oral prednisone in all CF patients presenting with PExs demonstrated a modest improvement in lung function (13). We believe that focusing this anti-inflammatory treatment on CF patients with PExs who are not responding to antibiotic treatment will maximize its potential benefit while minimizing any harmful side effects.

Hypothesis: We hypothesize that using oral corticosteroids as an adjunctive therapy in CF patients not responding to antibiotic treatment will improve lung function recovery during PExs by suppressing excessive inflammation in the lung.

Specific aim: To determine whether oral prednisone 2 mg/kg/day (max 60 mg) divided twice daily (based on previous study dosing (13)) for 7 days as an adjunctive therapy for PExs in CF patients who have not recovered their baseline FEV_1 after 7 days of IV antibiotic treatment, can increase the proportion of patients who recover FEV_1 at day 14 compared to placebo.

B. Background

1. Role of pulmonary exacerbations in CF- In the natural history of CF, there is a progressive decline in lung function with episodes of acute worsening of respiratory symptoms, called pulmonary exacerbations (PExs). PExs occur quite frequently in CF patients. In 2007, 38% of all CF patients seen in care centers in the US were treated with IV antibiotics for at least one PEx (2). Although there is no generally accepted definition of PEx, clinical features include increased cough or sputum, fever, weight loss, absenteeism from school or work due to illness, tachypnea, new crackles , decreased exercise tolerance, decreased pulmonary function tests or reduced oxyhemoglobin saturation, or a new finding on chest radiograph (14). The requirement for hospitalization or IV antibiotics is often used to determine the severity of a PEx (15-16). PExs requiring IV antibiotics have been associated with decreased lung function in children (17-18), with CF-related diabetes (19), sleep disturbances (20) and health-related quality of life (21). PExs have long term effects as well. In a large epidemiologic study using the Toronto CF database, we showed that over half of the decline in forced expiratory volume in 1 second (FEV_1) over the lifetime of an individual with CF is directly attributable to these episodes of exacerbation (6). In addition, investigators, including ourselves, have identified that a quarter to a third of CF patients do not regain >90% of baseline FEV_1 and only approximately 40% recover full lung function following an exacerbation (3-5). The average time from initiation of antibiotic treatment for a PEx to the highest observed FEV_1 has been shown to be 8.7 days with a median of 10 days (SD 4.0 days) (22). Regaining lung function following a PEx is key as low FEV_1 is one of the most significant predictors of mortality in CF. Using data from the Cystic Fibrosis Foundation Patient Registry, Liou et al. developed a multivariable logistic regression model to identify key clinical features of CF that determined 5-year survivorship (23). Annual number of acute PExs was identified as a characteristic of the disease that predicted survival.
Several investigators have examined the reasons for failing to recover lung function after treatment of PEx. Female gender, worse nutritional status and greater drop in FEV_1 from baseline to exacerbation have all been identified as risk factors for failing to recover lung function, but none of these factors are actually modifiable at the time of treatment of the exacerbation (3, 24). Studies that have investigated mechanisms by which these failures may occur have focused on serum markers of inflammation (eg CRP and WBC), measured either at the beginning or the end of antibiotic therapy, and shown that higher levels are associated with

treatment failure (25). Variables predicting response cannot be examined in isolation, however, as intravenous antibiotic treatment is known to have multiple effects in CF patients during PEx including decreasing sputum bacterial density, neutrophil counts, IL-8 and neutrophil elastase (8). Changes in these variables correlate, to varying degrees, with improvements in $FEV_{1,}$ but how the changes in these variables predict failure of treatment in any given patient has not previously been well characterized (9, 26). To answer this question, we did a secondary analysis of a randomized, double-blind study of IV antibiotic treatment of PEx in CF patients with chronic *P. aeruginosa* infection to identify predictors of response, including changes in microbiological, systemic and pulmonary markers of inflammation, to antibiotic treatment of pulmonary exacerbations. In a multivariable model, we found that decrease in sputum neutrophil elastase was an independent predictor of response to antibiotic treatment at day 14 (OR 5.40, 95% CI 1.04-28.01, p=0.04). In addition, higher CRP (HR 1.35 (95% CI: 1.01; 1.78), p=0.04) and sputum neutrophil elastase (HR 1.71 (95% CI: 1.02; 2.88), p=0.04) at day 14 of antibiotic therapy were associated with a greater risk of subsequent exacerbation (10). The observation that non-responders had higher pulmonary neutrophil elastase activity, associated with negative long-term consequences, raises the question as to whether treating this ongoing primary inflammation could improve lung function recovery in these patients and ultimately their long-term survival.

2. Use of anti-inflammatories, including steroids, in CF- One of the hallmarks of CF lung disease is excessive airway inflammation. There are many potential sources of the increased inflammatory cytokines observed in CF. Evidence suggests that CF airway epithelium may have inherent pro-inflammatory properties, as NF-κB activation and resulting IL-8 expression have been shown to be elevated in airway cells with the 508del CFTR mutation, even in the absence of infection (27). Infection of airway epithelial cells with typical CF pathogens, such as *P. aeruginosa* and *S. aureus*, has also been shown to stimulate significant IL-8 production (28). IL-8 is the principal chemokine attracting neutrophils in the CF lung, which perpetuate the inflammatory response and cause pulmonary damage through the release of proteases, for example. The primary sputum inflammatory markers that are elevated in CF patients are the measures of neutrophil activation: neutrophil count, IL-8 and neutrophil elastase. These markers are relevant given that multiple studies have shown that increased pulmonary neutrophil elastase activity is the most predictive biomarker for subsequent lung function decline and the development of bronchiectasis in young children with CF (29-30).

Several anti-inflammatory therapies have been used in CF in an attempt to mitigate the lung damage resulting from the excessive, continued pro-inflammatory response. These include alpha-1 antitrypsin, an endogenous inhibitor of neutrophil elastase, non-steroidal anti-inflammatory drugs such as ibuprofen and corticosteroids (12) (31-32). Corticosteroids are known to block the accumulation of neutrophils at sites of inflammation, likely secondary to reduced adherence, and thus have a potential role in the treatment of the predominantly neutrophilic driven inflammation seen in CF lung disease (11). Steroids have been best studied as an oral, chronic suppressive therapy to improve lung function in CF. Three randomized,

double-blind placebo controlled trials were included in a recent Cochrane review of the subject (33-35). These three studies were done in children with CF using prednisone doses of 1-2 mg/kg/day either daily or on alternate days; the duration of oral glucocorticoid administration and follow up ranged from 12 weeks to 4 years. Two of the three studies reported a statistically significant increase in FEV_1 compared to placebo (33, 35); the third study using a higher dose of 2 mg/kg on alternate days, was prematurely discontinued due to a high number of adverse events (34). Adverse events included glucose abnormalities, cataracts and growth retardation, especially linear growth retardation, occurring as early as 6 months with the 2 mg/kg alternate day dosing and after 24 months with the 1 mg/kg alternate day dosing. Thus, although long-term glucocorticoid treatment does improve lung function in individuals with CF, it is at a cost of significant side effects.

Systemic steroids have also been used in chronic obstructive pulmonary disease (COPD), a lung condition characterized, like CF, by episodes of acute exacerbation driven by a neutrophilic inflammatory response, frequently requiring hospital admission for antibiotic therapy. Although systemic corticosteroids have only been shown to increase FEV_1 in approximately 10% of stable COPD patients, it does have a role in the treatment of PEx (36). In a randomized, double-blind, placebo controlled trial patients with COPD exacerbations were randomly assigned to either oral prednisolone 30 mg once daily or placebo for 14 days in addition to the standard therapy of nebulised bronchodilators, antibiotics and oxygen (37). FEV_1 % predicted after bronchodilation rose from 25.7% (95% CI 21.0-30.4) to 32.2% (95% CI 27.3-27.1) in the placebo group compared to 28.2% (95% CI 23.5-32.9) to 41.5% (95% CI 35.8-47.2) in the corticosteroid treated group ($p<0.0001$). Additionally, hospital stays were shorter in the corticosteroid treated group. Although the groups did not differ at the 6 week follow up, these data suggest that corticosteroid treatment is an effective strategy in exacerbations of COPD.

There are limited data, however, on the use of systemic steroid treatment during periods of acute PEx in CF. IV steroid use has been reported predominantly in young infants with CF during episodes of respiratory illness and demonstrated modest improvements in flows as measured by functional residual capacity at follow up compared to the placebo group (38-39). Only one randomized, placebo controlled trial of oral prednisone in patients with CF has been published to date. The study by Dovey et al. randomized (in a 1:1 assignment, on the first day of antibiotic therapy) 24 CF patients age 10 years and older receiving IV antibiotics for a PEx to either oral prednisone 2 mg/kg/day divided twice daily for 5 days or placebo (13). The mean increase in FEV_1 % predicted in the prednisone vs placebo group was 12.2+5.2% vs 8.1+10.5% respectively, at day 6 and 14.7+8.8% vs 10.2+11.2% respectively, at day 14 of antibiotic therapy; these differences were not statistically significant. A limited number of subjects had sputum inflammatory markers assessed; in those that did, the mean change in sputum IL-8 from day 1 to day 14 of antibiotic therapy in the prednisone vs placebo group was -0.2 $\log_{10}$ pg/ml (SD 0.7) vs -0.5 $\log_{10}$ pg/ml (SD 0.3) which was not significantly different. In the subjects treated with prednisone, 6 developed glucosuria, two of whom also developed hyperglycemia. Two subjects

discontinued prednisone, one due to hypertension and one due to hyperglycemia. Based on the trend toward improvement in pulmonary function with prednisone therapy, the investigators calculated that 250 randomized subjects would be required to detect a 4% point difference in FEV_1 at day 14 of antibiotic treatment for a PEx. They concluded that the modest added benefit in lung function was not warranted given the side effects and lack of anti-inflammatory effect.

The study by Dovey et al. has significant limitations in its design that affect its power calculations and conclusions about the value of future randomized controlled trials of steroid treatment in PExs. Firstly, the investigators randomized all patients presenting with an acute exacerbation. It is well know that 75% of these individuals will respond to traditional antibiotic treatment alone, diluting any potential beneficial effect of adjuvant steroid therapy. We propose to randomize subjects who have not recovered their baseline FEV_1 at day 7 of antibiotic treatment to prednisone, thereby maximizing any possible improvements while limiting exposure to adverse side effects. Additionally, Dovey et al. used the change in FEV_1 as a continuous primary outcome measure whereas we have shown that CF patients treated for a PEx clearly separate into responders and non-responders to antibiotic therapy at day 14 (**Figure 1**). It is therefore more appropriate to perform power calculations dichotomizing the primary outcome and using a test of proportions. Finally, the study by Dovey et al. had measurements of sputum inflammatory markers in a very limited number of patients and the study was thus not powered to detect significant changes in this outcome which is known to be widely variable between patients. Furthermore, the authors only measured sputum WBC count, IL-8 and leukotriene B_4 concentrations and not neutrophil elastase which is a more stable and predictive measure of airway inflammation in CF.

Therefore, oral steroid therapy has the potential to improve lung function in individuals in CF. Despite limited data, 17.5% of CF patients in the United States received one or more courses of oral corticosteroids in 1998 according to the Cystic Fibrosis Foundation Registry; many of these are given during acute pulmonary exacerbations. In a survey of adult CF physicians in the United Kingdom, 100% of respondents reported having used oral glucocorticoids as an adjuvant to IV antibiotic treatment during CF PExs (40). A well-designed randomized controlled trial of short-term oral steroid use during CF PExs in those who do not respond to IV antibiotic treatment is thus needed to inform clinical practice as to its potential efficacy. Our study proposes to fill this knowledge gap.

C. Research Design and Methods

Study design

This will be a randomized, double blind, placebo controlled trial of 7 days of oral prednisone in CF patients receiving IV antibiotic treatment for a pulmonary exacerbation at the Hospital for Sick Children, and multiple sub-sites, both adult and paediatric populations across Canada..

Eligible patients will be randomized over a recruitment period of 3 years. Each patient can only be randomized in the study once.

Study inclusion criteria

1. Diagnosis of CF by newborn screening or at least one clinical feature of CF, AND either (a) or (b) as follows:
    a) A documented sweat chloride ≥ 60 mEq/L by quantitative pilocarpine iontophoresis
    b) A genotype with two identifiable CF-causing mutations
2. Age > 6 years old.
3. Acute pulmonary exacerbation treated with IV antibiotics as previously defined
    - 10% relative drop in FEV_1 from baseline at the time of exacerbation
4. Informed consent by patient or parent/legal guardian
5. Ability to reproducibly perform pulmonary function testing
6. Ability to comply with medication use including the ability to take capsules, study visits and study procedures as judged by the site investigator

Study exclusion criteria

1. A respiratory tract culture positive for *Burkholderia cenocepacia* in the 12 months prior to enrollment
2. A respiratory tract culture positive for *Mycobacterium abscessus* in the 12 months prior to enrollment
3. Treatment with IV or oral corticosteroids within 2 weeks of enrollment or from Day 0-Day 7 of the pulmonary exacerbation
4. Active allergic bronchopulmonary aspergillosis (ABPA) at the time of enrollment as determined by treating physician
5. Asthma related exacerbation at enrollment as defined by the treating physician based on clinically compatible symptoms (eg. wheeze)
6. History of avascular necrosis or pathologic bone fracture
7. Uncontrolled hypertension with end organ damage
8. Active gastrointestinal bleeding
9. Status post lung or other organ transplantation
10. Pregnancy
11. Lactose intolerance (contained in placebo)
12. On Lumacaftor-Ivacaftor (Orkambi) at the time of exacerbation
13. Investigational drug use within 30 days prior to enrollment visit
14. Physical findings that would compromise the safety of the subject or the quality of the study data as determined by site investigator

D. Study protocol

Research coordinators will approach all patients at day 0 of intravenous antibiotic treatment for a pulmonary exacerbation to obtain consent, determine eligibility and to complete a Data Collection Form. If the participant is female and post-menarchal, a pregnancy test will be done at day 7 before randomization to prednisone or placebo. If the test is positive, the participant is ineligible for randomization. If the test is positive, the participant and her primary care CF physician will be informed of the result in private. Every effort will be made to keep any pregnancy test results private.

All study sites will perform pulmonary function tests at day 7 of IV antibiotic therapy for a pulmonary exacerbation as part of clinical care. If based on day 7 pulmonary function testing (±2 days), a patient has not recovered >90% of their baseline FEV_1 % predicted (Non-Responders), they will be randomized into the placebo or prednisone treatment arm (**Figure 2** Randomization Flow Chart). Baseline lung function will be defined as the best FEV_1 in the 6 months prior to the PEx (or in the 12 months prior to PEx if there are no measurements in the 6 months prior) (3). Patients who achieve >90% of their baseline FEV_1 % predicted at day 7 (Responders) will not be randomized to prednisone or placebo but will continue in the study as outlined below.

Patients will be randomized to receive either oral prednisone 2 mg/kg/day (max 60 mg; rounded to the nearest 10 mg) (Table 1) divided twice daily or oral placebo twice daily for 7 days in addition to the IV antibiotics prescribed by their physician (**Randomization**). On Day 7 only, it may be necessary on some occasions when the study drug (prednisone or placebo) is not available until the late afternoon, to give the study drug as one full dose (as opposed to twice daily). The choice and duration of antibiotic therapy will be left completely to the discretion of their treating physician. Study outcome measures will be obtained for all enrolled subjects at day 0 (±2 days) and subsequently at day 7 (±2 days), day 14 (±2 days) of IV antibiotic treatment and at the 1 month follow up visit (approximately 1 month after day 14), as per usual standard of care (; **Table** 2 Study Design Schedule). However, day 0 may + 3 days when impacted by a Friday or long weekend admission/IV antibiotic start time. Patients who are receiving home IV antibiotic therapy for a PEx will also be eligible as they return to clinic weekly according to standard clinical care.

During the course of their admission, for patients willing to provide samples, the following specimens will be collected for the bio-repository for future analysis. All biological samples will be labeled using the patient's study number. The patient's study number will be linked to patient identifiers only by a master list that is managed by the PI and the Research Coordinator of this study:

- Sputum for inflammatory markers— For patients who can produce sputum, we will collect a research sputum sample which will be immediately sent on ice to collaborator Dr. Hartmut Grasemann's laboratory for processing for inflammatory markers and stored for future examination of biomarkers according to the standardized protocols developed by the Therapeutic Drug Network, US CF Foundation.

- Serum for biomarkers—Blood collection for future studies will occur at the time of clinical bloodwork or if a patient chooses to provide research blood separately. A total of 8 mls of blood will be drawn in two lavender top EDTA tubes in the phlebotomy clinic, stored on site and subsequently delivered to Dr. Bradley Quon's laboratory at St. Paul's Hospital (a sub-site for the study) for final processing and storage.

Randomization and distribution of drug/placebo

Randomization (1:1) will be done through central allocation of a block-randomization schedule prepared through a computer-generated random listing. Randomization will be done by the study data analyst at the Hospital for Sick Children. The analyst will be the only member of the investigative team aware of the randomization assignment and will not be directly doing the statistical analysis. He/she will not be involved in the care of the study patients or the measurement of outcomes in the study. No other research staff nor patients nor physicians will be aware of the randomization assignment. The pharmacist at each study site will call the analyst for randomization.

Once the patient is randomized, the analyst will inform the research pharmacy at each study site and they will distribute either prednisone or placebo (lactose, packaged in #0 capsules of identical appearance) to the hospital ward or the CF clinic according to dosing Table 1. Physicians and patients will be blinded to the treatment, and placebo will be produced to look identical to the treatment drug. The microbiology lab and pulmonary function lab will also be blinded to the treatment allocation. Compliance will be measured by nurses for hospitalized patients and by asking patients to return any unused medication for patients receiving homecare IV antibiotics.

Safety monitoring

Patients who are most at risk of developing side effects secondary to corticosteroid use will be excluded based on the above exclusion criteria. Subjects will be monitored with measurements of urine glucose on day 7, day 10 ($\pm$ 2 days) and on day 14 of antibiotic treatment while on prednisone (or placebo). If the urine glucose is > 2+, a serum glucose measurement will be performed. All the information will be provided to the treating physicians; they can decide at any time to withdraw their patient and request unblinding of the treatment allocation. The study will be monitored by a Data Safety Monitoring Board (DSMB) (Appendix 1.2-Data Safety Monitoring Plan).

E. Study outcomes:

Primary outcome:

The proportion of subjects who achieve >90% of their baseline FEV_1 % predicted at day 14 of IV antibiotic treatment for a PEx in each treatment arm. Recovery of lung function will be defined as previously as an FEV_1 of >90% of baseline FEV_1 at day 14 of antibiotic therapy (3). Baseline lung function will be defined as the best FEV_1 in the 6 months prior to the PEx (or in the 12 months prior to PEx if there are no measurements in the 6 months prior). Lung function measurements will be performed according to American Thoracic Society criteria (42) and absolute values will be converted to percent predicted using the Global Lung Function Initiative-2012 reference equation (43).

Secondary outcomes:

i) The proportion of subjects who achieve >90% of their baseline FEV_1 % predicted at the 1 month follow up visit after the PEx in each treatment arm.

Patients are typically seen in CF clinic 1 month following discharge from hospital for a PEx and pulmonary function tests are done at every CF clinic visit as part of standard clinical care. Lung function recovery will be defined as per the Primary Outcome.

ii) The absolute change in pulmonary function tests, including forced expiratory volume in 1 second (FEV_1), forced vital capacity (FVC) and maximal mid-expiratory flow rate (FEF_{25-75}), measured at day 7, day 14 of IV antibiotic treatment and the 1 month visit in each treatment arm.

iii) The change in the cumulative score on a quality of life questionnaire measured by the CFQ-R and the CFRSD at day 0, day 7, day 14 of IV antibiotic treatment and the 1 month follow up visit in each treatment arm.

The questionnaire that will be used is the Cystic Fibrosis Questionnaire-Revised (CFQ-R) Respiratory domain (44). The CFQ-R is a disease-specific instrument that measures health-related quality of life for adolescents and adults with CF with a developmentally appropriate version for children. The CFQ-R is a reliable and valid measure of quality of life for individuals with CF and is frequently used in clinical trials to assess the effects of new therapies (45). In addition, subjects will also be asked to complete the CF Respiratory Symptom Diary (CFRSD) on day 0, day 7, day 14 and at the 1 month follow up visit(46). The Chronic Respiratory Infection Symptom Score will be applied to the CFRSD to calculate a severity score from 0-100 (100 being most severe). The CRISS score has been previously shown to be a responsive measure of steroid treatment effect in CF pulmonary exacerbations(47).

iv) The length of hospitalization and duration of IV antibiotic treatment in each treatment arm.

Length of hospitalization (number of days in hospital) and duration of IV antibiotic treatment are clinically relevant and patient-meaningful outcomes that will be calculated for each patient and compared between treatment groups.

v) The time to subsequent PEx in each treatment arm.
Based on our previous study, we know that the time between exacerbations is one of the most important determinants of overall lung function decline in an individual with CF (6). Time to subsequent pulmonary exacerbation will be calculated from the last day of IV antibiotic therapy of the randomized exacerbation to the first day of re-starting IV antibiotic treatment for a new pulmonary exacerbation (minimum 21 days between events). We will compare the average (or median if data not normally distributed) duration between treatment groups. We will follow up patients for subsequent exacerbations for 1 year following their randomized exacerbation.

vi) The number of adverse events and withdrawals from study recorded in each treatment arm.
Subjects will be monitored for adverse events during hospitalization by the research coordinator and will be instructed to call the research coordinator with reports of adverse events once discharged. Adverse events will also be assessed at the 1 month follow up visit. Assessment of adverse events will include any mild events (transient event, no treatment change, e.g. cough), moderate events (treatment discontinued, e.g. hypertension, hyperglycemia) and severe events (gastrointestinal bleed, hospitalization or death). In the 1 year follow up, new infections with *P. aeruginosa*, *M. abscessus* or *B. cepacia* complex will be recorded in each group. Adverse events will be reported to the Research Ethics Board of each study site and to the study's Data Safety Monitoring Board (DSMB). Withdrawals from the study and the reason for it will also be recorded as an outcome measure.

vii) The change in the measurement of sputum (neutrophil count, neutrophil elastase and IL-8 levels) and serum (including neutrophil count and hsCRP) inflammatory markers measured at day 0, day 7, day 14 of IV antibiotic treatment and the 1 month follow up visit in each treatment arm.
The anti-inflammatory effects of steroid therapy will be examined in both the lung and serum. Sputum will be collected at all study sites and immediately shipped on ice to collaborator Dr Hartmut Grasemann's laboratory for processing as previously described according the standardized protocols developed by the Therapeutic Drug Network, US CF Foundation (48). Serum samples will also be collected at these time points for neutrophil count and CRP measurements through the hospital laboratory as part of clinical care and an aliquot will be banked for future serum biomarker studies at each study site (49-50).

F. Sample size calculation

To calculate the sample size for the primary outcome, we did an analysis using the Toronto CF Database of all the PExs treated with IV antibiotics from 1997-2012 at the Hospital for Sick Children and St Michael's Hospital (almost 3,000 PEx). When examining PExs for which all

pulmonary function data points were available (n=228), we determined that 50% of patients will recover >90% of their baseline FEV_1 at day 7 of antibiotic treatment, with the remaining 50% available for randomization to either prednisone or placebo treatment. Of the 50% who do not recover >90% of baseline FEV_1 at day 7, 30% of these will go on to recover FEV_1 at day 14 of antibiotic treatment. We therefore estimated a 30% placebo response rate. Using these estimates, we calculated the sample size (**power two proportions** command in Stata 13.1) for the comparison of two proportions (Table 3).

For this study, we are aiming for a clinically significant treatment effect size of approximately two and a half-fold (30% response in the placebo arm vs 60% in the treatment arm). In order to have 80% power (at a significance level of 5%) we would need a sample size of **84** (**42/arm**). Estimating a 50% participation rate and a 20% drop out rate, we will need to approach 210 patients.

Since 50% of all patients who present with a PEx recover their lung function by day 7 of antibiotic therapy, 30% of patients will not experience more than a 10% relative drop in FEV_1 (inclusion criteria) and 10% of CF patients have diabetes and are on insulin which is an exclusion criteria (CPDR 2012 and personal communication-CF clinic directors), we will need to screen approximately 650 exacerbation events to approach 210 patients and enroll 105 patients into the study (Table 3). Over the 3 year study period, we expect to enroll enough study partcipants from all study sites) which will give us a sufficient population from which to enroll our necessary study sample size. We have had face to face meetings and discussions with our team of co-investigators who have been involved in the design and are fully committed to participating in this study. We have successfully collaborated with this team of researchers in previous clinical trials (10, 51) and fully expect we will achieve our current stated aims.

G. Statistical analyses

When target sample size is reached, the study statistician (who will not be involved in the conduct of the study) will unblind the treatment allocation (identify who is in group A vs B but not identify the groups themselves as active drug or placebo) and estimate the treatment effect between the two groups based on the primary outcome (intention-to-treat). If there is a statistically significant treatment effect ($p<0.05$), the study statistician will do the following statistical analyses. For the primary outcome we will compare the proportion of randomized patients that responded at day 14 of therapy in the placebo arm compared with the treatment arm using a two sample test for proportions. We will perform intent-to-treat analysis according to the treatment arm subjects were randomized to. We will also perform a per-protocol analysis based on subjects who completed the 7 days of prednisone or placebo as per protocol. The same analysis will be performed for the proportion responding to therapy at 1 month. Logistic regression analysis will then be performed to adjust for potential confounders, such as age, gender, *Pseudomonas aeruginosa* or methicillin-resistant *Staphylococcus aureus* (MRSA) infection and lower FEV_1, as these factors have been associated with failure to recover lung function after an exacerbation (3, 25). For the additional secondary outcomes, absolute change in pulmonary function tests, length of hospitalizations and change in sputum measurements,

we will use parametric (t-test), or non-parametric (Mann-Whitney) two sample comparison test. Linear regression analysis will be then performed to adjust for potential confounders, as listed above. Time to subsequent exacerbation will be compared using a Cox proportional hazard model. Potential confounders will be adjusted for in the Cox-proportional hazard model. All secondary outcomes will be analyzed both as intention-to-treat and per-protocol. All outcomes will be obtained for all subjects irrespective of whether they completed the study per protocol. In the case of missing data, we will perform the analysis on complete (complete case analysis). To determine whether diabetic patients have a different response compared to non-diabetic patients, we will perform a sensitivity analysis comparing return to baseline lung function in diabetics vs non-diabetics according to treatment group.
If when the statistician estimates the treatment effect between the two groups (above), there is NOT a statistically significant treatment effect ($p>0.05$), the statistician will re-calculate the sample size based on the observed data from 84 participants, in order to determine what sample size is required to observe a statistically significant effect. This data will be provided to the DSMB who will decide whether or not to continue the study, based on feasibility and the clinical relevance of the treatment effect size. Appendix 1.1 includes detailed plans for the analysis of the extension study, which will be designed as an adaptive trial (52).

H. Potential Challenges and Alternative Approaches

The main challenge of this study is the large estimated treatment effect size. The future adaptive trial design, however, allows us the flexibility to calculate the necessary sample size based on observed data if the difference between groups is clinically but not statistically significant. These data will inform the decision whether to continue the trial with the addition of other centers in either Canada or the United States (Deliverables and Future Directions). Another challenge is the number of potential confounders influencing lung function recovery. We have designed this trial to be pragmatic, that is, reflective of real life conditions, as it is not possible to stratify based on all the variables that could affect response such as concomitant medications, hospital vs home IV therapy, choice and duration of antibiotic therapy. Therefore, we will collect all this information and sensitivity analyses will be done to determine whether these variables affect the results.

I. Personnel Requirements and Study Timeline:

We will require a research coordinator at each site over the study period (see Budget).

J. Knowledge Translation (KT):

We have developed a KT plan with the assistance of Dr Melanie Barwick, scientific director of KT at the Hospital for Sick Children (Appendix). Our target audiences for the results of this study are CF clinicians, patients and researchers. We will partner with CF Canada to implement the following KT strategies for disseminating our results: publishing in an open-access journal for all audiences, presenting at national (Canadian Clinics conference) and international CF

conferences (North American CF conference, European CF conference) and talking to patient communities at CF Chapter meetings. We will also develop a plain language summary for dissemination at conferences, on the CF Canada website and through CF newsletters. Through our strong collaboration with CF centers across Canada and with the assistance of CF Canada, we will disseminate results through teleconferences and other regular encounters with Canadian CF clinic directors.

K. Deliverables and Future Directions:

If the study subjects in this trial treated with oral prednisone have significantly better recovery of FEV_1 after antibiotic treatment for a pulmonary exacerbation compared to controls, without suffering more adverse effects, we will have identified an effective, available and inexpensive treatment option that can be readily instituted into clinical practice. However, if the observed differences are still clinically meaningful but not statistically significant, we will determine the required sample size and if feasible, expand the trial into other Canadian or US CF sites. We have also applied to the US CF Foundation for funding of this study and to gauge their interest in participation. In addition, this large, multi-center interventional study will serve as a platform for sub-studies of pulmonary exacerbations. Within our consortium of Canadian CF researchers, we have formed a sub-group of investigators to examine biomarkers of exacerbations using stored serum and sputum samples collected as part of this trial. We therefore expect this project to significantly expand our knowledge of CF pulmonary exacerbations. Our ultimate goal is to improve lung function recovery following these events in order to extend the survival of individuals with CF.

L. References

1. Gibson RL, Burns JL, Ramsey BW. Pathophysiology and management of pulmonary infections in cystic fibrosis. Am J Respir Crit Care Med. 2003;168(8):918-51.
2. Flume PA, Mogayzel PJ, Jr., Robinson KA, Goss CH, Rosenblatt RL, Kuhn RJ, et al. Cystic fibrosis pulmonary guidelines: treatment of pulmonary exacerbations. Am J Respir Crit Care Med. 2009;180(9):802-8.
3. Sanders DB, Bittner RC, Rosenfeld M, Hoffman LR, Redding GJ, Goss CH. Failure to recover to baseline pulmonary function after cystic fibrosis pulmonary exacerbation. Am J Respir Crit Care Med. 2010;182(5):627-32.
4. Waters V, Atenafu EG, Salazar JG, Lu A, Yau Y, Matukas L, et al. Chronic *Stenotrophomonas maltophilia* infection and exacerbation outcomes in cystic fibrosis. J Cyst Fibros. 2012;11(1):8-13.
5. Waters V, Stanojevic S, Klingel M, Chiang J, Sonneveld N, Kukkar R, et al. Prolongation of antibiotic treatment for cystic fibrosis pulmonary exacerbations. J Cyst Fibros. 2015;14(6):770-6.
6. Waters V, Stanojevic S, Atenafu EG, Lu A, Yau Y, Tullis E, et al. Effect of pulmonary exacerbations on long-term lung function decline in cystic fibrosis. Eur Respir J. 2012;40(1):61-6.
7. Corey M, Farewell V. Determinants of mortality from cystic fibrosis in Canada, 1970-1989. Am J Epidemiol. 1996;143(10):1007-17.
8. Ordonez CL, Henig NR, Mayer-Hamblett N, Accurso FJ, Burns JL, Chmiel JF, et al. Inflammatory and microbiologic markers in induced sputum after intravenous antibiotics in cystic fibrosis. Am J Respir Crit Care Med. 2003;168(12):1471-5.
9. Mayer-Hamblett N, Aitken ML, Accurso FJ, Kronmal RA, Konstan MW, Burns JL, et al. Association between Pulmonary Function and Sputum Biomarkers in Cystic Fibrosis. Am J Respir Crit Care Med. 2007;175(8):822-8.
10. Waters VJ, Stanojevic S, Sonneveld N, Klingel M, Grasemann H, Yau YC, et al. Factors associated with response to treatment of pulmonary exacerbations in cystic fibrosis patients. J Cyst Fibros. 2015;14(6):755-62.
11. Parrillo JE, Fauci AS. Mechanisms of glucocorticoid action on immune processes. Annu Rev Pharmacol Toxicol. 1979;19:179-201.
12. Cheng K, Ashby D, Smyth RL. Oral steroids for long-term use in cystic fibrosis. Cochrane Database Syst Rev. 2013;6:CD000407.
13. Dovey M, Aitken ML, Emerson J, McNamara S, Waltz DA, Gibson RL. Oral corticosteroid therapy in cystic fibrosis patients hospitalized for pulmonary exacerbation: a pilot study. Chest. 2007;132(4):1212-8.
14. Rosenfeld M, Emerson J, Williams-Warren J, Pepe M, Smith A, Montgomery AB, et al. Defining a pulmonary exacerbation in cystic fibrosis. J Pediatr. 2001;139(3):359-65.
15. Ellaffi M, Vinsonneau C, Coste J, Hubert D, Burgel PR, Dhainaut JF, et al. One-year outcome after severe pulmonary exacerbation in adults with cystic fibrosis. Am J Respir Crit Care Med. 2005;171(2):158-64.

16. Goss CH, Burns JL. Exacerbations in cystic fibrosis. 1: Epidemiology and pathogenesis. Thorax. 2007;62(4):360-7.
17. Konstan MW, Morgan WJ, Butler SM, Pasta DJ, Craib ML, Silva SJ, et al. Risk factors for rate of decline in forced expiratory volume in one second in children and adolescents with cystic fibrosis. J Pediatr. 2007;151(2):134-9, 9 e1.
18. Emerson J, Rosenfeld M, McNamara S, Ramsey B, Gibson RL. *Pseudomonas aeruginosa* and other predictors of mortality and morbidity in young children with cystic fibrosis. Pediatr Pulmonol. 2002;34(2):91-100.
19. Marshall BC, Butler SM, Stoddard M, Moran AM, Liou TG, Morgan WJ. Epidemiology of cystic fibrosis-related diabetes. J Pediatr. 2005;146(5):681-7.
20. Dobbin CJ, Bartlett D, Melehan K, Grunstein RR, Bye PT. The effect of infective exacerbations on sleep and neurobehavioral function in cystic fibrosis. Am J Respir Crit Care Med. 2005;172(1):99-104.
21. Britto MT, Kotagal UR, Hornung RW, Atherton HD, Tsevat J, Wilmott RW. Impact of recent pulmonary exacerbations on quality of life in patients with cystic fibrosis. Chest. 2002;121(1):64-72.
22. VanDevanter DR, O'Riordan MA, Blumer JL, Konstan MW. Assessing time to pulmonary function benefit following antibiotic treatment of acute cystic fibrosis exacerbations. Respir Res. 2010;11:137. PMCID: 2959026.
23. Liou TG, Adler FR, Fitzsimmons SC, Cahill BC, Hibbs JR, Marshall BC. Predictive 5-year survivorship model of cystic fibrosis. Am J Epidemiol. 2001;153(4):345-52.
24. Sanders DB, Hoffman LR, Emerson J, Gibson RL, Rosenfeld M, Redding GJ, et al. Return of FEV_1 after pulmonary exacerbation in children with cystic fibrosis. Pediatr Pulmonol. 2010;45(2):127-34.
25. Parkins MD, Rendall JC, Elborn JS. Incidence and risk factors for pulmonary exacerbation treatment failures in patients with cystic fibrosis chronically infected with *Pseudomonas aeruginosa*. Chest. 2012;141(2):485-93.
26. Regelmann WE, Elliott GR, Warwick WJ, Clawson CC. Reduction of sputum *Pseudomonas aeruginosa* density by antibiotics improves lung function in cystic fibrosis more than do bronchodilators and chest physiotherapy alone. Am Rev Respir Dis. 1990;141(4 Pt 1):914-21.
27. Weber AJ, Soong G, Bryan R, Saba S, Prince A. Activation of NF-kappaB in airway epithelial cells is dependent on CFTR trafficking and Cl- channel function. Am J Physiol Lung Cell Mol Physiol. 2001;281(1):L71-8.
28. Ratner AJ, Bryan R, Weber A, Nguyen S, Barnes D, Pitt A, et al. Cystic fibrosis pathogens activate Ca2+-dependent mitogen-activated protein kinase signaling pathways in airway epithelial cells. J Biol Chem. 2001;276(22):19267-75.
29. Sagel SD, Wagner BD, Anthony MM, Emmett P, Zemanick ET. Sputum biomarkers of inflammation and lung function decline in children with cystic fibrosis. Am J Respir Crit Care Med. 2012;186(9):857-65. PMCID: 3530222.
30. Sly PD, Gangell CL, Chen L, Ware RS, Ranganathan S, Mott LS, et al. Risk factors for bronchiectasis in children with cystic fibrosis. N Engl J Med. 2013;368(21):1963-70.

31. McElvaney NG, Hubbard RC, Birrer P, Chernick MS, Caplan DB, Frank MM, et al. Aerosol alpha 1-antitrypsin treatment for cystic fibrosis. Lancet. 1991;337(8738):392-4.
32. Lands LC, Stanojevic S. Oral non-steroidal anti-inflammatory drug therapy for lung disease in cystic fibrosis. Cochrane Database Syst Rev. 2013;6:CD001505.
33. Auerbach HS, Williams M, Kirkpatrick JA, Colten HR. Alternate-day prednisone reduces morbidity and improves pulmonary function in cystic fibrosis. Lancet. 1985;2(8457):686-8.
34. Eigen H, Rosenstein BJ, FitzSimmons S, Schidlow DV. A multicenter study of alternate-day prednisone therapy in patients with cystic fibrosis. Cystic Fibrosis Foundation Prednisone Trial Group. J Pediatr. 1995;126(4):515-23.
35. Greally P, Hussain MJ, Vergani D, Price JF. Interleukin-1 alpha, soluble interleukin-2 receptor, and IgG concentrations in cystic fibrosis treated with prednisolone. Arch Dis Child. 1994;71(1):35-9. PMCID: 1029909.
36. Callahan CM, Dittus RS, Katz BP. Oral corticosteroid therapy for patients with stable chronic obstructive pulmonary disease. A meta-analysis. Ann Intern Med. 1991;114(3):216-23.
37. Davies L, Angus RM, Calverley PM. Oral corticosteroids in patients admitted to hospital with exacerbations of chronic obstructive pulmonary disease: a prospective randomised controlled trial. Lancet. 1999;354(9177):456-60.
38. Tepper RS, Eigen H, Stevens J, Angelicchio C, Kisling J, Ambrosius W, et al. Lower respiratory illness in infants and young children with cystic fibrosis: evaluation of treatment with intravenous hydrocortisone. Pediatr Pulmonol. 1997;24(1):48-51.
39. Ghdifan S, Couderc L, Michelet I, Leguillon C, Masseline B, Marguet C. Bolus methylprednisolone efficacy for uncontrolled exacerbation of cystic fibrosis in children. Pediatrics. 2010;125(5):e1259-64.
40. Hester KL, Powell T, Downey DG, Elborn JS, Jarad NA. Glucocorticoids as an adjuvant treatment to intravenous antibiotics for cystic fibrosis pulmonary exacerbations: a UK Survey. J Cyst Fibros. 2007;6(4):311-3.
41. Waters V, Yau Y, Prasad S, Lu A, Atenafu E, Crandall I, et al. *Stenotrophomonas maltophilia* in cystic fibrosis: serologic response and effect on lung disease. Am J Respir Crit Care Med. 2011;183(5):635-40.
42. Miller MR, Hankinson J, Brusasco V, Burgos F, Casaburi R, Coates A, et al. Standardisation of spirometry. Eur Respir J. 2005;26(2):319-38.
43. Quanjer PH, Stanojevic S, Cole TJ, Baur X, Hall GL, Culver BH, et al. Multi-ethnic reference values for spirometry for the 3-95-yr age range: the global lung function 2012 equations. Eur Respir J. 2012;40(6):1324-43.
44. 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(4):2347-54.
45. Saiman L, Marshall BC, Mayer-Hamblett N, Burns JL, Quittner AL, Cibene DA, et al. Azithromycin in patients with cystic fibrosis chronically infected with *Pseudomonas aeruginosa*: a randomized controlled trial. Jama. 2003;290(13):1749-56.
46. Goss CH, Edwards TC, Ramsey BW, Aitken ML, Patrick DL. Patient-reported respiratory symptoms in cystic fibrosis. J Cyst Fibros. 2009;8(4):245-52.

47. Heltshe SL, Goss, C.H., Thompson V, Sagel S.D., Sanders D.B., Marshall B.C., Flume P.A. Short-term and long-term response to pulmonary exacerbation treatment in cystic fibrosis. Thorax. 2015.
48. Sagel SD, Kapsner R, Osberg I, Sontag MK, Accurso FJ. Airway inflammation in children with cystic fibrosis and healthy children assessed by sputum induction. Am J Respir Crit Care Med. 2001;164(8 Pt 1):1425-31.
49. Ratjen F, Saiman L, Mayer-Hamblett N, Lands LC, Kloster M, Thompson V, et al. Effect of azithromycin on systemic markers of inflammation in patients with cystic fibrosis uninfected with *Pseudomonas aeruginosa*. Chest. 2012;142(5):1259-66. PMCID: 3610595.
50. Sagel SD HS, Hamblett N, Emmett P, Hencken K, Chmiel J, Goss C, Montgomery G, Nasr S, Perkett E, Saavedra M, Slovis B., editor. Changes in plasma biomarkers in CF pulmonary exacerbations. North American CF Conference; 2010; Baltimore, MD: Pediatric Pulmonology.
51. Yau YC, Ratjen F, Tullis E, Wilcox P, Freitag A, Chilvers M, et al. Randomized controlled trial of biofilm antimicrobial susceptibility testing in cystic fibrosis patients. J Cyst Fibros. 2015;14(2):262-6.
52. Fodd and Dru Administration. Adaptive Design Clinical Trials for Drugs and Biologics. Silver Spring, Maryland: Food and Drug Administration; 2010.
53. Chow SC, Chang M. Adaptive design methods in clinical trials - a review. Orphanet J Rare Dis. 2008;3:11. PMCID: 2422839.
54. Jenkins BA, Glenn LL. Variability of FEV1 and criterion for acute pulmonary exacerbation. Front Pediatr. 2014;2:114. PMCID: 4201084.
55. Blood glucose monitoring [database on the Internet]. National Institute of Diabetes and Digestive and Kidney Diseases. 2016.
56. Blood pressure measures by age and height percentile [database on the Internet]. National Heart, Lung and Blood Institute. 2016.

Table 1. Prednisone doses by weight for enrolled study subjects

	Morning Dose	**Evening Dose**
10.0-14.5 kg	10	10
14.6-19.5 kg	20	10
19.6-24.5 kg	20	20
24.6-29.5 kg	30*	20
>29.6 kg	30*	30*

* 30 mg dose administered as 1 capsule of #0 capsule containing 6 x 5 mg (30 mg) prednisone tablets. Other doses will be administered as capsules of #0 capsule containing 2 x 5 mg (10 mg) prednisone tablets.

Table 2. Study Design Schedule

Study Design Schedule	Day 0	Day 7	Day 10	Day 14	1 Month Follow Up
Standard Clinical Assessments					
PFT	X	X		X	X
Medical History	X				
Height & Weight	X				
Study Drug					
Drug Dispensed		X*			
Drug Accountability				X*	
Study Safety Measures					
Urine Dip		X*	X*	X*	
Blood Pressure		X*	X*	X*	
Adverse Events				X	X
Bio Banking					
Serum for banking	X	X		X	X
Sputum for banking	X	X		X	X
Questionnaires					
CFQ-R	X	X		X	X
CFRSD-CRISS	X	X		X	X

*For randomized patients ONLY

Table 3. Required sample size per arm to detect FEV1 recovery at day 14 of antibiotic treatment

Response in the placebo arm	Response in the treatment arm	No. of patients needed/ arm	Total no. of Pexs needed	Total no. of Pexs needed to screen
30%	45%	163	326	2,533
30%	50%	93	186	1,445
30%	55%	61	122	948
30%	60%	42	84	652

Figure 1. Responders VS Non Responders

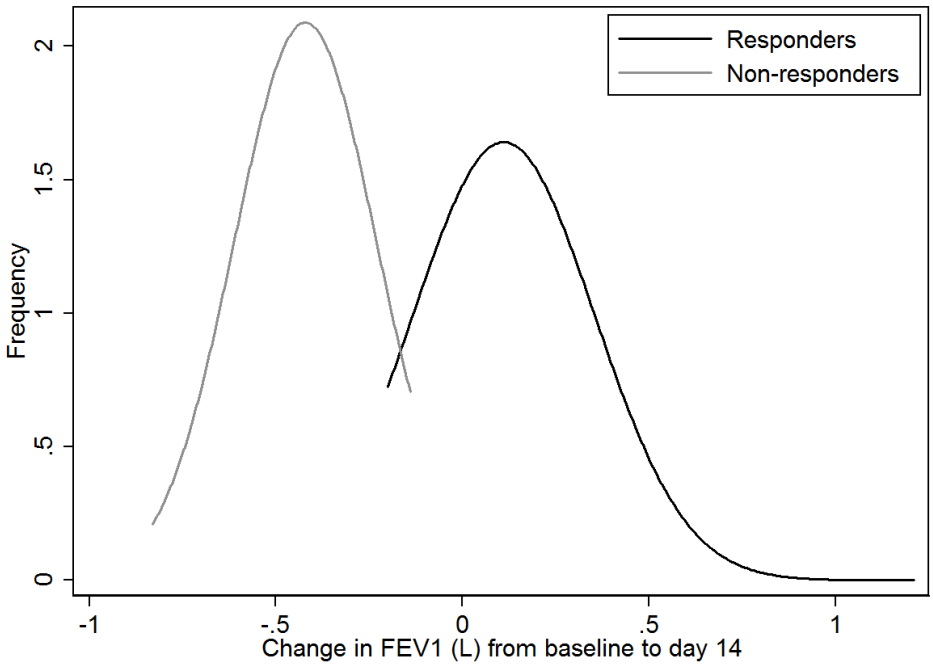
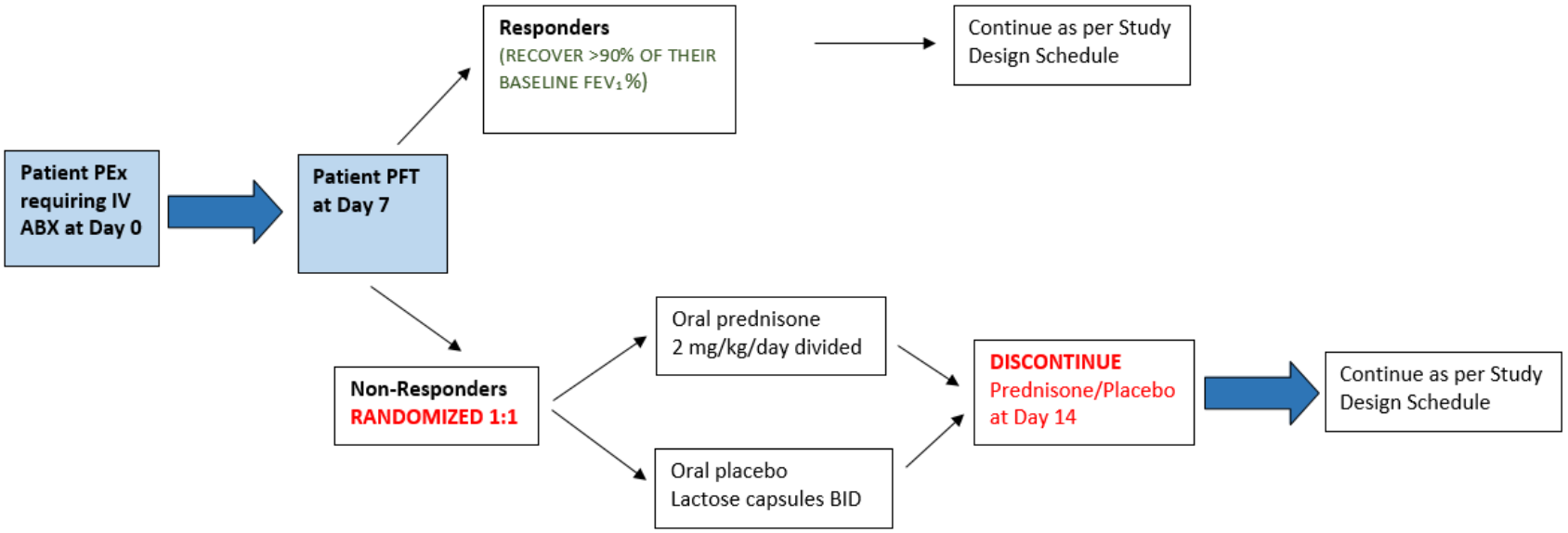


Figure 1. Change in forced expiratory volume in 1 second (FEV_1) measured in liters from baseline to day 14 of antibiotic treatment in patients who recovered >90% of their lung function (responders) compared to those who did not (non-responders) represented as distribution graphs.

Figure 2. Randomization Flow Chart



Appendix 1.1

Adaptive Study Design

We propose to complete the current study as planned until the target sample size is reached. Statistician S.S. will then unblind the treatment allocation (identify who is in group A vs B but not identify the groups themselves as active drug or placebo) and analyze the data to determine whether a significant treatment effect was observed. If the p value is significant ($p<0.05$) S.S. will complete the analysis as outlined in the Statistical analyses section.

If the p value is not significant ($p>0.05$) ,S.S. will re-calculate the sample size based on the observed data from 84 participants, in order to determine what sample size is required to observe a statistically significant effect. This data will be provided to the DSMB who will decide whether or not to continue the study, based on feasibility and the clinical relevance of the treatment effect size. If the DSMB decides it is feasible and the treatment effect is clinically relevant, the study will be continued until the new re-calculated sample size is reached and all study site investigators and personnel will remain blinded to treatment allocation. Additional study sites may be added and further funding may be sought for the extension study. The data collected as part of this current study will be used in the extension study. The extension study will be designed as an adaptive trial (52). Adaptive trial designs are study designs that allow for adaptations to be made to the trial after its initiation without undermining the validity and integrity of the results (53). They are increasingly being used to study rare diseases in which there are limited numbers of subjects and trial durations are frequently very long. The significance level (i.e. p value) of the extension study will be adapted to adjust for the fact that unblinded analysis was conducted at the end of this current study(54).

Appendix 1.2

Data Safety Monitoring Plan

Although subjects will be randomized to either oral prednisone or placebo, no investigational drug will be tested in this trial. Upon funding of the study, the study will be submitted to the Research Ethics Board (REB) at the Hospital for Sick Children and all other study sub-sites). REB comments and amendments will be distributed to all sites in order to ensure that the approved study protocol is identical at all sites.

Assessment of Risk

The assessment of risk is deemed to be **moderate** in this trial due to the known side effects of prednisone (eg. hyperglycemia, hypertension, gastrointestinal bleeding). Given that we will be treating subjects with a short course of oral prednisone (7 days), we do not expect to observe the long-term side effects noted with more prolonged steroid use (eg. growth retardation, osteopenia/avascular necrosis). Although certain subjects will receive placebo, they will still be receiving antibiotic treatment for the pulmonary exacerbation which is standard of care.

Anticipated Adverse Events and Grading Scale

In the previous study by Dovey et al. 12 CF subjects received oral prednisone (2 mg/kg/day x 5 days) and 12 subjects received placebo during treatment for a pulmonary exacerbation (13). The most common adverse events were glucosuria, hyperglycemia and hypertension. Hypertension was reported for 2 subjects in each arm, with one prednisone treated subject withdrawn on day 2 due to this. Glucosuria was reported among 6 subjects in the prednisone arm and 1 in the placebo arm (p=0.03); 2 of the subjects in the prednisone arm with glucosuria also had hyperglycemia (1 of these was withdrawn due to this). Additional adverse events included headache, restlessness, heartburn, nausea/epigastric pain, swelling, increased thirst and increased urination. There were no serious events in either treatment arm.

We therefore anticipate the most common adverse events to be hyperglycemia (random serum glucose > 200 mg/dL or 13 mmol/L)(55), glucosuria (> 1+ urine glucose) and hypertension (defined as the 99th percentile of blood pressure measurement according to gender, age and

height using the NIH guidelines(56)) and will monitor for these adverse events (see below- Safety Monitoring Plan). In addition, all untoward medical occurrences, whether or not deemed causally related to study participation, will be recorded and site investigators will be asked to assess both severity and whether or not the adverse event was related to study participation. We will use the following grading scale to assess adverse events:

Adverse Event Category	Description
Mild	transient event, no treatment change, e.g. glucosuria
Moderate	treatment discontinued e.g. severe/sustained hypertension or hyperglycemia
Severe	severe: death, unexpected hospitalization

Given that subjects will be randomized at the time of hospitalization for a pulmonary exacerbation, we will monitor the time to subsequent exacerbation. If we observe a shorter time to subsequent exacerbation in one of the treatment arms, this will be reported to the DSMB.

Reporting of AEs

All adverse events will be recorded by the research coordinator and reported to the study site investigator as well as the PI (Dr Waters), who will oversee close and continuous monitoring for safety concerns throughout the trial. All serious adverse events will be reported to Health Canada, based on the Health Canada Guidance: Adverse Drug Reactions (ADRs) Reporting Criteria. All serious and unexpected adverse events will also be reported to the REB at each study site as well the REB at the Hospital for Sick Children (as study sponsor) within 48 hours of their occurrence. Adverse events will also be reported to the Data Safety Monitoring Board (DSMB) (see below) in the form of regular reports submitted every 6 months. Serious adverse events will be reported to the DSMB within 48 hours.

Safety Monitoring Plan

Hospitalized subjects will be monitored on day 7, day 10 (+/- 2 days) and on day 14 with urine dip glucose measurements while on prednisone (or placebo). If the urine glucose is > 2+, a serum glucose measurement will be performed. All the information will be provided to the treating physicians; they can decide at any time to withdraw their patient and request unblinding of the treatment allocation. To unblind a subject, the research coordinator or physician will call the PI (Dr Valerie Waters) at 416-813-7654 ext 204541 or page 416-246-4487

who will then request unblinding from the study data analyst. Additionally, subjects will also have a blood pressure done on day 7, day 10 (+/- 2 days) and on day 14 as part of their vital signs assessment. The research coordinator will also visit the subject daily to record all adverse events.

For patients who receive their treatment at home with home intravenous antibiotic therapy, they will be taught how to test their urine glucose (or parents in the case of children) on day 7, day 10 (+/- 2 days) and on day 14 and told to call the research coordinator with the result and report their adverse events. If the urine glucose is > 2+, they will be asked to come to clinic to have a serum glucose drawn and reported to the research coordinator as well as responsible physician. The home care nurse (or via home ambulatory machine) will also do the blood pressure check on day 7, day 10 (+/- 2 days) and on day 14 and report it to the research coordinator as well as responsible physician. The decision to withdraw a subject based on adverse events of hypertension or hyperglycemia will be made by the treating physician. Once the 7 days of treatment (prednisone or placebo) are completed, the subjects will be instructed to call the research coordinator with serious adverse events such as a re-hospitalization for a pulmonary exacerbation. At the follow up clinic visit, the research coordinator will once again review adverse events with the subject.

Diabetic patients on insulin may have disruption of their glucose control while on prednisone. Therefore, as per standard clinical care, diabetic patients on insulin will do daily blood glucose monitoring and adjust their insulin accordingly as per published guidelines{Committee, 2018 #83}. This will be performed and monitored by the clinical team and treating physicians as per standard of care. Diabetic patients for whom glucose control has become unmanageable will be discontinued from the study as per the treating physician's assessment.

Safety Reviews

The conduct of the study will be monitored by an independent Data Safety Monitoring Board. We will appoint members, including CF physicians, from Canadian CF centers that are not participating in this trial. In addition, we will also appoint an independent statistician (not involved in this trial) to be on the DSMB. The DSMD will review safety reports on a semi-annual basis, or pre their requested frequency. Under the direction of the statistician on the DSMB, an interim analysis will be performed at the mid-way point of the trial (when 42 patients have been randomized (minimum 21 in each arm) and the primary outcome has been collected) to assess for safety. The DSMB can request unblinding of the data at any time. Serious adverse events will be reported to the DSMB by the PI within 48 hours of their occurrence. In addition,

all serious and unanticipated adverse events will be reported to the local REB as well as the REB at the Hospital for Sick Children with 48 hours of their occurrence, as stated above.