CLINICAL RESEARCH PROJECT

PROTOCOL #11-H-0068
Drug Name: aerosolized cyclosporine A in solution with propylene glycol (CIS)
IND Number: 109,707
Sponsor: Richard Childs, MD

Title: Phase II Trial of Cyclosporine Inhalation Solution (CIS) in Lung Transplant and Hematopoietic Stem Cell Transplant Recipients for Treatment of Bronchiolitis Obliterans

Other identifying words: Lung transplant, Single lung transplant, Double lung transplant, Peripheral blood stem cell transplant, Bronchiolitis obliterans syndrome, myeloablative stem cell transplant, nonmyeloablative stem cell transplant, inhaled cyclosporine

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Subject of Study:	Number	Sex	Age range
Hematopoietic stem cell transplant recipients (group A)	Screen up to 50 to get up to 39	Either	10-80
Lung transplant recipients (Group B) (No longer enrolling)	Screen up to 50 to get up to 39	either	10-80
Total subjects	Screen up to 100 to get up to 78	either	10-80

Project involves ionizing radiation?	Yes
Off site project?	No
Multi-institutional project?	No
DSMB involved	Yes

Precis

Bronchiolitis Obliterans (BO) is an obstructive lung disease that can affect individuals that have undergone a lung or hematopoietic stem cell transplant. BO has been studied most extensively in lung transplant recipients, where it is considered to represent chronic lung rejection. It is the leading cause of death after lung transplant, with mortality rates up to 55%. In hematopoietic stem cell transplantation, BO is thought to be a manifestation of chronic graft-vs-host disease (GVHD). Up to 45% of patients undergoing hematopoietic stem cell transplantation at the NHLBI develop a decline in pulmonary function. Conventional therapy for patients who develop BO consists of augmentation of systemic immunosuppressants. Systemic immunosuppression has limited efficacy for BO and is associated with deleterious consequences including increased risk of infections and decreased graft-versus tumor/leukemia effects.

Recently, cyclosporine inhalation solution (CIS) in solution with propylene glycol has been shown to improve overall survival and chronic rejection-free survival in lung transplant patients.[(8)] These findings suggest targeted delivery of immunosuppressive therapy to the diseased organ warrants further investigation as this may minimize the morbidity associated with systemic immunosuppression. However, there currently exists limited data regarding the overall efficacy of inhaled cyclosporine to treat established BO following lung transplantation. Furthermore, inhaled cyclosporine has not been studied in the treatment of BO following hematopoietic stem cell transplantation.

Here, we propose to evaluate the safety, efficacy, and pharmacodynamics of inhaled cyclosporine for the treatment of BO. Two distinct patient populations will be offered enrollment in this protocol: hematopoietic transplant recipients with BO (group A) and lung transplant recipients with BO (group B). Study participants will receive CIS at an initial dose of 150mg, three times weekly. Patients will undergo dose titration to a maximum dose of 300mg, three times weekly. Drug deposition and pharmacokinetic analyses will be performed at the initiation of treatment. Clinical parameters, including pulmonary function tests, will be measured in addition to laboratory markers of the anti-inflammatory response to CIS. Adverse events associated with treatment will be recorded.

The primary objective is to 1) assess the safety and efficacy of inhaled cyclosporine as a new therapy in hematopoietic transplant patients and lung transplant patients with established BO. Additionally, we seek to promote a better understanding of the pathogenesis of BO in these two transplant groups and to assess the anti-inflammatory effects of inhaled cyclosporine in patients that develop this complication.

The *primary endpoint* of each study group is the best response, FEV_1 improvement or stabilization from study baseline at week 18 for two successive measures, at least 1 week apart, no more than 2 weeks apart. *Secondary endpoints* include the toxicity profile as measured by CTCAE criteria (safety), the study of pharmacokinetics and lung deposition characteristics of inhaled cyclosporine, improvement in high resolution chest CT images, results of peripheral blood and bronchoalveolar cytokine arrays to assess secondary markers of inflammation, and functional capacity measurements using a six-minute walk test.

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1.0 OBJECTIVES

1.1 Primary objective:

To assess the safety and efficacy of inhaled aerosolized cyclosporine A solution in hematopoietic (group A) and lung transplant patients (group B) with bronchiolitis obliterans (BO) or BO syndrome (BOS). As of Amendment M, lung transplant patients are no longer being enrolled on this protocol.

1.2 Secondary Objectives

- To evaluate the lung deposition and pharmacokinetics of this CSA preparation
- To investigate the inflammatory pathways that lead to the development of BO
- To ascertain the anti-inflammatory effects of this CSA preparation ex vivo and in vivo

2.0 BACKGROUND

2.1 Introduction

After the first year post lung transplant, BO is the leading cause of death, accounting for 26% of all deaths between post transplant years 1 and 3[1]. Annually, approximately 15,000 allogeneic hematopoietic stem cell transplants are performed. BO as a manifestation of chronic graft-vs.-host disease (GVHD) can also occur after hematopoietic stem cell transplantation. Up to 45% of patients undergoing hematopoietic stem cell transplantation at the NHLBI develop a decline in pulmonary function, with a significant proportion of these patients being diagnosed with BO[2]. Although similarities and differences in the pathogenesis of BO following lung and hematopoietic stem cell transplantation have not been well characterized, both present with similar clinical manifestations and are treated similarly with increased doses of systemic immunosuppressants. The current treatment for BO is inadequate and involves increasing systemic immunosuppression or immune-modulating therapies, which are of marginal efficacy.

2.2 Hematopoietic Transplant and BO / BOS

Hematopoietic stem cell transplant is an established therapy for the treatment of many life-threatening diseases. However, there are many life-long complications associated with this treatment, especially lung disease. Pulmonary dysfunction after hematopoietic stem cell transplant has been well documented, and is one of the most frequent complications seen after transplant [2, 3, 4]. The incidence of BO after hematopoietic transplant has been estimated between 5 and 60% [4, 10]. The underlying pathophysiology is not completely understood, but it is thought that BO represents a form of chronic GVHD, with the lung epithelium serving as a target for immune-mediated injury induced by transplanted donor T lymphocytes [5]. In concert with this proposed mechanism is the observation that GVHD is the greatest risk factor for the development of BO after stem cell transplant [4]. This is also supported by the very low incidence of BO occurring after autologous stem cell transplant. It has been proposed that additional factors may have a role in the development of BO. Other factors may include recurrent aspiration due to GVHD induced esophagitis, infection, HLA mismatched transplant, use of busulfan or methotrexate, or lung disease prior to transplant [3, 4].

2.3 Lung transplant and BO / BOS

Bronchiolitis obliterans is the characteristic lesion seen on histology in chronic rejection after lung transplant. It is estimated that 50% of lung transplant recipients will develop BOS within five years of transplantation, with mortality rates upwards of 55% [10, 8]. The pathogenesis of BOS development after lung transplant is thought to have immune and non-immune facets. It has been postulated that airway inflammation and epithelial cell injury result in a disordered repair process that leads to the fibroproliferative obstruction of the small airways. The initial injury may result from allo-immune or immune-independent factors or both, but have a common final pathway, BO. Various immune mediated risk factors, which have been proposed as inciting events include: increased HLA mismatching, acute rejection, and lymphocytic bronchitis and bronchiolitis in the absence of infection. Non-immune mediated risk factors include chronic aspiration, infection, especially with CMV, and ischemic graft injury [6,10].

2.4 BO clinical findings and diagnosis

BO is an obstructive lung disease of the small airways. Patients may present with cough, dyspnea, wheezing, or may be asymptomatic at the time of diagnosis. Cough is present in 60-100% of patients and dyspnea in 50-70% [3]. In patients who have received a hematopoietic stem cell transplant, signs and symptoms of chronic graft versus host disease (GVHD) affecting other organs are often present.

The gold standard for the diagnosis of BO is histologic. On histology, there is fibrous proliferation affecting the small airways due to injury and inflammation of the epithelial cells. However, histologic evidence of disease is often difficult to obtain due to the patchy nature of the small airway involvement. As such, a set of clinical diagnostic criteria has been proposed by the NIH working group [7]. When histology is not available, the term Bronchiolitis Obliterans Syndrome (BOS) is used. The diagnostic criteria for bronchiolitis obliterans syndrome is largely based on the presence of obstructive pattern pulmonary function tests with exclusion of other causes of airway obstruction such as asthma, infection or emphysema.

Criteria for the diagnosis of BOS, as proposed by the NIH working group, are met when all of the following are met:

- FEV_1/FVC less than 0.7 and a FEV_1 less than 75% predicted
- Evidence of air trapping on high-resolution chest CT or residual volume ≥120%
- Absence of infection in the respiratory tract
- One or more manifestations of chronic GVHD in another organ system
- A 10% decline from pre-transplant baseline in the FEV_1 and one of the following: FEV1/FVC less than 0.7, evidence of air trapping on CT or residual volume ≥120%

It is generally thought that this definition is too restrictive, and it has on more than one occasion excluded patients with biopsy proven BO. As such, in this study we will require for diagnosis of BOS an FEV_1 less than 75% predicted, absence of infection or other causative etiology, a 10% decline from pre-transplant baseline in the FEV_1 and one of the following: FEV1/FVC less than 0.7, evidence of air trapping on CT or residual volume ≥120%, or in hematopoietic transplant recipients at least one other manifestation of cGVHD involving another organ system. In lung transplant recipients, the same criteria will be used, but the FEV_1 decline required will be greater than 20% compared to best post-transplant measurement.

For a determination of baseline values and diagnosis of BOS, FEV_1 measurements will be determined by the average of 2 sequential measurements taken at least 3 weeks apart up to 6 months apart. Patients can be on a bronchodilator; however, its use will be suspended during pulmonary function testing.

2.5 Current Treatment for BO

Current treatment for BO is inadequate and involves increasing systemic immunosuppression or immune-modulating therapies. In patients who are not already on immunosuppressive therapy, as may be the case in hematopoietic stem cell patients, prednisone is commonly used as an initial treatment. If there is no improvement, immunosuppression with cyclosporine or azathioprine is initiated. While on immunosuppression, prophylaxis for pneumocystis carinii and streptococcus pneumonia should be given [(3)]. Despite these treatments, improvement or stabilization in lung function is noted in only 10-60% of patients [(3, 4, 11)]. Additionally, increased immunosuppression is associated with increased risk of infection, morbidity, and disease relapse.

In lung transplant–associated BO, the response rates to conventional therapy, while varied, are quite disappointing. Generally, only stabilization of FEV1 or a less steep, but continued decline is noted in response to therapy[25]. Recently, the use of azithromycin has shown promise for the treatment of BO in lung transplant recipients. In a study by Gottleib et al, they were able to achieve an improvement, defined as FEV1 increase ≥ 10% in 30% of patients after 6 months of treatment[26]. In hematopoietic stem cell transplant recipients, the response after therapy has been less well characterized. One retrospective review by Sanchez et al demonstrated that with conventional immunosuppression, 30% improved (defined by normalization of PFTs), 30% had a partial response (improvement in PFTs, but not normalization), and 30% had no response, with continued decline in PFTs[27]. A study by Dudek et al, using more aggressive immunosuppressive regimens including ATG, thalidomide, methylprednisolone, cyclosporine, azathioprine, noted an improvement (10% increase in FEV1) rate of 49%, stabilization of 17%, and progression of 34%[28]. A precipitous decline in lung function is often consistent with the natural disease progression[29].

2.6 The Investigational Product: Cyclosporine Inhalation Solution

2.6.1 Background

Cyclosporine Inhalation Solution (cyclosporine A in solution with propylene glycol, CIS) is a novel therapeutic agent in late stage development designed to deliver cyclosporine topically to the airways of lung transplant recipients in combination with standard immunosuppressive regimens. By administering cyclosporine through an inhaled route, augmented immunosuppression is provided directly to the diseased, target tissue while minimizing the systemic exposure and subsequent toxicity of the parent compound.

2.6.2 Pharmacology

CIS consists of the active ingredient cyclosporine (USP) dissolved in propylene glycol. Cyclosporine is among the most common immunosuppressants used for the prevention and treatment of renal, liver, and heart allograft rejection. Cyclosporine has also been demonstrated to extend the graft survival of bone marrow, skin, small intestine, pancreatic, and lung transplants, as well as treats a variety of rheumatologic conditions. Cyclosporine suppresses the immune response by inhibiting evolutionarily conserved signal transduction pathways necessary for T lymphocyte activation and proliferation.

Cyclosporine is an 11 amino acid cyclic peptide derived from a naturally occurring fungus. It is highly lipophilic and can readily cross the cell membrane of T-lymphocytes without receptor activation. Once in the cytoplasm, cyclosporine forms a drug-protein complex with a specific immunophilin (cyclophilin) that can block the function of calcineurin, a T cell serine threonine phosphatase. When blocked, calcineurin cannot facilitate the translocation of a nuclear transcription activation factor (NFAT) from the cytoplasm to the nucleus where it would normally activate transcription of interleukin-2 (IL-2) and other cytokines. As a result, early T cell activation and proliferation are inhibited in a potent manner. By delivering cyclosporine directly to the affected airways, early lymphocyte activation is diminished and intra-graft immune events that can contribute to the development of BO are controlled.

In vivo preclinical pharmacology and pharmacokinetic experiments have not been performed with the current formulation of cyclosporine dissolved in propylene glycol. However, earlier experiments in dogs and rats conducted with cyclosporine dissolved in ethanol revealed that exposure to inhaled cyclosporine was well tolerated and resulted in lung concentrations that were several hundred-fold higher than concentrations in liver, kidney, heart and blood (12). In rat lung transplant models, treatment with inhaled cyclosporine provided protection from allograft rejection compared to untreated animals and led to a dose-dependent reduction in pro-inflammatory cytokine production. Furthermore, the degree of protection was superior to animals treated with cyclosporine via a systemic intramuscular route (13). Similar results were found when inhaled cyclosporine was evaluated in a canine orthotopic lung transplant model (14).

2.6.3 Animal models

Two 28-day inhalational toxicology studies sponsored by Chiron Corporation (Battelle studies N103752 in rats and N103751 in dogs) provide the core data for direct toxicity evaluation (both systemic and local) of both CIS and propylene glycol. These studies were performed with daily dosing at maximally tolerated (rats) or maximally feasible (dogs) doses of CIS. Exposure in these studies was in excess of the maximum clinical exposure, both in terms of overall dose given per day for both cyclosporine and propylene glycol as well as in frequency of dosing (daily in animals vs. three times/week clinically). These studies demonstrated that the local effects of cyclosporine in the respiratory tract should be tolerable in humans at the proposed clinical doses. There was no unexpected systemic toxicity or significant local respiratory system toxicity associated with inhalation exposures up to approximately 2.7 times the maximum human exposure per dose. Mortality associated with the pulmonary effects of CIS was observed in rats at pulmonary deposited doses more than 5 times the maximum human exposure per dose.

Toxicokinetic data generated in these animal studies indicate that after inhalation, lung exposure to cyclosporine is 10 to 20-fold greater than systemic exposure as measured by peak concentrations, trough concentrations, and area under the curve (AUC) analyses. Cyclosporine from the propylene glycol formulation is partially absorbed systemically and does not accumulate with daily repeated dosing. Half-life ($t_{1/2}$) in the blood was consistent with that observed with intravenous (IV) exposure in both rats and dogs. Thus, once the compound reaches the systemic circulation, the disposition should be similar to the disposition of the compound after IV and/or oral administration.

2.6.4 Previous Human Experience

The systemic toxicity of cyclosporine has been well characterized in humans and animals. The most common toxicities associated with systemic exposures include renal dysfunction, hypertension, dyslipidemia, hirsutism, headache, increased risk of infections, and tremor. Additional, less common toxicities include gum hyperplasia, hyperkalemia, thrombocytopenia, and seizure. Hypertension, dyslipidemia, and renal dysfunction are particularly prevalent in the lung transplant population with extended use.

As cyclosporine is partially systemically absorbed following inhalation, the risk of systemic toxicities in addition to the risk of local respiratory tract toxicities was evaluated in repeat dose animal studies.

After demonstrating efficacy in preclinical canine and rodent transplant models, ethanol-based inhaled cyclosporine was tested in a series of open-label protocols evaluating its safety and efficacy in lung transplant recipients with established BO and/or refractory acute rejection (AR). In patients with established BO, the administration of inhaled cyclosporine in conjunction with standard immunosuppressive therapy led to improvement in rejection histology, stabilization of pulmonary function, and improvement in survival compared to contemporary and historical controls [15,16]. In patients with AR that failed to respond to high-dose pulsed methylprednisolone, the administration of inhaled cyclosporine in conjunction with standard immunosuppressive therapy led to improvement in AR histology, improvement in pulmonary function, a decline in pro-inflammatory cytokine production in bronchoalveolar samples, and improvement in survival compared to contemporary control patients [17,18,19]. Although these studies demonstrated the safety of inhaled cyclosporine, the ethanol solvent was associated with substantial respiratory tract irritation and was subsequently changed to propylene glycol.

These open label protocols were followed by a randomized, double-blind, placebo-controlled study of CIS designed to test the efficacy of CIS in preventing lung allograft rejection and improving outcomes when given as prophylactic therapy [8]. A total of 56 subjects were enrolled within 7 to 42 days following their single- or double lung transplant and treated with either CIS or placebo (propylene glycol alone). Of these 56 subjects, a total of 26 subjects received CIS and 30 subjects received placebo. All subjects underwent an initial dose-titration period to find the maximum tolerated dose up to a protocol-specified maximum of 300 mg (or the equivalent volume of propylene glycol in the placebo group). After this 10-day period, subjects were to continue therapy 3 times per week for a period of 2 years. The study concluded on August 21, 2003, when the 56th subject completed the full 2 years of treatment. Follow-up in the randomized cohort ranged from 24 to 56 months.

Results of the study demonstrated that treatment with CIS was associated with a 79% decreased risk of death compared with treatment with placebo (hazard ratio [HR] = 0.213; log-rank $p = 0.007$). This statistically significant survival advantage was also observed when stratified by transplant type, primary diagnosis, cytomegalovirus (CMV) donor positive/recipient negative mismatch, or the occurrence of a grade 2 or higher AR episode prior to enrollment. The survival advantage from CIS treatment may have arisen from a decreased incidence of chronic rejection, consistent with the mode of delivery to the airway epithelium. Subjects treated with CIS had significantly improved chronic rejection-free survival (HR = 0.28; log-rank $p = .001$) compared with subjects treated with placebo. Chronic rejection was diagnosed either through biopsy by the presence of BO or clinically by the presence of bronchiolitis obliterans syndrome (BOS). Among the 30 placebo-treated subjects, only 8 (27%) survived free of chronic rejection compared with 18 (69%) CIS-treated subjects. Analyses of BOS-free survival and BO-free survival revealed comparable results. However, treatment with CIS did not lead to significant difference in the occurrence of AR ($p = 0.73$).

Further analysis of the study population revealed that CIS had a favorable safety profile, and long-term administration did not lead to exacerbations of known toxicities of oral and intravenous cyclosporine. In particular, there was no evidence that administration of CIS led to increased risk of nephrotoxicity, neurotoxicity, increased risk of infections, or increased risk of malignancies. Treatment with CIS was found to be associated with respiratory tract irritation and bronchospasm, but these events were generally mild/moderate, occurred early in subjects' treatment course, diminished with time, and were not associated with the development of more serious respiratory complications. These findings were consistent with the analysis of the larger, 70 subject, safety database.

In March 2005, an open label early access treatment IND was initiated. The purpose of this protocol was to provide CIS to lung transplant physicians for use in suitable lung transplant recipients. Since that time, over 60 lung transplant recipients have received CIS. Data collection is limited to early tolerability subject diaries and serious adverse events (SAEs). Analysis of this safety database has not led to the emergence of any new safety findings. However, in recipients with poor pulmonary reserve (such as those with advanced BOS), intolerance to dosing is more common, and dose titration to the maximal 300 mg dose is sometimes not possible.

The safety and efficacy of CIS in preventing chronic rejection and improving survival in this population of lung transplant subjects has been previously demonstrated in a randomized double-blind, placebo-controlled, clinical study.[(8)] This study was the basis for a new drug application (NDA) to regulatory authorities. This application received an approvable letter, with approval contingent on additional supporting clinical data. There is currently a multicenter, phase III, randomized, controlled trial designed to assess the efficacy of prophylactic inhaled cyclosporine for the prevention of bronchiolitis obliterans in lung transplant recipients. However, there currently exists limited data on the efficacy of CIS in propylene glycol to treat established BO/BOS occurring in lung transplant recipients and no data in HSCT recipients with established BOS.

2.7 Rationale for Dose, Route and Schedule

Most of the previous clinical trials have taken place at the University of Pittsburgh Medical Center (UPMC), which is a leading center in lung transplantation research and clinical management of lung transplant recipients. We will conduct this study using the same dose of CIS as was used in the Pittsburgh chronic lung rejection prevention trial, specifically 300 mg cyclosporine (or maximally tolerated dose up to 300 mg) administered three times per week after an initial 6-week titration period. The formulation is 325 mg cyclosporine in 5.2 mL of propylene glycol (62.5 mg/mL).

2.8 Rationale for including pediatric Subjects

There are no alternative treatment options for children who develop this often-fatal consequence of lung or hematopoietic stem cell transplantation. Therefore the potential efficacy of inhaled CsA would be relevant to children. We do not expect an extraordinary effort will be required to accrue pediatric subjects as our transplant program offers transplant procedures to minors. At present there is limited data on the use of CIS in children. CIS has been administered to two pediatric lung transplant recipients, but this is the extent of the pediatric data available. However, there are studies investigating inhaled medications in children vs. adults which suggest systemic absorption of inhaled drugs, even when administered at the same doses, are not higher in pediatric populations compared to adults. A study investigating lung deposition of inhaled steroids in children and adults (children 2-3 years, children 4-6 years, adults 10-41years) with asthma receiving the same fixed dose of inhaled budesonide (2 x 200 ug via Nebuchamber pMDI with mouthpiece and nose clip) showed that systemic absorption of budesonide (as measured by plasma AUC) was similar for all age groups[21]. This data suggests, from a safety standpoint, that the prescribed dose of inhaled budesonide need not be adjusted for age. A previous pharmacokinetic study of CIS in adults with BO/BOS found that the systemic exposure of CSA using comparable doses of CIS as used in this study, is about 7-fold lower than is achieved with CSA given orally (Neoral®) at comparable doses[22]. Maximum blood concentrations of cyclosporine after aerosol administration of CIS ranged from 119 to 402 ng/mL, while 24 hr concentrations ranged from 9 to 48 ng/mL (versus reported C_{max} concentrations of 1555 ng/mL and trough concentrations of 268 ng/mL in liver transplant recipients, according to the Neoral® label). From these data we infer that inhaled CIS in pediatric populations age 10 and older will not result in toxic systemic levels of CSA, however, adjustments will be made for pediatric dosing per APT recommendations. We will perform lung deposition and pharmacokinetic studies in all pediatric subjects and will closely monitor their plasma cyclosporine levels during the study. Pediatric subjects 10 years of age or older will be allowed to participate in this study as they are at an age where they can cooperate sufficiently with drug administration and required study tests. Although there is limited data to judge potential risk in children, the seriousness of BO in children we believe warrants their participation.

2.9 Rationale for pediatric dosing

(per Charles Johnson, MD, Chief Medical Officer, APT Pharmaceuticals)

The main safety concerns in this age group are to ensure that there is not excessive systemic exposure to cyclosporine and there is no impact on lung function. It is normal practice to dose the currently licensed forms of cyclosporine (eg Neoral) on the basis of weight and monitor blood levels of cyclosporine. In addition, data from Chua et al, 1994 in 8 children (median age 10.8 y) with cystic fibrosis showed that in this age group, age was not a factor in the amount of drug deposited following aerosol delivery. Therefore, it is proposed to use weight as the main determinant of dose and divide the pediatric population into two groups on the basis of weight. Current experience in the adult population indicates that six adult subjects weighing less than 45 Kg (range 34.5 - 44.3 Kg) have been exposed to CIS. All six achieved a dose of 300 mg in the titration phase and none have reported adverse events associated with study drug. The lightest subject had a peak serum cyclosporine level of 39.7 ng/mL one hour after the inhalation. This level is substantially below the therapeutic level set for systemic therapy (250 -350 ng/mL), suggesting that systemic exposure is not likely to be dose limiting at least in children above 35 kg.

Since the same nebulizer/compressor system will be used in the pediatric population, the proportion of nominal dose which is emitted at the mouth-piece will remain constant. The lung delivered dose is then dependent upon minute ventilation (slightly lower than adults across this age group) and air entrainment (less dilution of the dose due to a lower inspiratory flow rate in children) these factors will exist as a continuum across the population having a greater impact in the smaller younger subjects. Although the adult data suggests that there is a wide therapeutic margin (very low systemic cyclosporine exposure) it would seem prudent to reduce the nominal dose in the youngest, lightest population. The proposed dose for the pediatric age group is as follows:

Weight ≥ 35 Kg dose at 300 mg
Weight < 35 Kg dose at 200 mg

This dosing schedule is being used in a multicenter phase III Trial, NCT00755781.

2.10 Clinical and Scientific Justification

Bronchiolitis Obliterans (BO) is an obstructive lung disease that can affect individuals after lung transplant or hematopoietic stem cell transplant. Patients with BOS report more restrictions in their functional capacity than transplant patients without BOS. There are preliminary studies suggesting that depressive symptoms, fatigue, and decrements in physical function occur in association with bronchiolitis obliterans in lung transplant recipients [30]. However, the characteristics and correlates of adverse changes in quality of life domains, including mood disturbance, in patients with bronchiolitis obliterans following stem cell transplantation have not been examined in prior research. Bronchiolitis obliterans syndrome in both lung and hematopoietic stem cell transplant recipients is associated with significant mortality. Median survival after the development of BOS in hematopoietic stem cell transplant recipients is only 2.5 years[10]. Mortality related to BOS is greater than 60% in patients after hematopoietic stem cell transplant and 55% in lung transplant recipients [4,8,9].

Currently, there are very few treatment options for patients who develop bronchiolitis obliterans. Patients are managed with increasing systemic immunosuppressants with limited success. This is associated with increased infections and in the setting of a hematopoietic stem cell transplant can decrease the graft-versus tumor/leukemia effect. Recent studies by Iacono, et al have demonstrated that inhaled cyclosporine (CSA) in solution with propylene glycol when given prophylactically, can improve overall survival and chronic rejection-free survival in lung transplant patients[(8)]. However, data on the efficacy of inhaled CSA to treat established BOS in lung transplant recipients is limited. Because targeted delivery to the diseased organ that minimizes the morbidity associated with systemic immunosuppression warrants further investigation in both the lung transplant and hematopoietic transplant setting, we propose this phase II clinical trial designed to evaluate the safety, efficacy, and pharmacodynamics of inhaled cyclosporine in lung transplant and hematopoietic transplant recipients.

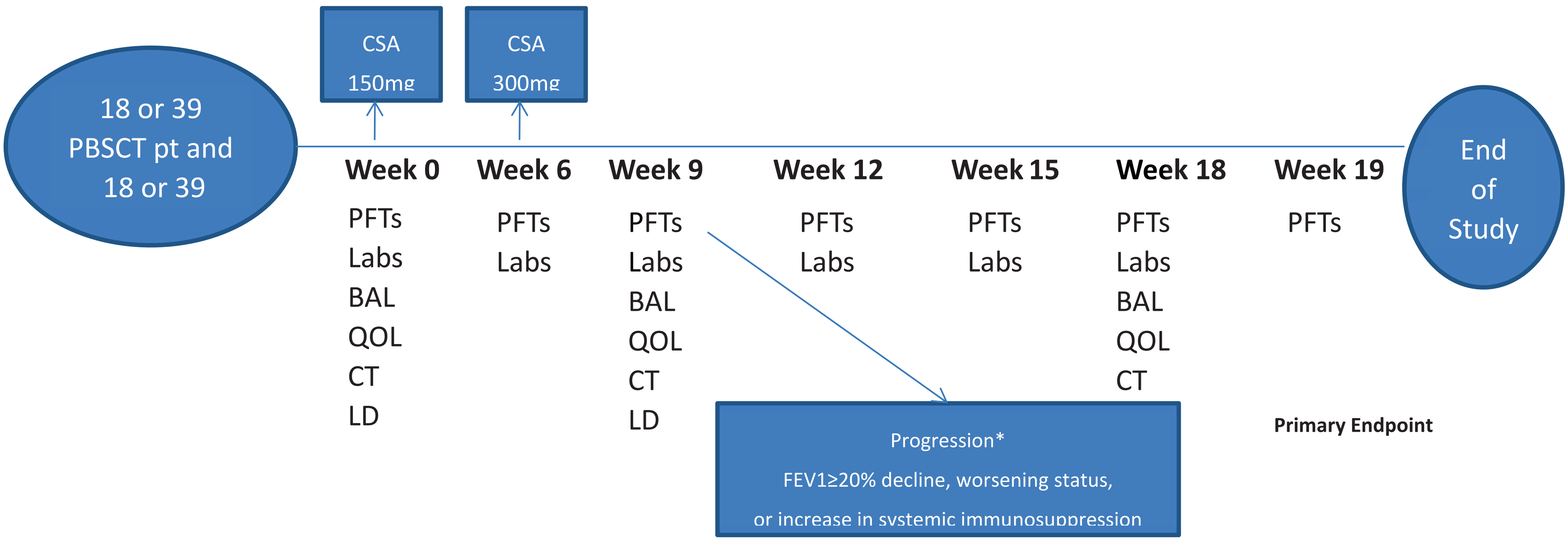
This study also seeks to further define the inflammatory pathways associated with BOS in stem cell transplant and lung transplant recipients and to investigate and compare similarities and differences in the pathogenesis of BOS in these two transplant groups. Bronchial epithelial cells and peripheral blood cells will be studied using multiplex cytokine arrays, flow cytometry, and gene expression profiling. These studies will be performed on samples collected from subjects and will promote further delineation of the underlying pathogenesis in BOS. Additionally, this study will examine the anti-inflammatory effects of cyclosporine on mediators of inflammation and differential gene expression in these populations. This has not been thoroughly studied in hematopoietic stem cell transplant recipients. This study evaluating aerosolized cyclosporine will contribute to a greater understanding of the pathogenesis of bronchiolitis obliterans, and set the stage for larger trials in hematopoietic stem cell transplant and lung transplant recipients.

3.0 INVESTIGATIONAL PRODUCT

Please refer to the Investigator's brochure for Cyclosporine Inhalation Solution (CIS).

4.0 STUDY DESIGN

The study is designed as a non-randomized, Phase II study of aerosolized cyclosporine A in solution with propylene glycol (CIS) in hematopoietic stem cell transplant recipients at least 99 days post transplant (Group A) or lung transplant recipients at least 6 months post transplant (Group B) who have been diagnosed with biopsy proven bronchiolitis obliterans or Bronchiolitis Obliterans Syndrome. The *primary endpoint* of each study group is FEV_1 improvement or stabilization from study baseline at week 18 for two successive measures, at least 1 week apart, no more than two weeks apart. Subjects who cannot tolerate the medication (at least 150mg dose), have disease progression (beginning at week 9) as defined by a 20% or more decline in FEV_1 on two successive measurements at least 2 weeks apart or require >25% increase in one or more existing immunosuppressive agents or the addition of new systemic immunosuppressive therapies due to worsening symptoms related to BOS will go off study. As of Amendment M, lung transplant patients are no longer being enrolled on this protocol.



* Based on two successive measures at least two weeks apart, beginning at week 9

5.0 ELIGIBILITY ASSESSMENT

5.1 Inclusion criteria

5.1.1 History of

- Hematopoietic stem cell transplant recipients at least 99 days post transplant (Group A)

5.1.2 Biopsy proven bronchiolitis obliterans (confirmed by NIH pathology department) or Bronchiolitis Obliterans Syndrome (BOS) as defined by:

- FEV_1 <75% predicted and
- No evidence of pulmonary infection as a causative etiology to lung dysfunction or other causative etiology and
- Decline in FEV_1 (The FEV_1 values used to determine BOS will be the average of 2 measurements of FEV_1 taken sequentially at least 3 weeks apart up to 6 months apart) compared to pre-transplant baseline for Group A.
    - For hematopoietic transplant patients, FEV_1 must have declined >10% from pre-transplant baseline (group A)
- And one of the following:
    - FEV_1/FVC <0.7
    - Air trapping seen on CT scan or RV≥120% predicted
    - Evidence of cGVHD affecting at least one other organ system (group A)

5.1.3 Age 10-80 years

5.1.4 Progressive disease or stable disease (active BOS, stable by FEV_1 criteria) on immunosuppressants at study entry

5.1.5 *Progressive disease at study entry:* Diagnosis of BO or BOS with evidence of a progressive decline in FEV_1. A documented decline (≥10%) in FEV_1 has occurred within 18 weeks (minimum documentation of 3 weeks) preceding study enrollment.

5.1.6 *Stable disease at study entry:* Diagnosis of BO or BOS on immunosuppressive therapy with evidence of stable disease (active BOS, stable by FEV_1 criteria), as documented by a stable FEV_1 (increase <5% and decrease <10%) within 18 weeks (minimum documentation of 3 weeks) preceding study enrollment.

5.1.7 Subjects on calcineurin inhibitors at study entry will be required to be on a stable dose of the calcineurin inhibitor for 4 weeks prior to study enrollment.

Prior to Amendment M, patients with lung transplant –associated BOS were eligible for the trial.

5.2 Exclusion criteria

5.2.1 Evidence of uncontrolled, pulmonary infection

5.2.2 Subjects with unstable coronary insufficiency, severe cardiac arrhythmias, and/or uncontrolled hypertension.

5.2.3 History of hypersensitivity to propylene glycol

5.2.4 History of allergic reaction or hypersensitivity to Technetium- 99m sulfur colloid, used in lung deposition studies

5.2.5 ECOG performance status greater than or equal to 3

5.2.6 Serum creatinine >2.5 mg/dl

5.2.7 Documented allergy or intolerance to cyclosporine

5.2.8 Women pregnant or breast feeding or not willing to use an approved method of birth control

5.2.9 Inability to comprehend the investigational nature of the study and provide informed consent

5.2.10 Life expectancy less than 18 weeks

5.2.11 An increase $\geq$ 5% and an absolute increase $\geq$ 0.05 in FEV1 in the 18 weeks (minimum documentation of 3 weeks) preceding study enrollment

5.2.12 Subjects who have had prior administration of inhaled cyclosporine.

6.0 TREATMENT PLAN

6.1 Pre-treatment Medication (albuterol)

Subjects will receive metered dose inhaler albuterol, 2 puffs as needed for prevention of bronchospasm or 3 ml solution for nebulization. Subjects may receive levalbuterol in place of albuterol or not receive pre-treatment.

6.1.1 Inhalation device: Subjects will be instructed regarding the use of the inhalation device as follows:

- Shake the inhaler well.
- Breathe out as completely as possible through your mouth.
- Hold the canister with the mouthpiece on the bottom, facing you and the canister pointing upward. Place the open end of the mouthpiece into your mouth. Close your lips tightly around the mouthpiece.
- Breathe in slowly and deeply through the mouthpiece. At the same time, press down once on the container to spray the medication into your mouth.
- Try to hold your breath for 10 seconds, remove the inhaler, and breathe out slowly.
- Wait 10 minutes before beginning cyclosporine nebulizer treatment.

6.1.2 Nebulizer: Subjects will be instructed regarding the use of the nebulizer device as follows:

- Before treatment, wash your hands with soap and water and dry completely.

- Place the air compressor on a sturdy surface that will support its weight. Plug the cord from the compressor into a properly grounded (three-prong) electrical outlet.
- Carefully measure medications exactly as you have been instructed and put them into the nebulizer cup.
- Assemble the nebulizer cup and mask or mouthpiece.
- Connect the tubing to both the aerosol compressor and nebulizer cup (with filter if beginning treatment with cyclosporine).
- Turn on the compressor to make sure it is working correctly. You should see a light mist coming from the back of the tube opposite the mouthpiece.
- Sit up straight on a comfortable chair. If the treatment is for your child, he or she may sit on your lap. If you are using a mask, position it comfortably and securely on you or your child's face. If you are using a mouthpiece, place it between you or your child's teeth and seal the lips around it.
- Take slow, deep breaths.
- Continue the treatment until the medication is gone (an average of 10 minutes for albuterol). The nebulizer will make a sputtering noise, and the cup will have just a little medication remaining.
- If dizziness or jitteriness occurs, stop the treatment and rest for about 5 minutes. Continue the treatment, and try to breathe more slowly. If dizziness or jitteriness continues to be a problem with future treatments, inform your doctor.
- During the treatment, if the medication sticks to the sides of the nebulizer cup, you may shake the cup to loosen the droplets.
- After each treatment, rinse the nebulizer cup thoroughly with warm water, shake off excess water, and let air-dry.
- Wait 10 minutes before beginning cyclosporine nebulizer treatment.

6.1.3 Concomitant Medications of Concern with albuterol (concurrent use will be per PI discretion and carefully monitored) Subjects will be queried as to what prescription medications, vitamins, nutritional supplements, and herbal products they are taking, or have stopped taking within the past two weeks as medication dose adjustments may be required or subjects carefully monitored for side effects with the following medications:

- beta blockers such as atenolol (Tenormin), labetalol (Normodyne), metoprolol (Lopressor, Toprol XL), nadolol (Corgard), and propranolol (Inderal); digoxin (Lanoxin); diuretics; epinephrine (Epipen, Primatene Mist); other inhaled medications used to relax the air passages such as metaproterenol (Alupent) and levalbuterol (Xoponex); and medications for colds

- antidepressants such as amitriptyline (Elavil), amoxapine (Asendin), clomipramine (Anafranil), desipramine (Norpramin), doxepin (Adapin, Sinequan), imipramine (Tofranil), nortriptyline (Aventyl, Pamelor), protriptyline (Vivactil), and trimipramine (Surmontil); and monoamine oxidase (MAO) inhibitors, including isocarboxazid (Marplan), phenelzine (Nardil), selegiline (Eldepryl, Emsam), and tranylcypromine (Parnate).

6.1.4 Subjects will also be instructed to discontinue albuterol and notify the research team immediately if they:

- become pregnant, plan to become pregnant, or are breast-feeding.

- experience severe wheezing and difficulty breathing immediately after it is inhaled.

6.1.5 Subjects will be instructed not to administer the albuterol and /or study drug on the day of assessments, as the albuterol and/or CsA may complicate the interpretation of the pulmonary function testing.

6.2 Study Drug (Aerosolized Cyclosporine A in solution with Propylene glycol) Administration

6.2.1 *Treatment plan:*

Week 1: Subjects will receive aerosolized cyclosporine A in solution with propylene glycol using a Sidestream nebulizer with the Invacare Mobilaire compressor at 30 psi. During the first week, subjects will receive 150 mg (total volume 2.4 ml), 3 times weekly. During the first and second doses of 150mg of study drug, subjects will be monitored for correct use of the aerosolizing device under direct supervision of the research team. An individualized maximum tolerated dose may be recommended. **Pediatric dosing** per APT recommendation: During the first week, subjects will receive 150 mg (total volume 2.4 ml), 3 times weekly. Subjects may not initiate aerosolized cyclosporine based on the results of the bronchoscopy, at the discretion of the PI.

Weeks 2-5: Subjects (adults and pediatric) will continue self administration of aerosolized cyclosporine A in solution with propylene glycol 150 mg (total volume 2.4 ml), 3 times weekly. A member of the study team will periodically (during weeks 2 – 5) call the subjects to discuss any concerns or issues related to study drug administration.

Weeks 6-8: Subjects will undergo a dose escalation to 300 mg inhalation dose (total volume 4.8ml) three times weekly. This is the dose that has been used previously in lung transplant recipients by Iacono et al (8). During dose escalation, subjects will be monitored under direct supervision of the research team. If a subject is unable to tolerate the dose escalation, they will be able to continue at the 150mg dose given three times per week or 125mg (total volume 2.0 mL) daily**.**
Pediatric dosing per APT recommendation:
Weight ≥ 35 Kg dose at 300 mg (total volume 4.8ml three times weekly).
Weight < 35 Kg dose at 200 mg (total volume 3.2 ml three times weekly). If the subject weighs less than 35 Kg and is unable to tolerate the dose escalation to 200 mg they can continue at 150 mg three times weekly or 85 mg (total volume 1.36 mL) daily.

Weeks 9-18: Continued dosing will be dependent on response and tolerance. Formal PFTs performed at NIH will be obtained to determine response to treatment. Subjects will continue at the 300mg inhalation dose (total volume 4.6ml) three times weekly or maximally tolerated dose.

Any subject that demonstrates disease progression (beginning at week 9) as defined by a 20% or more decline in FEV_1 on two successive measurements at least 2 weeks apart, or >25% increase in existing or addition of new immunosuppressive therapies (sustained for 3 weeks, excluding adjustments made for target drug levels), above baseline levels, due to worsening symptoms related to BOS, will be categorized as a treatment failure and will discontinue study drug administration and proceed with end of study drug assessment. For all others, study drug administration will end at week 19 assessment.
Deviation plan for a missed dose: Subjects will be treated with CIS three times weekly ideally on Monday, Wednesday, and Friday of the week. Should a subject miss a scheduled dose of CIS, we will attempt to alter the dosing regimen for that week so that the subject will still receive three doses of CIS over a week with a limit of 1 dose per day. Data will be captured and reported for subjects who deviate from the treatment plan.

Medication Holds: During study participation, subjects may be instructed to hold administration of CIS after development of an adverse event or other reason at the discretion of the PI. Information pertaining to the reason for and the duration of the medication hold will be captured in the subjects' medical record. If study drug is held for more than 7 days, the time of the hold will not be counted towards the overall study week count. Study drug can be held for up to 12 weeks.

6.2.2 Special Instructions Regarding the Administration of Aerosolized Cyclosporine A

Subjects will follow nebulizer instructions (section 6.1.2) with the following instructions specific to CsA: Continue the treatment until the medication is gone (approx. 20-30 minutes for cyclosporine).

A new nebulizer should be used after albuterol administration. Aerosolized CsA is provided in single use vials. Any CsA remaining in the vial after administration should be discarded immediately.

Aerosolized CsA should be administered such as to avoid others being exposed to CsA during the inhalation process.

Subjects will be given a medication log and asked to record their CIS administration on the log. Subjects will be encouraged to capture this information, but failure to comply with this request will not be reported as a protocol deviation.

6.2.3 Permitted Concomitant Medications

Post Transplant Medications: Subjects that require an increase in existing immunosuppressive meds by 25% due to worsening BOS symptoms (sustained for 3 weeks, excluding adjustments made for target drug levels) or need addition of new immunosuppressive therapies (sustained for 3 weeks), above baseline levels, due to worsening lung disease or BOS symptoms after week 9 will be categorized as treatment failures. However, if the increase or addition of new immunosuppressive therapies is for GVHD not involving the lung or to maintain baseline drug levels, this data will be captured and the subject will continue on study. Efforts will be made to make no changes in existing medical therapies other than tapering immunosuppressants in subjects who have PFTs showing stable or improved pulmonary function.

Systemic immunosuppressants will be tapered beginning at week 6, if subjects have an improvement or stabilization of FEV_1 compared to study baseline values, at the discretion of the PI, taking into account the subject's other manifestations of GVHD. Attempts will be made to taper prednisone first, before other systemic immunosuppressants. Prednisone will be decreased by 25% at the first taper. Subsequent tapers will be at the discretion of the PI. Lung transplant recipients will not have systemic immunosuppressants tapered.

Subjects that have improvement or stabilization of FEV_1, are not on prednisone, and are only on calcineurin inhibitors, will have their steady state calcineurin inhibitor (systemic cyclosporine or tacrolimus) dose tapered at the discretion of the PI. The calcineurin inhibitor dose will be decreased by 25% at the first taper. Subsequent tapers will be at the discretion of the PI.

Lung Infection Prophylaxis:
Lung infection prophylaxis will be at the discretion of the PI, taking into account the patient's other concomitant medications and medical history. The following are guidelines:

- For PCP pneumonia prophylaxis, subjects will take Bactrim, one double strength tablet (800/160mg) orally three times weekly or aerosolized pentamidine 300mg every four weeks by inhalation.

- For antiviral prophylaxis, subjects will receive acyclovir 800 mg twice daily. Pediatric subjects less than 40kgs will receive acyclovir 20mg/kg po twice daily to a maximum dose of 800mg twice daily.

- For antibacterial prophylaxis, subjects will receive penicillin 500mg twice daily or for penicillin allergic patients, azithromycin 250mg daily.

- Subjects who are already on adequate, alternative prophylaxis agents prescribed by their primary transplant teams can remain on their prior regimens.

6.2.4 Concomitant Medications of Concern with CsA (concurrent use will be per PI discretion and carefully monitored)

- Due to potential for drug interactions, other short acting sympathominetic aerosolized bronchodilators, beta-adrenergic receptor blocking agents, digoxin, monoamine oxidase inhibitors or tricyclic antidepressants will be allowed per PI discretion.

- Due to the potential for compromising the primary endpoint, subjects requiring an increase in systemic immunosuppression or who initiate new immunosuppressive agents, such as oral tacrolimus, CSA, prednisone, mycophenolate mofetil (MMF) or azathioprine due to symptoms of GVHD *not* involving the lung, will remain on study and will have concurrent use of these medications carefully tracked and accounted for during and at the end of study data analysis.

- Cyclosporine can interfere with or be affected by multiple drugs due to its metabolism through the CYP3A4 enzyme. As such, patients who are taking medications, which are either substrates for inducers of, or inhibitors of CYP3A4 (i.e. oral cyclosporine) will be closely monitored (see appendix c).

7.0 STUDY ASSESSMENTS

7.1 Screening Evaluation (Screening will be done under protocol 97-H-0041 and will be used to establish eligibility and baseline status)

- History and Physical with emphasis on symptom assessment
- ECOG
- Review of concomitant medications
- Vital signs
- CBC with differential
- Acute Care, Mineral and Hepatic Panels
- Serum Beta- HCG in female subjects
- When applicable (i.e. on oral CSA), a CSA trough level
- Pulmonary Function tests
  - Forced Spirometry
  - Slow Spirometry
  - Lung volumes with nitrogen washout

- Single Breath Diffusion
    - Exhaled Nitric Oxide (subset of subjects)
- Six minute walk test
- EKG
- High Resolution Chest CT (HRCT)
- Determination of baseline status
    - Progressive disease at study entry
        - Diagnosis of BO or BOS with evidence of a progressive decline in FEV_1. Documented decline (≥10%) in FEV_1 has occurred within 18 weeks (minimum documentation of 3 weeks) preceding study enrollment
    - Stable disease (active BOS, stable by FEV_1 criteria) at study entry on immunosuppressants
        - Diagnosis of BO or BOS on immunosuppressive therapy with evidence of stable disease (active BOS, stable by FEV_1 criteria), as documented by a stable FEV_1 (increase <5% and decrease <10%) within 18 weeks (minimum documentation of 3 weeks) preceding study enrollment

7.2 Enrollment

- The informed consent process will begin and the written informed consent document will be signed.

7.3 Week 1 Assessments

- Peak Flow measurement with instructions on home peak flow measurement
- Bronchoscopy with bronchoalveolar lavage (BAL) to be analyzed by
    - Gram stain
    - Routine cultures
    - Luminex cytokine arrays
    - Flow Cytometry
    - Gene expression profiles
    - Proliferative, cytotoxic, and apoptotic response of BAL lymphocytes to 3rd party epithelial cells and allogeneic lymphocyte population
    - T regulatory cell quantification by real time PCR on CD4+ selected T-cells using primers to fox p3
- Peripheral Blood sample for
    - Luminex cytokine arrays
    - Flow Cytometry
    - Gene expression profiles
    - Lymphocyte extended phenotypic analysis: Trail, Fas ligand, Perforin Granzyme B
    - Lymphocyte response to non-specific mitogens
    - Lymphocyte cytotoxic assays
    - T regulatory cell quantification by real time PCR on CD4+ T-cells using primers to fox p3
    - Proteomics
- Quality of life assessments
    - SF36
    - GVHD questionnaire (PBSC transplant subjects only)
    - HAP Questionnaire
    - CRQ-SAS questionnaire

The following assessments may be repeated at week one if the screening assessments were performed greater than 7 days prior to enrollment or if there are any changes in the subjects condition that may warrant repeat testing. The decision to repeat these assessments will be at the PI's discretion.

- Repeat History and Physical with emphasis on symptom assessment
- ECOG
- Review of concomitant medications
- Vital signs
- CBC with differential
- Acute Care, Mineral and Hepatic Panels
- Serum Beta- HCG in female subjects
- When applicable (i.e. on oral CSA), a CSA trough level
- Pulmonary Function tests
  - Forced Spirometry
  - Slow Spirometry
  - Lung volumes with nitrogen washout
  - Single Breath Diffusion
  - Exhaled Nitric Oxide (subset of subjects)
- Six minute walk test
- High Resolution Chest CT (HRCT)

7.4 End of Week 1 Assessments (+/- 3 days)

- Interim assessment with emphasis on symptomatology
- Vital signs
- ECOG performance status
- Assessment of comprehension of medication self-administration and adherence
- Review of concomitant medications
- CBC with differential (when possible done concurrently with post transplant monitoring)
- Acute Care, Mineral and Hepatic Panels (when possible done concurrently with post transplant monitoring)
- Repeat Serum Beta- HCG in women of childbearing potential
- Plasma cyclosporine level
- Radiolabelled lung deposition studies **(+/- 10 days; to be performed in a subset of patients**
- Pharmacokinetics (subset of subjects)
- Review Peak Flow measurements

7.5 Week 6 Assessments (+/- 5 days)

- Interim assessment with emphasis on symptomatology
- Vital signs
- ECOG performance status
- Review of concomitant medications and adherence
- CBC with differential (when possible, done concurrently with post transplant monitoring)
- Acute Care, Mineral and Hepatic Panels (when possible done concurrently with post transplant monitoring)
- Plasma cyclosporine level
- Repeat Serum Beta- HCG in women of childbearing potential
- Pulmonary Function tests
  - Forced Spirometry

  - Slow Spirometry
  - Lung volumes with nitrogen washout
  - Single Breath Diffusion
  - Exhaled Nitric Oxide (subset of subjects)
- Six minute walk test
- Review Peak Flow measurements
- Peripheral blood samples
  - Luminex cytokine arrays
  - Flow Cytometry
  - Gene expression profiles
  - Lymphocyte extended phenotypic analysis: Trail, Fas ligand, Perforin Granzyme B
  - Lymphocyte response to non-specific mitogens
  - Lymphocyte cytotoxic assays
  - T regulatory cell quantification by real time PCR using primers to fox p3
  - Proteomics

7.6 Week 9 assessments (+/- 5 days)

- Interim assessment with emphasis on symptomatology
- Vital signs
- ECOG performance status
- Review of concomitant medications and adherence
- CBC with differential (if possible, done concurrent with post transplant monitoring)
- Acute Care, Mineral and Hepatic Panels (if possible, done concurrently with post transplant monitoring)
- Plasma cyclosporine level
- Repeat Serum Beta- HCG in women of childbearing potential
- Pulmonary Function tests
  - Forced Spirometry
  - Slow Spirometry
  - Lung volumes with nitrogen washout
  - Single Breath Diffusion
  - Exhaled Nitric Oxide (subset of subjects)
- Six minute walk test
- Review Peak Flow measurements
- Bronchoscopy with bronchoalveolar lavage (BAL) to be analyzed by
  - Gram stain
  - Routine cultures
  - Luminex cytokine arrays
  - Flow Cytometry
  - Gene expression profiles
  - Proliferative, cytotoxic, and apoptotic response of BAL lymphocytes to 3rd party epithelial cells and allogeneic lymphocyte population
  - T regulatory cell quantification by real time PCR
- Peripheral blood samples
  - Luminex cytokine arrays
  - Flow Cytometry
  - Gene expression profiles
  - Lymphocyte extended phenotypic analysis: TRAIL, Fas ligand, Perforin Granzyme B

- Lymphocyte response to non-specific mitogens
  - Lymphocyte cytotoxic assays
  - T regulatory cell quantification by real time PCR
  - Proteomics
- High Resolution Chest CT
- Radiolabelled lung deposition studies (+/- 2 weeks) (subset of subjects)
- Pharmacokinetics studies (subset of subjects)
- Quality of life assessments
  - SF36
  - GVHD questionnaire (PBSC transplant subjects only)
  - HAP Questionnaire
  - CRQ-SAS questionnaire

7.7 Week 12 Assessments (+/- 7 days)

- Interim assessment with emphasis on symptomatology
- Vital signs
- ECOG performance status
- Review of concomitant medications
- CBC with differential (when possible, done concurrently with post transplant monitoring)
- Acute Care, Mineral and Hepatic Panels (when possible done concurrently with post transplant monitoring)
- Plasma cyclosporine level
- Repeat Serum Beta- HCG in women of childbearing potential
- Pulmonary Function tests
  - Forced Spirometry
  - Slow Spirometry
  - Lung volumes with nitrogen washout
  - Single Breath Diffusion
  - Exhaled Nitric Oxide (subset of subjects)
- Six minute walk test
- Review Peak Flow measurements
- Peripheral blood samples
  - Luminex cytokine arrays
  - Flow Cytometry
  - Gene expression profiles
  - Lymphocyte extended phenotypic analysis: TRAIL, Fas ligand, Perforin Granzyme B
  - Lymphocyte response to non-specific mitogens
  - Lymphocyte cytotoxic assays
  - T regulatory cell quantification by real time PCR
  - Proteomics

7.8 Week 15 Assessments (+/- 7 days)

- Interim assessment with emphasis on symptomatology
- Vital signs
- ECOG performance status
- Review of concomitant medications
- CBC with differential (when possible, done concurrently with post transplant monitoring)

- Acute Care, Mineral and Hepatic Panels (when possible done concurrently with post transplant monitoring)
- Plasma cyclosporine level
- Repeat Serum Beta- HCG in women of childbearing potential
- Pulmonary Function tests
    - Forced Spirometry
    - Slow Spirometry
    - Lung volumes with nitrogen washout
    - Single Breath Diffusion
    - Exhaled Nitric Oxide (subset of subjects)
- Six minute walk test
- Review Peak Flow measurements
- Peripheral blood samples
    - Luminex cytokine arrays
    - Flow Cytometry
    - Gene expression profiles
    - Lymphocyte extended phenotypic analysis: TRAIL, Fas ligand, Perforin Granzyme B
    - Lymphocyte response to non-specific mitogens
    - Lymphocyte cytotoxic assays
    - T regulatory cell quantification by real time PCR
    - Proteomics

7.9 Week 18 Assessments (+/- 7 days)

- Final assessment with emphasis on symptomatology
- Vital signs
- ECOG performance status
- Review of concomitant medications and compliance
- CBC with differential (if possible, done concurrent with post transplant monitoring)
- Acute Care, Mineral and Hepatic Panels (if possible, done concurrently with post transplant monitoring)
- Plasma cyclosporine level
- Pulmonary Function tests
    - Forced Spirometry
    - Slow Spirometry
    - Lung volumes with nitrogen washout
    - Single Breath Diffusion
    - Exhaled Nitric Oxide (subset of subjects)
- Six minute walk test
- Review Peak Flow measurements
- Bronchoscopy with bronchoalveolar lavage (BAL). BAL to be analyzed by
    - Gram Stain
    - Routine cultures
    - Luminex cytokine arrays
    - Flow Cytometry
    - Gene expression profiles
    - Proliferative, cytotoxic, and apoptotic response of BAL lymphocytes to 3rd party epithelial cells and allogeneic lymphocyte population
    - T regulatory cell quantification by real time PCR using primers to fox p3
- Peripheral blood samples

- Luminex cytokine arrays
    - Flow Cytometry
    - Gene expression profiles
    - Lymphocyte extended phenotypic analysis: Trail, Fas ligand, Perforin Granzyme B
    - Lymphocyte response to non-specific mitogens
    - Lymphocyte cytotoxic assays
    - T regulatory cell quantification by real time PCR using primers to fox p3
    - Proteomics
- High Resolution Chest CT
- Quality of life assessments
    - SF36
    - GVHD questionnaire (PBSC transplant subjects only)
    - HAP Questionnaire
    - CRQ-SAS questionnaire

7.10 Week 19 (+/- 1 week) Confirmation of Week 18 Assessment

- Pulmonary Function tests
    - Forced Spirometry
    - Slow Spirometry
    - Lung volumes with nitrogen washout
    - Single Breath Diffusion
    - Exhaled Nitric Oxide (subset of subjects)
- End of Study Assessment (see section 7.10)

After week 19 assessment is completed, subjects will end study drug administration. PFTs from week 19 assessments will be used to confirm 18-week response assessment. Subjects with stabilized or improved disease will be offered the option of continuing CsA on **an extended access protocol** (see section 10.5, Off study). Those that choose to enroll on the extended access protocol will not have study drug therapy interrupted.

7.11 End of Study Assessment

Before subjects can be taken off-study (see section 10.5), the following assessment will be done:

7.11.1 Subjects completing study drug (Week 19)

- CBC with differential (if possible, done concurrent with post transplant monitoring)
- Acute Care, Mineral and Hepatic Panels (if possible, done concurrently with post transplant monitoring)
- Plasma cyclosporine level

7.11.2 2 weeks (+/- 1 week) after subjects are taken off study drug early (see section 10.5)

- Pulmonary Function tests
    - Forced Spirometry
    - Slow Spirometry
    - Lung volumes with nitrogen washout
    - Single Breath Diffusion
    - Exhaled Nitric Oxide (subset of subjects)

- CBC with differential (if possible, done concurrent with post transplant monitoring)
- Acute Care, Mineral and Hepatic Panels (if possible, done concurrently with post transplant monitoring)
- Plasma cyclosporine level
- Quality of life assessments (except for subjects taken off study drug at the week 9 assessment)
    - SF36
    - GVHD questionnaire (PBSC transplant subjects only)
    - HAP Questionnaire
    - CRQ-SAS questionnaire

Note: These assessments may be obtained from the subject's home facilities, if feasible.

7.12 International and Long-Distance Subjects

Subjects that are travelling internationally or more than 50 miles away from the NIH, at the discretion of the principal investigator, may have their schedules modified. The week 9 assessment may be moved forward to as early as week 7. The week 12, week 15, and end of study assessments may be obtained from the subject's home facilities if feasible.

8.0 ASSESSMENT TOOLS and PROCEDURES

Other than the pre-study evaluations to determine eligibility/baseline status and subsequent PFTs used to guide therapy, subjects will be able to refuse any radiologic test or bronchoscopy procedure unless clinically indicated.

8.1 Pulmonary Function tests

Spirometry measures how much and how quickly air is moved in and out of the lungs. For these tests, the subject breathes into a mouthpiece attached to a recording device (spirometer). The information collected by the spirometer may be printed out on a chart called a spirogram. The more common lung function values measured used to assess the subjects on this trial will include:

Forced vital capacity (FVC). This measures the amount of air you can exhale with force after you inhale as deeply as possible.

Forced expiratory volume (FEV). This measures the amount of air you can exhale with force in one breath. The amount of air you exhale may be measured at 1 second (FEV_1).

Peak expiratory flow (PEF). This measures how quickly you can exhale. It is usually measured at the same time as your forced vital capacity (FVC).

Total lung capacity (TLC). This measures the amount of air in your lungs after you inhale as in your lungs after a normal exhale (FRC) and the amount after you exhale with force (RV).

Maximum voluntary ventilation (MVV) . This measures the greatest amount of air you can breathe in and out during one minute.

Exhaled Nitric Oxide. This measures the amount of nitric oxide in your breath after exhalation. This will be performed in a subset of subjects based on the availability of testing staff and scheduling.

Lung Volumes using Nitrogen Washout. This measures the total lung capacity and provides an estimate of residual volume of the lung.

Diffusing Capacity of the lungs for carbon monoxide (DLCO). This measures the ability of the lungs to transfer gas from inhaled air to the red blood cells in pulmonary capillaries.

Peak Flow Measurements: Subjects will be given a home peak flow meter that will measure their peak flow and FEV1. The results will be recorded, and subjects will be given a level below which they should call the research team. Subjects with a decline in peak flow of >20% compared to pretreatment baseline will be instructed to return to the NIH for formal pulmonary function testing and clinical evaluation. Subjects will be encouraged to perform peak flow testing daily, however, failure to perform peak flow measurements will not be considered a protocol deviation. .

8.2 Bronchoalveolar lavage (BAL) and Biopsy

Bronchoalveolar lavage (BAL) is a medical procedure in which a bronchoscope is passed through the mouth or nose into the lungs and fluid is squirted into a small part of the lung and then recollected for examination. A BAL sample (minimal 30 mL) will be obtained from upper and lower lobes using conventional techniques prior to initiating CIS as well as at the end of the week 9 and 18 assessments. The following research investigations will be performed: Gram stain, routine cultures, Luminex cytokine arrays, flow Cytometry, gene expression profiles, examination of proliferative, cytotoxic, and apoptotic response of BAL lymphocytes to 3rd party epithelial cells and allogeneic lymphocyte population, and T regulatory cell quantification by real time PCR using17 primers to fox p3.

8.3 High Resolution Chest CT

HRCT is performed using a conventional CT scanner. However, imaging parameters are chosen so as to maximize spatial resolution:

- A narrow slice width is used (usually 1-2 mm)
- A high spatial resolution image reconstruction algorithm is used
- Field of view is minimized, so as to minimize the size of each pixel
- Other scan factors (e.g. focal spot) may be optimized for resolution at the expense of scan speed

The aim of performing a HRCT is to assess generalized lung disease. As such the test is conventionally performed by taking thin sections 10-40 mm apart. The result is a few images which should be representative of the lungs in general, but which cover only approximately one tenth of the lungs.

Intravenous contrast agents are not used for HRCT as the lung inherently has very high contrast (soft tissue against air).

8.4 Six Minute Walk Test

The six-minute walk test is generally used at the start of a Pulmonary Rehabilitation program and/or in the evaluation of lung transplant and will be used in this study to evaluate the primary endpoint, which is change in BO status. The object of this test is to walk on a flat surface such as a hallway for 6 minutes during which time subjects will be asked to rate their degree of breathlessness and fatigue levels. The distance walked during the 6 minutes is measured.

Blood pressure, heart rate, respiratory rate, and resting blood saturation by pulse oximetry will be taken after the test. Oxygen saturation will be taken during the walk. Subjects will be asked to assess their breathing status using a 0-10 scale.

Subjects will be instructed to:

- Wear comfortable clothing
- Wear shoes that are comfortable to walk in, such as tennis shoes.
- Take their medications as prescribed.
- Eat a light meal before early morning or early afternoon tests.
- Not to exercise vigorously within 2 hours of beginning the test.

8.5 Quality of Life Measurements

Participants sixteen years and older that speak English will perform self-report questionnaires using the SF36, Human Activity Profile (HAP), Chronic Respiratory Disease Questionnaire Self-Administered Standardized CRQ-SAS), and GVHD questionnaire. These assessments will occur at baseline, 9 weeks, and the 18-week evaluation, or at study conclusion if that should occur earlier.

- The SF36 is a 36 item self-report questionnaire that measures the impact of physical and emotional health status on functional performance. It has been used extensively, and is accepted by the U.S. Food and Drug Administration as proof of therapeutic benefit for improved functioning and other subject-reported outcomes.
- The HAP is a 94-item questionnaire that assesses the frequency with which individuals perform common activities.
- The CRQ-SAS is a 20-item questionnaire that assesses dyspnea, fatigue, emotional functioning, and mastery.
- The GVHD questionnaire (PBSC transplant subjects, group A) designed by Lee and colleagues is a 30-item symptom scale with seven subscales to capture cGVHD symptom burden.

9.0 ANCILLARY LABORATORY RESEARCH STUDIES

Human Biologic materials will be collected as follows for the clinical evaluation and management of the subject. Samples will be ordered and tracked through the CRIS screens. Should a CRIS screen not be available, the NIH form 2803-1 will be completed and will accompany the specimen and be filed in the medical record. Lung deposition studies will be conducted and reviewed by the nuclear medicine department.

9.1 Sample Collection: During the course of participating on this study (screening appointment, 6, 9, 12, 15, and 18 week visits) blood and bronchoalveolar lavage samples will be collected for correlative laboratory research studies.

9.2 Intended Use: These specimens will not be used for diagnostic purposes. Studies conducted on these samples will not be used in assessing the primary endpoint but are undertaken for secondary endpoints (Tissue pathogenesis of BO as detailed below) and/or exploratory ancillary research, which are approved by the NHLBI IRB and listed in Appendix B of the protocol. As these tests are being performed for secondary or exploratory purposes, failure to obtain the tests will not be considered a protocol deviation.

9.2.1 Multiplex Cytokine Arrays

Biopsies, BAL, and peripheral blood samples will be prepared for multiplex cytokine array analysis. Multiplex bead arrays allow one to independently and quantitatively assay multiple analytes simultaneously in small volumes of material.

9.2.2 Flow Cytometry

BAL and peripheral blood samples will be prepared for analysis using flow cytometry. Samples will be analyzed for T cell, NK cell and neutrophils expression.

9.2.3 Gene Expression Profiling

BAL, and peripheral blood samples will be prepared for the gene array analysis. Specifically, arrays focused on Apoptosis, Dendritic cells/Antigen Presentation, Cytokines, and Chemokines/Adhesion Molecules will be used. The use of smaller, more focused arrays decreases statistical problems associated with multiple testing and simplifies data analysis. Most interesting and significant findings will be validated by PCR and protein based assays such as immunohistochemistry and ELISA.

9.2.4 Pharmacokinetic Studies

All pediatric subjects and 20 adult subjects will be asked to participate in this portion of the study: from group A: 5 PBSC transplant subjects that are not on oral cyclosporine, and 5 PBSC transplant subjects that are on oral cyclosporine and from group B: 10 lung transplant subjects.

For all subjects, 2ml of blood will be collected at the following approximate time points after administration of inhaled cyclosporine: 0 (prior to), immediately after, 40 min, and 1, 2, 4, 6, 8, 12, 18, and 24 h. For transplant subjects on oral cyclosporine, 2ml of blood will be collected at the following time points after administration of their steady state dose of oral cyclosporine but before their first administration of inhaled cyclosporine: 0, 1, 2, 3, 4, 6, 8, and 12 h. These subjects will then receive their first inhalation treatment with the inhaled cyclosporine, and will have the post inhalation measurements taken. Participation in this portion of the study will require a brief hospital admission. Pharmacokinetic studies will then be repeated during week 9 in subjects that have undergone the dose escalation. On the days that the pharmacokinetic study is performed, oral cyclosporine will be taken on an empty stomach and no food will be consumed for 4 hours after drug administration. For all other administration, oral cyclosporine should be taken on a consistent schedule with relation to meals and time of day to avoid significant changes in absorption.

9.2.5 Pharmacogenetic Analysis

For some subjects who participate in the pharmacokinetic study, a single 7 mL EDTA blood sample will be collected at baseline for pharmacogenetic analysis. For some stem cell transplant recipients frozen samples or buccal swab will be used for this analysis if they are available. Genotypes for drug metabolizing enzymes and transporters will be determined using PCR-based methods and correlated with pharmacokinetic parameters.

9.2.6 Lung Deposition Studies

After subjects have acclimated to the nebulized cyclosporine, we will perform lung deposition studies in a subset of patients, while the participants are taking 150mg of inhaled cyclosporine. The cyclosporine in propylene glycol will be mixed with 0.3ml of normal saline containing the radioisotope tracer Technetium- 99m sulfur colloid. The total aerosol deposition will be measured and SPECT/CT imaging will be performed. The lung deposition studies will be repeated again at 9 weeks in a subset of subjects.

9.3 Storage: Research specimens will be stored in the laboratories of Dr. Richard Childs (NHLBI) and/or Dr. Anthony Suffredini (Clinical Center/Critical Care Medicine Department) under the care and supervision of the principal investigator of this study. All laboratory personnel with access to samples or subject information will complete the NIH online course in Protection of Human Subjects. Efforts to ensure protection of subject information include:

- The laboratory is located in a controlled access building and laboratory doors are kept locked at all times. Visitors to the laboratory are required to be accompanied by laboratory staff at all times.
- Hard copy records or electronic copies of documents containing subject information are kept in the locked laboratory or other controlled access locations.
- An electronic database is used to store information related to subject samples processed by the laboratory.
- All samples collected will be assigned a unique code.
- Vials holding subject samples are labeled with the sequential laboratory accession ID number that does not contain any personal identifier information.
- Samples will be stored until they are no longer of scientific value or until the subject withdraws consent, at which time they will be destroyed.
- If a subject withdraws consent for their continued use, their sample will be destroyed.

Tracking: Samples will be ordered and tracked through the CRIS Research Screens. Should a CRIS screen not be available, the NIH form 2803-1 will be completed and will accompany the specimen and be filed in the medical record. Samples will not be sent outside NIH without IRB notification and an executed MTA.

9.4 End of study procedures: Samples from consenting subjects will be stored until they are no longer of scientific value or if a subject withdraws consent for their continued use, at which time they will be destroyed.

9.5 Loss or destruction of samples: Should we become aware that a major breech in our plan for tracking and storage of samples has occurred, the IRB will be notified.

10.0 BIOSTATISTICAL CONSIDERATIONS

10.1 Primary and secondary endpoints

The *primary objective* is to assess the safety and efficacy of inhaled aerosolized cyclosporine A solution in hematopoietic (group A) and lung transplant patients (group B) with bronchiolitis obliterans (BO) or BO syndrome (BOS). The *primary endpoint* of each study group is FEV_1 improvement or stabilization from study baseline at week 18 for two successive measures, at least 1 week apart, no more than two weeks apart.

Criteria for response:
For subjects with progressive disease at study entry,(subjects with a documented decline (≥10%) in FEV_1 within 18 weeks of study enrollment), improvement in BO is defined as an FEV_1 increase ≥10% compared to baseline and stabilization of BO is defined as less than 10% increase to less than 10% decline in FEV_1 as compared to baseline.

For subjects with stable disease (active BOS, stable by FEV_1 criteria) at study entry (a stable FEV_1, increase < 5% and decrease < 10% within the 18 weeks preceding study enrollment), improvement in BO is defined as an FEV_1 increase ≥ 10%compared to baseline and stabilization of BO is defined as less than 10% increase to less than 10% decline in FEV_1 with a minimum 25% decrease in the dose of one or more systemic immunosuppressive drugs(assessed at week 18, sustained for 3 weeks, excluding adjustments made for target drug levels) while being treated with CIS. Subjects with stable BO at study entry that have less than a 10% increase to less than a 10% decline in FEV_1 as compared to baseline that have no reduction in systemic immunosuppression while receiving CIS will be considered non-responders. Response will require a confirmatory PFT result at least 1 week after 18 week assessment. The average of these two measurements will be used for assessment of response.

The treatment will be considered successful in each cohort if there is a more than 30% improvement or stabilization. Adverse events associated with the treatment will also be assessed. (Please see the table below)

The secondary objectives of the study are to assess the lung deposition and pharmacokinetics of this CSA preparation, the inflammatory pathways that lead to the development of bronchiolitis obliterans and the anti-inflammatory effects of this CSA preparation ex vivo and in vivo. *Secondary endpoints* will be measured in the laboratory using the results of multiplex cytokine arrays, flow cytometry and gene expression profiling from peripheral blood and bronchoalveolar lavage samples, lung deposition studies and pharmacokinetic studies. Secondary endpoints will also include changes in the six-minute walk tests, high-resolution chest CT images, and quality of life measurements.

We will evaluate response for group A and group B subjects separately, in order to assess if the treatment is effective in each disease.

Response Criteria

Disease at study entry	FEV1	Systemic Immunosuppression	Final Response classification
Progressive (≥10% decline in preceeding 18 wks)	Improvement (≥10%)	N/A	Improvement
Progressive	Stabilization (<10% increase and decrease <10%)	N/A	Stabilization
Progressive or Stable*	Decline ≥10%	N/A	Non-responder
Stable (<5% increase and decrease <10% in preceeding 18 wks)	Improvement (≥10%)	N/A	Improvement

Stable	Stabilization (<10% increase and decrease <10%)	Minimum 25% decrease	Stabilization
Stable	Stabilization (<10% increase and decrease <10%)	N/A	Non-responder

* Subjects that have decline more than 20% are also non-responders

10.2 Sample size

For each study group, we determine the sample size using the Two-Stage Minimax Design of Simon (1989)[20]. We test the null hypothesis that the proportion of subjects that respond to therapy (improvement or stabilization) is 0.3 or less against the alternative hypothesis that the proportion of subjects that respond to therapy (improvement or stabilization) is 0.5 or more. With alpha = 0.05 and 80% power, a maximum of 39 subjects will be needed for each of study groups A and B. In each study group, at the first stage, up to 19 subjects will be accrued and if 6 or fewer subjects have a response to CIS at 18 weeks, we will conclude that the treatment is not effective and we will stop that study group. Note that all 19 subjects may not need to be accrued, as long as 13 subjects do not show improvement or stabilization. If 7 or more of the 19 subjects respond to therapy at 18 weeks at the first stage in one of the study groups, then an additional 20 subjects will be accrued, bringing the total number of subjects to a maximum of 39. If the total number of subjects responding is 16 or fewer, we conclude that the treatment is not effective. If at least 17 of 39 subjects respond we will consider this treatment successful. Once again, this may happen before all 39 subjects are accrued. A 95% confidence interval for the proportion of subjects improving or stabilizing will be given. Based on the sample size of the study, the two-sided confidence interval will be obtained with the binomial exact method or the normal approximation, if appropriate. We will test our null hypothesis (that the proportion of response is 0.3 or less) formally using the confidence interval approach. If the lower 95% limit of the confidence interval is greater than 0.3, we will conclude the null hypothesis is rejected and the study treatment is successful. If the lower 95% limit of the confidence is less than 0.3, we will not reject the null hypothesis.

Subjects who are unable to tolerate a dose escalation to 300 mg but are able to tolerate 150 mg inhalation for at least two weeks will be included in the primary analysis. Subjects who will be considered treatment failures include:
1) those who develop progression of baseline BO (>20% decline in FEV1) during the 18 week course of the study
2) those who are deemed to have progressed at the end of study as evidenced by an FEV1 decline ≥ 10%
3) subjects who require increases in existing or addition of new immunosuppressive therapies due to worsening symptoms related to BOS (sustained for 3 weeks, excluding adjustments made for target drug levels) after week 9
4) subjects with BO or BOS who had stable disease (active BOS, stable by FEV_1 criteria) at study entry and are deemed to have a stable FEV_1, increase < 10% and decrease < 10% at the end of study, with less than a 25% reduction in one or more systemic immunosuppressive medications (sustained for 3 weeks, excluding adjustments made for target drug levels) while receiving CIS.
5) subjects that aren't able to tolerate the 150mg dose for more than two weeks.

All subjects who begin treatment will be included in the primary analysis, except those subjects that are unable to tolerate the 150mg dose for at least two weeks. In order to maintain an adequate sample size, additional subjects will be offered enrollment in order to account for those subjects that were unable to tolerate the 150mg dose for at least two weeks.

10.3 Data Analyses

All subjects who begin treatment will be included in the primary analyses, except those subjects that are unable to tolerate the 150mg dose for at least two weeks. The subject who goes off study before the time when the primary endpoint is evaluated will be considered as a non-responder. Every effort will be made to reach and evaluate the subjects at the 6 week, 9 week, 12 week, 15 week, 18, and 19 week end of intervention period. Adverse events will be tabulated by severity and disease (lung versus hematopoietic). For secondary endpoints, especially, the endpoints that were measured at multiple times such as 6, 9, 12, 15, 18, 19 weeks or until study end, a mixed-effects regression approach will be used to estimate the change of the endpoints and the effect of prognostic factors.

Final statistical analysis will be performed once all subjects have completed the end of study assessments and all data have been archived for analysis. We will use stratified analyses to account for effects of systemic immunosuppressive agents and other prognostic factors.

Secondary endpoints (such as change of 6 minute walk and the quality of life, etc) will be studies by repeated measured mixed effects regression, multiple regression, logistic regression and nonparametric regression models, if deemed appropriate.

Pharmacokinetic analysis
Inhaled cyclosporine pharmacokinetics will be determined by non-compartmental or compartmental analysis using WINNOLIN (Pharsight, Mountain View, CA) or other software as appropriate. For subjects who are administered both oral and inhaled cyclosporine, concentrations will be analyzed using the superposition principle. Parameters estimated will include but not limited to eliminate rate constant, volume of distribution, and area under the concentration versus time curve.

10.4 Stopping Rule

The study will be monitored to ensure that the occurrence of a specified set of treatment related serious adverse events (TRSAEs) during the 18 weeks of intervention does not substantially exceed an anticipated rate. The following TRSAEs will be considered for early stopping of either one of groups A or B:

- Death considered to be probably or definitely related to the inhaled CsA or
- Any grade IV toxicity considered to be probably or definitely related to inhaled CsA, i.e. opportunistic infection such as tissue-invasive CMV or *Pneumocystis carinii*, with exception of temporary cytopenias.

We will monitor the numbers of subjects who have developed any of the above specified TRSAEs using the stopping rule outlined below. The trial will be seriously considered for early stopping if the number of subjects in the trial who develop TRSAEs is over the pre-specified threshold value in the Table.

From experience using this agent in other clinical settings as detailed in the investigator's brochure, we anticipate the rate of developing at least one of the above specified TRSAEs to be 10% or less. Following Geller et al., our stopping rule is determined by a Bayesian approach[23]. The stopping boundary for the trial is reached if the Bayesian posterior probability that the true probability of developing one or more of the above specified TRSAEs exceeds this benchmark rate of 10% is at least 90%. We take our prior distribution to be a beta distribution with the sum of the two beta parameters to be 3, i.e. the parameters of the beta prior distribution are 0.30 and 2.70. Since we have seen in the past that the first few subjects to be accrued are possibly sicker than the rest of the subjects in the sample, we will start safety monitoring when 3 subjects have developed specified SAEs. The following table summarizes the threshold numbers for stopping either group A or B:

Number of Subjects	Stop if the number of subjects who develop any of the number of specified TRSAEs reaches:
≤ 11	3
$12 \leq n \leq 18$	4
$19 \leq n \leq 25$	5
$26 \leq n \leq 32$	6
$33 \leq n \leq 39$	7

We investigated the performance of the above stopping rules by a simulation study. For the stopping rule, we generated a study with 39 independent Bernoulli trials, each had a probability p=.1 for having the above TRSAE and q=1-p for not having such TRSAE and compared the TRSAE outcomes with the above stopping boundary to determine whether the study was stopped. We repeated the simulation 100,000 times and computed the proportion of stopped studies (i.e. "number of stopped studies"/100,000), which were stopped using the above stopping rule.

The following table summarizes the proportions of stopped studies under a number of scenarios.

Prob of specified TRSAE	5%	10%	15%	20%	30%
Proportion of Stopped Studies	2.2%	20.2%	51. 5%	77.4%	98.1%
Average Number of Subjects	38.4	34.5	27.8	21.0	11.9
Average Number of Subjects Suffering TRSAE	1.93	3.47	4.18	4.18	3.54

The DSMB will evaluate all serious adverse events and have all the required information to implement the above-defined monitoring plan. In addition, the DSBM may recommend early study termination if other unforeseen adverse events necessitate this decision.

10.5 Off Study Criteria

10.5.1 Subject choice: Subjects may withdraw from the study at their request any time. The risks of withdrawing will be discussed, as will alternative treatment options. Those subjects who choose to withdraw will be strongly encouraged to participate in study assessments until he/she initiates alternative BO therapy. In this scenario, patients will be deemed off study-drug, but have the option to continue on-study.

10.5.2 Principal investigator decision:

Subjects will be taken off study drug during the 18 week period of intervention if they:

- Have disease progression (beginning at week 9) as defined by a 20% or more decline in FEV_1 on two successive measurements at least 2 weeks apart
- Have evidence of an NCI grade IV toxicity related to study drug administration

- Require >25% increase in one or more existing immunosuppressive agents or the addition of new systemic immunosuppressive therapies due to worsening symptoms related to BOS (sustained for 3 weeks, excluding adjustments made for target drug levels) after week 9 (see section 6.2.3).
- Develop worsening performance status, defined by ECOG score ≥ 3 (please see hematology supportive care guidelines)
- Develop disease relapse or a significant non-pulmonary transplant complication in which death is likely to occur prior to the conclusion of the 18 week study period
- Are unable to comply with the study visits or become severely ill and cannot comply with the intervention.
- Are unable to tolerate the 150mg dose of the inhaled CsA
- Develop severe pulmonary infection that fails to respond to conventional treatment or where no effective drug treatment exists
- Subject becomes pregnant

Subjects that have stopped study drug administration will be asked to complete the study assessments, as off-study drug participants, whenever feasible. Lung deposition studies and pharmacokinetic tests will not be performed in off-study drug participants. Chest CT and bronchoscopy will only be performed if clinically indicated. If off-study drug subjects are unable or unwilling to complete the study assessments, they will be referred back to their primary transplant team and will end study participation.

10.5.3 Completion of the study

Upon completion of the week 19 assessment, subjects will have completed study participation and be taken off study and referred back to their primary transplant team to continue post transplant monitoring and care. After Week 19 assessment is completed, subjects with stabilized or improved disease will be offered the option of continuing CsA on **an extended access protocol**.

11.0 DATA AND SAFETY MONITORING

11.1 Safety Monitoring

NHLBI DSMB: The NHLBI Data Safety and Monitoring Board will review the protocol at an interval to be determined by the DSMB. A progress report will be forwarded to the DSMB at these times and their recommendations will be expeditiously implemented. The DSMB may recommend early termination of the study for considerations of safety and efficacy.

Safety Monitoring:

Principal Investigator: Accrual, efficacy and safety data will be monitored by the Principal Investigator, Nicole Gormley, MD and Richard Childs, M.D., Medically Responsible Investigator.

NIH IRB. Prior to implementation of this study, the protocol and the proposed consent and assent forms will be reviewed and approved by the properly constituted Institutional Review Board (IRB) operating according to Title 45 CFR 46. This committee will also approve all amendments to the protocol or informed consent, and conduct continuing annual review so long as the protocol is open to accrual or follow up of subjects.

FDA: An annual progress report, any amendments to the protocol, and any change in the status of the protocol will be forwarded to FDA to:

June Germain, MS, Regulatory Officer
Renata Albrecht, MD, Division Director
Division of Special Pathogen and Transplant Products Central Document Room Center of Drug Evaluation and Research, FDA
5901-B Ammendale Road, Beltsville, MD 20705 (w) 301-796-4024

APT Pharmaceuticals, Inc.: A copy of the annual progress report to FDA, any amendments to the protocol, and any change in the status of the protocol will be forwarded to

Greg Baigent [gbaigent@aptbio.com]
APT Pharmaceuticals, Inc
700 Airport Blvd, Suite 350, Burlingame, California 94010
Telephone Number Fax Number

NIH Radiation Safety Committee (RSC): NIH Radiation Safety Committee (RSC): Because the technetium 99m silver colloid deposition studies are research procedures and because the frequency of CT imaging exceeds that which is standard in this subject population, some of the radiation exposure is therefore determined as indicated for research purposes. The Radiation Safety Form 88-23a, which includes dosimetry calculations for the high resolution CT scans and lung deposition studies utilizing radio-labeled drugs, was submitted to the Radiation Safety Committee for review (c/o Sarah Kindrick, M.D., Clinical Protocol Administrator, NIH Radiation Safety Committee 301-496-2253) for initial and triennial RSC review so long as the protocol remains open for subject accrual and intervention.

	SCREEN		**On study medication**			
PROTOCOL TIMEPOINT	**Pre-study**		**Day 7**	**Day 63**	**Day 126**	
High resolution chest CT	□			□	□	
Radiolabeled Lung disposition studies			□	□		

Data Management: The PI will be responsible for overseeing entry of data into an in-house password protected electronic system and ensuring data accuracy, consistency and timeliness. The principal investigator, associate investigators, research nurses and/or a contracted data manager will assist with the data management efforts. Data will be abstracted from Clinical Center progress notes as well as from progress notes forwarded from home physicians. Laboratory data from NIH will be imported electronically from CRIS into an in-house clinical trial database. Laboratory values from referring home physicians will be entered into the system.

In order to maintain subject confidentiality, all case report forms, study reports and communications relating to the study will identify subjects by an assigned unique identifier. Case report forms may serve as source data if needed. All human subjects personally identifiable information (PII) as defined in accordance to the Health Insurance Portability and Accountability, eligibility and consent verification will be recorded. Primary data obtained during the conduct of the protocol will be kept in secure network drives or in approved alternative sites that comply with NIH security standards. Primary and final analyzed data will have identifiers so that research data can be attributed to an individual human subject participant, e.g., a unique code, or minimum PII required for subject identification. All primary and analyzed data will be located on the secure P drive.

In accordance with local and federal regulations, the Investigator will allow APT Pharmaceuticals, Inc. personnel or their designee, access to all pertinent medical records in order to verify the data gathered on the case report forms and to audit the data collection process. The US Food and Drug Administration (FDA) may also request access to all study records, including source documentation for inspection.

End of Study Procedures: Data will be stored in locked cabinets and in a password protected database until it is no longer of scientific value. Federal law requires that an Investigator maintain all study records for the indication under investigation for two years following the date a Product Licensing Application is approved or, if no application is to be filed or if the application is not approved for such indication, until two years after the investigation is discontinued and the FDA is notified. The results will be posted to clinicaltrials.gov.

Loss or Destruction of Data: Should we become aware that a major breech in our plan to protect subject confidentiality and trial data has occurred, the IRB will be notified.

Source Documentation: Clinical data entered onto the Case Report Form (CRF) may serve as source documentation.

Clinical Trial Monitoring Plan: Monitoring Responsible Party/ Monitor: An experienced independent protocol monitoring group has been contracted by the NHLBI. They will objectively audit records for compliance with the protocol.

Monitoring will assure the adequate protection of human subjects, safety of subjects involved in clinical investigations and the integrity and quality of data. The monitor/ responsible party for monitoring will be trained to perform monitoring activities.

Monitoring may occur prior to the IRB approval of the study (protocol/ site initiation), routine monitoring (during the protocol life cycle), and study termination. The monitoring activities will verify regulatory compliance, NIH & DIR policy compliance, IRB regulatory compliance, subject record review, and protocol compliance. The monitoring activities will be documented and the reports will be made available to regulatory personnel.

12. NIH INTRAMURAL IRB AND NHLBI CD REPORTING

12.1 Expedited Reporting

Events requiring expedited reporting will be submitted to the IRB per Policy 801 “Reporting Research Events”.

12.2 Reports to the IRB at the time of Continuing Review:

The PI or designee will refer to HRPP Policy 801 “Reporting Research Events” to determine IRB reporting requirements and timelines.

12.3 Reports to the CD:

The PI or designee will refer to NHLBI DIR guidelines to determine CD reporting requirements and timelines.

12.2 Assessment of Adverse Event Severity and Relationship to Treatment

The AEs will be graded by severity utilizing CTC version 4.0. A copy of the criteria can be down-loaded from the CTEP home page at http://ctep.cancer.gov/reporting/ctc.html. The category that overall best "fits" the relationship between the adverse event and the study drug should be chosen and recorded on the CRF and SAE form, if appropriate.

The investigator is responsible for assessing the causal relationship between any events and the study treatment. AEs will be attributed as unrelated, unlikely, possibly, probably, or definitely related to study medication or procedures. Additionally, the investigator is responsible for providing appropriate treatment for the event and for adequately following the event until resolution.

Relationship Between Treatment and AE

Unrelated	No temporal association to study product. An alternate etiology has been established. The event does not follow the known pattern of response to study product. The event does not reappear or worsen with re-challenge.
Probably not related / remote	No temporal association to study product. Event could readily be produced by clinical state, environmental or other interventions. The event does not follow the known pattern of response to study product. The event does not reappear or worsen with re-challenge.
Possibly related	Reasonable temporal relationship to study product. The event is not readily produced by clinical state, environmental, or other interventions. The event follows a known pattern of response to the study product or as yet unknown pattern of response.
Probably related	There is a reasonable temporal association with the study product. The event is not readily produced by clinical state, environmental, or other interventions. The event follows a known pattern of response to the study product. The event decreases with de-challenge.
Definitely related	There is a reasonable temporal relationship to the study product. The event is not readily produced by clinical state, environmental, or other interventions. The event follows a known pattern of response to the study product. The event decreases with de-challenge and recurs with re-challenge.

Treatment related SAEs (TRSAEs) are those attributed as possibly, probably or definitely. As detailed in section 9.7 stopping rules, death and any grade IV toxicity considered to be possibly, probably or definitely related to CIS will be monitored and considered for early stopping the study according to statistically determined criteria.

Hospitalization (overnight admission) for routine supportive care (platelet or RBC transfusions) or admission from the NIH inpatient unit to the NIH ICU for routine monitoring will not be reported as a serious adverse event.

APT Pharmaceuticals, Inc.: A listing of all SAEs will be submitted to APT pharmaceutical quarterly. Safety reports will be reported as detailed in section 12.5.

12.4 IND Safety Reporting to the FDA (IND 109,707)

Because this study will be conducted under an IND, U.S. Government regulations 21CFR, Part 312.32 require the issuance of an IND Safety Report to the Food and Drug Administration (FDA) for all "serious" adverse events that are "unexpected" and for which there is a "reasonable possibility that the experience may have been caused by the drug." Such serious events that are characterized as fatal or life-threatening must be reported to the FDA within 7 calendar days of the sponsor's initial receipt of the information, whereas non-fatal and non-life threatening events must be reported within 7 calendar days.

No expedited IND Safety Report is required if the event is considered to be expected based either on the study drug product label, the underlying disease states, or the study procedures.

The Safety report will contain a full written summary detailing relevant aspects of the serious adverse events in question. In each written IND safety report, all safety reports previously filed with the IND concerning a similar adverse experience will be included and the significance of the adverse experience in light of the previous, similar reports will be discussed.

Where applicable, information from relevant hospital case records and autopsy reports will be included. The investigator will always provide an assessment of causality at the time of the initial report as described in 'Assessment of Causality'.

Follow up information regarding the subject's subsequent course will be submitted until the SAE has resolved, the subject's condition stabilizes (in the case of persistent impairment) or the subject dies.

12.5 Sponsor's Reporting Responsibilities

Serious and unexpected suspected adverse reactions (SUSARs) as defined in 21 CFR 312.32 and determined by the sponsor will be reported to FDA as IND Safety Reports. The Sponsor will also submit an IND Annual Report of the progress of the investigation to the FDA as defined in 21 CFR 312.33.

Telephone and facsimile transmission safety reports. The sponsor shall also notify IRB, DSMB and FDA by telephone or by facsimile transmission of any **unexpected fatal or life-threatening experience associated with the use of the drug** as soon as possible but in no event later than 7 calendar days after our initial receipt of the information.

Hard copy submission of safety reports: Each notification will be made as soon as possible and in no event later than 7 calendar days after the sponsor's initial receipt of the information.

If an adverse drug experience not initially determined to be reportable is later determined reportable, a written safety report will be submitted as soon as possible, but in no event later than 7 calendar days after the determination is made.

12.5.1 Report Recipients

Safety reports will be submitted according to the reporting time frame (section 12.5.) to

- NHLBI DSMB

- FDA: June Germain, MS, Regulatory Officer
  Renata Albrecht, MD, Division Director
  Division of Special Pathogen and Transplant Products Central Document
  Room Center of Drug Evaluation and Research, FDA
  5901-B Ammendale Road Beltsville, MD 20705 (w) 301-796-4024

- **APT** APT pharmacovigilance Officer
  APT Pharmaceuticals, Inc
  700 Airport Blvd, Suite 350, Burlingame, California 94010

12.6 Reporting of pregnancy

Subjects who become pregnant during the study will discontinue the study medication immediately. The investigator, or his/her designee, will collect pregnancy information on any subject who becomes pregnant while participating in this study and submit this information to APT and the IRB within two weeks of learning of a subject's pregnancy. Information on the status of the mother and fetus will be forwarded to APT and the IRB as available. While pregnancy itself is not considered to be an AE or SAE, any pregnancy complication, spontaneous abortion, or elective termination of a pregnancy for medical reasons will be reported to APT in an SAE format. Generally, follow-up will be no longer than 6 to 8 weeks following the estimated delivery date and will include information on outcome of the pregnancy.

13.0 HUMAN SUBJECTS PROTECTIONS

13.1 Rationale for Subject Selection

No subjects will be excluded from participation based on gender, race or ethnicity. The study will be open to all subjects who satisfy the inclusion criteria and provide an informed consent to the protocol.

From previous hematopoietic transplant protocol recruitment patterns from NHLBI transplant recipients, we expect the population may be distributed as follows:

- By gender: 40% females; 60% males
- By age: ages 11-73, median 38; 7% ages 10-17; 20% ages 18-30; 28% ages 31-40; 22% ages 41-50 and 23% ages 51-73
- By race: 11% Asian, 8% Black, 44% Hispanic, 37% White

The Lung transplant recipients will be referral from the University of Maryland, which according to their prior accrual demographics may be distributed as follows:

- By gender: 45% females; 55% males
- By age: ages 23-71, median 58
- By race: 45% Black, 55% White

Recruitment: Subjects will be recruited from NIH Clinical Center hematopoietic stem cell transplant clinics and from the Lung transplant center at the University of Maryland.

Competition with other Branch protocols: None. However, there is an actively accruing protocol at the Clinical Center 08-C-0097: A Multi-Institutional Prospective Phase II Study of Montelukast for the Treatment of Bronchiolitis Obliterans Following Allogeneic Stem Cell Transplantation in Children and Adults. They will refer subjects to our study that have completed/failed their protocol.

Reimbursement for protocol participation, travel, food, and lodging will be consistent with NIH guidelines. In determining reimbursement, the following factors are considered applicable to this protocol: the patients are diagnosed with a rare disease; the patient population is sick; the protocol offers the potential for direct benefit; the protocol regimen is demanding; and in order to complete accrual in a reasonable timeframe a geographically dispersed participant population is required.

13.2 Participation of Children

This study will be limited to subjects aged 10 or older. Various study tests and medication administration requires adequate understanding and ability to participate in care. As such, pediatric subjects 10 years of age or older will be allowed to participate in this study as they are at an age where they can cooperate sufficiently and children less than age 10 will be excluded.

13.3 Risks and Discomforts

13.3.1 Related to CIS

CIS is under development for the indication of improved BOS-free survival following lung transplantation. As of November 13, 2009, approximately 444 lung transplant recipients have received CIS under protocols ACS001, ACS002, ACS004, or CIS001. In ACS001, the following adverse events occurred significantly more often in subjects receiving CIS than in subjects receiving placebo:

- Events that occurred in 30 to 50% of subjects receiving CIS: cough, chest pain, back pain, increased urinary frequency, pharyngitis, exacerbation of shortness of breath, and lung consolidation.

- Events that occurred in 10 to 29 % of subjects receiving CIS: cardiac murmur, respiratory tract irritation, polyuria, somnolence, hyperlipidemia, respiratory disorder, and hemoptysis.

In the studies that were not placebo controlled, or are as yet unblinded, the following adverse events were seen:

1. Events that occurred in greater than 10% of subjects: pyrexia, nausea, diarrhea, dyspnea, cough, wheezing, chest pain, pharyngitis, renal impairment, peripheral edema, lung transplant rejection, headache, insomnia, and neutropenia.
2. Events that occurred in 5 to 10% of subjects: Pharyngolaryngeal pain, pleural effusion, rhinorrhea, constipation, vomiting, fatigue, chest pain, candidiasis, pseudomonas infection, sinusitis, pneumonia, hyperkalemia, leucopenia, anemia, and hypertension.

Although not previously observed, in the current study, one subject did develop a rib fracture associated with coughing.
Unknown side effects: In addition, as with other clinical studies using experimental medicines and in subjects with serious medical conditions, there may be adverse events or side effects that are currently unknown and certain of these unknown risks could be permanent, severe, or life threatening. Subjects will therefore be carefully monitored and instructed to seek medical attention in the event of serve adverse events and contact the research team as needed.

During treatment, the study medication should be administered in a private, well-ventilated area to minimize the risk of other people being unnecessarily exposed to the drug particles in the air. Pregnant women, children, and individuals with asthma or other diseases with significant respiratory symptoms should avoid being in the room during or immediately after treatment.

Cyclosporine, when taken by mouth can cause kidney damage especially if blood levels are high. So far, use of CIS has not caused this problem in previous human research studies and should not increase this risk since blood levels have been very low

APT Pharmaceuticals remains attentive to safety throughout all ongoing studies. Additional unexpected safety findings will be provided to investigators upon receipt and evaluation.

13.3.2 Related to albuterol/levalbuterol

Severe allergic reactions (rash; hives; itching; difficulty breathing; tightness in the chest; swelling of the mouth, face, lips, or tongue); chest pain; ear pain; fast or irregular heartbeat; new or worsened trouble breathing; pounding in the chest; red, swollen, blistered, or peeling skin; severe headache or dizziness; unusual hoarseness; wheezing. Subjects will be instructed to discontinue albuterol/levalbuterol use, seek medical attention right away, and notify the research teams should any severe side effect occur.

The most common side effects include: Dizziness; headache; nausea; nervousness; sinus inflammation; sore or dry throat; tremor; trouble sleeping; vomiting.

13.3.3 Related to Blood Collection

Blood collection my cause some pain or bruising at the site on the arm from which the blood was drawn. There is a small possibility of fainting and infection. Similarly, there is a small risk of bleeding, blockage, or inflammation or infection of the vessel. Discomfort does not usually last long and permanent damage is extremely rare.

13.3.4 Related to the pharmacokinetic studies: Subjects will have blood collected at the aforementioned collection times. A brief hospital stay will be required to ensure correct timing of blood draws.

13.3.5 Related to Bronchoalveolar Lavage (BAL)

Subjects will sign a separate procedure consent for the BAL which will further detail the potential risks of this procedure which include:

- Mild discomfort due to coughing. This can be controlled by topical medication.

- A decrease in the amount of oxygen in the blood. Subjects will receive additional oxygen during the procedure and they will be closely monitored for oxygen levels, heart rate and blood pressure. The risk of a serious problem occurring is very small (less than 1 out of 10,000 procedures in published studies).

- A slow or irregular heartbeat occurs (rare). If it does not correct itself, we can treat this with medication. A heart rhythm monitor (electrocardiograph) will be used throughout the procedure.

- Mild bleeding from the nose can occur because the bronchoscope tube is sometimes placed through the nose to reach the large airways. Placing medication and lubricant inside the nose will be done before the procedure to lower the risk of this occurring.

- A sore throat for several hours after the procedure (Less than 10% of subjects)

- A bad reaction to the numbing medication (lidocaine), rarely. Side effects can include confusion or, very rarely, seizures. We have never seen this problem in over 1500 bronchoscopies performed in the Medical Intensive Care Unit at NIH over an 8-year period. This potential problem is minimized by using small, frequent doses of the medication.

- Fever has been reported to develop in less than 5% of healthy volunteers six to eight hours after the bronchoscopy.

- Infection in the lungs (very low risk) resulting from swallowing saliva into the lung airways. To minimize this risk, subjects do not eat or drink for at least six hours before the bronchoscopy.

- Pneumothorax can occur as a result of bronchoscopy, but is a very infrequent complication. It has been estimated that the risk of pneumothorax with trans-bronchial biopsy is between 1-3%.

- Individuals with lung disease may be at greater risk of complications from bronchoscopy, although bronchoscopies with bronchoalveolar lavage are performed commonly for such subjects without any serious complications or effects.

- There are risks associated with the conscious sedation medicine. These risks will be explained to the subjects and they will be consented separately at the time of the procedure.

13.3.6 Related to the Lung Function Tests:

These tests are very safe and side effects are unlikely. During the test, subjects are asked to breathe deeply or rapidly which may occasionally cause brief lightheadedness or soreness of the chest. In extremely rare cases, this may result in the release of a small amount of air from the lung into the lung cavity, which would be treated appropriately.

13.3.7 Related to the 6 Minute Walk

Subjects may develop shortness of breath or fatigue. Participants will be encouraged to walk at a pace that is comfortable for them.

13.3.8 Related to the Gene Expression Profiling Studies

Although genetic information can have implications regarding health, identity, paternity, employability and/or insurability for the research participant as well as family members, **No such relationship has been found for the genes we are testing.** Therefore the nature of the genetic testing (gene expression profiling studies) detailed in the protocol will not put participants at any further risk.

13.3.9 Related to Pregnancy and Nursing Mothers

The effects of albuterol and/or aerosolized cyclosporine A in solution with propylene glycol on the developing human fetus are unknown. For this reason and because it is unknown whether these drugs are teratogenic, women of childbearing potential and men must agree to use adequate contraception prior to (hormonal or barrier method of birth control; abstinence) and for the duration of study participation. If a woman becomes pregnant or suspects she is pregnant while on study, her treating physician should be informed immediately. Nursing mothers must be willing to discontinue nursing.

13.3.10 Related to radiation exposure:

- ***Related to the high resolution CT:*** CT (computed tomography), sometimes called CAT scan, uses special x-ray equipment to obtain image data from different angles around the body and then uses computer processing of the information to show a cross-section of body tissues and organs. Oral and/or intravenous contrast agents will NOT be used.

- ***Related to the lung deposition studies:*** Radioisotope tracer sulfur colloid will be inhaled along with the cyclosporine dissolved in propylene glycol for one treatment session. This is generally well-tolerated. Planar and SPECT/CT imaging will be performed to quantify the amount of drug deposited in the lungs and assess distribution.

13.4 Risks in relation to benefits

13.4.1 For Adult Subjects

The risks of participating in this trial are limited to side effects of the albuterol/levalbuterol and inhaled CsA, and the risks of standard diagnostic procedures (BAL and biopsy, the high resolution CT, and pulmonary function tests), the 6 minute walk, the lung deposition study, and the additional peripheral blood sample to be used strictly for research purposes. Samples for clinical monitoring will be collected during sample collection procedures that are part of their routine post transplant care and therefore pose no additional burden or risk.

The benefits to the subjects could be improvement in BO resulting in improved quality of life, decreased mortality associated with severe BO (should it develop), and potentially, treatment with other more toxic systemic therapies could also be avoided or postponed. Subjects will also receive direct health benefits due to thorough post transplant pulmonary examinations, early diagnosis of progression of BO and therefore earlier access to appropriate BO management.

Therefore, for adult subjects participating on this study, the research involves greater than minimal risk to subjects with the prospect of direct benefit (45 CFR 46.102*)*.

13.4.2 For Pediatric Subjects

DHHS will conduct or fund research in which the IRB finds that more than minimal risk to children is presented by an intervention or procedure that holds out the prospect of direct benefit for the individual subject, only if the IRB finds that:

(a)"The risk represents a minor increase over minimal risk". The risks of participating in this trial are limited to the side effects of the albuterol and inhaled CsA, the risks of standard diagnostic procedures (BAL and biopsy, the high resolution CT, the pulmonary function tests and the 6 minute walk), lung deposition studies, and the additional peripheral blood sample to be used strictly for research purposes. Samples for clinical monitoring will be collected concurrently with samples that are part of their routine post transplant care. Only those laboratory tests approved by the IRB and involving not greater than minimal risk will be conducted (See Appendix B).

(b) *The relation of the anticipated benefit to the risk is at least as favorable to the subjects as that presented by available alternative approaches*. The risk associated with the monitoring that will be done on this ancillary protocol exceeds only minimally that would be done routinely in post transplant subjects who have developed BO. This risk is most favorable given that pediatric subjects could derive benefit in improvement in BO, resulting in improved quality of life, decreased mortality associated with severe BO (should it develop), and avoidance of treatment with other more toxic systemic therapies. Subjects will also receive direct health benefits due to thorough post transplant pulmonary examinations, early diagnosis of BO progression, and therefore earlier access to appropriate BO management.

(c) *Adequate provisions are made for soliciting the assent of the children* and permission of their parents or guardians, as set forth in 46.408. An assent is available.

Therefore, the inclusion of children satisfies the criteria set forth in 45 Code of Federal Regulations 46, Subpart D: 46.405 Research involving greater than minimal risk but presenting the prospect of direct benefit to the individual subjects.

13.5 Informed Consent Processes and Procedures

The investigational nature and research objectives of this trial, the procedure and its attendant risks and discomforts will be carefully explained to the subject and when applicable the subjects' parents. The potential subject will be educated regarding the nature of the condition, proposed intervention, and outcome measures. Study subjects will be informed that participation is entirely voluntary and that withdrawal from the study can be made at any time without penalty of benefits to which they may be entitled. No consenting will be done by outside investigators or at outside institutions.

At any time during participation in the protocol, if new information becomes available relating to risks, adverse events, or toxicities, this information will be provided orally or in writing to all enrolled or prospective subject participants. Documentation will be provided to the IRB and if necessary the informed consent amended to reflect relevant information.

Informed consent of non-English speaking research participants: We anticipate the enrollment of Spanish-speaking research participants into our study. The IRB approved full consent document will be translated into that language in accordance with the Clinical MAS Policy M77-2. If there is an unexpected enrollment of a research participant for which there is no translated extant IRB approved consent document, the principal investigator and or those authorized to obtain informed consent will use the Short Form Oral Consent Process as described in MAS Policy M77-2, 45 CFR 46.117 (b) (2), and 21 CFR50.27 (b) (a). The summary that will be used is the English version of the extant IRB approved consent document.

We request prospective IRB approval of the use of the short form for up to a minimum of **10** participants in a given language and will notify the IRB at the time of continuing review of the frequency of the use of the Short Form. Should we reach the threshold of **10**, we will notify the IRB of the need for an additional use of the Short Form and that we will have that consent document translated into the given inherent language.

Minor subjects: If the patient is a minor, a minor assent will be sought. Where deemed appropriate by the clinician, and the child's parent or guardian, the child will also be included in all discussions about the trial and a minor's assent will be obtained. The parent who signs the consent for the minor must be a legally recognized parent or guardian. The parent or guardian will sign on the designated line on the informed consent attesting to the fact that the child had given assent. When the assent is not age appropriate, the study will be explained to the child and the assent will be obtained verbally from the child. Once the minor reaches 18, he/she will be consented for continued protocol participation using the adult consent.

Because this research holds a prospect of direct benefit to the health or well-being of pediatric participants and is available only in the context of the research, the assent of the pediatric participant is not a necessary condition for proceeding with the research. However, in the case of dissent, an independent pediatric care team (social worker and mental health specialist (psychologist or psychiatrist) will meet with the minor and his family to re-emphasize the importance of treatment on protocol; if the child continues to dissent, an ethics consult will be requested prior to enrollment.

When a pediatric subject reaches age 18, continued participation will require re-consenting of the now adult with the standard protocol consent document to ensure legally effective informed consent has been obtained. Should sample or data analysis continue following completion of active participation and the subject has reached 18 years of age, we will attempt to contact the subject using the last known contact information to obtain consent for continued use of data or samples collected during their prior visit. Given the length of time that may have transpired for some of the subjects since their last visit for this study, we request waiver of informed consent for those individuals who after good faith efforts to contact them, we are unable to contact.

Requirements for Waiver of Consent consistent with 45 CFR 46.116 (d), each of which must be addressed in relation to the protocol:

(1) The research involves no more than minimal risk to the subjects;
   a) Analysis of samples and data from this study involves no additional risks to subjects.

(2) The waiver or alteration will not adversely affect the rights and welfare of the subjects;
   a) Samples and data will be kept in secure locations in the laboratory of Dr. Young. Retention of samples or data does not affect the welfare of subjects.

(3) The research could not practicably be carried out without the waiver or alteration; and

a) Considering the length of time between a minor's enrollment and their age of majority, it is possible that more than a few subjects may be lost to follow up. A significant reduction in the number of samples analyzed could impact the quality of the research.

(4) Whenever appropriate, the subjects will be provided with additional pertinent information after participation.

a) We only plan to request a waiver of re-consent for those subjects who have been lost to follow-up.

In cases where parents share joint legal custody in making medical decisions of their child (e.g. by a custody agreement or court order) both parents must give their parental permissions regardless of level of risk of the research. Exceptions may be made if one parent is deceased, becomes incompetent or is not reasonably available (e.g. in prison).

Consenting to Pregnancy Testing in Minors of Childbearing Age: We will inform the minor during the assent process that for safety, we need to do a pregnancy test. She will also be told that if it is positive, we will counsel her and help her tell her parents. If the minor does not want to proceed she will be advised not to sign the assent and her enrollment on this screening protocol will end.

13.6 Conflict of Interest

The Principal Investigator assured that each associate investigator listed on the protocol title page received a copy of the NIH's Guide to preventing conflict of interest. Investigators added subsequent to the initial circulation were provided a copy of the document when they were added. No initial or subsequent members of the research team reported a potential conflict of interest.

This protocol has no associated patents, however, outside associate investigator Aldo Iacono, MD holds a US patent application A32130-072396.0162 11/11/1999 for Uses of aerosol cyclosporine for prevention and treatment of pulmonary Disease. Dr. Timothy Corcoran has received research grant funding from APT to the University of Pittsburg. To avoid any conflict of interest, Dr Iacono and Dr Corcoran will not participate in the consent process nor will they participate in any of the endpoint evaluations.

13.7 Technical Transfer Agreements

An MTA with APT Pharamceuticals, Inc was previously signed that allowed for in vitro assays to be performed using the cyclosporine in propylene glycol.

A CRADA between APT Pharmaceuticals, Inc has been fully executed.

14.0 PHARMACEUTICAL

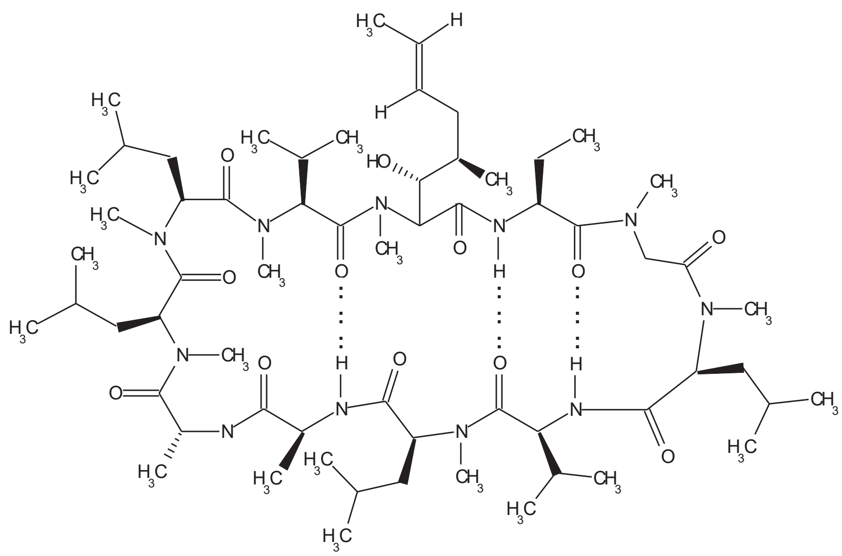
14.1 Cyclosporine Inhalation Solution (CIS)

Physical, chemical, and pharmaceutical properties CIS is a sterile, clear, colorless, preservative-free solution of cyclosporine (USP) in propylene glycol developed specifically for administration by oral inhalation. The active principle of CIS is a cyclic polypeptide immunosuppressant agent consisting of 11 amino acids. It is produced as a metabolite by the fungus species Beauveria nivea. The molecular formula is $C_{62}H_{111}N_{11}O_{12}$ and the molecular weight is 1202.63.

Chemical name: The chemical name for cyclosporine is [R-[R*,R*-(E)]]-cyclic(L-alanyl-D-alanyl-N-methyl-L-leucyl-N-methyl-L-leucyl-N-methyl-L-valyl-3-hydroxy-N,4-dimethyl-L-2-

amino-6-octenoyl-L-α-amino-butyryl-N-methylglycyl-N-methyl-L-leucyl-L-valyl-N-methyl-L-leucyl).

Chemical Structure



Supply: CIS is manufactured and filled into a sterile, single-use clear 6 mL glass vial sealed with a sterilized, siliconized light gray bromobutyl rubber stopper secured by a crimped tear-off aluminum overseal. This container closure system provides protection of the formulation from microbial contamination, moisture ingress, and other environmental contaminants during storage. Each vial contains 5.2 mL of solution containing 325 mg cyclosporine. This vial contains a sufficient amount of cyclosporine, USP in propylene glycol, USP to deliver 300 mg in 4.8 mL. CIS is designed specifically for aerosol inhalation via a single use SideStream® disposable nebulizer (Respironics, Inc., Murrysville, PA) with the Mobilaire compressor (Invacare, Elyria, OH) at 30 psi. Previous studies have used the AeroTech™ II nebulizer with a high flow compressor such as the Sunrise DeVilbiss 8650D set at 40 pounds per square inch (PSI). At 40 PSI, the average flow rate from the compressors is approximately 12.5 L/min.

Storage: CIS vials should be stored in the upright position at controlled room temperature at controlled room temperature, 25ºC (77°F), excursions permitted to 15°-30°C (59-86°F). Do not store in the refrigerator or freezer. CIS vials are single use. Any remaining CIS in the vial after dosing should be discarded.

Drug Product Composition

Component	Quality Standard	Function	Amount per vial	Concentration (% w/vol)
Cyclosporine	USP	Active ingredient	325 mg	62.5%
Propylene Glycol	USP	Solvent	5.2 mL	-

At a formulation of 62.5 mg/mL, the cyclosporine concentration in CIS is well below the saturation solubility of 400 mg/mL in propylene glycol. Cyclosporine is freely soluble at the manufacturing conditions of 20 - 40ºC and during long-term storage at 30ºC. Therefore, the risk of crystallization during its shelf life is low.

Aerosol Properties: An in vitro study was performed to assess the aerosol characteristics of CIS in the Sidestream® Disposable nebulizer. The mass median aerodynamic diameter (MMAD) particle size was determined by NGI impaction to be 1.9 µm. The geometric standard deviation (GSD) was 1.9. These aerosol particle properties are well suited for both large airway and small airway deposition.

Shipping: The NIH Pharmaceutical Development Services will be responsible for receiving, storing, dispensing and accounting for drug product. The shipping address for APT Pharmaceuticals, Inc supplied investigational agent is:

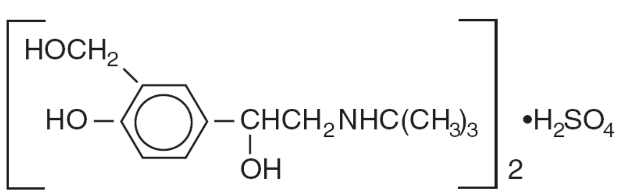
National Institutes of Health
PHARM DEV SVC, Room 1D35
10 Center Drive, MSC 1196, Building 10
Bethesda, Maryland 20892-1196
Shipping Designee Name: Judith Starling, RPh
Shipping Designee Phone No: (301) 496-1031
Shipping Designee FAX No: (301) 402-3268
Shipping Designee e-mail: jstarling@NIH.gov

Accountability Procedures: Drug accountability records will be maintained for all clinical supplies. All empty and partially used vials and clinical trial supplies will be destroyed locally according to the institution's standard operating procedures for drug destruction. The pharmacy will maintain detailed documentation of the number and identification of vials, which are destroyed, and copies of these documents will be provided to the Sponsor. Disposition of all unused boxes of study drug will be carried out according to instructions provided by the Sponsor at the end of the study after drug accountability is performed by the study monitor.

14.2 Albuterol

Physical, chemical, and pharmaceutical properties: Albuterol is a white or practically white powder, freely soluble in water and slightly soluble in alcohol. It is a relatively selective beta-2 adrenergic bronchodilator. The molecular formula is $(C_{13}H_{21}NO_3)_2 \cdot H_2SO_4$ and the molecular weight is 576.71.
Chemical name: The chemical name for albuterol is α^1[(*tert*-Butylamino)methyl]-4-hydroxy-m-xylene-α,α′diol sulfate (2:1) (salt).

Chemical Structure:



Supply: Albuterol is supplied in unit-dose vials containing albuterol sulfate inhalation solution 0.083%, 2.5mg/3ml.

Storage: Albuterol should be protected from light and stored in the pouch until time of use. It should be stored between 2^o and 25^o C (36^o and 77^o F).

15.0 Role of Collaborators

Aldo T. Iacono, M.D., Medical Director, Lung Transplant Program
Role: Will provide advice regarding administration of the CIS, trial design and clinical management of patients who have received a lung transplant.
Michael Terrin, MD, Professor of Epidemiology and Medicine
Role: Will provide trial design advice.
Timothy Corcoran, PhD, Professor of Medicine and Bioengineering, Pulmonary, Allergy and Critical Care Medicine,
Role: Will provide advice regarding the lung deposition studies.
The collaborators will not have access to Personally Identifiable Information (PII) in the study.

16.0 REFERENCES

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APPENDIX A MEDWATCH FORM FDA 3500A

http://www.fda.gov/Safety/MedWatch/HowToReport/DownloadForms/default.htm

APPENDIX B NHLBI HEMATOLOGY BRANCH LABORATORY RESEARCH STUDIES

	DESCRIPTION OF LABORATORY STUDY BY BRANCH SECTION	Does this test pose a greater than minimal risk to pediatric subjects per 45 CFR 46.404?	Does this test pose a greater than minimal risk to healthy pediatric donors per 45 CFR 46.404?
A	**Stem Cell Allotransplantation Section (Dr. A. John Barrett)**		
A.1	Measurement of lymphocyte function and immune responses directed toward allogeneic tissues, malignant cells, and infectious agents. Assay of a variety of antigens, including standard proliferation, cytotoxicity, and intracellular cytokine detection including GVHD predictive markers. Measurement of antigen-specific responses including employment of tetramers, ELISPOT technique, gene amplification-based assays, and flow cytometry. Selection of cells using immunomagnetic beads or flow cytometry. Culture, expansion, and selection of cells. Surface marker analysis of PB MC using flow cytometry. Cytokine/chemokine analysis of plasma/serum samples using ELISA and/or Luminex techniques.	No	No
A.2	Generation of cell lines for the study of immune cell interactions with other cells. Transformation of B-lymphocytes using Epstein-Barr virus. Derivation of malignant cell lines from patient leukemic or solid tumor samples.	No	No
A.3	Infection of cells and cell lines with recombinant genes to ascertain the effects of expressed molecules on immune responses and on growth and development. Transfection of cell lines with specific molecules to study antigen-specific responses.	No	No
A.4	Assays of peripheral blood and bone marrow progenitor cells including primitive and late erythroid progenitor-derived colonies, myelomonocytic colonies, and primitive multi- potential progenitor-derived colonies.	No	No
A.5	Injection of human cells into experimental animals to study the immune system and the growth of normal and malignant cells under varying conditions.	No	No
A.6	Testing of selection methods, cell isolation, and cell expansion leading to the development of new cell-based therapies requiring scale-up for clinical application.	No	No
A.7	Identification of individual T cell clones by their T cell receptor sequence.	No	No
A.8	Measurement of tumor and tissue specific antigens in cells of subjects and donors by mRNA,protein, or peptide expression in cells or fluids.	No	No
A.9	Laser capture micro dissection of cells from biopsies for GVHD to determine clonotypes.	No	No
A.10	DNA and RNA typing of genes that control immune responses in lymphocytes.	No	No
A.11	Microassay studies utilizing cellular DNA, cDNA, and RNA for neoplasia and host-tumor interactions.	No	No
B	**Molecular Hematopoiesis Section (Dr. Cynthia Dunbar)**		
B.1	Flow cytometric analysis of cell surface and cytoplasmic proteins, including cell adhesion molecules, putative retroviral receptors, and markers of differentiation, using bone marrow and mobilized peripheral blood cells.	No	No

B.2	Hematopoietic progenitor-derived colony ascertainment in vitro (as described above), and engraftment of immunodeficient mice for detection of human stem cell number and function.	No	No
B.3	Testing ability of hematopoietic progenitor cells to be transduced with retroviral, lentiviral, and novel gene transfer vectors in vitro.	No	No
B.4	Reprogramming of adult mature cells, including skin fibroblasts and blood cells, into induced pluripotent stem cells in vitro.	No	No
C	**Cell Biology Section (Dr. Neal Young)**		
C.1	Studies of blood and bone marrow hematopoietic progenitor numbers, including early and late erythroid progenitors, myelomonocytic progenitors, and multi-potential progenitor cells. In addition, bone marrow may be placed in long-term bone marrow culture to assess the function of stroma and stem cells and to assay more primitive progenitors, as well as organelle culture. Whole or selected bone marrow populations are cultured short-term for CD34 cell expansion.	No	No
C.2	Assays of apoptosis in hematopoietic cells and their progeny, using flow cytometric methods such as annexin and caspase-3 staining, propidium iodide uptake, and mitochondrial permeability tests.	No	No
C.3	Separation and functional study of cell populations characteristic of paroxysmal nocturnal hemoglobinuria, identified by absence of glycosylphosphatidylinositol anchored proteins.	No	No
C.4	Studies of mutation rates in hematopoietic cells and in buccal mucosa cells, using conventional hypoxanthine phosphoribosyltransferase activity functional assays, sequencing of mitochondrial DNA after specific gene amplification, and measurement of GPI-anchored deficient cells in blood and bone marrow.	No	No
C.5	Assays of immune function of T-cells, including intracellular cytokine staining, ELISPOT, semiquantitative gene amplification for gamma-interferon, tumor necrosis factor, interleukin-2, and other cytokines, and functional assessment in co-culture using specific neutralizing monoclonal antibodies. In addition, peripheral blood lymphocytes are subjected to spectratyping for CDR3 size distribution as well as nucleotide sequence of CDR3 peaks obtained.	No	No
C.6	Studies of engraftment of human normal and diseased bone marrow and peripheral blood in immunodeficient mice in order to determine the presence of hematopoietic repopulating stem cells as well as functional differences among selected populations.	No	No
C.7	Flow cytometric analysis of blood and bone marrow for lymphocyte phenotype, especially for evidence of activation of lymphocytes, for markers of apoptosis, and for antigens associated with primitive and mature hematopoietic cell populations.	No	No
C.8	Flow cytometric analysis of blood and bone marrow for hematopoietic stem cell progenitors and CD34 positive cells.	No	No
C.9	Studies of chromosomal instability in myelopdysplastic syndromes including BM cell and CD34 cell response to PAS crosslinking and examination of the cytotoxic effect of lymphocytes to the abnormal clone of cells.	No	No
C.10	Surface Enhanced Laser/Desorption Ionization (SELDI) time-of-flight mass spectrometry (Ciphergen) (proteomics methodology).	No	No
C.11	Mitochondrial DNA (mtDNA) sequence heterogeneity.	No	No
C.12	Measurement of EBV viral load.	No	No
C.13	Measurement of EBV LMP-1 via RT-PCR for LMP-1 RNA or flow cytometry for LMP-1.	No	No
C.14	Outgrowth assay of EBV transformed B cells.	No	No
C.15	Quantification of serumchemokines and cytokines (e.g. SDF-1, IL-10, IL-6, CXCR4, CXCL12).	No	No
C.16	Quantification of EBV cytotoxic T cells (tetramerstaining).	No	No

C.17	Telomere length measurement by Southern blot, Q-PCR, flow-fish, in situ hybridization and STELA	No	No
C.18	Telomere repair complex gene mutations by nucleotide sequencing of some or all of the following: *DKC1* ,*TERC*, *TERT*, *SBDS*, *NOp10* , *NHP2.*	No	No
C.19	Analysis of inflammatory markers and/or bacterial, viral, fungal or protozoal elements in plasma or serum using molecular, colorimetric, enzymatic, flow cytometric or other assays in subjects receiving immunosuppressive therapy, chemotherapy and/or bone marrow transplantation.	No	No
C.20	Confocal microscopic imaging of bone marrow.	No	No
C.21	Characterization of intracellular signaling proteins by cell permeabilization and flow cytometry, and quantitative immunoblots.	No	No
C.22	Assays for chromosomal aneuploidy by florescence in situ hybridization (FISH) and other molecular techniques.	No	No
C.23	Conversion of human dermal fibroblasts into hematopoietic progenitors using Oct4 transfection.	No	No
D	**Virus Discovery Section (Dr. Neal Young) THESE ASSAYS WILL NOT BE PERFORMED ON SAMPLES FROM HEALTHY PEDIATRIC DONORS**		
D.1	Assays of serum, blood cells, and bone marrow cells for B19 parvovirus and possible B19 variants using gene amplification, cell culture, and hematopoietic colony inhibition assays.	No	N/A
D.2	Assays of blood, bone marrow, liver, and other tissues for potentially novel viruses, using a variety of techniques including RNA and DNA assays, differential display, gene amplification with conserved and random primers, cell culture assays, immunohistochemical methods, and inocculation of mice, rabbits, and monkeys, as well as antibody measurements.	No	N/A
D.3	Assays of blood, bone marrow, and liver for known viruses, including herpesviruses such as cytomegalovirus, human herpesviruses 6, 7, and 8, enteric viruses such as A-6, circiviruses, and parvoviruses, using assays as in (2).	No	N/A
D.4	Spectra-typing of blood cells to determine response to known or putative viral infections.	No	N/A
D.5	HLA typing or subtyping to determine risk factors/determinants for hepatitis-AA studies.	No	N/A
D.6	Cytotoxic lymphocyte assays with intracellular cytokine measurement for determining anti-viral response and lymphocyte cloning to obtain clones with specific antiviral activity.	No	N/A
E	**Solid Tumor Section (Dr. Richard Childs)**		
E.1	Cr51 cytotoxicity assay to evaluating killing of patient tumor cells by patient NK cell clones and T-cells.	No	No
E.2	ELISA for IL-12 maturity of DC's made from subjects monocytes.	No	No
E.3	ELISA for IFN ã to evaluate specificity of CTL clones.	No	No
E.4	H thymidine uptake to evaluate proliferation potential of antigen specific T-cells.	No	No
E.5	PCR of STR to assess chimerism status of cellular subsets grown in-vitro or retrieved from subjects post-transplant.	No	No
E.6	Flow sorting of PBL and/or tissue samples to evaluate chimerism of different subsets.	No	No
E.7	Surface marker analysis of peripheral blood mononuclear cells using flow cytometry.	No	No
E.8	cDNA expression arrays to evaluate T-cells expression/gene patterns in subjects with GVHD and a GVT effect.	No	No
E.9	Geno typing of tumor or tissue samples by high density cDNA arrays.	No	No
E.10	VHL mutation analysis on kidney cancer tissue.	No	No

E.11	Transduction of dendritic and tissue cells with tumor antigens using plasmids, viral vectors and hybrid fusions.	No	No
E.12	Lasar capture microdisection of cells from tumor biopsies and tissue samples to determine origin (donor vs patient).	No	No
E.13	Quantification of polyoma virus BK exposure by serology and PCR in stem cell transplant donors and recipients from blood and urine samples.	No	No
E.14	Quantification of polyoma virus BK specific T cells in stem cell transplant donors and recipients from peripheral blood samples.	No	No
E.15	Determination of origin of neovasculature endothelial cells in tumor and tissue samples obtained from subjects post transplant.	No	No
E.16	Quantification of lymphocyte subsets CD34 progenitors and endovasculator progenitors in G-CSF mobilized peripheral cell allografts.	No	No
E.17	Testing for polyoma virus BK latency in CD34 progenitors, B cells and T cells in the G-CSF mobilized peripheral cell allografts.	No	No
E.18	Determination of etiology of membraneous nephropathy using serum from subjects.	No	No
E.19	Serum Proteomic patterns analysis to diagnose complications related to allogeneic transplantation.	No	No
E.20	Determine cell origin (donor vs patient) of tissue samples using IHC, IF, sorting, and FISH.	No	No
F	**Lymphoid Malignancies Section (Dr. Adrian Wiestner)**		
F.1	Culture of cells from research subjects to investigate molecular disease mechanisms, model host tumor interactions, and to test effect of drugs on cell survival and cellular functions.	No	No
F.2	Generation of stable cell lines for the study of hematologic malignancies.	No	No
F.3	Modifications of cells using standard expression systems or biologic molecules, e.g. interfering RNA, to investigate the effects of candidate genes on cellular functions.		
F.4	Identification and monitoring of B or T cell populations as identified by flow cytometry and by their B cell or T cell receptor expression.	No	No
F.5	Measurement of gene expression in cells or tissues. Techniques frequently used include gene expression profiling on microarrays, quantitative RT-PCR, Western blotting, flow cytometry and ELISA assays.	No	No
F.6	Analysis of chromosomal abnormalities or mutations in malignant cells and non-malignant cells including FISH technology and DNA sequencing.	No	No
F.7	Assays of immune function of B-cells and T-cells, including intracellular cytokine staining, ELISPOT, quantitative RT-PCR for cytokines or other immune regulatory genes.	No	No
F.8	Analysis of antibody specificities in serum and antigen specificity of the B-cell receptor on cells. Techniques may include expression of antibodies in phage display systems, generation of antibodies in cell culture systems and use of such antibodies to screen for cognate antigens.	No	No
F.9	Transplantation of human cells into mice (xenograft model) to study disease biology and to investigate the effect of experimental therapy.	No	No
F.10	Measurements of drug concentrations, biologic molecules and disease markers in blood, serum, and plasma.	No	No

APPENDIX C: DEFINITIONS

ACE inhibitors: Ace inhibitors may increase the nephrotoxicity of cyclosporine; monitor.

Allopurinol: Allopurinol may increase the levels/effects of cyclosporine; monitor cyclosporine concentrations.

Amiodarone: Amiodarone may increase the levels/effects of cyclosporine; monitor cyclosporine concentrations.

Androgens: Androgens may increase the levels/effects of cyclosporine; monitor cyclosporine concentration and for signs/symptoms of renal and/or hepatic toxicity (seen with danazol, fluoxymesterone, methyltestosterone, nandrolone, oxandrolone, oxymetholone, stanozolol, testolactone, and testosterone).

Antacids: Antacids may decrease the levels/effects of cyclosporine.

Antibiotics: Concomitant use may potentiate renal dysfunction (seen with ciprofloxacin, gentamicin, tobramycin, vancomycin, trimethoprim and sulfamethoxazole); increased cyclosporine concentrations by inhibiting cyclosporine metabolism (seen with azithromycin, clarithromycin, erythromycin, and norfloxacin, quinupristin/dalfopristin); may decrease cyclosporine concentrations by inducing cyclosporine metabolism (seen with nafcillin, and rifampin); may decrease immunosuppressant effects (seen with ciprofloxacin); CNS disturbances, seizures (seen with imipenem); imipenem may increase the levels/effects of cyclosporine (monitor for neurotoxicity).

Anticonvulsants: Anticonvulsants may decrease the levels/effects of cyclosporine (seen with carbamazepine, oxcarbazepine, phenobarbital, and phenytoin); monitor cyclosporine concentrations.

Antifungals: Concomitant use may potentiate renal dysfunction (seen with amphotericin B, ketoconazole). May increase cyclosporine concentrations by inhibiting cyclosporine metabolism (seen with fluconazole, itraconazole, ketoconazole, and voriconazole); monitor serum concentrations and renal function.

Antimalarials: Antimalarials may increase the levels/effects of cyclosporine; monitor (seen with chloroquine, hydroxychloroquine, primaquine).

Antineoplastics: Antineoplastics may increase the levels/effects of cyclosporine; monitor for renal dysfunction (seen with melphalan). Cyclosporine may increase the levels/effects of antineoplastics (seen with doxorubicin, etoposide and etoposide phosphate); consider reducing the dose of etoposide and etoposide phosphate by 50% with concomitant administration.

Bosentan: Cyclosporine may increase the levels/effects of bosentan. Bosentan may decrease the levels/effects of cyclosporine. Concurrent use is contraindicated.

Bromocriptine: Increases cyclosporine concentrations by inhibiting cyclosporine metabolism

Calcium channel blockers (eg, diltiazem, nicardipine, verapamil): Calcium channel blockers may increase the levels/effects of cyclosporine; monitor cyclosporine concentrations. Nifedipine has been reported to increase the risk of gingival hyperplasia.

Carbonic anhydrase inhibitors: Carbonic anhydrase inhibitors may increase the levels/effects of cyclosporine (seen with acetazolamide, dichlorphenamide, methazolamide; exceptions are brinzolamide, dorzolamide); monitor.

Caspofungin: Cyclosporine may increase the levels/effects of caspofungin; monitor for hepatotoxicity.

Colchicine: Colchicine may increase the levels/effects of cyclosporine; monitor for nephrotoxicity. Cyclosporine may increase levels/effects of colchicine; monitor for hepatotoxicity and myopathies.

Corticosteroids: Systemic corticosteroids may increase the levels/effects of cyclosporine (reported with methylprednisolone). Cyclosporine may increase the levels/effects of systemic corticosteroids. Convulsions have been reported with high-dose methylprednisolone.

CYP3A4 inducers: CYP3A4 inducers may decrease the levels/effects of cyclosporine. Example inducers include aminoglutethimide, carbamazepine, nafcillin, nevirapine, oxcarbazepine, phenobarbital, phenytoin, and rifamycins.

CYP3A4 inhibitors: May increase the levels/effects of cyclosporine. Example inhibitors include azole antifungals, clarithromycin, diclofenac, doxycycline, erythromycin, imatinib, isoniazid, nefazodone, nicardipine, propofol, protease inhibitors, quinidine, telithromycin, and verapamil.

CYP3A4 substrates: Cyclosporine may increase the levels/effects of CYP3A4 substrates. Example substrates include benzodiazepines, calcium channel blockers, cyclosporine, mirtazapine, nateglinide, nefazodone, sildenafil (and other PDE-5 inhibitors), tacrolimus, and venlafaxine. Selected benzodiazepines (midazolam and triazolam), cisapride, ergot alkaloids, selected HMG-CoA reductase inhibitors (lovastatin and simvastatin), and pimozide are generally contraindicated with strong CYP3A4 inhibitors.

Digoxin: Cyclosporine may increase the levels/effects of digoxin; severe digitalis toxicity has been observed.

Estrogen derivatives: Estrogen derivatives may increase the levels/effects of cyclosporine; monitor cyclosporine concentrations and for signs/symptoms of hepatotoxicity.

Ezetimibe: Ezetimibe may increase the levels/effects of cyclosporine. Cyclosporine may increase the levels/effects of ezetimibe.

Griseofulvin: Griseofulvin may decrease the levels/effects of cyclosporine.

H_2 blockers: H_2 blockers may increase the levels/effects of cyclosporine; monitor cyclosporine concentrations (seen with cimetidine, famotidine, ranitidine).

HMG-CoA reductase inhibitors: Cyclosporine may increase levels/effects of HMG-CoA reductase inhibitors, resulting in myalgias, rhabdomyolysis, acute renal failure; dosage adjustments of HMG-CoA reductase inhibitors are recommended.

Imatinib: May increase cyclosporine serum concentrations by inhibiting cyclosporine metabolism; monitor.

Metoclopramide: Metoclopramide may increase the levels/effects of cyclosporine.

Methotrexate: May increase cyclosporine concentrations and toxicity. Cyclosporine may increase the levels/effects of methotrexate and decreases plasma levels of its metabolite; monitor closely for signs of methrotrexate toxicity.

Minoxidil: Cyclosporine may increase the adverse/toxic effects of minoxidil; may lead to severe hypertrichosis.

NSAIDs: May increase the levels/effects of cyclosporine; concomitant use may potentiate renal dysfunction, especially in dehydrated patients (seen with diclofenac, naproxen, sulindac). Cyclosporine may increase levels/effects of diclofenac; the lowest possible dose of diclofenac should be used

Octreotide: Octreotide may decrease the levels/effects of cyclosporine; monitor cyclosporine concentrations.

Orlistat: Orlistat may decrease the levels/effects of cyclosporine; orlistat may decrease the absorption of oral cyclosporine formulations.

Pimecrolimus: Cyclosporine may increase serum levels/effects of pimecrolimus; monitor.

Probucol: Probucol may decrease the levels/effects of cyclosporine; monitor.

Progestins: Progestins may increase the levels/effects of cyclosporine; monitor for hepatotoxicity.

Protease inhibitors: Formal interaction studies have not been done; protease inhibitors are known to inhibit CYP3A4 and may increase the levels/effects of cyclosporine; use caution when using cyclosporine with indinavir, nelfinavir, ritonavir, or saquinavir.

Repaglinide: Cyclosporine may increase levels/effects of repaglinide; monitor.

Rifamycin derivatives (rifabutin, rifampin, rifapentine): May increase the metabolism, via CYP isoenzymes, of cyclosporine.

Sirolimus: Cyclosporine may increase serum levels/effects of sirolimus. Sirolimus may increase the adverse/toxic effects of cyclosporine. Concurrent therapy may increase the risk of HUS/TTP/TMA. Administer sirolimus 4 hours after cyclosporine to minimize the increase in sirolimus blood levels. Interaction may also occur with concomitant administration of temsirolimus (a pro-drug of sirolimus),

Sitaxsentan [CAN]: Cyclosporine may increase the levels/effects of sitaxsentan; concurrent use is contraindicated.

Sulfasalazine: Sulfasalazine may decrease the levels/effects of cyclosporine.

Sulfinpyrazone: Sulfinpyrazone may decrease the levels/effects of cyclosporine; monitor.

Ticlopidine: Ticlopidine may decrease the levels/effects of cyclosporine.

Vaccines: Vaccination may be less effective; avoid use of live vaccines during therapy.

APPENDIX D: Schedule of events

1. van Den Berg JW, Geertsma A, van Der Bij W, et al. Bronchiolitis obliterans syndrome after lung transplantation and health-related quality of life. American journal of respiratory and critical care medicine 2000;161(6):1937-1941.