Chiron Corporation

Pulmonary-Allergy Drugs Advisory Committee

Briefing Document

Pulminiq™

(Cyclosporine, USP)

Inhalation Solution (CyIS)

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AVAILABLE FOR PUBLIC DISCLOSURE WITHOUT REDACTION

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LIST OF ABBREVIATIONS

AE	adverse event
AR	acute rejection
AUC	area under the curve
BOS	bronchiolitis obliterans syndrome
BUN	blood urea nitrogen
CMV	cytomegalovirus
COPD	chronic obstructive pulmonary disease
CyIS	cyclosporine inhalation solution
CYP	cytochrome P
EFS	event-free survival
FDA	US Food and Drug Administration
FEF_{25-75}	forced expiratory flow between 25% and 75% of FVC
FEV_1	forced expiratory volume in 1 second
F/F	female/female
FPF	Fine particle fraction
FVC	forced vital capacity
GERD	gastroesophageal reflux disease
GSD	geometric standard deviation
ICU	intensive care unit
IV	intravenous
IFN	interferon
IL	interleukin
IND	Investigational New Drug
IPF	idiopathic pulmonary fibrosis
ISS	integrated safety summary
L	ligand
MedDRA	Medical Dictionary for Regulatory Activities
MMAD	mass median aerodynamic diameter
MMF	mycophenolate mofetil

MW	molecular weight
NDA	New Drug Application
NFAT	nuclear factor of activated T cell
NOS	not otherwise specified
OB	obliterative bronchiolitis
PG	propylene glycol
PLC	phospholipase C
PPH	primary pulmonary hypertension
PSD	particle size distribution
SAE	serious adverse event
SC	serum creatinine
SIL	squamous intraepithelial lesion
SOC	system organ class
$t_{1/2}$	half-life
TBB	transbronchial biopsy
TCR	t-cell receptor
t_{max}	time to maximum concentration
TNF	tumor necrosis factor
UPMC	University of Pittsburgh Medical Center
USP	United States Pharmacopeia

1. EXECUTIVE SUMMARY

1.1 Introduction and Clinical Rationale

Lung transplantation is the final therapeutic option for a variety of end-stage pulmonary diseases including emphysema, cystic fibrosis, and idiopathic pulmonary fibrosis. Although 40 years have passed since the first human lung transplant in 1963, long-term survival remains poor, and is considerably lower than survival after transplantation of other solid organs. Despite aggressive care, only 45% of lung transplant recipients will be alive 5 years following the transplant procedure. By contrast, 70% to 90% of heart, kidney, and liver transplant recipients will be alive at 5 years, and over 50% of recipients will still be alive 10 or more years following the transplant procedure.[1] The primary cause for the lower survival rate after lung transplantation is the development of progressive allograft failure as a result of chronic rejection. The progression of lung transplant chronic rejection involves histologic evidence of small airway damage leading to the diagnosis bronchiolitis obliterans (OB). As OB progresses, this small airway damage compromises pulmonary function, leading to the diagnosis of bronchiolitis obliterans syndrome (BOS). Despite augmented immunosuppressive therapy, OB is generally unresponsive, progressive, irreversible, and fatal, with a median survival of approximately 3 years after diagnosis.[2] Therefore, a pharmacologic intervention to prevent the development of OB and thereby improve survival is urgently needed for the lung transplant population.

The pathophysiology of OB is poorly understood, but multiple lines of evidence suggest that OB arises from immune-mediated injury to the epithelial cells lining the small airways of the allograft. There is no single insult that is thought to trigger chronic rejection; rather, the current theory suggests that chronic rejection may develop as a result of many different risk factors, which lead to the same final pathway of airway inflammation, cytokine release, epithelial sloughing, fibroproliferation, and ultimately to progressive scarring with obliteration of the small airways. Clinically, the end result is progressive shortness of breath, airflow obstruction, recurrent pulmonary infections, and, ultimately, death.

Although current approaches to immunosuppression after lung transplantation have failed to adequately prevent chronic rejection and OB, survival following lung transplantation has markedly improved since the introduction of cyclosporine in 1983. Cyclosporine is isolated from a naturally occurring fungus and belongs to a class of immunosuppressive agents called calcineurin inhibitors. The mechanism of action of these agents is the competitive binding to calcineurins, a family of cytosolic proteins important in cell signaling. The cyclosporine/calcineurin complex prevents downstream transcription factors from translocating to the nucleus and activating transcription of early cytokine genes involved in T-cell activation, proliferation, and differentiation. As such, cyclosporine is a potent inhibitor of T cell-mediated immune responses and its use has substantially reduced the incidence of acute rejection (AR) in lung transplant recipients. However, cyclosporine has a narrow therapeutic window, and significant adverse events including nephrotoxicity, neurotoxicity, hepatotoxicity, and malignancies are common with oral and parenteral administration.

The introduction of inhaled therapeutics has been a major advance in the treatment of chronic lung diseases. Use of inhaled beta-agonist and anticholinergic bronchodilators has become first-line therapy for exacerbations of chronic obstructive pulmonary disease, replacing earlier oral and subcutaneous formulations. Inhaled corticosteroids have a central role in minimizing exacerbations in stable patients with asthma, and have reduced patients' overall exposure to oral corticosteroids. Inhaled tobramycin has resulted in improved lung function and decreased sputum density of *Pseudomonas aeruginosa* in patients with cystic fibrosis and bronchiectasis. In each case, the goal of inhaled therapy is to effectively deliver the therapeutic agent in high concentrations in the lower respiratory tract while minimizing systemic exposure and subsequent toxicity of the parent compound.

With similar goals in mind, investigators at the University of Pittsburgh Medical Center (UPMC) began a program to develop an inhaled formulation of cyclosporine for lung transplant recipients. After more than a decade of clinical trials, investigators demonstrated that inhaled cyclosporine significantly improved survival and prevented chronic rejection when given in conjunction with patients' standard immunosuppressive regimens. In keeping with the company's commitment to develop novel pharmaceutical agents for the treatment of

chronic pulmonary conditions, Chiron subsequently became involved with the clinical development of CyIS. This involvement led to the submission of a New Drug Application (NDA) for marketing approval of Pulminiq™ (CyIS), a sterile, clear, colorless, solution of cyclosporine (cyclosporine, USP) drug substance in propylene glycol (propylene glycol, USP) for the indication of improved survival and prevention of chronic rejection in lung transplant recipients. If approved, CyIS would be the first drug indicated for lung transplant recipients and would represent an important advance in outcomes for this orphan patient population with a severe unmet medical need.

1.2 Development History of CyIS

The clinical development of CyIS began in 1988 at UPMC, one of the largest lung transplant centers in the United States. The hypothesis was that topical delivery of cyclosporine could achieve potent local immunosuppression with relatively low additional systemic exposure, resulting in decreased allograft rejection and improved overall outcomes. Initial experiments in canine and rodent transplant models confirmed that administration of CyIS led to 10- to 100-fold higher concentrations of cyclosporine in the lung than in heart or kidney tissue[3] and suggested a benefit in decreasing proinflammatory cytokine production and early allograft rejection with low systemic exposure.[4,5]

Initial open-label clinical trials were started in 1991 and conducted in lung transplant recipients with either established chronic rejection or AR refractory to standard therapy. In patients with chronic rejection, the addition of CyIS to standard immunosuppressive therapy was demonstrated to lead to improvement in rejection histology, stabilization of pulmonary function, and overall improved survival compared with contemporary UPMC controls and historical controls from a transplant registry.[6] In patients with refractory AR (who are at high risk of subsequently developing chronic rejection), the administration of CyIS in addition to standard immunosuppressive therapy led to improved rejection histology, reduction in proinflammatory cytokine production, dose-dependent improvement in pulmonary function, and improved survival compared with contemporary UPMC controls.[7-9] These open-label trials suggested that the addition of CyIS to these patients' standard immunosuppressive regimens could prevent or delay the progression of chronic rejection, but their interpretation

was limited by the lack of an adequate control group.[6,10] However, results of these studies acted as the foundation for the pivotal trial. In 1997, investigators began a randomized, prospective, double blind, placebo-controlled trial of CyIS in lung transplant recipients (ACS001). Unlike the previous protocols that evaluated CyIS in lung transplant recipients with established rejection refractory to therapy, ACS001 was designed to test the efficacy and safety of CyIS in preventing rejection and improving outcomes in patients shortly after their single- or double-lung transplant procedure.

It should be noted that the Investigational New Drug (IND) holder for the studies has been UPMC and as such the studies were not conducted by Chiron or any company. Although the protocol design and any changes to the conduct of the study were submitted to the FDA, prospective Case Report Forms (CRFs) were not in place during the study. This is not to imply, however, that adequate records were not kept, as this has been verified by post-study auditing both by Chiron and the FDA. To perform its independent analysis, Chiron subsequently created a CRF as a vehicle for going back to the medical records to record and verify all relevant clinical data.

1.3 ACS001: The Pivotal Study

1.3.1 Study Design

ACS001 was a single center, randomized, double-blind clinical trial designed to test the efficacy of CyIS in reducing allograft rejection and improving outcomes in lung transplant recipients. The trial had two phases: an initial open label pilot phase and a subsequent randomized blinded phase. In the pilot phase, 10 patients received open-label CyIS and were followed prospectively to monitor safety and tolerability in this patient population. In the randomized phase, 56 patients were enrolled within 7 to 42 days following their single- or double-lung transplant and treated with either CyIS or inhaled placebo. Of these 56 patients, a total of 26 patients received CyIS in propylene glycol (PG) and 30 patients received PG alone.

All patients 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 PG). After this 10-

day period, patients were to continue therapy three times per week for a period of 2 years. The study concluded on August 21, 2003, when the 56th patient completed the full 2 years of treatment. Follow-up in the randomized cohort ranged from 24 to 56 months.

1.3.2 Summary of Efficacy Results

A total of 47% of the placebo-treated patients died during the study compared with only 12% of CyIS-treated patients. As can be seen by the Kaplan-Meier estimates of survival duration **(Figure 1.3.2-1)** the results represent both a clinically meaningful and a statistically significant (log-rank *P*= .007) improvement. The unadjusted estimate of the risk of death among CyIS patients relative to placebo was 0.213 (95% confidence interval = 0.061, 0.743), representing a 79% reduction in mortality in patients treated with CyIS. This statistically significant survival advantage was also observed when stratified by transplant type, primary diagnosis, cytomegalovirus (CMV) donor positive/recipient negative match or mismatch, or the occurrence of a grade 2 or higher AR episode before the start of study medication **(Table 1.3.2-1).** When controlling for all four of these factors simultaneously in a single model, the survival advantage (relative risk = 0.238; 95% confidence interval = 0.064, 0.885; *P*= .032) remains significant.

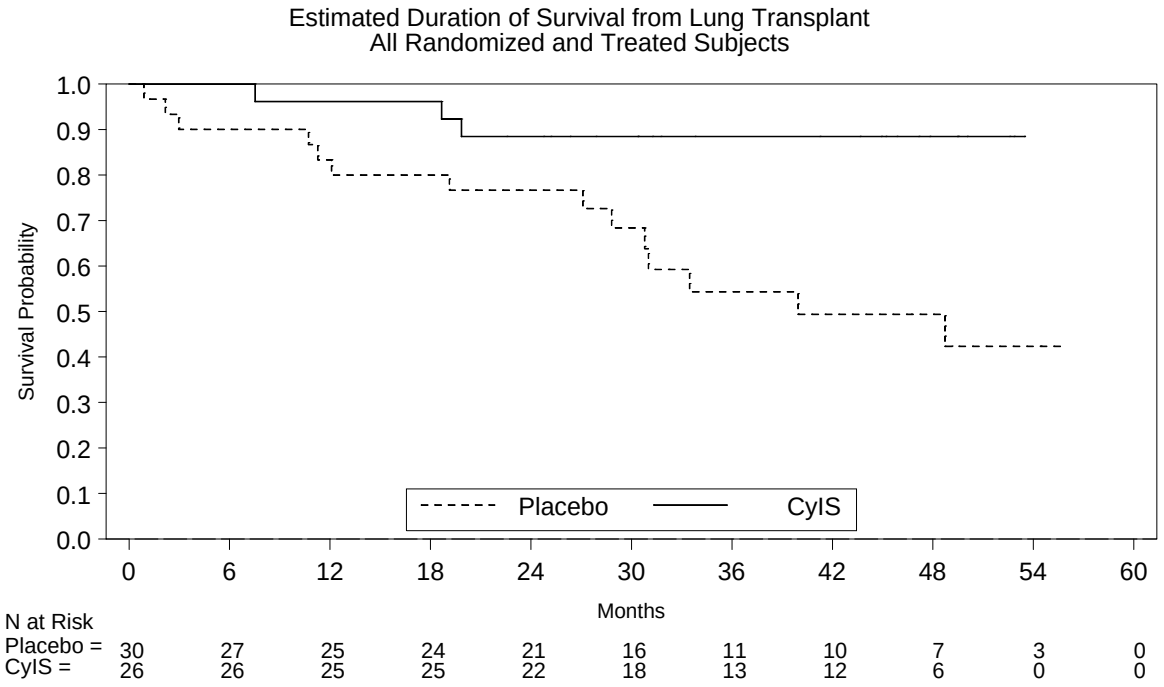


Figure 1.3.2-1. Kaplan-Meier plots of survival duration in patients treated with CyIS or placebo measured from date of transplant.

Table 1.3.2-1. Summary of Survival Duration (ACS001)

	Number of Deaths/Number in Strata (%)		
Strata	**Placebo (n = 30)**	**CyIS, Randomized (n = 26)**	**Log-rank *P* value**
All	14/30 (47)	3/26 (12)	.007
Transplant Type			.014[a]
Single	11/24 (46)	2/15 (13)	
Double	3/6 (50)	1/11 (9)	
Primary Diagnosis			.011[b]
Emphysema	8/19 (42)	2/9 (22)	
Other	6/11 (55)	1/17 (6)	
Grade 2+ AR Prior to Dosing			.009[c]
Yes	8/13 (62)	1/8 (13)	
No	6/17 (35)	2/18 (11)	

Table 1.3.2-1. Summary of Survival Duration (ACS001)

	Number of Deaths/Number in Strata (%)		
Strata	**Placebo (n = 30)**	**CyIS, Randomized (n = 26)**	**Log-rank *P* value**
CMV match / mismatch			.017[d]
Match	9/23 (39)	3/21 (14)	
Mismatch	5/7 (71)	0/5 (0)	

[a] *P* value from log-rank test stratified by transplant type (ie, single- or double-lung).
[b] *P* value from log-rank test stratified by primary diagnosis (emphysema or other).
[c] *P* value from log-rank test stratified by grade 2+ AR prior to dosing (yes/no).
[d] *P* value from log-rank test stratified by CMV match/mismatch.
CyIS = Cyclosporine inhalation solution; AR = Acute rejection; CMV = Cytomegalovirus.

The efficacy of CyIS is believed to arise from a decreased incidence of chronic rejection, consistent with its mode of delivery to the airway epithelium. Patients treated with CyIS had markedly improved chronic rejection-free survival (log-rank *P*= .001; **Figure 1.3.2-2**) compared with patients treated with placebo. Chronic rejection was diagnosed either through biopsy by the presence of OB or clinically by the presence of BOS—an unexplained and sustained 20% or larger reduction in forced expiratory volume in 1 second.[11] Among the 30 placebo-treated patients, only 8 (27%) survived free of chronic rejection compared with 18 (69%) CyIS-treated patients. The risk of chronic rejection or death among CyIS patients was only 28% [95% confidence interval = 0.124, 0.630] relative to placebo. Data stratified by major risk factors reveal the robustness of these results **(Table 1.3.2-2).** Analyses controlling for the same four risk factors simultaneously reach the same conclusion (relative risk = 0.318; 95% confidence interval = 0.137, 0.742; *P*= .008).

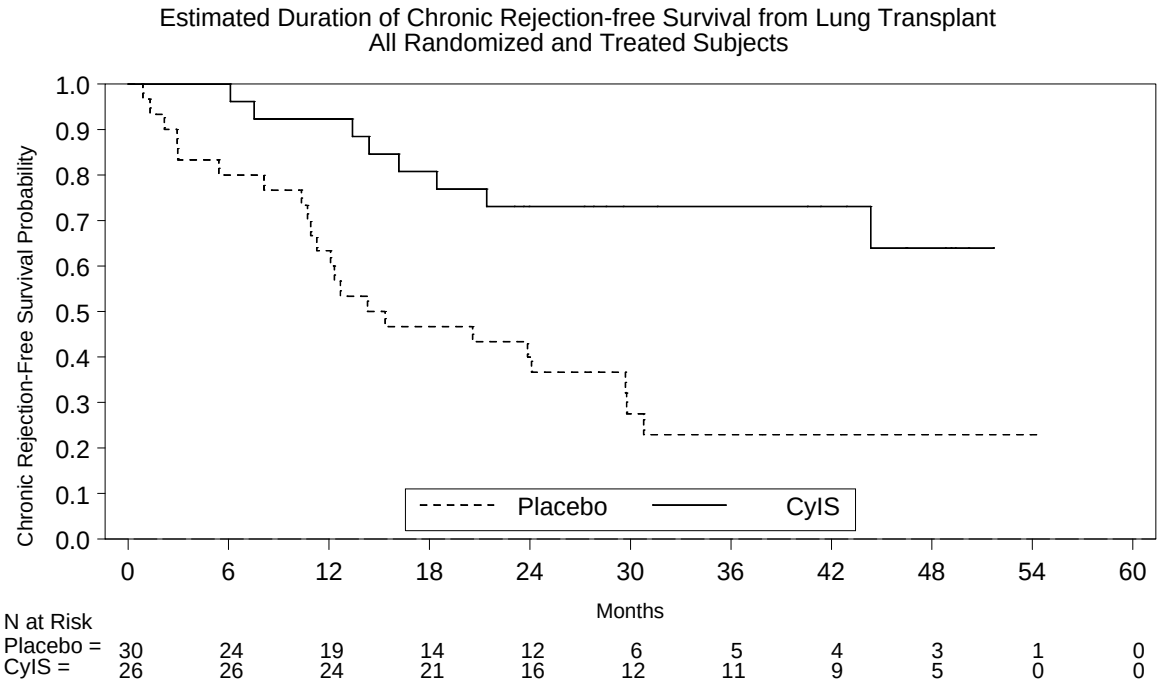


Figure 1.3.2-2. Kaplan-Meier estimates of chronic rejection-free survival duration.

Table 1.3.2-2. Summary of Chronic Rejection-Free Survival Duration (ACS001)			
	Number of Events/Number in Strata (%)		
Strata	**Placebo (n = 30)**	**CyIS, Randomized (n = 26)**	**Log-rank *P* value**
All	22/30 (73)	8/26 (31)	.001
Transplant Type			.002[a]
Single	18/24 (75)	5/15 (33)	
Double	4/6 (67)	3/11 (27)	
Primary Diagnosis			.005[b]
Emphysema	15/19 (79)	3/9 (33)	
Other	7/11 (64)	5/17 (29)	
Grade 2+ AR Prior to Dosing			.002[c]
Yes	12/13 (92)	2/8 (25)	
No	10/17 (59)	6/18 (33)	
CMV match / mismatch			.001[d]
Match	15/23 (62)	8/21 (38)	
Mismatch	7/7 (100)	0/5 (0)	

[a] *P* value from log-rank test stratified by transplant type (ie, single- or double-lung).
[b] *P* value from log-rank test stratified by primary diagnosis (emphysema or other).
[c] *P* value from log-rank test stratified by grade 2+ AR prior to dosing (yes/no).
[d] *P* value from log-rank test stratified by CMV match/mismatch.
CyIS = Cyclosporine inhalation solution; AR = Acute rejection; CMV = Cytomegalovirus.

This favorable outcome did not occur only due to improved survival among CyIS patients. A sensitivity analysis that censors deaths was performed to determine if CyIS has an effect on preventing chronic rejection beyond survival. Although such an analysis includes informative censoring and is biased against the CyIS group due to the survival advantage, it yielded a log-rank *P* value of .015, demonstrating the clear and overwhelming effect of CyIS in preventing chronic rejection.

1.3.3 Summary of Safety Results

Analysis of the ACS001 study population revealed that CyIS 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 CyIS led to increased risk of nephrotoxicity, neurotoxicity, increased risk of infections, or increased risk of malignancies. Cyclosporine inhalation solution was found to be associated

with respiratory tract irritation and bronchospasm, but these adverse events were generally mild/moderate, occurred early in patients' treatment course, diminished with time, and were not associated with the development of more serious respiratory complications. These findings were consistent with analysis of a larger safety database that included 70 patients treated with CyIS within one of the open-label protocols. In total, Chiron has analyzed safety data from 102 patients treated with CyIS.

1.4 Conclusions

The administration of CyIS led to a major improvement in overall survival and chronic rejection-free survival and was safe when given to lung transplant recipients shortly after their transplant procedure. Limitations to the data include their origin from a single center and the small sample size. Therefore, it is important to consider the approvability of this application in the context of a risk/benefit analysis. If one considers the demonstrated benefit against the maximum potential unknown risk (e.g., "risk uncertainty"), the case for approval is strong based on the following five points:

1. There is clear evidence of improved survival and decreased chronic rejection in a patient population for which there are no approved drugs or treatment alternatives.

2. The administration of CyIS minimizes systemic exposure to cyclosporine with no evidence of increased systemic toxicity.

3. The target patient population is a very small, well-defined population of approximately 1100 lung transplant recipients per year

4. CyIS is an inhaled formulation of a well known drug that has been part of the standard care for solid-organ transplant recipients for over 20 years.

5. Chiron has committed to a large 5-year postapproval study to confirm data generated from the pivotal trial.

Therefore, in light of the unmet medical need in this orphan patient population and the strong benefit/risk ratio, approval at this time is appropriate contingent on implementation of a postapproval confirmatory study.

2. INTRODUCTION

2.1 An Overview of Lung Transplantation

Multiple challenges have been overcome to make lung transplantation an acceptable therapeutic option for a diverse array of end-stage lung diseases. The first challenges to overcome were surgical and early efforts were aimed at reducing the frequency and severity of graft dehiscence at the bronchial anastomosis. After the development of adequate surgical techniques to minimize this complication, it became clear that lung transplant recipients were particularly susceptible to allograft rejection and pulmonary infections. Despite major advances in the field of immunology and the availability of several immunosuppressant medications, over the first 20 years following the first human lung transplant procedure, there were no long-term survivors. During this period there was decreasing enthusiasm for lung transplantation and only 40 lung transplant procedures were performed worldwide.

The introduction of cyclosporine as an immunosuppressant markedly changed these dismal results and marked a new era for lung transplantation. The first lung transplant recipient treated with cyclosporine in 1983 lived for 6.5 years following the procedure, compared with a maximum of only 10 months in the pre-cyclosporine era. For the next 20 years, overall numbers of lung transplants performed began to increase dramatically; however, over the past 2 years, these numbers have started to plateau as a result of a fixed donor population that is insufficient to meet the increasing demand. In 2004, approximately 1100 lung transplant procedures were performed in the United States and approximately 1800 lung transplant procedures worldwide.[12] Early estimates for 2005 suggest that overall numbers will likely be comparable.

2.2 The Problem of Chronic Rejection

Despite the introduction of several new immunosuppressants and improved peri- and postoperative management, long-term survival following lung transplantation has not increased substantially since 1983, and remains considerably lower than after other solid-organ transplants. Whereas heart, kidney, and liver transplant recipients can expect a 5-year survival rate of approximately 70% to 90% and a 10-year survival rate of >50%, lung

transplant recipients can only expect a 45% 5-year survival rate and a 10-year survival rate of approximately 25% **(Figure 2.2-1)**. The primary reason for this poor long-term prognosis is that lung transplant recipients develop chronic rejection at far higher rates than other solid-organ transplant recipients. The incidence of chronic rejection increases steadily with time, and it is estimated to occur in 60% to 70% of recipients who live 5 or more years following the transplant procedure.[13] Although insidious at onset, once established, chronic rejection is irreversible, unresponsive to therapy, and ultimately fatal. Chronic rejection is the primary cause of late mortality in lung transplant recipients and accounts for at least 30% of all deaths 3 or more years following the transplant procedure.[14]

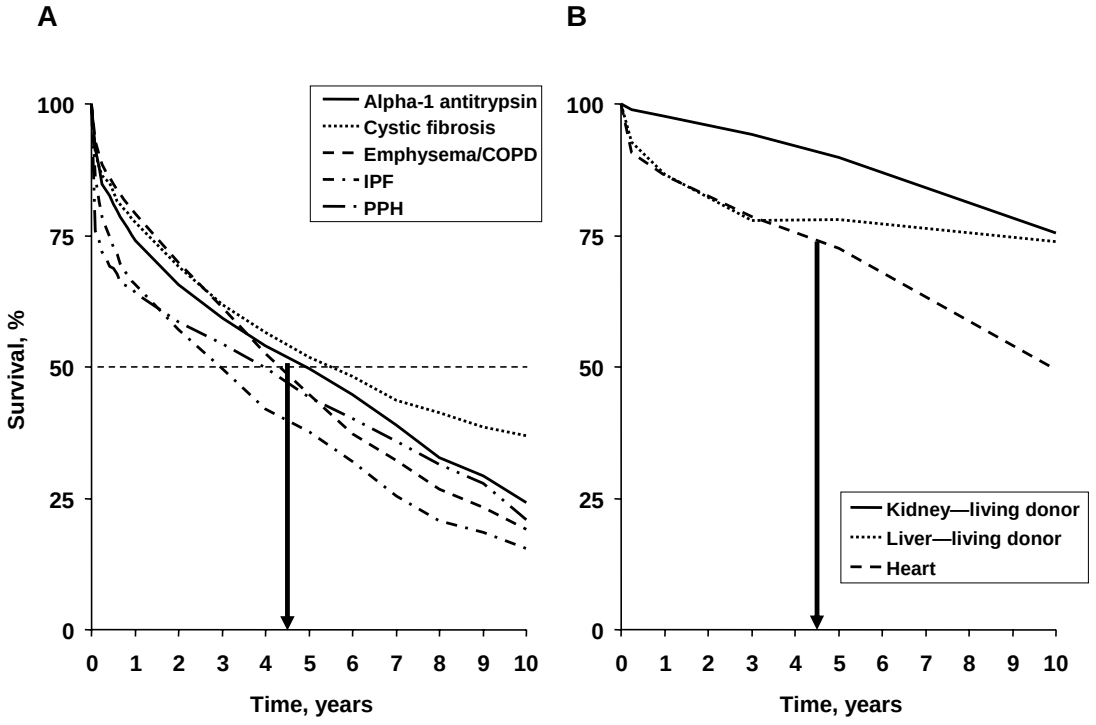


Figure 2.2-1. Comparative transplantation survival rates. (A) Primary lung transplant by underlying diagnosis. (B) Primary kidney, liver, and heart transplant. *Kidney, liver, and heart data were extrapolated from OPTN Annual Report, 2004.

Why lung transplant recipients develop chronic rejection at such high rates is an area of active research. One hypothesis is that, unlike other organs, the lung is continually exposed to the external environment with its many pathogens and irritants, leading to chronic low-

level inflammation. A second hypothesis is that, unlike other organs, the lung receives 100% of the blood flow and therefore has increased exposure to circulating immune and inflammatory cells. A third hypothesis is that the lung allograft contains substantial donor lymphatic tissue and thus large numbers of immunocompetent "passenger" lymphocytes that display major histocompatibility complex class II surface antigens and result in immune stimulation. Many other hypotheses exist, such as microaspiration from decreased cough reflex, decreased mucociliary clearance, and increased susceptibility to graft ischemia. It is likely that most of these hypotheses contribute to a multifactorial explanation.

Although chronic rejection was initially thought to be predominantly an immune-triggered process, recent research has demonstrated that chronic rejection may develop as a result of many different risk factors that may be either alloimmune-dependent or alloimmune-independent **(Figure 2.2-2)**. Meta-analyses have consistently shown that the most important risk factors for the development of chronic rejection are prior episodes of AR, CMV pneumonitis, the presence of anti-human leukocyte antigen antibodies, and lymphocytic bronchitis.[15] More recently, airway ischemia and gastroesophageal reflux disease (GERD) have emerged as likely risk factors for chronic rejection.

Regardless of the source of its initiation, chronic rejection leads to immunologic injury of the epithelial cells that line the small airways of the allograft. At its onset, lymphocytes infiltrate the submucosa of the airways and disrupt the epithelium. Over time, epithelial sloughing, ongoing cytokine release, and aberrant tissue repair lead to fibroproliferation and scarring resulting in obliteration of the small airways. This lesion is referred to as bronchiolitis obliterans (OB), and is the histologic manifestation of chronic rejection. Therefore, the pathophysiology of chronic rejection proceeds from evidence of inflammation to evidence of scarring (OB) and ultimately to clinical signs and symptoms (bronchiolitis obliterans syndrome or BOS). Clinically, chronic rejection presents gradually with dyspnea and airflow obstruction, but patients invariably develop graft dysfunction, recurrent pulmonary infections, and ultimately die unless they undergo repeat transplantation. In addition to its effect on survival, chronic rejection has also been shown to adversely affect quality of life by reducing mobility, energy level, and general sense of well being.[16]

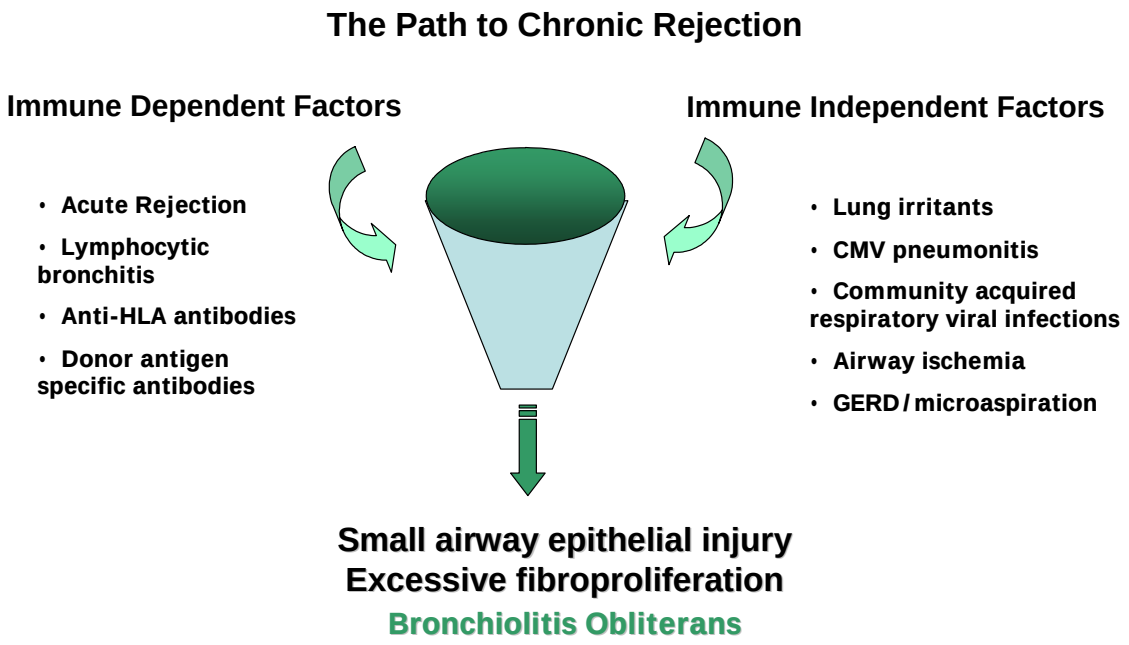


Figure 2.2-2. Proposed risk factors for chronic rejection.[2,15] HLA = Human leukocyte antigen; CMV = Cytomegalovirus; GERD = Gastroesophageal reflux disease.

There are two methods for diagnosing chronic rejection. The first method is through a transbronchial biopsy demonstrating the classic histologic findings of OB. This method has a reported specificity of 95% but sensitivity of only 17% because OB is not uniformly distributed in the early stages.[17] As a result of this poor sensitivity, the International Society of Heart and Lung Transplantation (ISHLT) convened a group of experts in 1993 to devise a second method for the diagnosis of chronic rejection[18] that was subsequently revised in 2001.[11] This method defines BOS as a persistent decline in graft function (as measured by forced expiratory volume in one second [FEV_1]) of at least 20% from a patient's posttransplant maximum value in the absence of another clinical explanation. The second method has improved sensitivity and does not require an invasive procedure, but is far less specific than transbronchial biopsy. Once chronic rejection is diagnosed either histologically or clinically, there are no known therapies that have consistently demonstrated benefit in delaying its progression or improving its prognosis. Therefore, in the absence of any treatment options, efforts should focus on prevention of chronic rejection in order to significantly improve long-term survival.

2.3 Limitations of Current Therapy

Although there are an increasing number of immunosuppressive drugs available to lung transplant recipients, the two most common causes of death at all times after the transplant procedure are chronic rejection and infection. These two causes of death illustrate the difficult task of managing immunosuppression in the posttransplant setting. Physicians need to continually weigh the risk of over-immunosuppression (and subsequent development of infection and other drug-related toxicities) with the risk of under-immunosuppression (and subsequent development of rejection). There is a clear need for a new type of drug that can provide additional immunosuppression without increasing the risk of infection and other life-threatening toxicities.

Following lung transplantation, patients are placed on an immunosuppressive regimen that generally consists of a backbone of three medications: a calcineurin inhibitor, an antimetabolite, and a corticosteroid. Each of these medications acts to modulate the reactivity to the allograft through nonspecific suppression of T-cell activation, proliferation, and anti-inflammation pathways. However, all of these medications has a very narrow therapeutic window (a range of systemic levels between which they are effective but not toxic), and serious adverse events are common. It is noteworthy that none of these immunosuppressants are specifically approved for lung transplantation **(Table 2.3-1)**. Each was developed and approved for indications in which there has been a significantly higher demand such as kidney, liver, and heart transplantation (with corresponding 15-fold, 5-fold, and 2-fold higher number of transplant recipients). Treatment regimens for lung transplant recipients have been developed empirically and adopted from data for other solid-organ transplants recipients, despite clearly different immunosuppressive needs.

Table 2.3-1. Immunosuppressant Therapies Indicated for the Treatment of Organ Transplant Recipients

	Kidney	Heart	Liver	Lung
Cyclosporine	X	X	X	
Mycophenolate	X	X	X	
Tacrolimus	X		X	
Azathioprine	X			

Cyclosporine and tacrolimus are the two available calcineurin inhibitors, and although structurally distinct, they have the same pharmacologic mechanism of action and similar side-effect profiles. Although cyclosporine has been available for a longer period of time, tacrolimus has now surpassed cyclosporine for both initial and maintenance immunosuppression. Nephrotoxicity is the most common and clinically significant adverse effect of both medications. This effect may be manifested by acute azotemia, chronic renal insufficiency, and renal tubular dysfunction. Data for cyclosporine suggest that rates of nephrotoxicity reach 38%.[19] Although there are fewer accumulated data for tacrolimus, overall rates are similar. The other major toxicities of both drugs are hypertension, glucose intolerance/diabetes, nausea, vomiting, diarrhea, and neurotoxicity manifested by headaches, visual changes, and tremor, with rare progression to encephalopathy and seizures.

Azathioprine is the oldest antimetabolite and was developed in the 1940s. Its immunosuppressive activity results from the drug's ability to inhibit synthesis of DNA, RNA, and proteins; interfere with cellular metabolism; and inhibit mitosis. The end results are decreased numbers of circulating lymphocytes, decreased immunoglobulin synthesis, and decreased IL-2 secretion. The primary significant adverse reactions associated with azathioprine are fever, nausea, vomiting, diarrhea, bone marrow suppression, arthralgias, and retinopathy. More recently, mycophenolate mofetil (MMF) has become available and has gained increased acceptance. The most common adverse events associated with MMF are hypertension, fluid retention, electrolyte and lipid abnormalities, nausea, abdominal pain, diarrhea, back pain, tremor, headache, dyspnea, and cough.

Corticosteroids in the form of prednisone and methylprednisolone are used in nearly all lung transplant patients and are associated with many serious short- and long-term side effects.

The most notable of these are glucose intolerance/diabetes, osteopenia, cataracts, hypertension, accelerated atherosclerosis, memory loss, insomnia, agitation, fatigue, and weakness. In the majority of cases, the development of these effects is related to dose and cumulative duration of therapy.

2.4 Rationale for an Inhaled Immunosuppressant

The narrow therapeutic window along with the failure of current immunosuppressive therapies to prevent chronic rejection highlights the clinical dilemma for the need of higher intragraft immunosuppression without higher risk of life-threatening toxicities. When immunosuppressants are given orally or intravenously, blood concentrations increase, but concentrations within the graft remain low and are limited by diffusion of the drug across the vascular endothelium **(see Section 3.2.3)**. By contrast, when an immunosuppressant is given via inhalation, there is direct intrapulmonary availability and high graft concentrations. Furthermore, the mode of delivery allows for direct topical application to the epithelial cells that line the small airways of the graft—precisely the site of chronic rejection.

The rationale for the development of an inhaled immunosuppressant follows logically from the prior successes of inhaled therapeutics for other chronic pulmonary conditions. The use of inhaled corticosteroids in stable patients with moderate or severe asthma has led to important decreases in the rate of asthmatic exacerbations while limiting patients' systemic exposure and risk of long-term consequences such as bone loss, hypertension, and glucose intolerance. Similarly, the use of inhaled antibiotics has led to improved pulmonary function and decreased sputum concentration of *Pseudomonas aeruginosa* in patients with cystic fibrosis without increasing the risk of the renal dysfunction and ototoxicity that are commonly seen with intravenous (IV) administration. In each case, the local delivery of drug allowed for an important pharmacologic effect while minimizing systemic exposure to the parent compound and thereby minimizing the risk of systemic toxicity. The intrapulmonary drug concentrations achieved with these inhaled therapeutics could not have been reached with oral or IV administration without the development of life-threatening toxicities. As described in **Section 3.2.3, Clinical Pharmacokinetics**, the intrapulmonary concentration of

cyclosporine achieved with CyIS would require a doubling of the standard oral dose of cyclosporine.

2.5 Clinical Development of Cyclosporine Inhalation Solution (CyIS)

The clinical development of CyIS has not followed the typical process for an industry-sponsored program. The preclinical and clinical trials have taken place at the University of Pittsburgh Medical Center (UPMC), which has been a leading center in lung transplantation research and performed an average of one lung transplant procedure per week in 2004.

Initial work on developing an inhaled formulation of cyclosporine began in the 1980s with attempts to find an adequate solvent. Due to the extremely lipophilic nature of cyclosporine, aqueous solubility of cyclosporine was less than 10 µg/mL. After attempts with multiple other solvents, initial experiments used cyclosporine in ethanol, which has a solubility of approximately 100 mg/mL. However, early human trials with ethanol-based CyIS showed that this formulation was difficult to tolerate due to airway irritation. Ultimately, propylene glycol (PG) was chosen as the solvent for CyIS given its higher solubility (>100 mg/mL) and less irritating effects. Propylene glycol also has a long history of use in pharmaceutical products and cosmetics and is generally regarded as safe. Additional data on the safety of PG are provided in **Section 3.3.3** and **Section 3.3.4**.

The first preclinical experiments in nontransplanted dogs given 200 mg CyIS through an endotracheal tube demonstrated that the dose was well tolerated and did not cause a change in lung function, alveolar-arteriolar gradient, or tissue histology. In addition, giving CyIS through an inhalation route was highly effective at delivering a targeted dose—pulmonary concentrations of cyclosporine were 10- to 100-fold higher than concentrations in heart or kidney tissue.[3] In the next set of experiments, using an orthotopic canine transplant model, dogs were given single-agent immunosuppression with CyIS. The results demonstrated a dose-dependent reduction in allograft rejection.[4] Subsequently, in a rat transplant model, rats were given either CyIS or an identical dose of intramuscular cyclosporine. Results demonstrated that CyIS was at least as effective as intramuscular cyclosporine at decreasing allograft rejection and proinflammatory cytokine production, but at 3-fold lower blood concentrations.[5]

Encouraging preclinical results led to a series of open-label protocols at UPMC beginning in 1991. These protocols were designed to evaluate CyIS in lung transplant recipients who had either established chronic rejection or refractory AR. In the first series of protocols, lung transplant recipients with established chronic rejection were given CyIS in addition to their standard immunosuppressive regimens. As a result, investigators reported improvement in rejection histology, stabilization of pulmonary function, and improved survival relative to contemporary nonenrolled UPMC lung transplant recipients as well as relative to historical controls from the Stanford Lung Transplant database.[6] In the second series of protocols, lung transplant recipients with AR that failed to respond to at least two courses of immunosuppressive intensifications (termed refractory AR) were given CyIS in addition to their immunosuppressive regimens. The rationale for testing in these patients was the hypothesis that CyIS could impact AR and thereby decrease the risk of chronic rejection and improve overall outcomes. As a result of these studies, investigators reported that CyIS-treated patients had improved histology (by at least one grade), a reduction in proinflammatory cytokine production, dose-dependent improvement in pulmonary function, and improvement in overall survival relative to UPMC nonenrolled controls.[7-9]

These experiments produced results suggesting that the administration of CyIS was safe and could delay the progression of chronic rejection, but their interpretation was limited as a result of the lack of an adequate control population. However, they set the stage for a confirmatory prospective, randomized, double blind, placebo-controlled clinical trial to test the efficacy and safety of CyIS in preventing allograft rejection and improving outcomes in lung transplant recipients. The results of this pivotal trial (ACS001) are provided in **Section 4** and **Section 5** of this Briefing Document, and formed the basis for the NDA, the subject of this advisory committee.

Chiron became involved with the clinical development of CyIS just before the conclusion and unblinding of ACS001. Given the limited commercial incentives for this small patient population, Novartis sold the rights to develop CyIS for a nominal fee. Chiron has significant experience in the field of inhaled therapeutics delivered by nebulizer since it holds the license for TOBI®, an inhaled formulation of tobramycin used in the treatment of pseudomonal infections in patients with cystic fibrosis. After the conclusion of ACS001,

Chiron independently source-verified all of the data and performed an independent analysis of efficacy and safety. The clear and compelling survival results in this orphan population with poor long-term prognosis led Chiron to pursue the FDA approval of CyIS under the proposed trade name PULMINIQ.

To provide additional support for the safety data generated in ACS001, Chiron also retrospectively collected safety data on all 70 patients enrolled in one of UPMC's open-label protocols after January 1, 1995. This safety dataset was analyzed collectively and is referred to as ACS002 (see **Section 4** for further discussion of the differences between ACS001 and ACS002). The decision to use January 1, 1995, as the study initiation date for ACS002 was a result of the need for a cohort of patients with comparable baseline immunosuppressive regimens. In 1994 tacrolimus was approved, and shortly after approval, this immunosuppressant supplanted oral cyclosporine at UPMC for both initial and maintenance immunosuppression. In total, Chiron has collected and verified efficacy data on 56 patients and safety data on 102 patients.

3. PHARMACOLOGY, PHARMACOKINETICS, METABOLISM, PRECLINICAL TOXICOLOGY, AND PHARMACEUTICS

3.1 Introduction

Cyclosporine is a potent immunosuppressive agent that, in animals, prolongs survival of allogeneic transplants involving skin, kidney, liver, heart, pancreas, bone marrow, small intestine, and lung. Cyclosporine has been demonstrated to suppress some humoral immunity and, to a greater extent, cell-mediated immune reactions involved in allograft rejection, delayed hypersensitivity, experimental allergic encephalomyelitis, Freund's adjuvant arthritis, and graft-versus-host disease in animals. Cyclosporine also has been shown to prolong the survival of allogeneic transplants in humans.

3.2 Mechanism of Action

3.2.1 Summary of Pharmacologic Effects

The exact mechanism of action of cyclosporine has not been fully elucidated. However, experimental evidence suggests that the effectiveness of cyclosporine results from the specific and reversible inhibition of immunocompetent T lymphocytes. No effect on phagocytic function (e.g., changes in enzyme secretion, chemotactic migration of granulocytes, macrophage migration, or carbon clearance in vivo) has been detected in animals. Cyclosporine does not cause bone marrow suppression in animal models or humans.

3.2.2 Nonclinical Pharmacology

Cyclosporine binds to cyclophilin, and the complex is a potent inhibitor of calcineurin, which regulates expression of a variety of cytokines, including interleukin-2 **(Figure 3.2.2-1)**.[20] In the presence of cyclosporine, transcription factors of the NFAT family are not activated, resulting in a failure to activate expression of interleukin-2, an important T-cell growth factor.[21] The cellular immune response is inhibited at the lymphocyte proliferation stage. Therefore, local immunosuppressive treatment with cyclosporine may control early intragraft immune events and prevent episodes of AR.

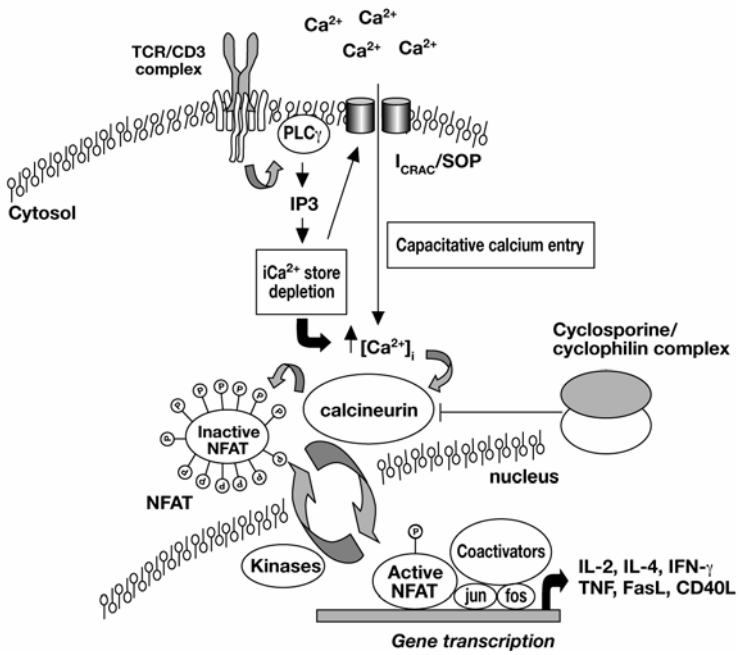


Figure 3.2.2-1. Cyclosporine mechanism of action. TCR = T-cell receptor; PLC = Phospholipase C; NFAT = Nuclear factor of activated T cell; IL = Interleukin; IFN = Interferon; TNF = Tumor necrosis factor; L = Ligand.

3.2.3 Clinical Pharmacokinetics

The principal rationale for the development of CyIS was the hypothesis that "delivery of cyclosporine to the transplanted lung by aerosol deposition achieves higher concentrations of cyclosporine in the graft than when it is delivered by whole blood, and higher concentrations in the graft will reverse rejection without unacceptable toxicity."[22] Although our understanding of the mechanism of action has advanced since then, the central argument remains intact.

During the course of the ACS001 study a deposition substudy was performed wherein 15 CyIS patients and 7 placebo patients were given their study drug mixed with technetium-99m–labeled diethylenetriaminepentaacetic acid (DTPA).[50] Representative gamma scintigraphy images obtained during this substudy in a double-lung transplant recipient and single-lung transplant recipient are provided in **Figure 3.2.3-1** and

Figure 3.2.3-2, respectively. Detailed results on the estimated dose deposition and dose distribution are provided in Appendix 8.1.

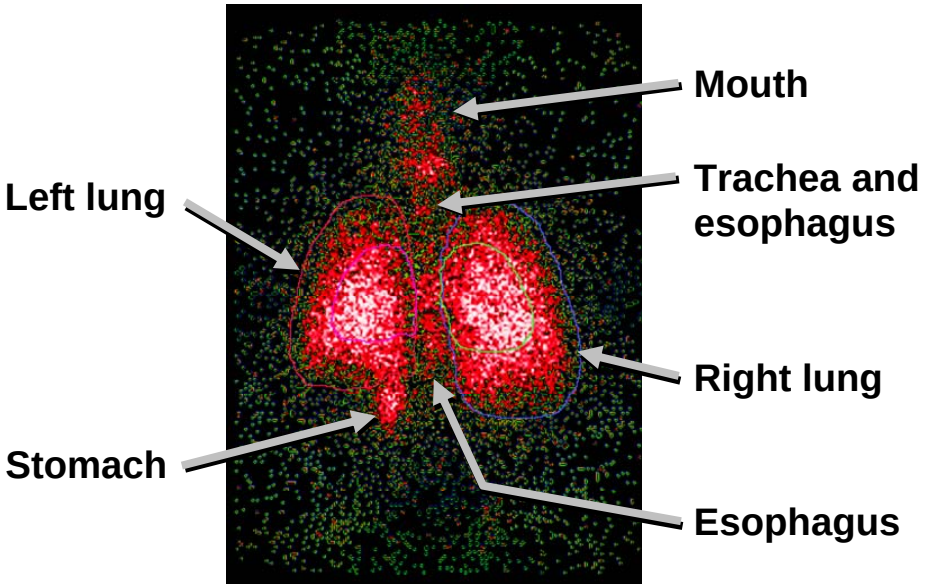


Figure 3.2.3-1. Representative gamma scintigraphy in a double-lung transplant recipient.

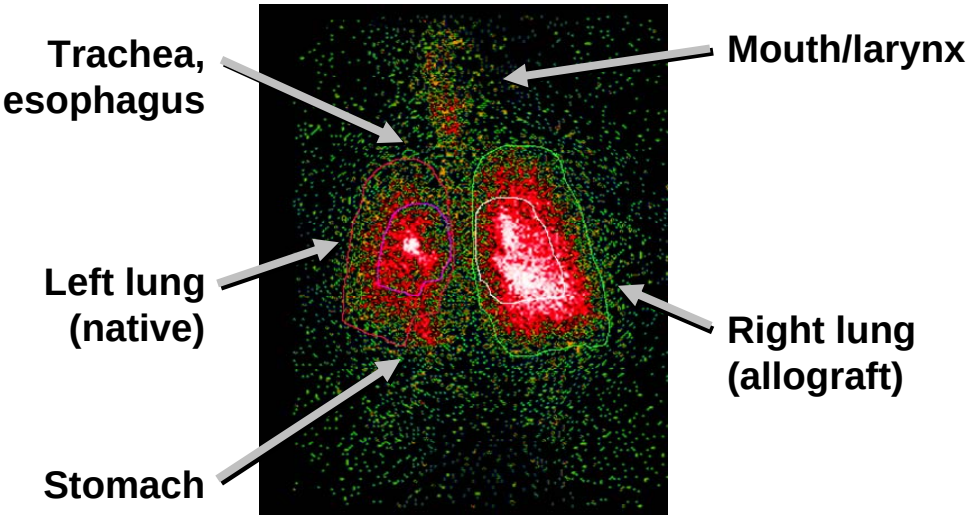


Figure 3.2.3-2. Representative gamma scintigraphy in a single-lung transplant recipient.

Burckart et al.[23] characterized the lung deposition and systemic absorption of the same formulation of CyIS as was used in the pivotal clinical study ACS001 in 8 lung-transplant patients. They demonstrated that the mean absolute systemic bioavailability of a 300-mg dose of CyIS was 8.2% (range, 5.4% to 11.2%) of the dose *placed in the nebulizer*—that is, 24.6 mg (range, 17.8 to 39.3 mg). Furthermore, lung scintigraphy and pharmacokinetic studies demonstrated an almost exact 1:1 ratio between the systemically absorbed dose and the dose deposited in the lung. Absorption via the GI tract following incidental ingestion was estimated to be negligible.

In view of the narrow therapeutic window of cyclosporine and its significant dose-related toxicity, it is relevant to consider systemic exposure to cyclosporine following CyIS in context of systemic exposure following standard oral regimens used in organ transplantation.

In Burckart's[23] series of recent lung transplant recipients, peak blood concentrations of cyclosporine after a single 300-mg dose of CyIS by inhalation ranged from 119 to 402 ng/mL with a mean value of 206.3 ng/mL, whereas blood levels 24 hours after dosing ranged from 9 to 48 ng/mL (mean, approximately 30 ng/mL), with a mean $AUC_{0\text{-}24}$ of 1033.9 ng·hr/mL.

In **Table 3.2.3-1**, these exposure levels are compared with exposures following standard Neoral® (oral cyclosporine) regimens in recent kidney and liver transplant recipients.[24] It is reasonable to compare single doses of CyIS against steady-state Neoral® exposures because CyIS is given 3 times per week, which results in negligible accumulation. Systemic trough levels immediately before the next dose are very low. Peak and trough blood levels and $AUC_{0\text{-}24}$ are all 7- to 10-fold lower after 300 mg CyIS by inhalation compared with standard Neoral® regimens in kidney and liver transplant patients.

Table 3.2.3-1. Comparison of Blood Levels of Cyclosporine Following a Single 300-mg dose of CyIS by Inhalation and Steady-State Blood Levels Following Standard Oral Neoral® Regimens in Recent Solid-Organ Transplant Patients

	Dose	Population	Mean Peak Blood Concentration, ng/mL	Mean Trough Blood Concentration, ng/mL	Mean $AUC_{0\text{-}24h}$, ng·hr/mL
CyIS	300 mg	Recent Lung Transplant	206	< 30[b]	1034
Neoral®	597 mg/d[a]	Recent renal Transplant	1802	361	8772
Neoral®	458 mg/d[a]	Recent liver transplant	1555	268	7187

[a] Given orally as divided doses 12 hours apart.
[b] Approximation: range was 9 to 48 ng/mL measured 24 hours postdose.
CyIS = Cyclosporine inhalation solution; AUC = Area under the curve.

Although systemic blood levels of cyclosporine are very low 24 hours after inhalation of 300 mg CyIS, there is evidence for persistence of higher levels of cyclosporine in lung tissue following inhalation. Burckart et al[23] estimated the terminal half-life ($t_{½}$) of cyclosporine in lung tissue at about 40.7 hours following CyIS by inhalation. This contrasts to terminal elimination $t_{½}$ measured in blood of 6.5 hours after IV or 8.4 hours after oral administration.[23,24]

Two mechanisms have been postulated for prolonged persistence of cyclosporine in lung tissue following inhalation:

1. Uptake and slow release by pulmonary macrophages.[25]

2. Direct binding of cyclosporine to phospholipids in the alveolar epithelial membrane and lung surfactant.[26]

Whatever the mechanism, this prolonged lung residency time is compatible with the three-times-weekly dosing regimen proposed for CyIS.

It is informative to estimate the oral dose of cyclosporine required to achieve the same lung levels as aerosol administration of 300 mg CyIS. In the determination of lung levels, whether after systemic or aerosol administration, it is customary to express it in terms of weight of tissue. For the case of aerosol administration of cyclosporine, because of the diffusion control and the low lung clearance, the deposited dose per lung weight is a reasonable estimate of an average lung concentration across the lung tissue, for comparison purposes. Using a human lung weight of 700 g and a deposited dose of 25 mg cyclosporine (see above), lung concentrations of cyclosporine are then approximately 25 mg dose/700 g lung, or 35714 ng/g.

Studies in rats suggest that the lung has a particular avidity for cyclosporine,[5] such that trough lung concentrations are about 10-fold greater than trough blood concentrations when cyclosporine is given daily via the subcutaneous or intramuscular route.[5,27] Allowing for this, to obtain a lung concentration of 35714 ng/g a trough systemic blood concentration of approximately 4000 ng/mL is needed. Assuming a similar lung-to-blood partition ratio after systemic administration in humans, we can then estimate the oral dose equivalent.

As shown in **Table 3.2.3-1**, in renal transplant patients the indicated regimen (equivalent to 597 mg/day given as two doses 12 hours apart) gives a maximum concentration (C_{max}) of about 1800 ng/mL—less than half the target level. Hence, the dose and target blood concentrations of cyclosporine would need to be more than doubled to achieve the same lung concentration as CyIS. In other words, an extra 600 mg oral cyclosporine per day would be needed to achieve the same levels as 25 mg given directly by the inhalation route—a dose ratio of 24:1.

Oral cyclosporine has somewhat variable bioavailability, mainly due to limited absorption. Therefore, it may be more appropriate to consider systemic exposure rather than dose for this comparison. As an index of relative systemic exposure, CyIS achieves an estimated lung concentration of 35714 ng/g with a blood C_{max} of 206 ng/mL. Following oral administration,

a blood C_{max} of 4202 ng/mL is needed to achieve the same lung concentration—about a 19-fold increase in peak blood level.

Giving twice the standard oral dose of cyclosporine would not be an appropriate clinical option, because the therapeutic window for cyclosporine is narrow, and even the standard dose is associated with significant dose-related toxicity including renal dysfunction, hepatotoxicity, hypertension, and dyslipidemia on chronic administration.

Finally, it is relevant to comment on the potential safety implications of variations in administered dose due to errors in formulation that were noted during the clinical investigation. As previously stated, the "lung dose" of cyclosporine is about 25 mg following 300 mg CyIS by inhalation. A variation of as much as 20% in dose would result in a range from about 20 to 30 mg. The change in blood levels of cyclosporine following such an error (about 41 ng/mL at peak, about 6 ng/mL at trough) would not be clinically important in comparison with levels routinely used in the management of transplant patients as well as the fact that cyclosporine is clearly concentrated in the lung.

3.2.4 Preclinical Pharmacokinetics

Animal studies were carried out to determine the efficacy of aerosolized cyclosporine in acute lung transplantation models. Healthy rats exposed to a single 3-mg/kg dose of aerosolized cyclosporine demonstrated several-hundred-fold higher cyclosporine tissue levels in lung compared with liver, kidney, heart, and blood. At 24 hours postdose, only the lung parenchyma demonstrated residual cyclosporine. The area under the curve $(AUC)_{0-\infty}$ was approximately 46,000 ng•hr/g in lung and 2,500 ng•hr/g in blood, which is a 20-fold higher exposure in lung compared with blood. In a fully allogeneic, orthotopic left lung transplantation study using rats, aerosolized cyclosporine at 18 mg/kg total daily dose deposited a pulmonary dose of 3.6 mg/kg/day.[27] After a total of 7 doses, rats were protected from allograft rejection (mean grade, 1.2 ± 0.4; $p < .05$) and the result was superior to intramuscular-administered cyclosporine (mean grade, 1.8 ± 0.35). Grading scale was from 0, none, to 4, vascular thrombosis and massive intra-alveolar hemorrhage. Finally, a study performed in rats examined the ability of aerosol or systemic cyclosporine to prevent rejection.[5] The results showed that a systemic dose of 15 mg/kg intramuscular cyclosporine

gave a similar rejection score as an aerosol dose of 30 mg/kg (3 mg/kg pulmonary deposited dose) cyclosporine.

3.3 Preclinical Toxicology

3.3.1 Cyclosporine: Systemic Exposure

The systemic toxicity of cyclosporine has been well characterized in humans and animals. Pharmacologic activity and toxicity associated with systemic exposure in animals are consistent with effects observed with human use.[28-31] The major adverse effects observed in humans at clinical exposures include renal dysfunction, hepatotoxicity, hypertension, dyslipidemia, hirsutism and tremor. Additional, less-common toxicities include gum hyperplasia, hyperkalemia, and thrombocytopenia.

3.3.2 CyIS: Pulmonary Exposure

Limited published data are available in animals to address the potential local (respiratory system) toxicities of inhaled cyclosporine. The primary data for pulmonary exposure are based on two pivotal 28-day inhalation toxicology studies in rats and dogs. In these studies animals were dosed daily at maximally tolerated (rat) or maximally feasible (dog) doses. This provides safety evaluation at multiples to the maximum clinical exposure in terms of overall dose given per day for cyclosporine as well as in frequency of dosing (daily in animals vs. three times/week in humans) and provides an estimate of a “worst case” toxicity from use with CyIS.

In the 28-day rat inhalation study, rats were dosed with total inhaled doses of 7.4, 24.3, and 53.8 mg/kg cyclosporine. There were treatment-related hematologic, serum chemistry, and kidney findings that were consistent with systemic cyclosporine toxicity in all dose groups but no pulmonary histologic findings.

In the 28-day dog inhalation study, dogs were dosed daily with total inhaled doses of 4.4, 7.6, and 9.6 mg/kg cyclosporine. Respiratory rates were lower in the high-dose group males compared with other groups which resulted in the high-dose males having similar estimated deposition of the test material as the mid-dose males. This suggests that additional dosing

duration would not result in significantly increased exposures. There were no test-article–related adverse findings. In dogs, there were no clinically significant effects on the respiratory system at maximally feasible doses.

Microscopically, minimal and focal ulceration of the laryngeal mucosa, with inflammation, was observed in all three mid-dose male dogs from primary necropsy and both high-dose recovery males. These ulcers were generally pinpoint in nature with the rest of the larynx appearing normal. No other dogs had evidence of laryngeal ulceration. As no ulcers were observed in concurrent vehicle control animals or low dose dogs, this finding was classified as equivocally related to treatment.

3.3.3 Propylene Glycol: Systemic Exposure

Propylene glycol is Generally Recognized as Safe (GRAS 21 CFR 182) at levels of up to 2% of composition for most food categories, 5% in alcoholic beverages and 97% in seasonings and flavorings. Based on metabolic and toxicologic data, the World Health Organization (WHO) has set 25 mg/kg/day as an acceptable daily intake. However, a National Toxicology Program-Center for the Evaluation of Risks to Human Reproduction (NTP-CERHR, 2004) Expert Panel Report estimates that the general population is exposed at 2400 mg/day or 34 mg/kg/day for a 70-kg person. No effect was detected in rats dosed up to 50000 ppm PG in the diet (equivalent to 2.5 g/kg/day) for 2 years and dogs were unaffected by daily oral doses of 2 g/kg in the diet for 2 years.[32,33]

Propylene glycol is not a novel excipient and is used in many different approved pharmaceutical formulations administered by oral, buccal, intravenous, intramuscular, topical, transdermal, ophthalmic, otic, rectal, vaginal, intranasal and inhalation routes (FDA's Inactive Ingredients for Approved Drug Products). No limit currently exists for PG in pharmaceutical products and the Inactive Ingredient list does not provide information on product names or use patterns, so a direct comparison to PG exposure from approved products is not possible. While no other products are identified that use 100% PG as an excipient, approved intravenous/intramuscular products contain up to 82% PG and one inhaled product contains 25% PG.

The maximum amount of PG potentially absorbed systemically in humans from a vial of CyIS based on the specific gravity of PG (1.036 g/mL) is 8.6 mg/kg pulmonary deposited dose.

The 8.6 mg/kg estimated to be the maximum human pulmonary deposited exposure/dose three times per week is approximately three times less than the WHO recommended daily exposure (25 mg/kg/day). Therefore, the likelihood of systemic toxicity of PG from CyIS for the proposed use is low.

3.3.4 Propylene Glycol: Pulmonary Exposure

A 28-day PG study was performed giving 14 mg/mL or 418 mg/kg/day to 15 male rats, and 14 mg/mL or 539 mg/kg/day to 15 female rats over a period of 90 minutes. Ten from each group were then necropsied on day 29 with the remaining five in each group being sacrificed after a 32-day recovery period. Assuming that 50% of the administered dose was deposited in the lung, this equates to a daily dose of 209 mg/kg for the males and 270 mg/kg for the females. If it is assumed that 8% of the total inhaled dose is in fact deposited in the lungs, this results in a daily dose of 34 mg/kg in the males and 43 mg for the females. No vehicle-associated histopathologic abnormalities were found in any of the groups immediately following 28 days of exposure or with a further 32-day recovery period.

A 28-day PG study was performed giving 12 mg/mL or 183 mg/kg/day to five male dogs, and 12 mg/mL or 154 mg/kg/day to five females over a period of 60 minutes. Three from each group were then necropsied on day 29 with the remaining two in each group being sacrificed after a 28-day recovery period. Assuming that 50% of the administered dose was deposited in the lung, this equates to a daily dose of 91 mg/kg for the males and 77 mg/kg for the females. If it is assumed that 20% of the total inhaled dose is in fact deposited in the lungs, this results in a daily dose of 37 mg/kg in the males and 31 mg for the females. No vehicle-associated histopathologic abnormalities were found in any of the groups immediately following 28 days of exposure or with a further 28-day recovery period.

Additional data to support the safety of repeat dose pulmonary administration of PG have been reported from a 3-month inhalation study performed in rats.[34] Sprague-Dawley rats

(19/sex/group) were dosed 6 hr/day, 5 days/week for 90 days at concentrations of 0, 0.16, 1.0, or 2.2 mg PG/L air (corresponding to 20, 120, and 270 mg/kg total inhaled doses). The no observed adverse effect level was determined to be the 1.0 mg/L aerosol concentration (approximately 120 mg/kg total inhaled dose). Effects included nasal hemorrhaging and ocular discharge (attributed to the dehydrating effects of PG), a modest decrease in body weights at the high dose, and an increase in the number of goblet cells and mucin content of goblin cells in the nasal turbinates. There were no histologic changes in the trachea, lungs, or larynx. There were no changes in hematology, clinical chemistry, or organ weights that were related to treatment.

3.3.5 Relationship Between Lung Exposure in Animals and Humans

Assumptions made in determining the relative exposures in animals compared with the human exposure for CyIS and PG are listed in **Table 3.3.5-1**. The average amount of CyIS deposited in human lungs (weight range, 600 to 800 g; average weight, 700g [excluding bronchial stem]) following a 300-mg inhalation is 25 mg **(see Table 3.3.5-2)**.[23]

Table 3.3.5-1. Animal Data Derived From 28-Day Inhalation Studies

Animal	Sex	Average weight	Average lung weight
Rat	Male	340 g	2.1 g
	Female	220 g	1.7 g
Dog	Male	10 kg	93 g
	Female	8.5 kg	75 g

Table 3.3.5-2. Exposure to CyIS and Propylene Glycol

	CyIS		
Species	**Admin. Dose, mg**	**Amount (µg) CyIS/g of lung tissue**	**Lung exposure relative to humans**
Human	300	35.7	1
Rat/kg, male/female	4.5/4.1	714/529	20/14.8
Dog/kg, male/female	1.7/2.2	183/249	5.1/7

	PG		
Species	**Admin. Dose (g 300 mg CyIS)**	**Amount (mg) PG/g of lung tissue**	**Lung exposure relative to humans**
Human	4.67	0.56	1
Rat/kg, male/female	0.06/0.06	10/7.7	17.9/13.8
Dog/kg, male/female	0.02/0.03	2.5/4	4.5/7.1
CyIS = Cyclosporine inhalation solution; PG = Propylene glycol.			

3.4 Distribution

No specific distribution studies were performed with CyIS; however, since the drug is administered directly into the lungs, it is expected that local lung concentrations would be higher compared with other routes of administration. Cyclosporine is absorbed through the lungs and distributed widely throughout the body. It crosses the placenta and is found in breast milk. Preferential uptake of cyclosporine occurs in the liver, pancreas, and adipose tissue. Cyclosporine penetrates the central nervous system poorly. In plasma, the distribution of cyclosporine is inversely dependent on the hematocrit concentration in the blood. Approximately 33% to 47% of systemic cyclosporine is found in plasma, 4% to 9% in lymphocytes, 5% to 12% in granulocytes, and 41% to 58% in erythrocytes. In plasma, approximately 90% of cyclosporine is bound to lipoproteins. Any medications that affect the binding of cyclosporine to lipoproteins may modify the clinical response to cyclosporine.

3.5 Metabolism

Cyclosporine is extensively metabolized by cytochrome P450 3A4 in the liver, and to a lesser extent in the gastrointestinal tract, kidney, and lung. At least 25 cyclosporine metabolites have been identified, and most show 10% to 20% of the immunosuppressive activity of the parent drug. They also contribute to the toxicity profile of cyclosporine. The major metabolites of cyclosporine are M1, M9, and M4N, which at steady state for blood concentrations have a mean AUC of 70%, 21%, and 7.5%, respectively.

No specific metabolism studies were performed for CyIS, but published studies indicate that biotransformation pathways in isolated lung slices are similar to those observed in the liver.[35]

However, the absolute amount of M1 in lung slices is lower compared with the liver. No regional differences in metabolism were observed between different areas of the lung, and, after aerosol administration, cyclosporine lung metabolism is expected to be equal to that of liver at first contact. However, with time, liver metabolism would be expected to exceed lung metabolism (approximately 8-fold higher). These results indicate that cyclosporine is extensively metabolized and that the major route of elimination is via bile and fecal excretion.

3.6 Drug-Drug Interactions

No specific drug interaction studies were performed with CyIS. However, low systemic exposures (5% to 11% of the administered dose) are expected after CyIS use, and drug interactions after IV and oral dosing are well documented in the labeling and literature. Agents that affect the CYP3A4 system may alter the metabolism of cyclosporine. In addition, agents that induce CYP450 activity may increase metabolism and decrease cyclosporine concentrations.

Based on information from the Neoral® label, the following is a summary of drugs reported to interact with cyclosporine. Drugs that increase cyclosporine levels include calcium channel blockers (diltiazem, nicardipine, and verapamil), antifungals (fluconazole, itraconazole, and ketoconazole), antibiotics (clarithromycin, erythromycin, and quinupristin/dalfopristin), glucocorticoids (methylprednisolone), allopurinol, bromocriptine, danazol, metoclopramide, colchicines, and amiodarone. Drugs that decrease cyclosporine levels include antibiotics (nafcillin and rifampin), anticonvulsants (carbamazepine, phenobarbital, and phenytoin), octreotide, ticlopidine, and St. John's Wort. Orlistat decreases the absorption of cyclosporine approximately 33% in healthy volunteers, and reductions in exposure have been reported in transplant patients. Some drugs that are able to interact with cyclosporine and also potentiate renal dysfunction include nonsteroidal anti-inflammatory agents, aminoglycosides, melphalan, amphotericin B, tacrolimus, cimetidine, and ranitidine. Patients with psoriasis receiving immunosuppressive agents or radiation therapy should not receive concurrent cyclosporine because of the possibility for excessive immunosuppression.

Cyclosporine may also interfere with the levels of other drugs. Methotrexate concentrations increase 30% and the 7-hydroxymetabolite concentrations decrease 80% with concurrent cyclosporine administration. The reduced clearance of prednisolone, digoxin, and lovastatin was reported when these drugs are administered with cyclosporine. Furthermore, cyclosporine should not be used with potassium-sparing diuretics because hyperkalemia may result. Moreover, vaccination may be less effective during treatment with cyclosporine, and the use of live vaccine should be avoided.

3.7 Special Population Studies

No special population studies were performed with CyIS. Cyclosporine inhalation solution is not intended for pediatric use. Based on information from the Neoral label, renal failure or dialysis does not notably alter cyclosporine clearance. In addition, no relevant differences in pharmacokinetic parameters have been demonstrated in the elderly population.

3.8 Chemistry

Cyclosporine, USP, the active pharmaceutical ingredient in CyIS, is a cyclic polypeptide immunosuppressant agent consisting of 11 amino acids with a molecular formula of $C_{62}H_{111}N_{11}O_{12}$ and molecular weight of 1202.63. Cyclosporine, USP is a crystalline powder. The chemical structure of cyclosporine (cyclosporine A) is provided below in **Figure 3.8-1.**

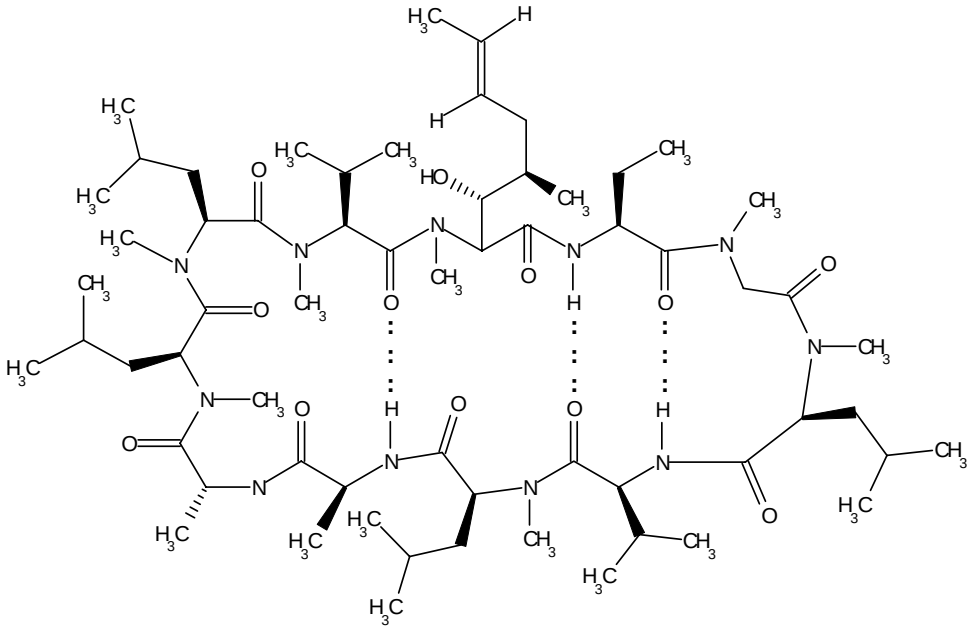


Figure 3.8-1. Cyclosporine, USP, is stable at room temperature (15ºC to 30ºC) and is freely soluble in propylene glycol, USP, with a saturation solubility of 400 mg/mL.

Cyclosporine inhalation solution drug product is a simple formulation of cyclosporine dissolved in PG, the only excipient in the formulation. Propylene glycol was chosen because of its ability to solubilize cyclosporine (the target formulation concentration of 62.5 mg/mL is well below the drug substance solubility limit), it is nonirritating and has a nonconstricting effect on human airways even when used undiluted.[36] The nebulization and aerosol properties of solutions containing different levels of PG are also well characterized.[37]

Cyclosporine inhalation solution is formulated as a sterile, clear, colorless solution of cyclosporine, USP drug substance in PG, USP, filled into a sterile, single-use clear glass vial sealed with a sterilized rubber stopper, secured by an aluminum overseal. The commercial presentation of CyIS is a 6-mL vial containing 325 mg of cyclosporine, USP, in a 5.2-mL solution of PG to deliver 300 mg of cyclosporine at the highest dose of 4.8 mL. Cyclosporine inhalation solution may also be prescribed as a 1.5-mL (100 mg) and 3.0-mL (300 mg) dose from the single-use vial. Cyclosporine inhalation solution is administered as an aerosol for inhalation with an AeroTech™ Nebulizer (CIS-US, Inc.; Bedford, Mass).

Cyclosporine inhalation solution is demonstrated to be stable for up to 24 months at room temperature. The proposed label storage statement is "Store at 25ºC (77ºF); excursions permitted to 15-30ºC (59-86ºF)." Cyclosporine inhalation solution is not recommended for reuse or refrigerated storage.

4. SUMMARY OF CYIS EFFICACY

The efficacy data for CyIS were generated from a prospective, randomized, double-blind, placebo controlled trial (ACS001) performed at UPMC between 1997 and 2003. The safety data for CyIS were generated from ACS001 as well as a retrospective analysis of data from seven open label studies performed at UPMC between 1995 and 2003 (ACS002). **Table 4-1** summarizes the design, objectives, and endpoints of ACS001 and ACS002.

Table 4-1. Summary of Pivotal Efficacy and Safety Studies

Study	Design	Objectives	Study Endpoints
ACS001 N = 66	Randomized, double-blind, placebo-controlled study of CyIS for 2 years in patients 7 to 42 days following lung transplantation	• Primary: To determine whether CyIS lowers the rate of acute cellular rejection • Secondary: To determine whether CyIS therapy results in improved survival, decreased rate of chronic rejection, and improved lung function • To determine the safety of CyIS when started 7 to 42 days following transplantation and given for up to 2 years	• Rate of biopsy-proven AR • Survival duration • Chronic rejection as manifested by obliterative bronchiolitis and bronchiolitis obliterans syndrome • Maintenance of lung function • Deaths, treatment-emergent adverse events, serious adverse events, changes in laboratory values
ACS002 N = 70	Collection, verification, and analyses of UPMC studies performed under IND No. 36,417	• To collect, verify, and analyze demographic and safety data from all 70 University of Pittsburgh transplant recipients from Jan 1995 to Aug 2003 who received at least 1 dose of open-label CyIS (treated outside Study ACS001)	• Demographic and transplant characteristics • Dosing information/exposure to study drug • Deaths, treatment-emergent adverse events, serious adverse events, changes in laboratory values

CyIS = Cyclosporine inhalation solution; UPMC = University of Pittsburgh Medical Center; AR = Acute rejection; CR = Chronic rejection; IND = Investigational New Drug.

4.1 ACS001 Study Design

Study ACS001 was a randomized, double blind, placebo-controlled study of CyIS given for up to 2 years as an adjunct to standard immunosuppressive therapy in patients following a single- or double-lung transplant. The study was conducted by UPMC, one of the largest lung transplant centers in the United States.

The primary efficacy endpoint was the rate of biopsy-proven AR of grade 2 or higher (2+) during the 2-year treatment period. Important prospectively defined secondary endpoints included overall survival duration, chronic rejection as manifested by OB and BOS, and maintenance of lung function. The first randomized patient began dosing in November 1998 and the last in August 2001. The study ended on August 21, 2003, when the last patient enrolled completed the protocol-specified 2 years of dosing. Thus follow-up on all randomized patients ranged from 24 months to 56 months.

The trial began in 1997 with a 10-patient open-label pilot phase prior to the randomized phase. These 10 patients were dosed with CyIS for a minimum of 12 weeks and followed prospectively to form a cohort that could be monitored for safety and tolerability prior to the initiation of the randomized study phase. During the randomized, double-blind, placebo-controlled phase beginning in 1998, 56 patients were enrolled and treated with CyIS or placebo (inhaled PG).

Figure 4.1-1 summarizes the inclusion and exclusion criteria for ACS001. All patients were enrolled between 7 and 42 days following their transplant procedure. Randomization was carried out by the Department of Biostatistics at UPMC. Four separate randomization lists were prepared as the randomization was stratified by planned start of study treatment (7 to 21 days or 22 to 42 days after transplant surgery) and CMV mismatch (donor positive/recipient negative) or match (all other donor/recipient combinations).

Inclusion Criteria:

- **Single or double lung transplant recipients**
- **18 years or older**

Exclusion Criteria:

- **Active fungal or bacterial pneumonia or anastomotic infections prior to initiation of appropriate treatment**
- **Untreated bronchial stenosis >80%**
- **Failure to wean from mechanical ventilation**
- **Women of childbearing potential unwilling to use appropriate birth control and avoid pregnancy**

- **Subjects enrolled 7 - 42 days post transplant**
- **26 patients treated with inhaled cyclosporine vs. 30 patients treated with placebo—PG**
- **Initial 10 day dose escalation phase to maximal dose tolerated up to 300 mg in 4.8 mL PG**
- **Three times per week maintenance dose for two years**
- **Study termination date 21AUG03**

Figure 4.1-1. Study ACS001 inclusion/exclusion criteria. CMV = Cytomegalovirus; PG = Propylene glycol.

All patients underwent an initial 10-day dose-determination period before beginning the maintenance phase of dosing. Patients were started on a 100-mg dose of CyIS (or equivalent volume of placebo) and over the 10-day period, the dose was gradually titrated up in no more than 100-mg increments as tolerated to a protocol-specified maximum dose of 300 mg CyIS. The maximally tolerated dose (or equivalent volume of placebo) reached on day 10 was then continued three times per week (for example, Mondays, Wednesdays, and Fridays) for an intended period of 2 years. All patients were then followed for survival and chronic rejection up through the study closure date of August 21, 2003.

A total of 68 patients were enrolled in study ACS001. The first 10 patients received open-label CyIS during the study pilot phase. During the randomized trial phase, 58 additional patients were randomized to receive either CyIS (n = 28) or placebo (n = 30) in addition to standard care. Among these 58, two patients withdrew consent before receiving a single dose of study medication. Only the 56 patients who were randomized and received at least one dose of study medication were included in the analysis of efficacy or safety. All 66 patients

who received study medication were included in the safety analysis **(Figure 4.1-2; Section 5)**.

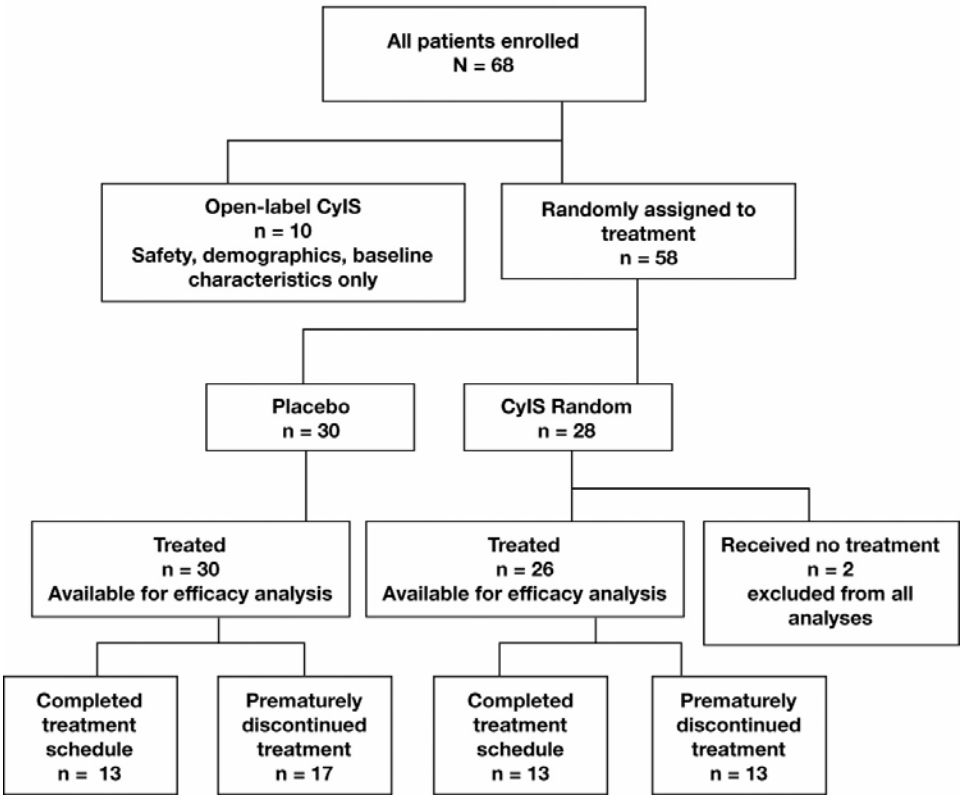


Figure 4.1-2. Patient disposition in Study ACS001. CyIS = Cyclosporine inhalation solution.

Details of the overall extent of exposure to CyIS can be found in **Section 5.2**.

Although the single-center design of ACS001 limits the generalizability of results, it provides a clearer scientific interpretation of the efficacy of CyIS by preventing center-to-center variability in patient management and outcomes from confounding the efficacy determination.

4.2 Baseline Demographics of Study Population

Patient baseline demographics for the 56 randomized and treated patients are summarized in **Table 4.2-1** and indicate that Placebo and CyIS patients were similar with respect to most

baseline characteristics with several notable exceptions. Double-lung transplants were more common in the CyIS group, which could potentially introduce bias favoring the CyIS group. However emphysema was less common and idiopathic pulmonary fibrosis was more common in the CyIS group, which could potentially introduce bias favoring the placebo group.

Table 4.2-1. Demographics and Baseline Characteristics

Demographic or Baseline Characteristic	**Placebo (n = 30)**	**CyIS (n = 26)**
Mean Age, years (range)	51.6 (19 - 65)	50.4 (18 - 65)
Sex Distribution, n (%)		
Female	12 (40)	10 (38)
Male	18 (60)	16 (62)
Mean Height, cm (range)	168 (150 - 188)	170 (150 - 185)
Mean Weight, kg (range)	65 (36 - 112)	70 (49 - 91)
Ethnic Origin, n (%)		
Asian	1 (3)	0
Black	1 (3)	1 (4)
Caucasian	28 (93)	24 (92)
Hispanic	0	1 (4)
Randomization Strata, n (%)		
Open label	0	0
CMV match/7-21 day start	13 (43)	13 (50)
CMV mismatch/7-21 day start	4 (13)	2 (8)
CMV match/22-42 day start	9 (30)	7 (27)
CMV mismatch/22-42 day start	4 (13)	4 (15)
Smoking History, mean pack years	36.6	35.7
Single Lung Transplant Type, n (%)	24 (80)	15 (58)
Grade 2+ AR prior to 1st dose of study drug, n (%)	13 (43)	8 (31)
Baseline FEV_1, mean L[a]		
Single-lung transplants	1.38	1.43
Double-lung transplants	1.73	2.08
CMV Donor+/Recipient–, n (%)	7 (23)	5 (19)
Primary Diagnosis, n (%)		
Cystic fibrosis	4 (13)	5 (19)
Emphysema	19 (63)	9 (35)
IPF/mixed connective tissue	3 (10)	6 (23)
Other	4 (13)	6 (23)
Panel Reactive Antibodies > 10%, n (%)	0	2 (8)

Table 4.2-1. Demographics and Baseline Characteristics		
Demographic or Baseline Characteristic	**Placebo (n = 30)**	**CyIS (n = 26)**
Recipient Diagnosis of Diabetes Mellitus, n (%)	1 (3)	3 (12)
Recipient Diagnosis of Obesity, n (%)	3 (10)	5 (19)
Donor Age > 35 years	18 (60)	16 (62%)
Donor Weight > 70 kg, n (%)	19 (63)	13 (50)
Donor/Recipient Sex Other Than F/F, n (%)	22 (73)	18 (69)
Planned Dosing > 21 days Posttransplant, n (%)	13 (43)	11 (42)
ICU Stay > 4 days	11 (37)	12 (46)
ICU Stay >7 days	9 (30)	3 (12)
[a] Missing baseline FEV-1 values imputed with first on-study results. CyIS = Cyclosporine inhalation solution; CMV = Cytomegalovirus; AR = Acute rejection; FEV_1 = Forced expiratory volume in 1 second; IPF = Idiopathic pulmonary fibrosis; F/F = Female/female; ICU = Intensive care unit.		

Table 4.2-2 illustrates the distribution of other baseline donor and recipient baseline characteristics that have been demonstrated in the literature to potentially affect outcome. While randomization attempts to achieve balance between treatment groups at baseline, randomized trials can have differences between groups in one or more baseline characteristics. However, as is apparent in **Table 4.2-2**, the majority of factors are either balanced or potentially bias results in favor of the placebo group.

Table 4.2-2. Documented Baseline and Disease Characteristics Affecting Clinical Outcome

Imbalances in Baseline Characteristics Favoring CyIS Cohort	**Balanced Baseline Characteristics**	**Imbalances in Baseline Characteristics Favoring Placebo Cohort**
Single-lung transplant[12] (80% Placebo vs. 58% CyIS)	CMV D+/R– mismatch[12] (23% Placebo vs. 19% CyIS)	Primary diagnosis other than emphysema[12] (37% Placebo vs. 65% CyIS)
Prior grade 2+ AR prior to dosing[a 15] (43% Placebo vs. 31% CyIS)	Gender Mismatch[b 12,38] (33% Placebo vs. 27% CyIS)	Primary diagnosis of idiopathic pulmonary fibrosis[12] (3% Placebo vs. 23% CyIS)
	Female donor/female recipient[12] (27% Placebo vs. 31% CyIS	Donor weight > 70 kg[12] (63% Placebo vs. 50% CyIS)
	Female recipients[12] (40% Placebo vs. 38% CyIS)	Panel reactive antibodies >10%[39] (0% Placebo vs. 8% CyIS)
	Recipient age > 35 years[12] (87% Placebo vs. 88% CyIS)	Recipient diagnosis of diabetes mellitus (3% Placebo vs. 12% CyIS)[12]
	Prior grade 1+ AR prior to dosing[a 15] (57% Placebo vs. 54% CyIS)	Recipient diagnosis of obesity[40,41] (10% Placebo vs. 19% CyIS)
	Donor bacterial colonization[42] (40% Placebo vs. 35% CyIS)	
	Donor history of diabetes[12] (0% Placebo vs. 0% CyIS)	

[a] We are extrapolating data on the effect of early AR to the presence of AR prior to dosing.
[b] There are conflicting reports regarding the affect of gender mismatch with one report suggesting association with no significant outcomes and one report suggesting association with positive outcomes.
CyIS = Cyclosporine inhalation solution; D = Donor; R = Recipient; AR = Acute rejection.

Aerosolized treatment was administered over 15 to -45 minutes using a commercially available jet nebulizer (AeroTech II, CIS-US, Bedford, Mass). Patients were premedicated by inhalation of 2% lidocaine and 0.5 mg albuterol as needed to minimize any irritation from CyIS or placebo treatment. In addition to the study drug or placebo, all patients received standard immunosuppressive therapy, which in most cases consisted of tacrolimus, azathioprine, and prednisone for both baseline and maintenance immunosuppression **(Tables 4.2-3 and 4.2-4)**. Review of tacrolimus and cyclosporine blood levels as well as prednisone doses demonstrated that groups were comparable in terms of degree of immunosuppression.

Table 4.2-3. Summary of Reported Baseline Immunosuppressive Treatment in Study ACS001

Immunosuppressive Medications, n (%)	Placebo (n = 30)	CyIS (n = 26)
Prednisone	30 (100)	26 (100)
Tacrolimus	30 (100)	26 (100)
Azathioprine	30 (100)	22 (85)
Mycophenolate mofetil	5 (17)	5 (19)
Cyclosporine	1 (3)	1 (4)

CyIS = Cyclosporine inhalation solution.

Table 4.2-4. Summary of Reported Maintenance Immunosuppressive Treatment in Study ACS001

Immunosuppressive Medication, n (%)	Placebo (n = 30)	CyIS (n = 26)
Prednisone	30 (100%)	26 (100%)
Tacrolimus	29 (97%)	25 (96%)
Azathioprine	27 (90%)	21 (81%)
Mycophenolate mofetil	15 (50%)	15 (58%)
Cyclosporine	9 (30%)	7 (27%)
Rapamycin	4 (13%)	1 (4%)

CyIS = Cyclosporine inhalation solution.

Although dosing in each patient continued for a maximum of 2 years, treatment assignments remained blinded until the last patient completed dosing in August 2003. All patients continued to be followed for efficacy endpoints postdosing until the trial was closed on

August 21, 2003. Thus, follow-up on all randomized patients ranged from 24 months to 56 months.

4.3 Summary of Efficacy Data

The results of ACS001 demonstrated that, in patients treated comparably with standard immunosuppression therapy, administration of CyIS prevented chronic rejection and led to a statistically significant and clinically meaningful improvement in survival compared with placebo. Specifically, this pivotal study demonstrated the following:

- **CyIS significantly improved survival duration relative to the date of lung transplant compared with placebo (*P*= .007; relative risk (CyIS/placebo) = 0.213)**
- **CyIS significantly increased chronic rejection-free survival duration compared with placebo (*P*= .001; relative risk = 0.279)**
- **CyIS significantly increased OB-free survival compared with placebo (*P*= .003; relative risk = 0.249)**
- **CyIS significantly increased BOS-free survival compared with placebo (*P*= .019; relative risk = 0.385)**
- **CyIS did not significantly reduce the rate of grade 2+ AR compared with placebo (*P*= .73)**

4.3.1 Survival Duration

Treatment with CyIS resulted in a statistically significant and clinically meaningful improvement in survival duration relative to the date of lung transplantation compared with placebo. Among placebo-treated patients, only 53.3% survived through the study end date compared with 88.5% of patients randomized to CyIS (log-rank *P*= .007; **Figure 4.3.1-1**). The estimated relative risk of death (CyIS/Placebo) was 0.213 (95% CI = 0.061, 0.743]), representing a 79% decrease in the risk of death in patients treated with CyIS compared with placebo.

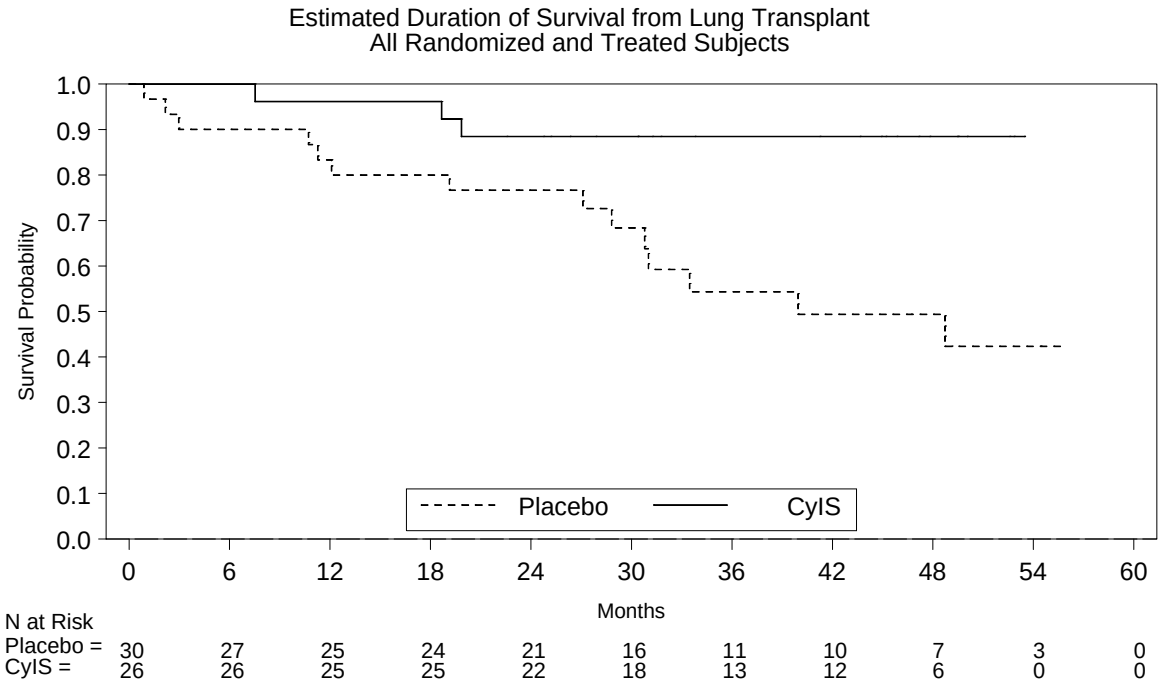


Figure 4.3.1-1. Kaplan-Meier plots of survival duration in patients treated with CyIS or placebo measured from date of transplant. CyIS = Cyclosporine inhalation solution.

While the objective of randomization is to control for variability between treatment groups at baseline, randomized trials can show differences in one or more baseline characteristics between groups. The survival advantage of CyIS cannot be explained by the differences between groups in terms of baseline donor and recipient characteristics. **Figure 4.3.1-2** displays the association between each major risk factor and the survival outcome when the effect of treatment group (CyIS vs. placebo) is ignored. In addition to those well documented in the literature, we are also including the risk factor of ICU length of stay >7 days. Although not previously demonstrated to be an independent risk factor, the FDA suggested including this factor in the analysis. On the vertical axis are listed each factor and on the horizontal axis the estimated relative risk is shown as a small vertical line. The associated 95% confidence interval for the relative risk is shown as a horizontal line. Although not shown, the upper 95% confidence limit for panel reactive antibodies > 10% is 32.32. Each estimate was obtained by fitting a Cox regression model with just the single risk factor as a

covariate in the model. The magnitude by which the relative risk estimate and confidence interval are > 1 indicates the strength of the relationship between the presence of that factor and death. Conversely a relative risk estimate and confidence interval much < 1 would indicate an inverse relationship between the presence of that factor and death.

Interestingly, in ACS001, a diagnosis of emphysema was associated with poorer outcome, which directly conflicts with the majority of the published literature. The higher proportion of placebo patients who had a diagnosis of emphysema may explain this outcome. In other words, the benefit of CyIS therapy to those patients who entered study with diagnoses other than emphysema may have outweighed the higher risk associated with their clinical condition requiring lung transplant. Nevertheless, the survival analyses we present include results both adjusted and unadjusted for diagnosis other than emphysema.

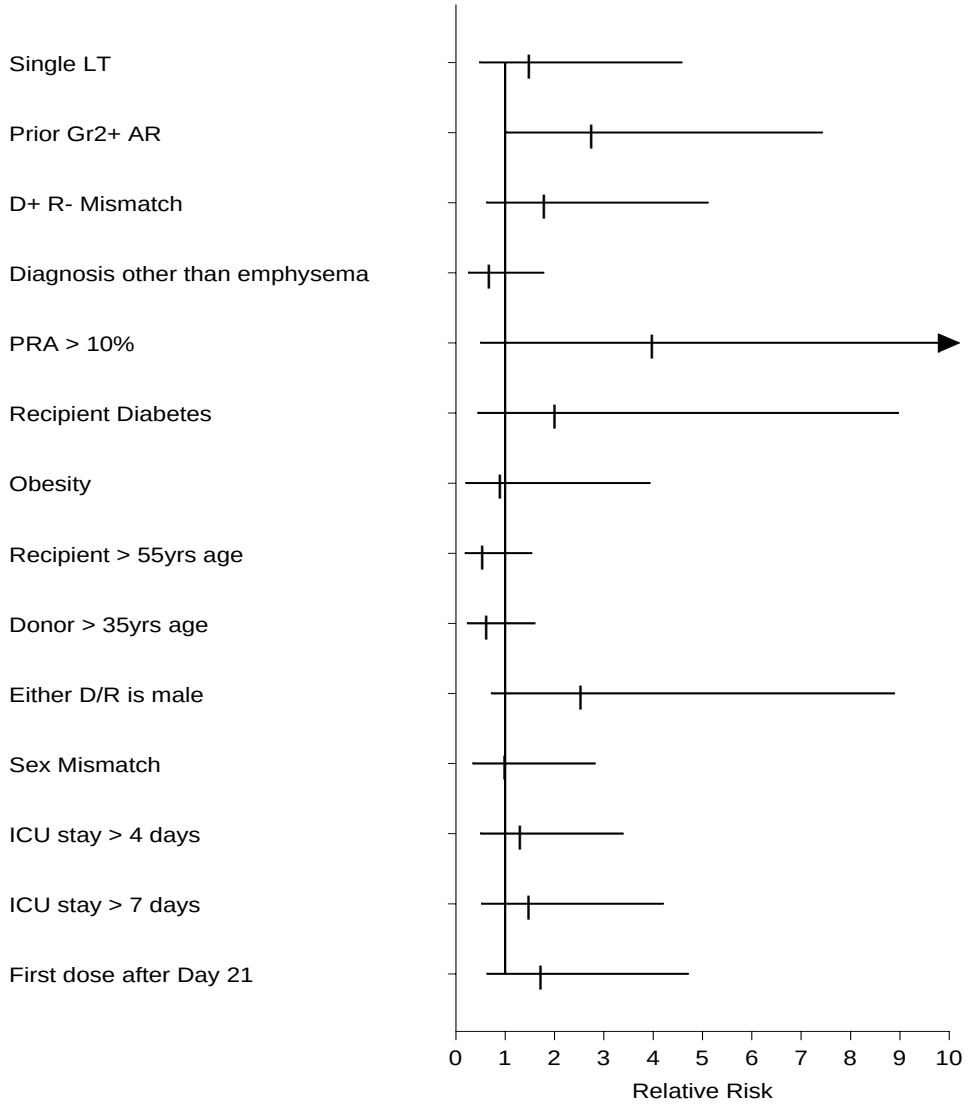


Figure 4.3.1-2. Estimate and 95% confidence interval of the relative risk of death in ACS001 by donor/recipient characteristic. LT = Lung transplant; AR = Acute rejection; D = Donor; R = Recipient; PRA = Probabilistic risk assessment; ICU = Intensive care unit.

Figure 4.3.1-3 shows the resulting relative risk estimate (CyIS/placebo) after adjusting for each donor or recipient characteristic. For every risk factor, the upper bound of the 95% confidence intervals for the relative risk is < 1, indicating that CyIS significantly improved survival even after adjusting for the factor in a Cox regression model.

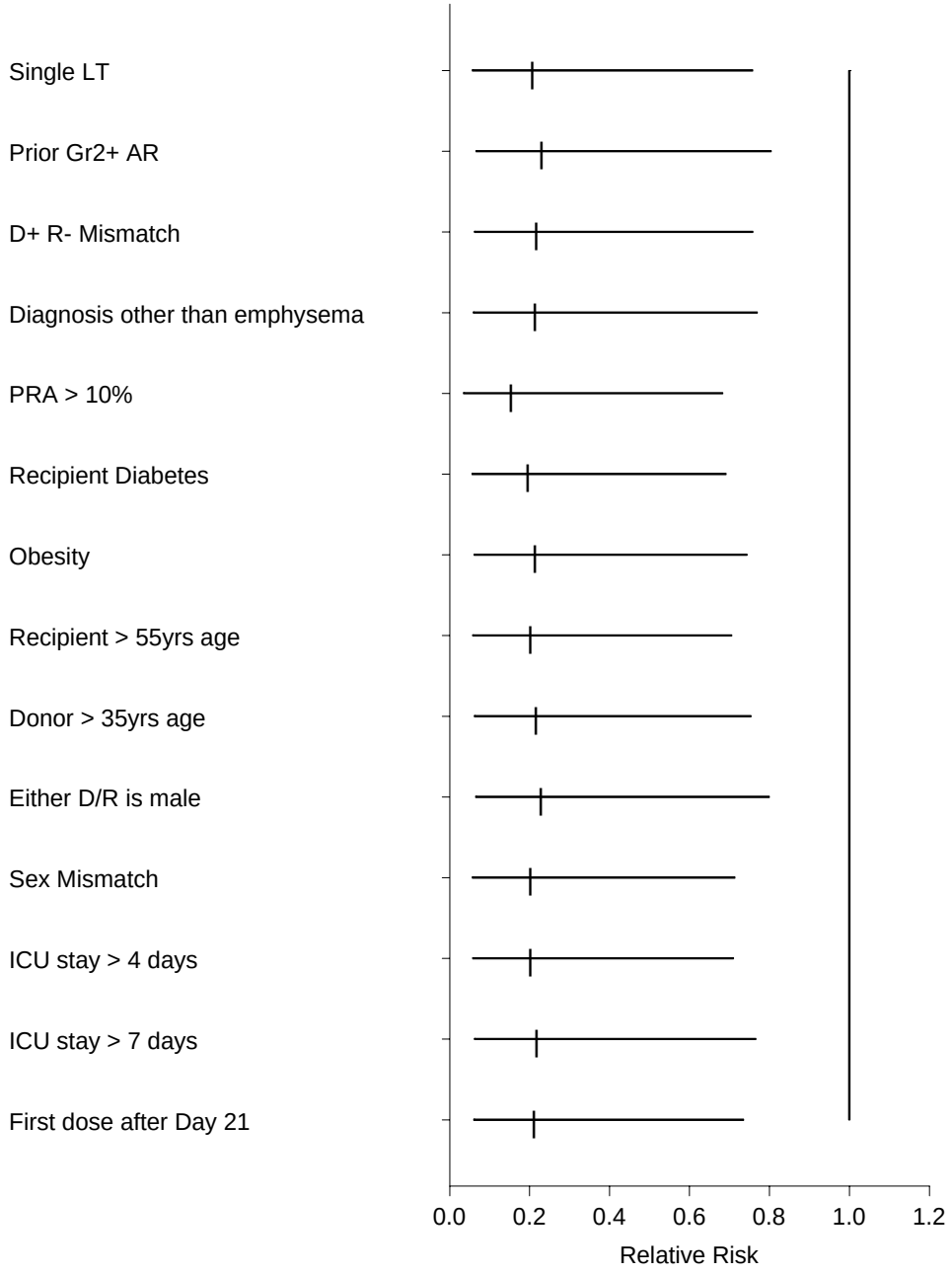


Figure 4.3.1-3. Estimate and 95% confidence interval of the relative risk (CyIS/Placebo) of death in ACS001 adjusted by donor/recipient characteristic. LT = Lung transplant; AR = Acute rejection; D = Donor; R = Recipient; PRA = Probabilistic risk assessment; ICU = Intensive care unit.

In **Table 4.3.1-1** we present details of the survival advantage when adjusted for the major risk factors documented in the literature as having a significant association with survival in lung transplant patients: single-lung transplant, prior grade 2+ AR, diagnosis other than emphysema, and CMV donor positive/recipient negative (D+/R–) mismatch. The number of patients having the other major risk conditions in the literature, probabilistic risk assessment (PRA) > 10% and diagnosis of diabetes mellitus, are too few to present meaningful adjusted analyses. However, because these conditions were seen nearly exclusively among CyIS patients, they would not contribute a bias in favor of the CyIS group. The results in **Table 4.3.1-1** indicate that the survival benefit associated with CyIS was consistent when data were stratified either by type of lung transplant (ie, single- or double-lung; stratified log-rank *P*= .014), primary diagnosis of emphysema (stratified log-rank *P*= .011), prior grade 2 or higher AR (stratified log-rank *P*= .009), or CMV match/mismatch (stratified log-rank *P*= .017).

Table 4.3.1-1. Summary of CyIS Effect on Survival Duration

	Number of Deaths/Number in Strata (%)		
Strata	**Placebo (n = 30)**	**CyIS, Randomized (n = 26)**	**Log-rank *P* value**
All	14/30 (47)	3/26 (12)	.007
Transplant Type			.014[a]
Single	11/24 (46)	2/15 (13)	
Double	3/6 (50)	1/11 (9)	
Primary Diagnosis			.011[b]
Emphysema	8/19 (42)	2/9 (22)	
Other	6/11 (55)	1/17 (6)	
Grade 2+ AR Prior to Dosing			.009[c]
Yes	8/13 (62)	1/8 (13)	
No	6/17 (35)	2/18 (11)	
CMV match/mismatch			.017[d]
Match	9/23 (39)	3/21 (14)	
Mismatch	5/7 (71)	0/5 (0)	

[a] *P* value from log-rank test stratified by transplant type (ie, single- or double-lung).
[b] *P* value from log-rank test stratified by primary diagnosis (emphysema or other).
[c] *P* value from log-rank test stratified by grade 2+ AR before dosing (yes/no).
[d] *P* value from log-rank test stratified by CMV match/mismatch.
CyIS = Cyclosporine inhalation solution; AR = Acute rejection; CMV = Cytomegalovirus.

4.3.1.1 Sensitivity Analyses Around the Survival Duration Endpoint

In order to investigate the robustness of this observed survival benefit, the survival data were subjected to a series of sensitivity analyses. In each case, the survival advantage remained statistically significant and in favor of patients treated with CyIS. When survival was measured from the time of the first dose of study drug rather than from the date of transplant, the results were nearly identical, with an estimate of relative risk of 0.216 (log-rank *P*= .008; **Figure 4.3.1.1-1**).

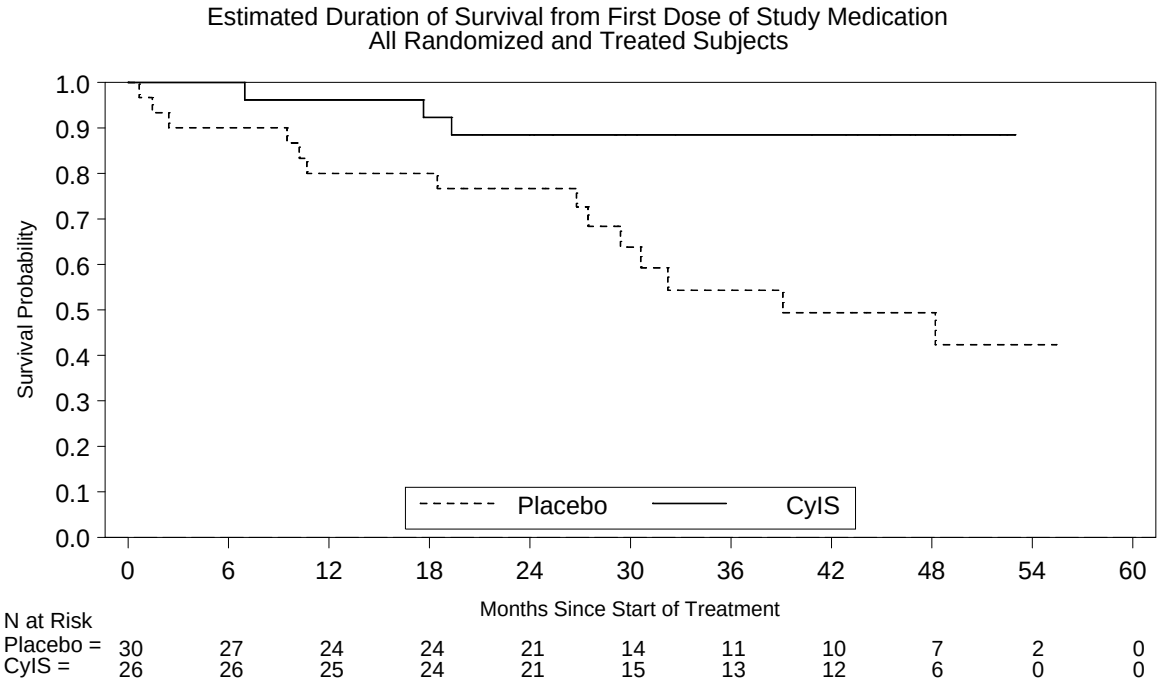


Figure 4.3.1.1-1. Kaplan-Meier plots of survival duration in patients treated with CyIS or placebo measured from first dose of study medication. CyIS = Cyclosporine inhalation solution.

Additional sensitivity methods involved exclusion of specific patients from analysis. Although the resulting *P* value is important, when excluding selected patients from analysis the corresponding effect on the relative risk is most important. If the treatment effect is

constant across the study population, analysis of subsets would be expected to yield similar estimates of the treatment effect, but larger standard errors and hence wider confidence bounds and larger *P* values due to the reduced sample size. This was the pattern that was observed with the sensitivity analyses of subsets from ACS001. The results of these analyses are shown in **Table 4.3.1.1-1.**

The original analysis was performed on 56 randomized and treated patients. When the three placebo patients who died during the first 3 months posttransplant are excluded from analysis, the resulting relative risk remains small (relative risk = 0.265, *P*= .029). In a second sensitivity analysis, the full amount of study drug a patient could receive per protocol adjusted for death was determined. The 14 patients who received less than 20% of this protocol maximum dose were removed from analysis (6 placebo vs. 8 CyIS). The resulting relative risk estimate of 0.243 is consistent with the overall unadjusted result. A third analysis consisted of removing 15 patients who developed pneumonia within 1 month of initiation of study medication. While the resulting estimate of relative risk of 0.250 is very similar to the overall result, it is not surprising that after removing 26% of the patients from analysis the *P* value crosses 0.05. A fourth analysis consisted of excluding from analysis five patients who were in the ICU more than 14 days following the transplant procedure and yielded results very similar with the overall results (relative risk = 0.275, log-rank *P*= .036). Finally, when adding the survival data from the 10 open-label pilot patients to the randomized CyIS group (N = 36) the survival advantage of this therapy remained clear.

Table 4.3.1.1-1. Summary of Sensitivity Analyses Around the Survival Endpoint

	Sample Size			
Sensitivity Analysis	**Placebo**	**CyIS**	**Log-rank *P* value**	**Relative Risk (CyIS/Placebo)**
Analysis on full dataset	30	26	.007	0.213
Survival relative to date of first dose	30	26	.008	0.216
Patients (n = 3) removed from placebo arm because of death with 3 months	27	26	.029	0.265
Only patients who received ≥ 80% of maximum CyIS or placebo dose, adjusted for death	24	18	.048	0.243

Table 4.3.1.1-1. Summary of Sensitivity Analyses Around the Survival Endpoint				
	Sample Size			
Sensitivity Analysis	**Placebo**	**CyIS**	**Log-rank *P* value**	**Relative Risk (CyIS/Placebo)**
Patients (n = 15) who developed pneumonia within 1 month of initiation of study medication removed from analysis	20	21	.058	0.250
Patients (n = 5) who were in the ICU more than 14 days removed from analysis	26	25	.036	0.275
Addition of 10 patients administered open-label CyIS	30	36	.018	0.349
CyIS = Cyclosporine inhalation solution; ICU = Intensive care unit.				

4.3.1.2 Comparison of Mortality With Other Databases

In order to investigate whether the placebo patient population in ACS001 was representative of what would be expected in a larger patient population, the mortality observed in ACS001 was compared against mortality trends observed in two patient groups obtained from outside the study. The first of these groups was the nonenrolled patients ("screen failures") who underwent lung transplantation at UPMC during the same period of time as the ACS001 study enrollment. To mimic the ACS001 enrollment window, patients who died within 30 days of transplant surgery were excluded. **(Figure 4.3.1.2-1)**. This comparison reveals that the two populations were similar.

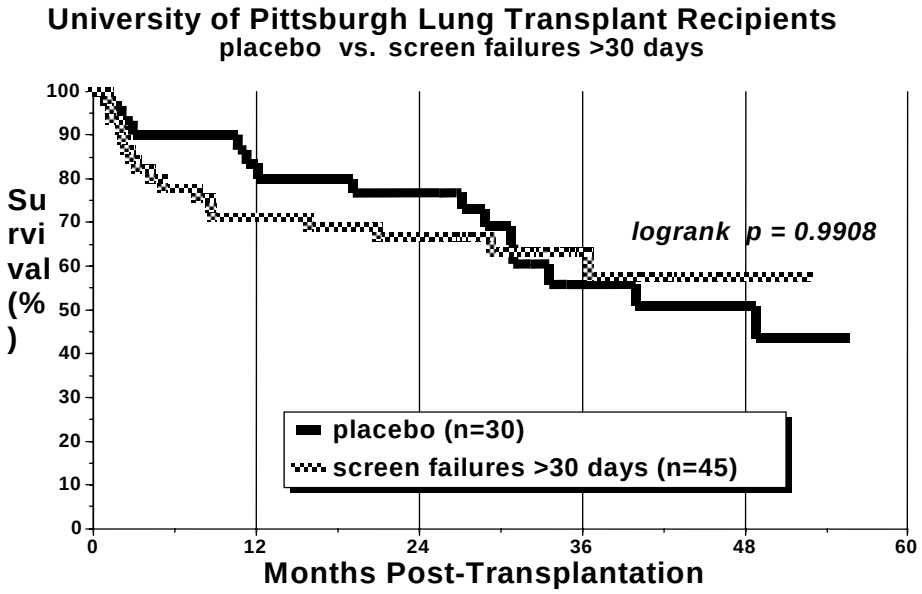


Figure 4.3.1.2-1. Kaplan-Meier plots for survival of nonenrolled lung transplantation recipients at University of Pittsburgh Medical Center surviving at least 30 days versus patients in the ACS001 placebo group.

A second comparison was made with US patient data obtained from the United Network for Organ Sharing (UNOS) transplant registry. The UNOS provided Chiron with data on a cohort of lung-only transplants performed in adult recipients between January 1, 1998, and December 31, 2001, at US centers other than UPMC. Only those recipients reported to have survived a minimum of 7 days were included. Patients were matched with ACS001 patients in both groups according to transplant type (single or double), sex, CMV D+/R– mismatch, age at transplant (18 to 40, 41 to 50, 51 to 60, 61+ years), primary diagnosis, and AR. The UNOS patients without a corresponding ACS001 match were deleted from analysis. To avoid introducing any bias due to early deaths, UNOS patients whose survival time did not exceed the time to first dose from a potential ACSOO1 match were excluded from possible matching. Using a stratified Cox proportional hazards regression model, in which strata were defined as the cohort of matches, the UNOS patients were compared separately to the two ACS001 study arms.

To best compare long-term survival, data from both ACS001 placebo patients and UNOS patients followed through March 1, 2005, were used. The 30 ACS001 Placebo patients had 1038 UNOS matches. The Kaplan-Meier estimates of survival duration are shown in **Figure 4.3.1.2-2**. The stratified proportional hazards regression model yielded an estimate of the relative risk (UNOS/Placebo) of 0.837 (*P*= .518) implying that the ACS001 Placebo patients were representative of all US transplants conducted between 1998 and 2001.

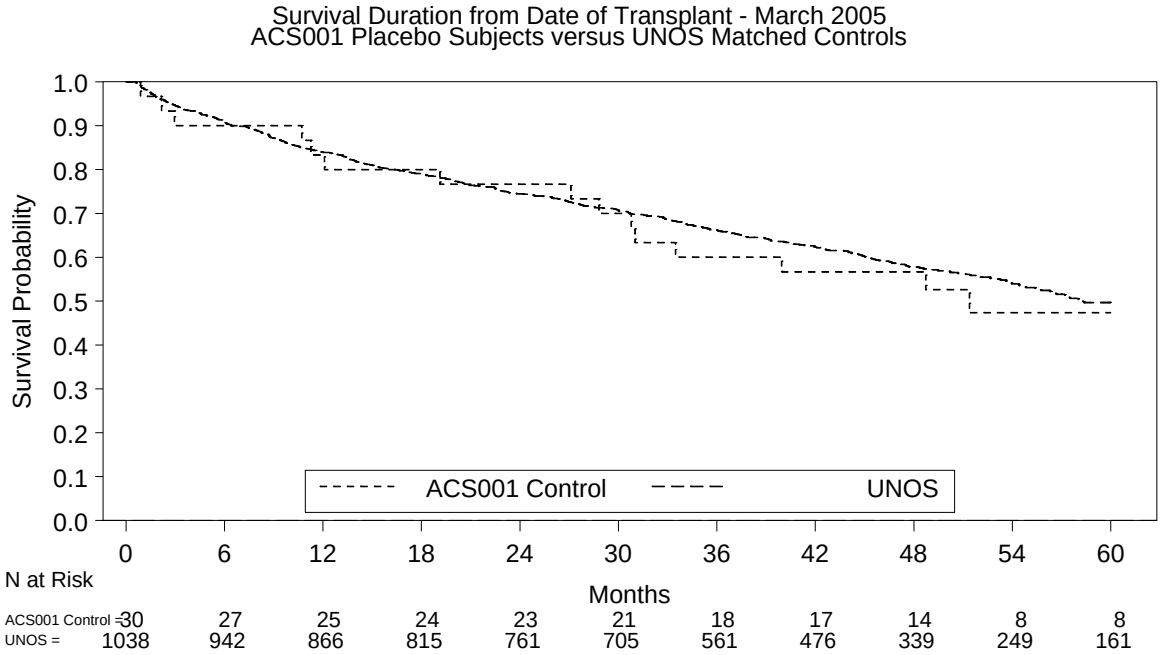


Figure 4.3.1.2-2. Kaplan-Meier survival estimate of ACS001 placebo patients versus UNOS-matched controls. UNOS = United Network for Organ Sharing.

When comparing the 26 CyIS Random patients with UNOS, there were 1058 UNOS-matched controls. Because CyIS therapy was not offered following study closure, comparisons with the UNOS were made through August 21, 2003. The Kaplan-Meier estimates of survival are shown in **Figure 4.3.1.2-3**. The stratified proportional hazards regression model yielded an estimate of the relative risk (CyIS/UNOS) of 0.252 (*P*= .019). These results are very similar to the ACS001 comparison of CyIS to placebo, and further

reinforce the generalizability of the CyIS survival benefit observed in ACS001 to other lung transplant centers in the United States.

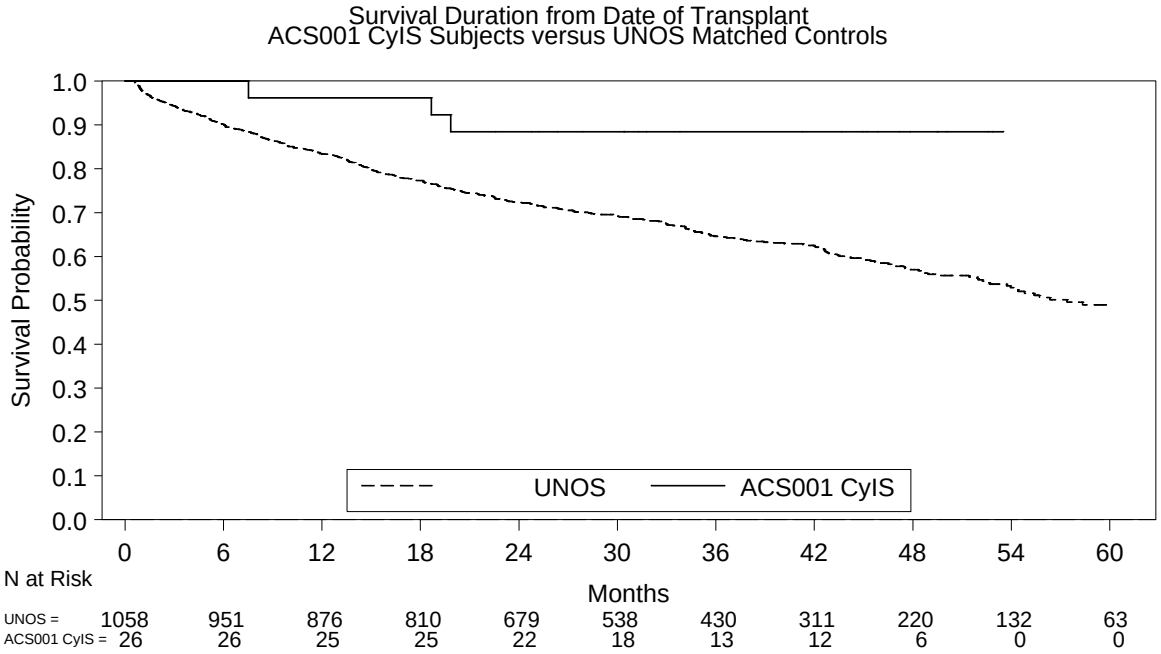


Figure 4.3.1.2-3. Kaplan-Meier survival estimates of ACS001 CyIS patients versus UNOS-matched controls. CyIS = Cyclosporine inhalation solution; UNOS = United Network for Organ Sharing.

These comparisons demonstrate that (1) the placebo group had similar survival to cohorts of lung transplant recipients both at the same institution and elsewhere, and (2) that treatment with CyIS offers a substantial survival benefit to US lung transplant patients.

4.3.1.3 Poststudy Survival Results

A poststudy follow-up for survival was conducted in June 2004. At this time point treatment group was unblinded and patients had been removed from the study drug for a minimum of 10 months and a maximum of 3.5 years. Results showed that 2 of the 23 surviving CyIS patients and 1 of the 16 surviving placebo-treated patients died during this period. Since

neither treatment arm received assigned study drug during this follow-up period, but could receive open-label CyIS (as did two placebo patients and three CyIS patients), results are expected to trend toward the null. The Kaplan-Meier plot of survival duration including this follow-up period is shown in **Figure 4.3.1.3-1.** As expected, because CyIS therapy was not widely offered poststudy to the active group, the corresponding log-rank *P* value of .017 is larger than the .007 obtained at study closure. But because it is still statistically significant, this result supports the inferences drawn at study closure on the effect of CyIS on improved survival.

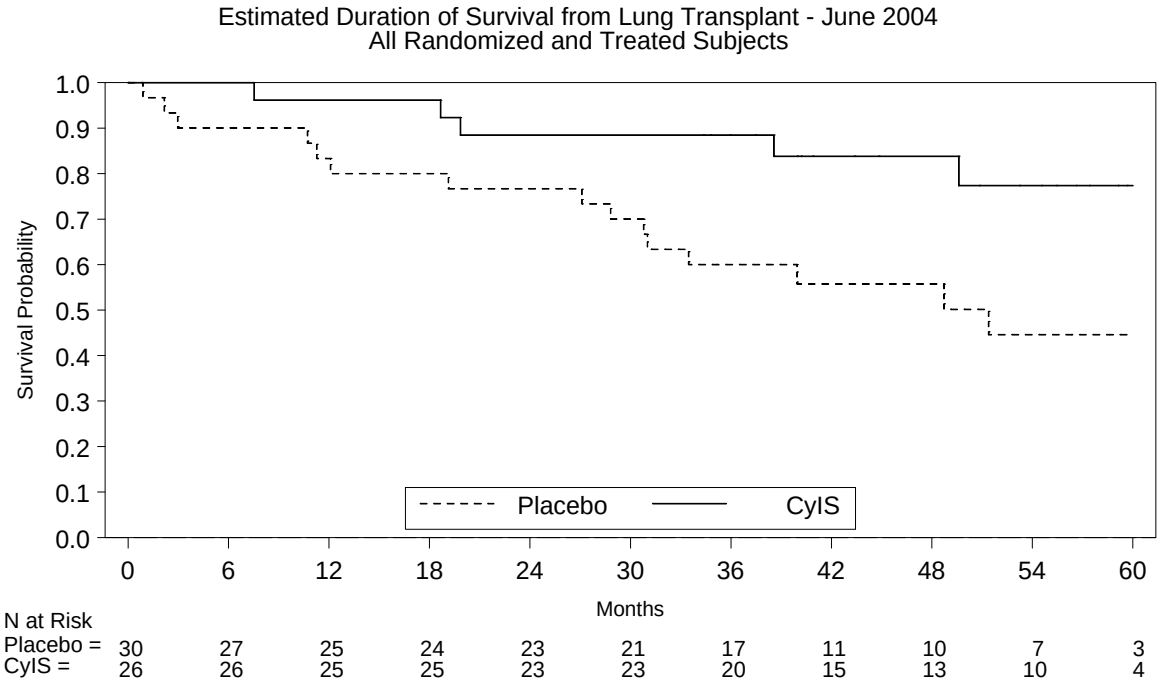


Figure 4.3.1.3-1. Kaplan-Meier estimate of survival including data 10 months after study closure. CyIS = Cyclosporine inhalation solution.

4.3.2 Chronic Rejection-Free Survival Duration

The advantage of CyIS therapy in reducing mortality can be attributed to its effect in preventing chronic rejection. In ACS001, CyIS therapy resulted in a substantial and

significant increase in chronic rejection-free survival (log-rank *P*= .001; **Figure 4.3.2-1**). Chronic rejection was diagnosed either through biopsy by the presence of OB or clinically by the presence of BOS. Among the 30 placebo-treated patients, only 8 (27%) survived free of chronic rejection compared with 18 (69%) CyIS-treated patients. Since the relative risk of chronic rejection or death (CyIS/Placebo) was 0.280 (95% CI = 0.124, 0.630), the risk of chronic rejection or death was only 28% as great on the CyIS arm when compared with the placebo arm, resulting in 72% reduction in the risk of chronic rejection or death due to CyIS.

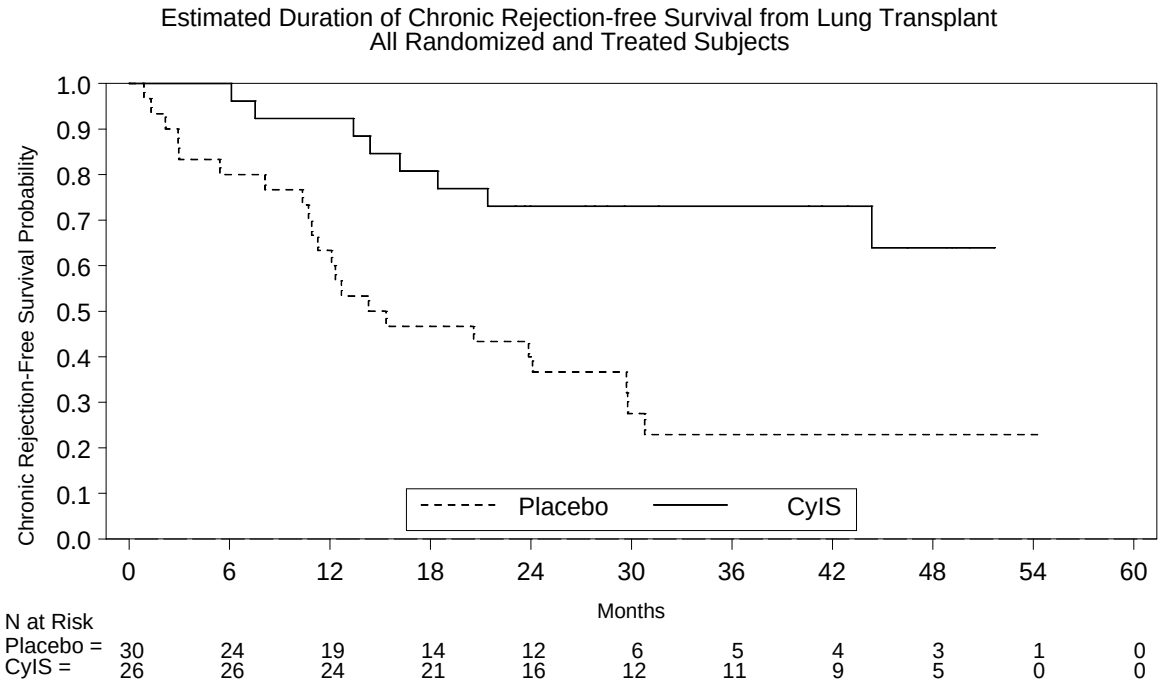


Figure 4.3.2-1. Kaplan-Meier estimates of chronic rejection-free survival duration. CyIS = Cyclosporine inhalation solution.

The effect of CyIS on the risk of chronic rejection or death after adjusting for major risk factors in a Cox regression model reveals the robustness of this result. **Figure 4.3.2-2** shows the estimate of the relative risk (CyIS/placebo) after adjusting for each donor or recipient characteristic. For every risk factor, the upper bound of the 95% confidence intervals for the relative risk is substantially < 1, indicating that CyIS significantly improved chronic rejection-free survival relative to placebo.

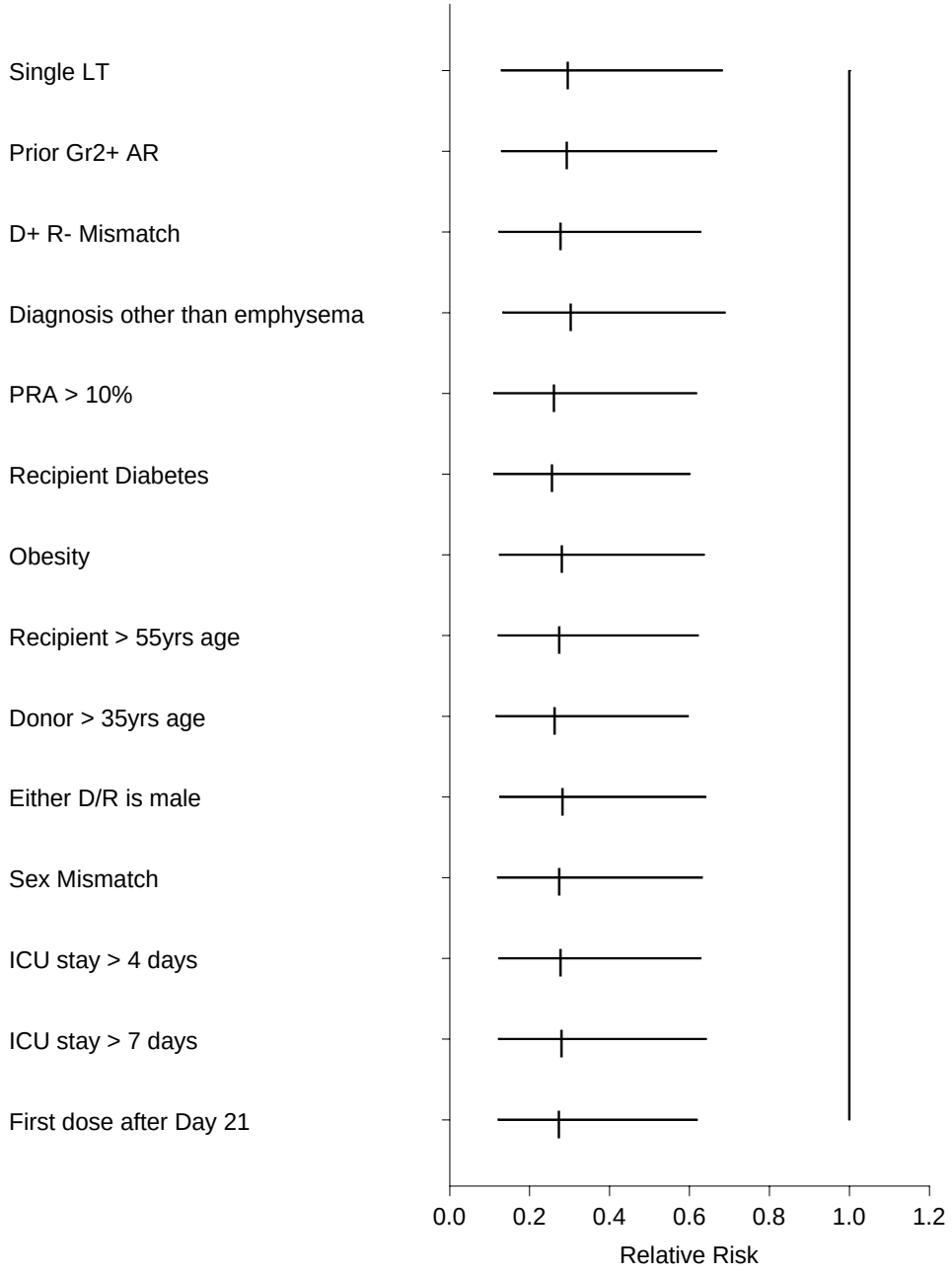


Figure 4.3.2-2. Estimate and 95% confidence interval of the relative risk (CyIS/Placebo) of chronic rejection or death in ACS001 adjusted by donor/recipient characteristics. LT = Lung transplant; AR = Acute rejection; D = Donor; R = Recipient; PRA = Probabilistic risk assessment; ICU = Intensive care unit.

Further details of the effect of CyIS on chronic rejection-free survival after adjusting for either transplant type, primary diagnosis of emphysema, prior grade 2+ AR, or donor/recipient CMV mismatch are found in **Table 4.3.2-1**. The clinical effect did not appear to be limited to any subgroup. Analysis stratified by each factor shows significant differences between arms consistent with the unadjusted results.

Table 4.3.2-1. Summary of CyIS Effect on Chronic Rejection-Free Survival Duration

	Number of Events/Number in Strata (%)		
Strata	**Placebo (n = 30)**	**CyIS, Randomized (n = 26)**	**Log-rank *P* value**
All	22/30 (73)	8/26 (31)	.001
Transplant Type			.002[a]
Single	18/24 (75)	5/15 (33)	
Double	4/6 (67)	3/11 (27)	
Primary Diagnosis			.005[b]
Emphysema	15/19 (79)	3/9 (33)	
Other	7/11 (64)	5/17 (29)	
Grade 2+ AR Prior to Dosing			.002[c]
Yes	12/13 (92)	2/8 (25)	
No	10/17 (59)	6/18 (33)	
CMV match/mismatch			.001[d]
Match	15/23 (65)	8/21 (38)	
Mismatch	7/7 (100)	0/5 (0)	

[a] *P* value from log-rank test stratified by transplant type (ie, single- or double-lung).
[b] *P* value from log-rank test stratified by primary diagnosis (emphysema or other).
[c] *P* value from log-rank test stratified by grade 2+ AR prior to dosing (yes/no).
[d] *P* value from log-rank test stratified by CMV match/mismatch.
CyIS = Cyclosporine inhalation solution; AR = Acute rejection; CMV = Cytomegalovirus.

This favorable outcome on chronic rejection-free survival did not occur solely as a result of improved survival among CyIS patients. A sensitivity analysis that censors deaths was performed to determine if CyIS has an effect on preventing chronic rejection independent of survival. The resulting Kaplan-Meier plot is shown in **Figure 4.3.2-3.** Although such an analysis includes informative censoring and is clearly biased against the CyIS group due to the survival advantage, it yielded a log-rank *P* value of .015, demonstrating the clear and pronounced effect of CyIS in preventing chronic rejection.

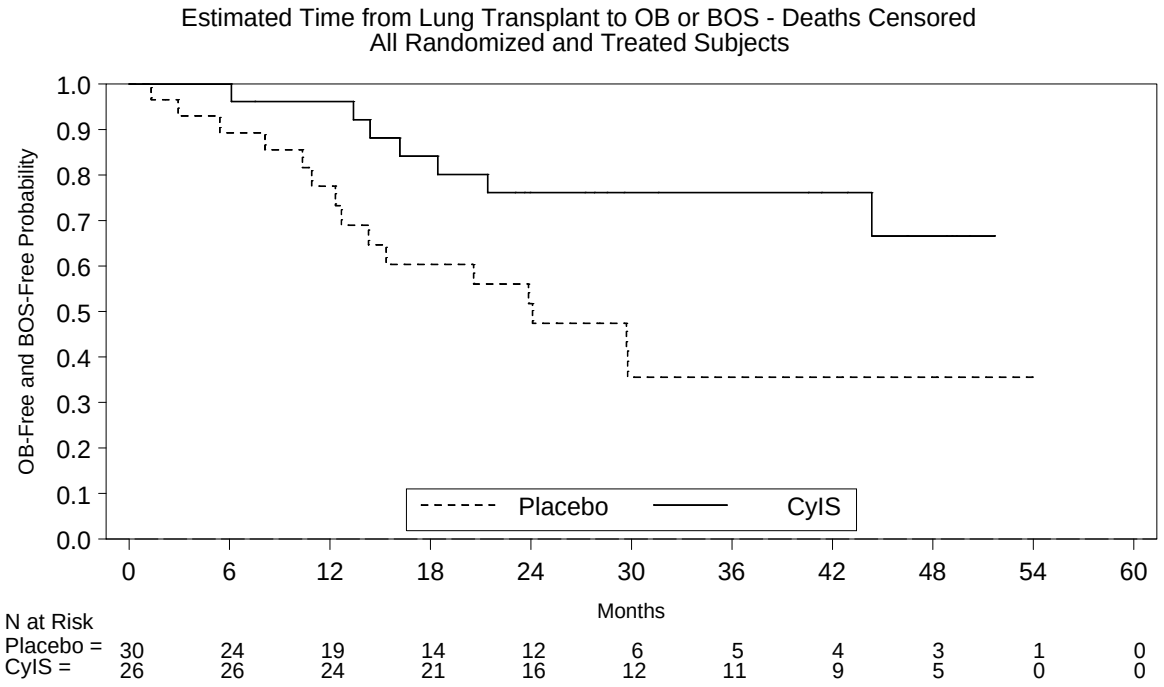


Figure 4.3.2-3. Kaplan-Meier estimates of time from lung transplant to OB or BOS. OB = Obliterative bronchiolitis; BOS = Bronchiolitis obliterans syndrome.

Although chronic rejection is a composite endpoint of OB and BOS, in the subsequent sections we will see that CyIS therapy has a significant effect on the individual components of this endpoint.

4.3.2.1 Multivariate Analyses of Survival and Chronic Rejection-Free Survival

In the preceding sections the effect of CyIS on both survival duration and chronic rejection-free survival duration was observed in unadjusted analyses as well as in analyses that adjusted for an individual risk factor. In this section we show that the beneficial effect of CyIS therapy on these endpoints was observed in analyses that control for multiple risk factors simultaneously. A Cox regression model was fit with treatment and these additional risk factors as covariates. Although a somewhat greater proportion of placebo patients had a single lung transplant and had at least one episode of grade 2+ AR prior to dosing than CyIS

patients, the results shown in **Table 4.3.2.1-1** demonstrate that when adjusting for these two factors simultaneously, the effect of CyIS remains significant. Furthermore, when adjusting simultaneously for these risk factors and CMV D+/R– mismatch or when adjusting for the four major risk factors cited in the literature simultaneously yields a significant result in favor of the CyIS arm. Thus the large and significant advantages in survival and chronic rejection-free survival cannot be explained by differences in prognostic factors between groups.

Table 4.3.2.1-1 also includes results of multivariate analyses that adjust for an ICU stay of duration longer than 1 week. Although duration of ICU stay is not documented in the literature as a prognostic factor for death among lung transplant recipients, we include this factor here as FDA has noted the imbalance between treatment arms on this baseline condition and expressed their concern that this could indicate placebo patients had a worse clinical prognosis at baseline. The results of the multivariate analyses in **Table 4.3.2.1-1** demonstrate that when one adjusts for the inclusion of an ICU stay longer than 1 week in addition to the recognized risk factors, the estimate of the treatment effect and the *P* value does not change in any meaningful way.

The consistent results one sees on overall survival duration and chronic rejection-free survival duration in these various models indicates the robustness of the efficacy result.

Table 4.3.2.1-1. Results of Multivariate Analyses

Covariates in Multivariate Model in Addition to Treatment Group	**Adjusted Relative Risk (CyIS/Placebo) [95% Confidence Interval] *P* value**	
	Survival Duration	**Chronic Rejection-Free Survival Duration**
• Transplant Type • Prior Grade 2+ AR	0.227 [0.062, 0.835] *P* = .026	0.314 [0.135, 0.727] *P* = .007
• Transplant Type • Prior Grade 2+ AR • CMV D+/R– Match/Mismatch	0.234 [0.063, 0.867] *P* =.030	0.314 [0.136, 0.728] *P* = .007
• Transplant Type	0.238 [0.064, 0.885]	0.318 [0.137, 0.742]

Table 4.3.2.1-1. Results of Multivariate Analyses		
Covariates in Multivariate Model in Addition to Treatment Group	**Adjusted Relative Risk (CyIS/Placebo) [95% Confidence Interval] *P* value**	
	Survival Duration	**Chronic Rejection-Free Survival Duration**
• Prior Grade 2+ AR • CMV D+/R– Match/Mismatch • Primary Diagnosis (emphysema or other)	*P* = .032	*P* = .008
• Transplant Type • Prior Grade 2+ AR • ICU Stay > 7 days	0.217 [0.057, 0.833] *P* = .026	0.306 [0.127, 0.735] *P* = .008
• Transplant Type • Prior Grade 2+ AR • CMV D+/R– Match/Mismatch • ICU stay > 7 days	0.225 [0.057, 0.879] *P* = .032	0.308 [0.128, 0.741] *P* = .009
• Transplant Type • Prior Grade 2+ AR • Primary Diagnosis (Emphysema or other) • ICU stay > 7 days	0.224 [0.058, 0.871] *P* = .031	0.316 [0.130, 0.766] *P* = .011
• Transplant Type • Prior Grade 2+ AR • Primary Diagnosis (emphysema or other) • CMV D+/R– Match/Mismatch • ICU stay > 7 days	0.230 [0.058, 0.909] *P* = .036	0.315 [0.129, 0.767] *P* = .011
CyIS = Cyclosporine inhalation solution; AR = Acute rejection; CMV = Cytomegalovirus; D = Donor; R = Recipient; ICU = Intensive care unit.		

4.3.2.2 Histologic Obliterative Bronchiolitis-Free Survival

One of the nonsurvival components of the chronic rejection-free survival endpoint is histologic evidence of OB by transbronchial biopsy. Patients were carefully evaluated after

transplantation, and received regular surveillance transbronchial biopsies to identify histologic evidence of OB. The median number of biopsies performed per patient was 11 in each arm. A Poisson regression analysis of the biopsy rate indicates no difference between treatment arms in the frequency of biopsies (*P*= .879). Treatment with CyIS significantly delayed the onset of OB **(Figure 4.3.2.2-1)**. During the study, OB or death occurred in only 19% (5 of 26) of patients randomized to CyIS compared with 60% (18 of 30) of placebo-treated patients (log-rank *P*= .003).

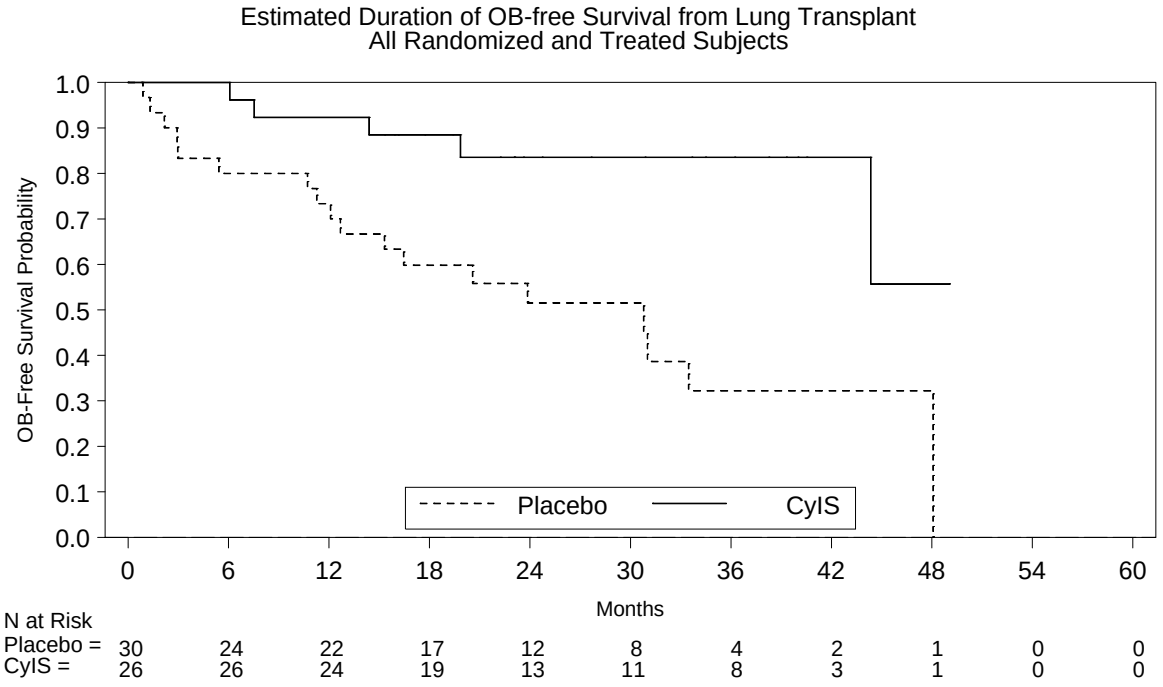


Figure 4.3.2.2-1. Kaplan-Meier plots of OB-free survival of patients treated with CyIS compared with placebo. OB = Obliterative bronchiolitis; CyIS = Cyclosporine inhalation solution.

Of the 18 events in the placebo group, 9 were OB and 9 were deaths. Of the 5 events in the CyIS arm, 3 were OB and 2 were deaths. Consistent effects were observed when the analysis was stratified either by transplant type, primary diagnosis of emphysema, grade 2+ AR prior to first dose, or CMV match/mismatch status **(Table 4.3.2.2-2)**. When comparing the results

across these subgroups, there was a consistent pattern in favor of improved OB-free survival in the patients treated with CyIS.

Table 4.3.2.2-2. Summary of CyIS Effect on OB-Free Survival Duration

	Number of Clinical Events/Number in Strata (%)		
Strata	**Placebo (n = 30)**	**CyIS, Randomized (n = 26)**	**Log-rank *P* value**
All	18/30 (60)	5/26 (19)	.003
Transplant Type			.004[a]
Single	14/24 (58)	3/15 (20)	
Double	4/6 (67)	2/11 (18)	
Primary Diagnosis			.010[b]
Emphysema	11/19 (58)	3/9 (33)	
Other	7/11 (64)	2/17 (12)	
Grade 2+ AR Prior to Dosing			.007[c]
Yes	10/13 (77)	2/8 (25)	
No	8/17 (47)	3/18 (17)	
CMV Match/Mismatch			.008[d]
Match	11/23 (48)	5/21 (24)	
Mismatch	7/7 (100)	0/5 (0)	

[a] *P* value from log-rank test stratified by transplant type (ie, single- or double-lung).
[b] *P* value from log-rank test stratified by primary diagnosis (emphysema or other).
[c] *P* value from log-rank test stratified by grade 2+ AR prior to dosing (yes/no).
[d] *P* value from log-rank test stratified by CMV match/mismatch.
CyIS = Cyclosporine inhalation solution; OB = Obliterative bronchiolitis; AR = Acute rejection; CMV = Cytomegalovirus.

4.3.2.3 Bronchiolitis Obliterans Syndrome-Free Survival

The other nonsurvival component of chronic rejection-free survival is the presence of BOS, a clinical diagnosis based on a decrease in lung function (as determined by a 20% or greater drop in FEV_1) from a maximum posttransplant measurement. A 20% decrease (i.e., grade 1 BOS) is the earliest accepted definition of BOS. This study specifically analyzed BOS (grade 1 and higher)-free survival as a secondary endpoint.

Bronchiolitis obliterans syndrome-free survival was significantly longer in patients randomized to CyIS compared with placebo (log-rank *P*= .019; **Figure 4.3.2.3-1**). A total of

63% (19 of 30) of patients treated with placebo developed grade 1 or higher BOS or died compared with only 31% (8 of 26) of patients randomized to CyIS.

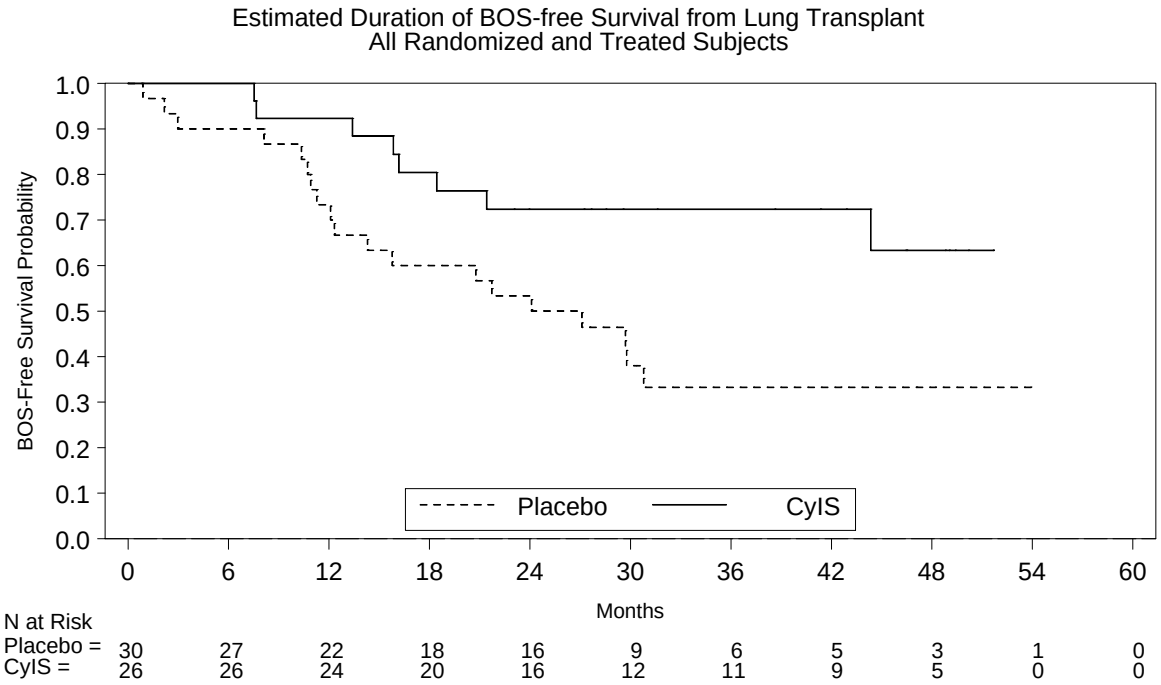


Figure 4.3.2.3-1. Kaplan-Meier plots of BOS-free survival of patients treated with CyIS compared with placebo. BOS = Bronchiolitis obliterans syndrome; CyIS = Cyclosporine inhalation solution.

Of the 19 events in the placebo group, 11 were BOS and 8 were deaths. Of the 8 events in the CyIS arm, 7 were BOS and 1 was death. Analysis across the various subgroup strata showed a consistent benefit in terms of BOS-free survival for patients in the CyIS group **(Table 4.3.2.3-1)**.

Table 4.3.2.3-1. Summary of CyIS Effect on BOS-Free Survival Duration			
	Number of Clinical Events/Number in Strata (%)		
Strata	**Placebo (n = 30)**	**CyIS, Randomized (n = 26)**	**Log-rank *P* value**
All	19/30 (63)	8/26 (31)	.019
Transplant Type			.025[a]
Single	15/24 (63)	5/15 (33)	
Double	4/6 (67)	3/11 (27)	
Primary Diagnosis			.036[b]
Emphysema	12/19 (63)	3/9 (33)	
Other	7/11 (64)	5/17 (29)	
Grade 2+ AR Prior to Dosing			.027[c]
Yes	11/13 (85)	2/8 (25)	
No	8/17 (47)	6/18 (33)	
CMV match/mismatch			.021[d]
Match	13/23 (57)	8/21 (38)	
Mismatch	6/7 (86)	0/5 (0)	

[a] *P* value from log-rank test stratified by transplant type (ie, single- or double-lung).
[b] *P* value from log-rank test stratified by primary diagnosis (emphysema or other).
[c] *P* value from log-rank test stratified by grade 2+ AR prior to dosing (yes/no).
[d] *P* value from log-rank test stratified by CMV match/mismatch.
CyIS = Cyclosporine inhalation solution; BOS = Bronchiolitis obliterans syndrome; AR = Acute rejection; CMV = Cytomegalovirus.

4.4 Acute Rejection

Seventy percent (21 of 30) of placebo-treated patients and 73% (19 of 26) CyIS-treated patients experienced at least one AR episode of grade 2 or higher. There were a total of 36 grade 2+ AR episodes among placebo patients and a total of 35 in the CyIS arm. Because individual patients may have had several episodes of AR during the trial, the rate of AR following the start of dosing was analyzed in a Poisson regression model that accounted for a variety of factors that are associated with lung function. These factors included treatment group, CMV match/mismatch, transplant type, and the development of a grade 2 or higher AR between time of transplant and start of dosing. Based on the Poisson regression model, the rate of grade 2 or higher AR was 9% higher in patients treated with CyIS compared with

placebo (*P*= .73) during the study. Based on this analysis, it does not appear that CyIS prevents development of AR when added to a standard immunosuppression treatment regimen. However, it is interesting to note that 16 (46%) of the 35 episodes of AR that occurred in the CyIS arm were among the 8 patients who received less than 20% of their protocol-specified dose.

The initial hypothesis of ACS001 was that CyIS would be able to decrease the rate of AR, and thereby improve outcomes by decreasing the rate of chronic rejection. However, since the initial design of the study, there has been a clear shift in the way AR and chronic rejection are viewed clinically and physiologically. Acute rejection (also termed "vascular rejection") is mediated in the vasculature and manifested by a perivascular lymphocytic infiltrate. By contrast, chronic rejection (also termed "airway rejection") is mediated in the airway epithelial compartment and manifested by a peribronchiolar lymphocytic infiltrate. Given the mode of delivery of CyIS directly to the airway epithelium, and given the known low systemic exposure, the primary effect on chronic rejection and lack of effect on AR are consistent with current views of these two processes. Therefore, using AR as the primary endpoint for an aerosolized immunosuppressant delivered to the airway epithelium might not have been appropriate. Survival and chronic rejection-free survival are the more relevant endpoints.

4.5 Lung Function

The study was not designed in a manner to allow for a meaningful comparison between groups in pulmonary function test results. Predosing spirometry values were unavailable for 42% of CyIS-treated patients and 43% of placebo-treated patients. Those analyses conducted did not reveal a significant difference between arms in pulmonary function test results.

4.6 Supporting Data for the Efficacy of CyIS

Data supporting the efficacy of CyIS can be derived from preclinical and clinical results in the published literature. In 1988, the lung transplantation group at the University of Pittsburgh began to investigate the potential benefit of inhaled cyclosporine for regional immunosuppression of the lung allograft. It was postulated that high concentrations of

cyclosporine introduced into the transplanted lung could be used with systemic immunosuppression to improve rejection control or possibly even replace systemic immunosuppression.[43]

The initial preclinical studies, described in more detail in **Section 3.2.3**, demonstrated that aerosol delivery of cyclosporine could reduce AR, compared to control animals without immunosuppression.[4,44] These studies did not address the efficacy of the addition of aerosol cyclosporine to standard immunosuppression. These positive preclinical results led to evaluation of aerosolized cyclosporine in patients with OB. In 1991, the first clinical evaluation was a study of the deposition of cyclosporine in the rejecting lung. In most patients, inhaled cyclosporine was found to be deposited in the peripheral lung allograft, although interpatient variability was apparent.[45] The results of this trial led to the initiation of an open-label study in patients who met the criteria for refractory rejection or OB. The investigators concluded that patients in this trial showed improvement in rejection histology and stabilization or improvement of lung function without pulmonary or systemic toxicity.[6] Between 1991 and 2001, 90 transplant recipients who developed OB were identified from the University of Pittsburgh Lung Transplant Registry. Of these 90 patients, 39 had met criteria for treatment with open-label aerosolized cyclosporine for refractory AR. The 51 recipients who did not received aerosolized cyclosporine were used as controls, along with another set of all recipients who developed OB selected from the Novartis Lung Transplant Registry (N = 100). The control patients from UPMC had a median survival of 2.4 years after diagnosis of OB and the multicenter controls had a median survival of 2.3 years, indicating that the control group from UPMC matched an independent transplant database. Patients who received aerosolized cyclosporine had a median survival of 4.5 years. The investigators reported, using a Cox proportional hazards model, that treatment with aerosolized cyclosporine was associated with improved survival compared to both UPMC (hazard ratio, 0.49; $P < .05$) and an independent registry control group (hazard ratio, 0.42; $P < .05$).[46]

The early results in patients with OB led to the use of aerosolized cyclosporine in patients with refractory AR that failed to respond to conventional immunosuppression in 1993. Results from 18 cases treated with aerosolized cyclosporine, compared with 23 controls matched by histologic severity and treated with standard care, demonstrated improved lung

function.[7] An updated analysis included 59 patients, and demonstrated histologic improvement in 63% of individuals.[8] The updated population had a 1-year survival of 95% compared to 75% for contemporary UPMC controls matched by rejection events and immunosuppressive cycles, and 5-year survival was 75% versus 55% respectively ($P < .05$).

The following observations on aerosolized cyclosporine were made from these early trials:

- CyIS was generally well tolerated without excessive toxicity.
- CyIS was associated with increased survival when used in patients who already had developed OB.
- CyIS was associated in better outcome in patients with refractory AR, a significant risk factor for the development of OB.

These results were the basis for UPMC to conduct ACS001 where the hypothesis that aerosolized cyclosporine is effective at preventing chronic rejection and prolonging survival was tested.

4.7 Conclusion

ACS001 was a randomized, double-blind, placebo-controlled trial designed to test the efficacy of CyIS in preventing allograft rejection and improving outcomes in lung transplant recipients. Although the study did not meet the primary endpoint of decreased AR, from a scientific standpoint, this was not surprising given what has been learned about the pathophysiology of these two processes. Rather, ACS001 demonstrated that the administration of CyIS directly to the airway epithelium markedly improved survival and decreased chronic rejection. A 79% decrease in the risk of death in patients treated with CyIS represents both a statistically significant and clinically meaningful improvement in outcomes for the lung transplant patient population, which up to the present has a 5-year survival rate of only 45%. For the first time, lung transplant recipients were able to achieve survival rates comparable with other solid-organ transplant recipients.

5. SUMMARY OF SAFETY DATA ON CYIS

The CyIS formulation used in clinical studies and proposed for commercialization is a formulation of cyclosporine, USP, drug substance and propylene glycol, USP. The safety data for cyclosporine are well established as it has been approved since 1983 and is part of standard care for immunosuppression following all solid-organ transplantations as well as regularly used for severe active rheumatoid arthritis and severe recalcitrant plaque psoriasis. As described in **Section 2.3**, the most common and clinically significant adverse events with cyclosporine are nephrotoxicity, hepatotoxicity, neurotoxicity, increased risk of infections, and increased risk of malignancies. These events are generally correlated with magnitude and duration of systemic exposure. The rationale for the development of CyIS was to deliver topical immunosuppression and thereby increase the drug concentration locally while limiting systemic exposure and corresponding systemic toxicities.

Propylene glycol (PG) is the only excipient in the formulation. Propylene glycol is a pharmaceutically accepted solvent that has been used in marketed drug products that are administered via enteral, parenteral, and nasal inhalation routes. Propylene glycol is listed on the FDA's List of Inactive Ingredients for Approved Drug Products. In **Section 3.3.4**, reference is made to data obtained in dogs and rats that were exposed to daily inhalation of high dose PG for 28 days with essentially no ill effect. This section will describe the adverse event (AE), serious adverse event (SAE), and death data generated in study reports ACS001 and ACS002.

5.1 Safety Data Collection and Analysis

Safety data to support the approval of the NDA for Cyclosporine, USP, Inhalation Solution (CyIS), were generated from two study populations: 66 patients enrolled and treated with CyIS or placebo in study ACS001, and 70 patients enrolled in 1 of 7 different open-label protocols that formed the safety study ACS002 **(see Section 4, Table 4-1)**. Although the open-label protocols first began enrolling patients in 1991, only patients enrolled after January 1, 1995, were included in ACS002. The decision to use January 1, 1995, as the study initiation date for ACS002 was a result of the 1994 approval of tacrolimus. Shortly after approval, this immunosuppressant supplanted oral cyclosporine at UPMC for both

initial and maintenance immunosuppression. A third study population (the integrated safety summary or ISS) consists of the 102 unique patients treated with CyIS within ACS001 or ACS002. This 102 value accounts for four patients treated initially with CyIS as part of ACS001 who subsequently withdrew from the study in order to enroll in ACS002. Data from these crossover patients were counted only once.

Safety was assessed in ACS001 and ACS002 by the collection of all AEs and SAEs that occurred during the study from the date of first dose of study drug up through 90 days after the last dose of study drug, death, or study closure on August 21, 2003. Chiron representatives reviewed all source data and recorded events onto Chiron-generated case report forms (CRFs), which were subsequently source-verified by Chiron data monitors. As these data were retrospectively collected, no attempts were made to assess severity or relatedness. Seriousness was assessed using standard regulatory definitions for events that resulted in death; were considered life threatening; required hospitalization or prolonged an existing hospitalization; or resulted in a persistent or significant disability.

All patients in both studies were treated with standard immunosuppressive therapy prior to and during treatment with CyIS or placebo. The primary therapies for immunosuppression were tacrolimus, azathioprine, and prednisone, with occasional use of MMF cyclosporine, and rapamycin **(see Section 4, Table 4.2-3)**. All of these agents have side effect profiles that can confound analysis of safety data arising from patients receiving CyIS **(see Section 2.3)**; therefore, a safety analysis plan was developed in which the CyIS safety data were reviewed in three stages. The first stage reviewed safety data from the 66 treated patients in ACS001, comparing AEs in the 36 patients treated with CyIS (pilot and randomized) with the 30 patients treated with placebo. The goal of this stage was to detect safety signals within the environment of concurrent immunosuppressive therapy and chronically ill patients. In the second stage, safety data from the 70 patients in ACS002 were reviewed with the goal of comparing existing signals and detecting additional signals in this different patient population. In the third stage, data from the ISS were reviewed to ensure that no safety signals were missed in this larger population. The rationale behind this stepwise approach was to minimize the risk of diluting small safety signals as a result of combining safety datasets from two different study populations.

5.2 Overall Extent of Exposure

The overall extent of exposure for patients enrolled in ACS001 and ACS002 and who received at least one dose of study drug (CyIS or placebo) is described below. In ACS001, 10 patients received open-label CyIS in a pilot-phase, followed by 56 patients randomized and treated with either CyIS (n = 26) or placebo (n = 30). In ACS002, 70 patients were enrolled in 1 of 7 different open-label protocols. In both studies, patients were dosed with 100 mg CyIS (or equivalent volume of placebo) on the first study day. Over the next nine days the dose was titrated in no more than 100-mg increments, according to tolerability, to a protocol-specified maximum daily dose of 300 mg CyIS. The dose reached on day 10 was then continued three times a week. For ACS001, the specified dosing duration for randomized patients was 2 years; however, the 10 pilot patients had the option to continue dosing after the 2-year period. For ACS002, there was no protocol-specified dosing duration, and dosing duration was based largely on physician discretion. **Table 5.2-1** summarizes the extent of dosing received by patients in ACS001 and ACS002.

Table 5.2-1. Summary of Exposure to Study Drug

	ACS001 Placebo	ACS001 CyIS Random	ACS001 All CyIS	ACS002
Number of Patients Treated With Study Drug	30	26	36	70
Completed Therapy	13 (43%)	13 (50%)	19 (53)	NA[b]
Completed or Died During Follow-up (through Aug 21, 2003)	28 (93%)	23 (88%)	30 (83%)	NA[b]
Final Dose, mg (mean/median)	NA	211/250	233.3/300	212.1/200
Minimum/Maximum Dose, mg	NA	100/300	100/300	100/300
Duration of Dosing, months (mean/median)	16/20.8	16/22.5	18.9/23.9	22.1/10.5
Number of Doses Received (mean/median)	190.1/227.0	189.0/260.5	225/267.5	NA[b]
Total Cumulative Dose, mg[a] (mean/median)	N/A	44753.9/ 53825.0	58719/ 57450	NA[b]

[a] Based on target concentration of 62.5 mg/mL.
[b] University of Pittsburgh Medical Center did not record all doses for Study ACS002 and therefore drug exposure is limited to final dose information only.
CyIS = Cyclosporine inhalation solution; NA = Not applicable.

Within each study population, there was a wide range of tolerated doses, from the initial 100 mg to the protocol-specified maximum dose of 300 mg. Additionally, there was a wide range of duration of dosing, from 1 day to 7.1 years. As seen from this table, overall duration of exposure to CyIS was longest within the ACS002 population as a result of the lack of prespecified duration of dosing as well as the larger size of the study. At least one patient from each study discontinued after one dose. Events leading to premature CyIS discontinuation are discussed further in **Section 5.5.**

5.3 Adverse Events

Adverse events and SAEs are common in lung transplant patients as a result of postoperative complications, chronic immunosuppression, frequent infections, and acute and chronic graft rejection. Furthermore, these events often result in hospitalization leading to an increased risk of iatrogenic complications and nosocomial infections. Accordingly, all study patients experienced multiple AEs. The following section will highlight those that were statistically significant ($P < .1$) between CyIS and placebo groups and those that appeared clinically relevant.

5.3.1 Adverse Events in ACS001

Safety data for the 66 patients treated in ACS001 were collected beginning on the first day of administration of the study drug up through 90 days after the last dose administered (survival permitting) or August 21, 2003, whichever came first. For the safety discussion, the CyIS group will refer to the 10 CyIS-treated patients in the pilot phase and the 26 CyIS-treated patients in the randomized phase for a total of 36 CyIS-treated patients vs. 30 placebo-treated patients.

Given the extended duration of the study combined with the underlying health status of all patients enrolled, it was expected that a substantial number of AEs would be reported. In fact, all 66 patients reported at least one AE, and AEs were reported in 24 of 26 MedDRA System Organ Classes (SOCs), with most SOCs including more than 35% of patients. **Table**

5.3.1-1 displays all SOCs and individual AEs in which the CyIS group had a statistically significant ($P \leq .1$) increase compared with placebo.

Table 5.3.1-1. ACS001: Common Adverse Events (Events With CyIS Rates Greater Than Placebo Rates and $P \leq .10$)

MedDRA System Organ Class	Common Adverse Events	Placebo (n = 30)	All CyIS (n = 36)	Fisher's Exact *P* value
Eye	Vision Blurred	8 (27%)	15 (42%)	.0935
Gastrointestinal	Retching	0	4 (11%)	.0817
General/ Administrative Site	Chest Pain	6 (20%)	15 (42%)	.0371
Investigations	Cardiac Murmur	0	6 (17%)	.0214
Metabolism/ Nutrition	Hyperlipidemia NOS	0	5 (14%)	.0422
Musculoskeletal/ Connective Tissue/ Bone	Back Pain	3 (10%)	12 (33%)	.0189
Nervous System	Somnolence	0	6 (17%)	.0214
Psychiatric	Confusion	0	4 (11%)	.0817
Renal/Urinary	Any	19 (63%)	29 (81%)	.0666
	Urinary Frequency	5 (17%)	14 (39%)	.0313
	Polyuria	1 (3%)	6 (17%)	.0750
Reproductive System	Any	4 (13%)	10 (28%)	.0900
Respiratory/ Thoracic/ Mediastinal	Acute Respiratory Failure	0	4 (11%)	.0817
	Cough	8 (27%)	18 (50%)	.0321
	Dyspnea Exacerbated	4 (13%)	11 (31%)	.0614
	Hemoptysis	0	5 (14%)	.0422
	Lung Consolidation	4 (13%)	11 (31%)	.0614
	Pharyngitis	5 (17%)	14 (39%)	.0313
	Respiratory Disorder NOS	0	7 (19%)	.0107
	Respiratory Tract Irritation	1 (3%)	6 (17%)	.0750

CyIS = Cyclosporine inhalation solution; MedDRA = Medical Dictionary for Regulatory Activities; NOS = Not otherwise specified.

A full listing of adverse events occurring in ≥ 10% of ACS001 patients is provided in **Appendix 8.2.** The following subsections will highlight the pertinent findings.

5.3.1.1 Respiratory, Thoracic, and Mediastinal Disorders

The largest group of AEs came from this SOC (97% placebo vs. 100% CyIS). In reviewing the data, a syndrome of bronchial irritation and airway obstruction emerged in the treatment group. Cyclosporine inhalation solution-treated patients had a higher incidence of bronchospasm, cough, exacerbated dyspnea, excessive bronchial secretions, respiratory tract irritation, hemoptysis, respiratory disorder NOS, and wheezing irritant when compared with placebo-treated patients. This most likely represents a direct effect of cyclosporine in the airways and was not unexpected given the mode of delivery. The preponderance of patients noted this cluster of AEs at the beginning of the study, during the dose-escalation phase. Most of these patients initially required premedication with bronchodilator and local anesthetic, but premedication use was reported to decline after 10 days of therapy. Additionally, there was no evidence of progression from these events to more serious episodes of acute respiratory failure, pneumonitis, or acute respiratory distress syndrome as a result of CyIS. The observation of bronchial reactivity early in the treatment course appears to be the most significant AE of CyIS. Interestingly, there was no significant difference in these events in single-lung transplant recipients vs. double-lung transplant recipients despite the presence complete pulmonary denervation in the latter group. These findings are consistent with prior experiments demonstrating that local bronchial reactivity can occur in the absence of afferent input.[47]

Lung consolidation was noted with significant increased frequency in the CyIS group (13% placebo vs. 31% CyIS, P= .061), associated with less prominent increases in lung infiltration and rales. The underlying etiology is not clear as rates of atelectasis, pulmonary mass, lung infection NOS, pneumonia NOS, acquired bronchomalacia, and pneumonitis NOS, pleural effusion, and pneumothorax NOS were all reported with increased frequency in the placebo group. Acute respiratory failure was noted with increased frequency in the CyIS group, but this finding was balanced by increased reporting of respiratory failure and respiratory arrest in the placebo group.

5.3.1.2 Blood and Lymphatic System Disorders

Adverse events in this SOC were higher in the placebo group than in the CyIS-treated group (80% vs. 58%). There was no evidence of increased lymphoproliferative disorders or bone marrow suppression.

5.3.1.3 Cardiac Disorders

Adverse events in this SOC were evenly split between groups (57% placebo vs. 53% CyIS) and evenly distributed by treatment group

5.3.1.4 Eye Disorders

During the course of the study 50% of placebo-treated patients reported an eye disorder vs. 61% of CyIS-treated patients. These numbers were largely driven by reports of blurred vision (27% placebo vs. 42% CyIS, P= .094) and cataracts (13% placebo vs. 14% CyIS). The cataracts were likely due to concomitant corticosteroid use, but the etiology of increased blurry vision was not apparent. Given the association of blurry vision with possible cyclosporine and tacrolimus toxicity, the individual patient reports of blurry vision in both placebo and CyIS-treated groups were reviewed. Of the 15 cases of blurry vision in the CyIS-treated group, 6 were temporally associated with confusion, tremor, short-term memory loss, or headache, suggesting a possible neurotoxic etiology; 5 were isolated reports without other indicators of primary central nervous system etiology; and 4 cases had alternate explanations for the finding (2 cases of hyperglycemia, and 2 cases of cataracts were reported concurrently). Of the 8 cases in the placebo group, 4 were associated with other neurologic symptoms described above and 4 were isolated reports. Therefore, a similar percentage of patients in both groups reported blurry vision associated with other neurologic symptoms (13% placebo vs. 17% CyIS). In summary, despite an increase in the reporting of blurry vision in the CyIS-treated group, there is no clear indication that this represents neurotoxicity beyond that expected from patients' baseline immunosuppression.

5.3.1.5 Gastrointestinal Disorders

Gastrointestinal events were reported frequently in both groups (100% placebo vs. 83% CyIS). Only retching was noted to be significantly increased in the CyIS group, but overall numbers were low, and rates of nausea/nausea aggravated, vomiting, and gastrointestinal upset were comparable between the two groups.

5.3.1.6 General Disorders and Administrative Site Conditions

These events were reported at similar frequency in both groups of patients (97% placebo vs. 92% CyIS). The greatest difference between placebo and CyIS groups was found in patients reporting chest pain (20% placebo vs. 42% CyIS, $P = .037$). In reviewing individual case reports, patients reporting chest pain frequently reported associated pharyngitis, suggesting a common etiology. This difference is likely related to inhalation of active drug with subsequent irritation of bronchoalveolar tree. The patients reporting chest pain did not overlap substantially with those reporting concurrent chest tightness, wheezing, and exacerbated dyspnea, suggesting that some patients may experience irritation as a separate phenomenon from bronchospasm.

5.3.1.7 Infections and Infestations

Given the nature of the study population, a preponderance of bronchopulmonary infections was expected. All placebo-treated patients and 86% of CyIS patients reported AEs in this SOC. There were no differences between treatment groups with regard to other sites of infections or pathogens. Taken together, these results show no evidence that long-term treatment with CyIS contributes to increased risk of infection.

5.3.1.8 Investigations

There were many instances of clinical and laboratory abnormalities in both treatment groups (73% placebo vs. 75% CyIS), but specific AEs were evenly distributed in most cases.

5.3.1.9 Metabolism and Nutrition Disorders

Adverse events in this SOC were common and evenly distributed between placebo (77%) and CyIS (75%) groups. The most common AEs of dehydration, diabetes mellitus, and fluid retention showed no differences between the two groups. Hyperlipidemia was seen with increased frequency in CyIS-treated patients (0% placebo vs. 14% CyIS, *P*= .042) but equal numbers of placebo and CyIS-treated patients reported hypercholesterolemia (17% placebo vs. 19% CyIS), suggesting that this finding is unlikely to be clinically relevant.

5.3.1.10 Musculoskeletal Connective Tissue and Bone Disease

There were similarly high rates of AEs reported in both groups (73% placebo vs. 78% CyIS). The most prominent findings were increased back pain (10% placebo vs. 33% CyIS; *P*= .019) and muscle cramps (17% placebo vs. 28% CyIS). The relevance of these nonspecific reports is unclear given the frequent association of myalgias, arthralgias, and pain syndromes with other immunosuppressive agents.

5.3.1.11 Nervous System Disorders

Adverse events in this SOC were common in both groups (80% placebo vs. 86% CyIS). These numbers are driven largely by a high frequency of headaches in both groups. Somnolence was not reported in any placebo-treated patients but seen in 17% of CyIS-treated patients (*P*= .021). The relevance of these findings is unclear, but in review of individual cases, somnolence was rarely temporally associated with other neurologic symptoms such as blurry vision, confusion, memory impairment, or headache.

5.3.1.12 Psychiatric Disorders

Adverse events in this SOC were relatively frequent with a slightly higher incidence in the treatment group (57% placebo vs. 67% CyIS). There was a significant increased reporting in the treatment group of confusion (0% placebo vs. 11% CyIS, *P*= .082) but overall numbers were low and this AE has previously been reported for prednisone, especially with prolonged use.

5.3.1.13 Renal and Urinary Disorders

A significant increase in the reporting of AEs in this SOC was found in the treatment group compared with placebo (63% placebo vs. 81% CyIS, *P*= .067). These increases were driven primarily by higher rates of urinary frequency (17% placebo vs. 39% CyIS, *P*= .031) and polyuria (3% placebo vs. 17% CyIS, *P*= .075). Review of individual patient data failed to uncover a common etiology for these symptoms. Rates of renal failure and renal insufficiency were comparable between groups.

5.3.1.14 Reproductive System and Breast Disorders

Adverse events were noted in 28% of CyIS-treated patients and 13% of placebo-treated patients. The difference in reporting was due almost entirely to single case reports in the CyIS group with no significant differences when stratified by gender.

5.3.1.15 Summary of ACS001 AE Data

In summary, there were only a few safety signals that emerged from review of the AEs reported. The syndrome of bronchial irritation and bronchospasm is not surprising and is most likely a direct result of the inhaled drug. In general, these AEs were noted early in the treatment course and diminished with time. Although these symptoms and signs were noted, they did not appear to lead to more serious episodes of respiratory failure, and overall mortality was significantly reduced in the CyIS-treated group. The addition of aerosolized cyclosporine to patients' immunosuppressant regimens did not appear to exacerbate any of the known toxicities of enteral/parenteral cyclosporine. Overall, the safety data obtained in ACS001 reveal a favorable safety profile with relatively minor potentially CyIS-related effects.

5.3.2 Adverse Events in ACS002

Data from the 70 patients enrolled in ACS002 were derived from seven different open-label noncomparative protocols designed to evaluate the safety of CyIS in lung transplant patients with established refractory rejection, active infections, and other serious complications. As the demographics of this patient population (with refractory AR and chronic rejection at

baseline) were more heterogeneous than the ACS001 population, safety data review of these 70 patients was used to substantiate the findings noted in ACS001 as well as to screen for additional safety signals, but a direct comparison with the ACS001 population was not performed.

Review of the safety data from the 70 ACS002 patients confirmed the safety signals found in ACS001 but did not yield new signals. The most common individual AEs of pyrexia (54%), nausea (50%), diarrhea NOS (43%), and dyspnea NOS (41%) are within expected ranges for lung transplant patients with recurrent infections, AR and chronic rejection, and chronic immunosuppression. The findings of cough (36%), dyspnea exacerbated (40%), wheezing (27%), chest pain (20%), and pharyngitis (14%) are likely reflective of treatment-related airway irritation and bronchial reactivity. Rates of renal impairment NOS (36%) were not elevated beyond expected ranges for patients with refractory rejection who have received augmented immunosuppression with tacrolimus and enteral/parenteral cyclosporine.[12] Rates of respiratory infections were within expected ranges, and there was no indication of increased malignancy or lymphoproliferative disorders. A table of all AEs reported in ≥ 10% of ACS002 CyIS patients is provided in **Appendix 8.3.** Review of the combined ISS population did not reveal any additional safety findings.

5.4 Serious Adverse Events

Serious adverse events are common in patients following lung transplantation as a result of underlying cardiopulmonary disease, chronic immunosuppression, frequent infections, and recurrent hospitalizations. As in the previous section, data from ACS001 were analyzed first, followed by data from ACS002.

5.4.1 Serious Adverse Events in ACS001

The SAE data from ACS001 were analyzed in two phases. The first phase examined all SOCs and individual SAEs in order to extract those for which numbers in the CyIS group exceeded those in the placebo group and were statistically significant based on a Fisher's Exact P value ≤ .1. The second phase examined all SAEs to find those having potential clinical relevance, even if statistical significance was not reached.

Serious adverse events were reported in 93% of patients receiving placebo (n = 30) and 89% of patients receiving CyIS (n = 36). In the placebo cohort, the number of SAEs reported by patients ranged from 0 (2 patients) to 21 (1 patient). In the CyIS cohort the number of SAEs reported by patients ranged from 0 (4 patients) to 16 (1 patient). The types of SAEs reported were broad, covering 20 different MedDRA SOCs, with only eight individual SAEs reported in more than 10% of patients treated with CyIS. The majority of SAEs reported occurred in only one patient. A full listing of SAEs occurring in ≥ 10% of ACS001 patients is provided in **Appendix 8.4**.

Of all SAEs reported, only one occurred in the CyIS group with an incidence that was significantly greater than the placebo group. Hypotension NOS was noted in six CyIS patients and 1 placebo patient (17% vs. 3%, $P = .075$). Given that hypotension is not a commonly reported AE for any of the immunosuppressives used in this study, each of the six cases in the CyIS group was analyzed separately. In 5 of the 6 cases, hypotension was reported concurrently with serious infections, and often as a premorbid condition. Therefore, it is unlikely these episodes were a direct result of CyIS administration.

Safety analysis from reported AEs revealed that CyIS administration might be associated with a syndrome of bronchospasm and airway irritation. Review of the SAE data revealed a similar but less prominent trend. Although SAE reporting in the Respiratory, Thoracic, and Mediastinal Disorders SOC was relatively equal between the two groups (53% placebo vs. 47% CyIS), exacerbated dyspnea was noted with higher frequency in the CyIS group (3% placebo vs. 14% CyIS, $P= .12$). Reports of chest tightness, chest pain, and wheezing were also slightly more frequent in the CyIS group than the placebo group. These findings are consistent with trends noted in the AE section and likely reflect treatment-induced airway irritation and obstruction.

5.4.2 Serious Adverse Events in ACS002

The lack of a comparison group precludes any definitive statement about the SAEs in the ACS002 patient population. However, a global review of reported SAEs in this larger group can potentially detect unusual or unexpected events that may have been missed in a smaller

patient sample. There were 176 separate SAEs reported by 91% of ACS002 patients. Reporting was broad and even for SOCs and individual SAEs, with only one case report occurring in 70% of individual SAEs. Serious adverse events reported from ≤ 3 patients (≤ 4% of total) accounted for 87% of all SAE terms.

Most SAEs were reported within the Respiratory, Thoracic, and Mediastinal Disorders (67%) and Infections and Infestations (63%) SOCs. This high level of reporting was expected given the study population of lung transplant patients on chronic immunosuppressives with established complications. Within the Respiratory SOC, the major SAEs were clustered in the following groups: dyspnea exacerbated (20%)/dyspnea NOS (11%); and respiratory failure (19%)/respiratory distress (10%). Given the previous AE and SAE data from ACS001, it could be expected that exacerbated dyspnea arose as a direct result of inhalation of active drug, although bronchospasm (9%) and wheezing aggravated (3%) were not reported as frequently. Dyspnea and exacerbated dyspnea were also expected given the progressive nature of OB and the frequent episodes of pneumonia documented in a significant number of ACS002 patients. The relationship between dyspnea/exacerbated dyspnea and subsequent respiratory distress/respiratory failure is less clear as a substantial number of these SAEs occurred in the setting of respiratory infections.

Respiratory tract infections from bacterial, viral, and fungal pathogens represented the overwhelming majority of SAEs in the Infections and Infestations SOC. Only single case reports exist for infection SAEs from other sites. Pneumonia NOS (23%) was the most frequently documented SAE of the SOC followed by an aggregate of Bronchitis Bacterial NOS (4%)/Bronchitis NOS (6%). Review of the ISS SAE listings did not lead to any new safety findings.

Overall, in review of the ACS001 placebo-controlled study population and the ACS002 open-label study population, there was no evidence that CyIS was associated with a statistically significant increased incidence of SAEs. It is possible that CyIS may be associated with a trend toward increased an increased incidence of bronchospasm, but these events did not progress to more serious respiratory complications.

5.5 Adverse Events Leading to Study Drug Discontinuation

A comparison of AEs leading to premature study drug discontinuation within the ACS001 study population did not reveal any new safety findings. Within ACS001, a higher percentage of placebo patients withdrew due to AEs (27%) compared with CyIS patients (17%). In both groups, there were two patients that withdrew within the first 2 weeks of dosing. Additionally, of those who withdrew dosing, the percentage of patients withdrawing due to SAEs (as opposed to an AE) was similar (63% placebo vs. 67% CyIS).

Within the combined ACS001 and ACS002 CyIS populations, a combined total of 40 patients receiving CyIS in ACS001 and ACS002 prematurely discontinued treatment as a direct result of AEs and SAEs. Overall, a pattern emerged of patients discontinuing therapy secondary to events causing symptoms of airway irritation, airway obstruction, and shortness of breath. Taken together, 26 of 40 patients fell into this syndrome (65%), and the majority of these patients' symptoms were recorded as AEs rather than SAEs (76% versus 24%, respectively). Although many of these patients may have had concurrent illness and events other than airway irritation and bronchial reactivity to explain these premature discontinuations, it is likely that a significant number of patients had to prematurely discontinue study medication as a result of CyIS. Patients who discontinued CyIS as a result of these symptoms had decreased mean duration of dosing compared to those who discontinued for other AEs (8.0 months vs. 10.6 months). For the 9 CyIS patients that discontinued CyIS prior to 1 month, 8 (89%) were a result of these symptoms.

Although a substantial number of patients could not tolerate prolonged use of CyIS due to large and small airway effects, it is noteworthy that there was no evidence that these effects led to any premorbid or morbid events. Although premature study drug discontinuation was common in both studies, ACS001 demonstrated a greater number of early discontinuations in the placebo group than the CyIS-treated group. These findings suggest that, overall, CyIS is fairly well tolerated in lung transplant recipients.

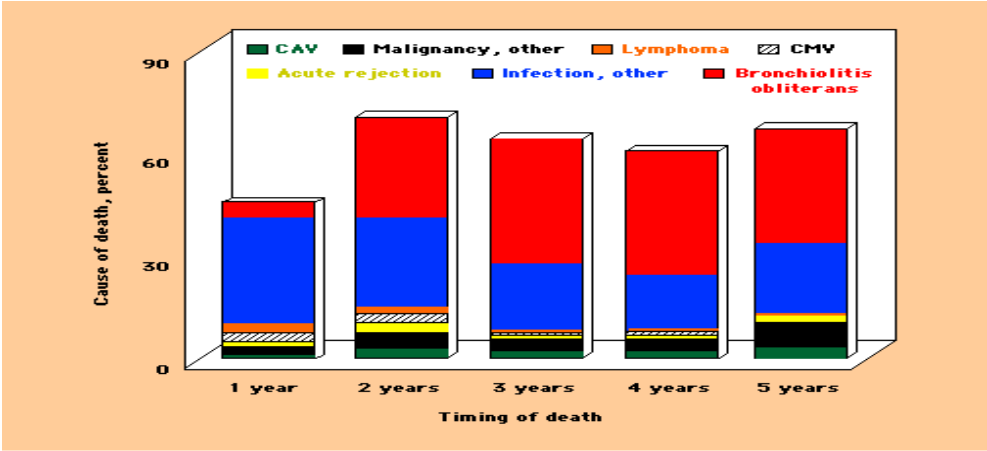
5.6 Deaths

5.6.1 Deaths Occurring During ACS001

Data regarding patient deaths in the ACS001 trial were initially obtained from the investigator at the UPMC. Data were then collected by site monitors and entered into case report forms from the source documents. When available, these sources also contained autopsy reports. In four cases, the primary cause of death was unknown. These included one patient randomized to CyIS and three patients randomized to placebo. **Appendix 8.5** demonstrates the primary and secondary causes of death for all 22 patients that died either during the study period. These included 5 patients in the pilot phase, 3 patients in the randomized CyIS group, and 14 patients in the placebo group.

Patient deaths after transplant ranged from 28 days to 4.1 years in the placebo group and 211 days to 5.3 years in the CyIS-treated group. There were several important findings from an analysis of ACS001 deaths. First, treatment with CyIS was associated with a significantly lower risk of death compared with placebo, and this finding is discussed in greater detail in **Section 4**. Second, in the period of greatest risk of death—the first 100 days after transplant—three deaths occurred in the placebo group vs. no deaths in the CyIS-treated group. Third, aside from the few cases in which the primary cause of death was unknown, nearly all recorded deaths were either primarily or secondarily a result of infection or OB.

Figure 5.6.1-1 illustrates the predominant causes of death in lung transplant patients as a function of time after transplant derived from the 1999 *Journal of Heart and Lung Transplantation*. For 6 of the 7 CyIS-treated patients for whom a primary cause of death was recorded, the primary cause of death matched the most common cause of death for the year after transplant. This includes 3 patients (01S0501, 01S0506, 01S101B) who died from infectious causes within the first year, 1 patient (01S305B) who died from infectious causes in the second year, 1 patient (01S0507) who died of OB in the third year, and 1 patient (01S0510) who died of OB in the fifth year. The seventh patient (01S0508) died of an undifferentiated carcinoma, which represented the third-most-common cause for the year of the patient's death.



Cause of death following lung transplantation The predominant causes of death vary according to the time elapsed since lung transplantation, although infection and rejection account for the greatest proportion of deaths in all groups. Data from Hosenpud, JD, Bennett, LE, Keck, BM, et al. The Registry of the International Society for Heart and Lung Transplantation: Sixteenth official report – 1999. J Heart Lung Transplant 1999; 18:611.

Figure 5.6.1-1. Causes of death following lung transplantation.

Therefore, there is no indication that administration of CyIS in post-lung transplant patients led to any unexpected or untimely deaths. This assertion is supported by the data presented for SAEs in **Section 5.4**, which did not reveal any evidence of treatment-related SAEs.

5.6.2 Deaths Occurring in ACS002

Thirty-five of the 70 patients in ACS002 (50%) died prior to the study termination date of August 21, 2003. Twenty-eight patients died after withdrawing from the study due to AEs **(see Section 2.7.4.2.1.4)** and four patients died after withdrawing from the study for other reasons (i.e., protocol deviations/violations, lost to follow up, unknown). Of the 35 deaths, 16 occurred prior to the 90th day after the last treatment, and 19 deaths occurred after the 90th day, but prior to the study completion date of August 21, 2003. Given the wide range of settings in which ACS002 deaths occurred, no definitive statements about the role of CyIS in these deaths could be made. However, after review of the individual cases, there were no unexpected deaths or trends not predicted by the patients' underlying pathology.

Appendix 8.6 lists the primary and contributing causes of deaths for all 35 patients. In two cases, the cause of death is unknown (01R085, 01R106). Of the 35 patients' primary cause of death, 33 were common causes of death in post-lung transplant patients: 13 (37%) had infections; 11 (31%) had graft failure due to AR/chronic rejection/OB; 4 (11%) had nonspecific "respiratory failure" "shock" or "multi-organ failure" from poorly characterized causes; 2 (6%) had malignancies (lung carcinoma and posttransplant lymphoproliferative disease); 2 (6%) had unknown causes; and 1 (3%) had a pulmonary embolism. Two deaths occurred from central nervous system causes, which are not expected or common in this patient population. However, review of these cases failed to reveal any likely association with CyIS and neither patients was receiving therapy at the time of the event.

5.7 Conclusions

Review of the safety data from 102 lung transplant recipients enrolled within ACS001 or ACS002 provides strong support for the initial hypothesis that topical administration of cyclosporine could achieve a therapeutic effect without exacerbating the known toxicities of oral or intravenous cyclosporine. Despite prolonged use in the majority of patients, there was no evidence that CyIS led to any increased risk of nephrotoxicity, neurotoxicity, hepatotoxicity, increased risk of infections, or increased risk of malignancies.

Consistent with its mode of delivery, the safety signals noted with CyIS were locally within the respiratory tract. The most notable safety signal was probable treatment-related bronchospasm manifested primarily by cough, wheezing, and exacerbated dyspnea. These events were most often during or immediately after treatment, and generally most apparent in the early weeks of dosing. Importantly, these events did not lead to more serious respiratory complications such as acute respiratory failure, acute respiratory distress syndrome, or hypersensitivity pneumonia, even in the ACS002 population who had diminished pulmonary reserve at baseline. Additionally, CyIS appeared to result in respiratory tract irritation manifested primarily by pharyngitis and chest pain. Both bronchospasm and respiratory tract irritation are well-documented adverse events of other inhaled therapeutics[48,49] and therefore were not unexpected. Bronchodilators and/or topical anesthetics as premedication were

offered to patients upon initiation with CyIS; however, use of these medicines was reported to decline after the first 10 days of treatment.

6. CONCLUSIONS

Cyclosporine inhalation solution has the potential to address a significant unmet medical need by improving the survival of patients receiving lung transplants. The weight of evidence presented for benefit and the limited risk of harm support approval at this time.

6.1 The Unmet Need Is Clear

Despite best current care, the long-term survival after lung transplantation remains considerably lower compared with other solid-organ transplants. Whereas heart, kidney, and liver transplant recipients can expect a 5-year survival rate of approximately 70% to 90% and a 10-year survival rate of greater than 50%, lung transplant recipients can only expect a 45% 5-year survival rate and a 10-year survival rate of approximately 25%.

6.2 The Scientific Rationale for CyIS by Inhalation Is Sound

Lung transplant recipients develop chronic rejection at far higher rates than other solid-organ transplant recipients. Chronic rejection occurs in 60% to 70% of lung transplant recipients by 5 years after transplant. Once established, chronic rejection is irreversible, unresponsive to therapy, and ultimately fatal. Rejection episodes are managed by "dose intensification," but conventional oral immunosuppressive regimens are associated with dose-limiting systemic toxicity such that optimally efficacious doses are often unachievable.

Administration of CyIS significantly increases lung exposure to cyclosporine relative to systemic exposure. We estimate that pulmonary delivery decreases systemic exposure of a given dose about 7-fold **(Section 3-3)**.

Finally, pulmonary delivery of drugs by aerosol to increase lung-to-systemic exposure ratio and to deliver them directly to the effect compartment is a well-recognized and very successful strategy in chronic lung diseases such as asthma (beta agonists, steroids) and cystic fibrosis (tobramycin, DNAse)

6.3 The Evidence for Benefit of CyIS Is Strong

Study ACS001, a blinded, randomized, placebo-controlled study, demonstrated a major improvement in both overall survival and chronic rejection-free survival for CyIS over placebo in lung transplant patients **(Figure 4.3.1-1)**. Treatment was initiated soon after transplantation, and all patients received standard care including a full-dose, multiple-drug cocktail of immunosuppressive agents.

Although Study ACS001 was a relatively small study, conducted at a single site in 56 randomized patients (30 placebo, 26 CyIS), close scrutiny of the data suggests the outcome is robust and generalizable.

First, the mortality and clinical course of the placebo group in Study ACS001 closely matches that of screen failures **(Figure 4.3.1.2-1)** and contemporaneous patients treated at other transplant centers **(Figure 4.3.1.2-2)**.

Second, the magnitude of the observed benefit is significant with the hazard ratio for risk of death estimated at 0.213 (95% CI = 0.061, 0.743). The benefit is maintained when allowing for baseline imbalances between treatment groups **(Figure 4.3.1-2)** and confounding factors that could potentially affect outcome **(Table 4.3.1.1-1)**.

Study ACS001 is supported by preclinical evidence showing inhaled cyclosporine to be effective in controlling rejection in lung transplants.[4,44]

Furthermore, there is supportive evidence of benefit from other published clinical studies conducted with aerosolized cyclosporine in lung transplantation **(Section 4.6)**. This includes 90 patients documented in the University of Pittsburgh lung transplant registry between 1991 and 2001 with pre-existing rejection.[46] The 39 patients who received open-label aerosolized cyclosporine had improved survival (hazard ratio, 0.49; $P < .05$) compared both with the 51 patients in this series who did not and also compared with an independent registry control group.

6.4 The Risks Are Limited

6.4.1 Known Risks

Systemic exposure following CyIS by inhalation is low: <15% of the AUC and C_{max} of standard oral doses of cyclosporine used in solid-organ transplants; therefore, significant systemic toxicity would not be expected. This is supported by the safety profile in the 102 patients included in ACS001 and ACS002. Despite prolonged use in the majority of patients, there was no evidence that CyIS led to any increased risk of nephrotoxicity, neurotoxicity, hepatotoxicity, increased risk of infections, or increased risk of malignancies.

As expected, the adverse events associated with CyIS were locally within the respiratory tract. Most significant was a constellation of cough, wheezing, and dyspnea probably reflecting bronchospasm; and pharyngitis and chest pain probably reflecting direct irritation induced by CyIS treatment. These events were usually seen in the early weeks of treatment and did not lead to more serious respiratory complications.

Further reassurance regarding the local (lung) effects of high-dose inhaled cyclosporine and PG is provided by 28-day inhalation toxicology studies in rats and dogs. No significant lung toxicity was observed at exposure levels about 5-fold higher than human exposure in dogs and about 15-fold higher in rats.

6.4.2 Unknown Risks

Cyclosporine inhalation solution is an inhaled formulation of a well-known drug that has been part of the standard care for solid-organ transplant recipients for over 20 years. The tolerability profile of cyclosporine is well characterized, so it is unlikely that new problematic systemic toxicities will emerge in the future.

The target patient population is very small (approximately 1100 patients per year), and the diagnosis of lung transplant is very clear. Therefore, the potential for widespread use of CyIS in patients unlikely to benefit is small.

6.5 The Sponsor Is Committed to Further Study to Address Outstanding Questions

Although, as stated previously, the weight of evidence supports approval of CyIS at this time, several areas remain appropriate for future study. In particular, it will be important to document the magnitude of the benefit and safety profile of CyIS in a broader population of lung transplant patients in different treatment centers. Therefore, Chiron Corporation is committed to a large 5-year postapproval study of CyIS.

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Notes: CORPORATE NAME: Pulmonary Council, International Society for Heart and Lung Transplantation.

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8. APPENDICES

Appendix 8.1 Estimated CyIS Dose Deposition and Distribution in Lung Transplant Recipients

During the course of ACS001, a substudy was performed to estimate CyIS dose deposition and distribution in both single- and double-lung transplant recipients.[50] In this substudy, 15 patients treated with CyIS (6 double-lung transplant recipients and 9 single-lung transplant recipients) and 7 patients treated with placebo were given their study drug mixed with technetium-99m–labeled diethylenetriaminepentaacetic acid (DTPA). Prior bench testing demonstrated that the radionuclide fully mixed in solution and that the radioactivity associated with the tag was linearly proportional to the mass of active drug. The radiolabeled study drug was given in a closed system so that all radiation counts could be assessed within the delivery equipment and an exhalation filter. All radioactivity not accounted for in the external system was subtracted from the total dose and assumed to be in the patient. Following inhalation of the radiolabeled study drug, all patients underwent gamma scintigraphy imaging to visualize dose distribution. In addition to estimating total intrapulmonary dose of study drug, an analysis of the scintigraphy images allowed for estimation of relative distribution within the transplanted lung in an attempt to distinguish central deposition (i.e., within the larger airways) from peripheral distribution (i.e., within the smaller airways and alveoli).

Figure 8.1-1 illustrates that the overwhelming majority of radiolabel deposited in the lower respiratory tract with relatively even dose distribution between the two transplanted lungs of a double-lung transplant recipient. Lesser amounts were deposited in the mouth, esophagus, trachea, and stomach.

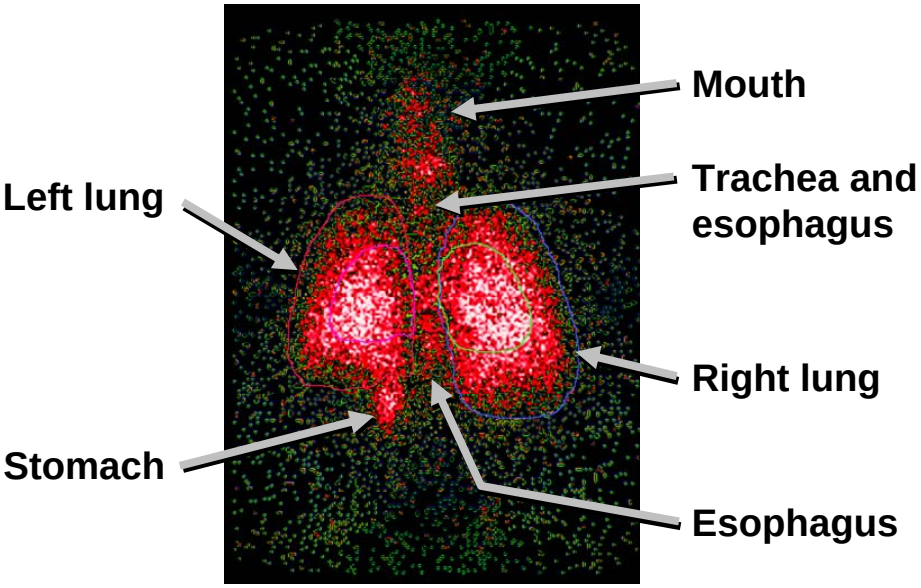

Figure 8.1-1. Representative gamma scintigraphy in a double-lung transplant recipient**.**

Figure 8.1-2 illustrates that approximately half of the deposited dose in the lower respiratory tract was deposited peripherally, consistent with the known favorable aerosol characteristics of CyIS. The estimated dose was averaged between the two lungs so that the transplant dose could be represented by a single quantity. For the case of these 6 double-lung transplant recipients, the average estimated deposited dose of CyIS was 16.2 mg per transplanted lung, corresponding to an average of 5.4% of the administered dose per transplanted lung (range, 3.3 to 7.1% per transplanted lung).

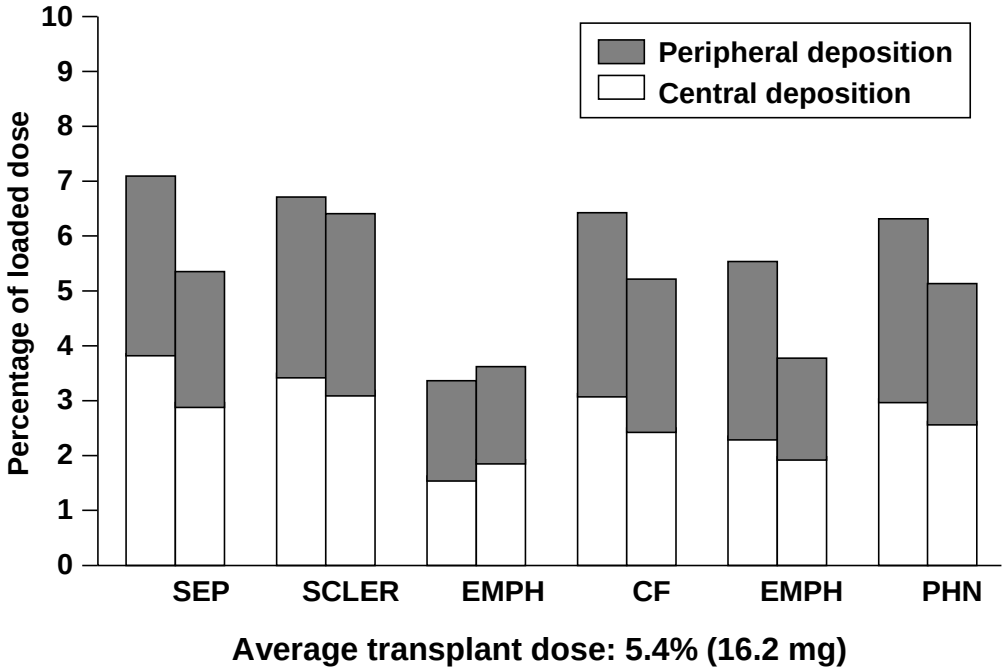

Figure 8.1-2. Central versus peripheral deposition in a double-lung transplant. SEP = Septic lung disease; SCLER = Scleroderma; EMPH = Emphysema;
CF = Cystic fibrosis; PHN = Pulmonary hypertension.

Figure 8.1-3 illustrates the gamma scintigraphy image obtained from a single-lung transplant recipient and illustrates clear preferential dose deposition in the allograft compared with the native diseased lung. These findings were confirmed in 8 of 9 single lung transplant recipients.

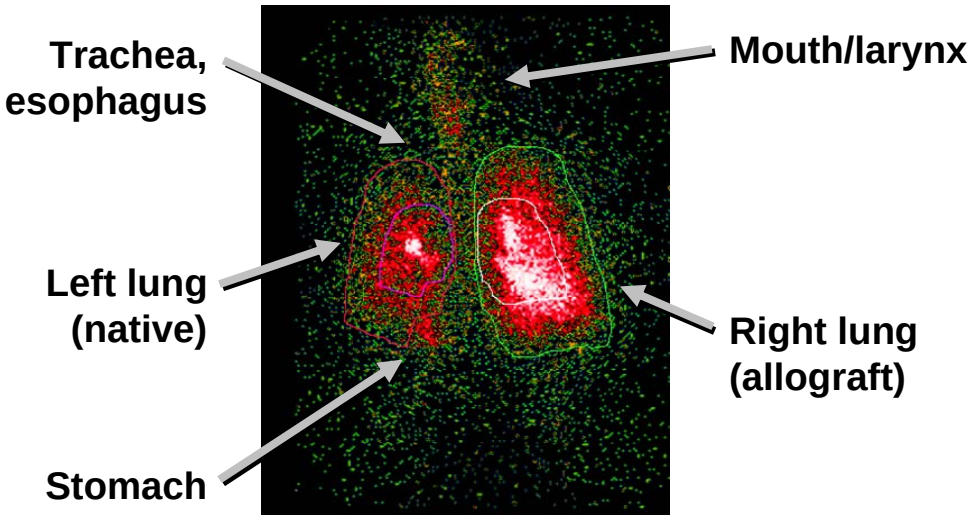

Figure 8.1-3. Representative gamma scintigraphy in a single-lung transplant recipient.

Figure 8.1-4. In these patients the average estimated deposited dose of CyIS was 18.6 mg per transplanted lung, corresponding to an average of 6.2% of the administered dose per transplanted lung (range, 2.2% to 9.2% per transplanted lung). IPF = Idiopathic pulmonary fibrosis; SIL = Silicosis; EMPH = Emphysema.

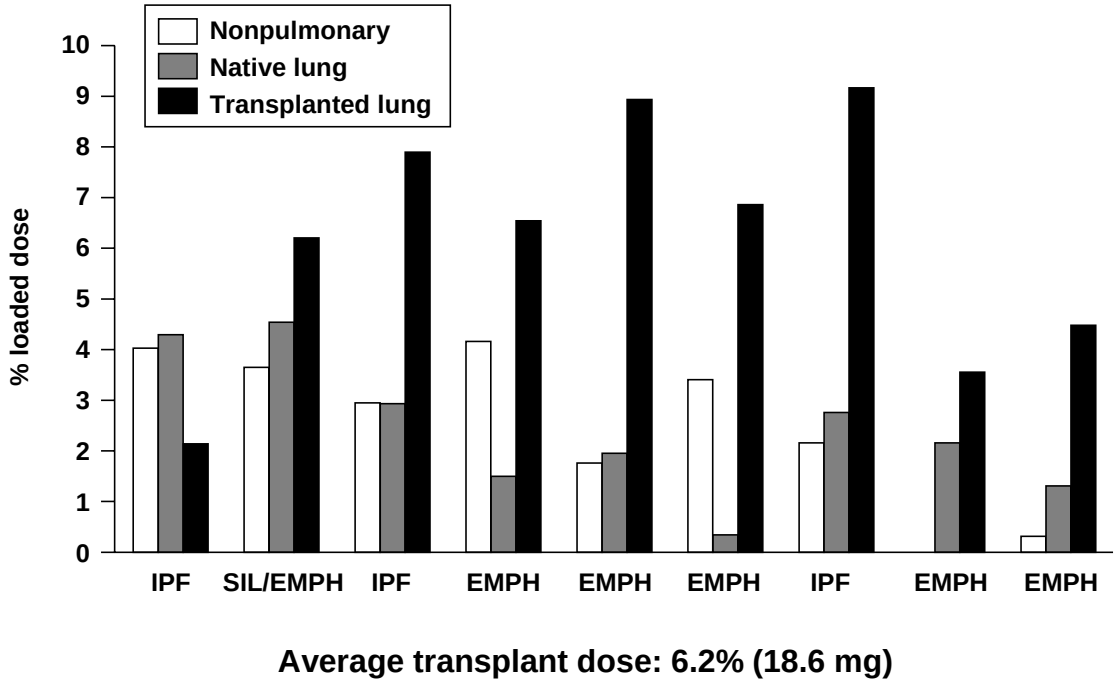

Figure 8.1-4. Allograft versus native deposition in single-lung transplant.

Taken together, the deposition substudy confirmed early findings from Burckart et al.[23] that the majority of CyIS was deposited in the lower respiratory tract. The previous estimated lower respiratory tract deposited dose of 5.4% to 11.2% (of administered dose in the nebulizer) compares favorably with this second estimated deposited dose of 2.2% to 14.2% (of administered dose in the nebulizer). In addition, CyIS was found to be distributed evenly between lungs in double-lung transplant recipients and preferentially to the allograft in single-lung transplant recipients. Finally, it is noteworthy that although patient-to-patient variability in dose deposition was substantial, there was no apparent relationship between deposited dose and underlying pulmonary disease.

Appendix 8.2. Adverse Events Reported by ≥ 10% of ACS001 Patients		
System Organ Class **Adverse Event**	**Placebo, n (%)** **(n = 30)**	**CyIS, n (%)** **(n = 36)**
Blood and Lymphatic System Disorders	24 (80)	21 (58)
Anemia NOS	17 (57)	12 (33)
Leukopenia NOS	6 (20)	8 (22)
Lymphadenopathy	1 (3)	5 (14)
Neutropenia	9 (30)	1 (3)
Thrombocytopenia	3 (10)	0 (0)
Cardiac Disorders	17 (57)	19 (53)
Atrial Fibrillation	4 (13)	3 (8)
Bradycardia NOS	2 (7)	4 (11)
Cardiac Failure Congestive	3 (10)	0 (0)
Cardiomegaly NOS	2 (7)	4 (11)
Clubbing	3 (10)	1 (3)
Dilatation Atrial	2 (7)	4 (11)
Palpitations	1 (3)	5 (14)
Pericardial Effusion	3 (10)	1 (3)
Pulmonary Edema NOS	4 (13)	6 (17)
Tachycardia NOS	3 (10)	4 (11)
Ventricular Hypertrophy	3 (10)	4 (11)
Ear and Labyrinth Disorders	10 (33)	10 (28)
Ear Pain	4 (13)	3 (8)
Hearing Impaired	2 (7)	4 (11)
Tinnitus	7 (23)	3 (8)
Endocrine Disorders	2 (7)	4 (11)
Eye Disorders	15 (50)	22 (61)
Cataract	4 (13)	5 (14)
Vision Blurred	8 (27)	15 (42)
Visual Disturbance NOS	5 (17)	2 (6)
Gastrointestinal Disorders	30 (100)	30 (83)
Abdominal Pain NOS	8 (27)	7 (19)
Abdominal Distension	2 (7)	4 (11)

Appendix 8.2. Adverse Events Reported by ≥ 10% of ACS001 Patients		
System Organ Class **Adverse Event**	**Placebo, n (%)** **(n = 30)**	**CyIS, n (%)** **(n = 36)**
Abdominal Pain Upper	3 (10)	3 (8)
Ascites	4 (13)	4 (11)
Colonic Polyp	3 (10)	1 (3)
Constipation	4 (13)	7 (19)
Diarrhea NOS	18 (60)	16 (44)
Dyspepsia	6 (20)	11 (31)
Dysphagia	6 (20)	7 (19)
Gastritis NOS	2 (7)	5 (14)
Gastroesophageal Reflux Disease	5 (17)	7 (19)
Hemorrhoids	3 (10)	0 (0)
Nausea	15 (50)	20 (56)
Retching	0 (0)	4 (11)
Toothache	1 (3)	4 (11)
Vomiting NOS	11 (37)	14 (39)
General Disorders and Administrative Site Conditions	29 (97)	33 (92)
Anasarca	3 (10)	0 (0)
Asthenia	1 (3)	5 (14)
Chest Pain	6 (20)	15 (42)
Chest Tightness	3 (10)	8 (22)
Fatigue	10 (33)	6 (17)
Fatigue Aggravated	9 (30)	8 (22)
Lethargy	1 (3)	5 (14)
Malaise	2 (7)	5 (14)
Edema Peripheral	11 (37)	12 (33)
Pyrexia	14 (47)	15 (42)
Rigors	7 (23)	9 (25)
Thirst	7 (23)	9 (25)
Weakness	4 (13)	4 (11)
Hepato-Biliary Disorders	6 (20)	6 (17)
Cholelithiasis	4 (13)	2 (6)
Immune System Disorders	4 (13)	1 (3)
Infections and Infestations	30 (100)	31 (86)

Appendix 8.2. Adverse Events Reported by ≥ 10% of ACS001 Patients		
System Organ Class **Adverse Event**	**Placebo, n (%)** **(n = 30)**	**CyIS, n (%)** **(n = 36)**
Aspergillosis	4 (13)	4 (11)
Bronchitis Acute NOS	3 (10)	3 (8)
Bronchitis NOS	11 (37)	15 (42)
Bronchopneumonia	3 (10)	2 (6)
Candidal Infection NOS	1 (3)	5 (14)
Colitis Pseudomembranous	4(13)	3(8)
Cytomegaloviral Infection	3 (10)	7 (19)
Enterococcal Infection NOS	3 (10)	0 (0)
Fungal Infection NOS	3 (10)	2 (6)
Gastroenteritis NOS	3 (10)	3 (8)
Herpes Simplex	3 (10)	4 (11)
Herpes Zoster	1 (3)	4 (11)
Lung Infection Pseudomonal	5 (17)	4 (11)
Nasopharyngitis	5 (17)	8 (22)
Oral Candidiasis	8 (27)	3 (8)
Pneumonia Cytomegaloviral	8 (27)	4 (11)
Pneumonia NOS	8 (27)	9 (25)
Pneumonia Staphylococcal	3 (10)	0 (0)
Septic Shock	3 (10)	3 (8)
Sinusitis NOS	6 (20)	8 (22)
Stenotrophomonas Infection	3 (10)	2 (6)
Upper Respiratory Tract Infection NOS	7 (23)	9 (25)
Injury and Poisoning	14 (47)	15 (42)
Drug Toxicity NOS	3 (10)	1 (3)
Post Procedural Pain	1 (3)	4 (11)
Investigations	22 (73)	27 (75)
Blood Creatinine Increased	4 (13)	1 (3)
Cardiac Murmur NOS	0 (0)	6 (17)
Cytomegalovirus Antigen Positive	4 (13)	3 (8)
Weight Increased	5 (17)	9 (25)
Weight Decreased	8 (27)	5 (14)
Metabolism and Nutrition Disorders	23 (77)	27 (75)

Appendix 8.2. Adverse Events Reported by ≥ 10% of ACS001 Patients		
System Organ Class **Adverse Event**	**Placebo, n (%)** **(n = 30)**	**CyIS, n (%)** **(n = 36)**
Anorexia	5 (17)	2 (6)
Dehydration	7 (23)	5 (14)
Diabetes Mellitus NOS	3 (10)	6 (17)
Fluid Retention	4 (13)	7 (19)
Hypercholesterolemia	5 (17)	7 (19)
Hyperglycemia	3 (10)	4 (11)
Hyperkalemia	5 (17)	9 (25)
Hyperlipidemia NOS	0	5 (14)
Hypomagnesemia	3 (10)	1 (3)
Hyponatremia	4 (13)	1 (3)
Malnutrition	3 (10)	2 (6)
Musculoskeletal, Connective Tissue and Bone Disorders	22 (73)	28 (78)
Arthralgia	3 (10)	6 (17)
Back Pain	3 (10)	12 (33)
Muscle Cramps	5 (17)	10 (28)
Myalgia	7 (23)	8 (22)
Osteoporosis NOS	4 (13)	4 (11)
Pain in Jaw	4 (13)	3 (8)
Pain in Limb	7 (23)	7 (19)
Neoplastic, Benign/Malignant Disorders	11 (37)	12 (33)
Lung Nodule	7 (23)	5 (14)
Nervous System Disorder	24 (80)	31 (86)
Amnesia	4 (13)	2 (6)
Dizziness	6 (20)	10 (28)
Headache NOS	15 (50)	21 (58)
Hypoesthesia	2 (7)	4 (11)
Memory Impairment	3 (10)	6 (17)
Somnolence	0 (0)	6 (17)
Tremor	12 (40)	14 (39)
Tremor Aggravated	2 (7)	5 (14)
Psychiatric Disorders	17 (57)	24 (67)
Agitation	3 (10)	3 (8)

Appendix 8.2. Adverse Events Reported by ≥ 10% of ACS001 Patients		
System Organ Class **Adverse Event**	**Placebo, n (%)** **(n = 30)**	**CyIS, n (%)** **(n = 36)**
Anxiety	4 (13)	2 (6)
Confusion	0 (0)	4 (11)
Depression	3 (10)	1 (3)
Insomnia	6 (20)	5 (14)
Mental Status Changes	3 (10)	2 (6)
Short Term Memory Loss	5 (17)	8 (22)
Sleep Disorder NOS	4 (13)	5 (14)
Renal and Urinary Disorders	19 (63)	29 (81)
Dysuria	5 (17)	3 (8)
Polyuria	1 (3)	6 (17)
Renal Failure Acute	5 (17)	3 (8)
Renal Failure NOS	2 (7)	6 (17)
Renal Impairment NOS	10 (33)	7 (19)
Urinary Frequency	5 (17)	14 (39)
Reproductive System and Breast Disorders	4 (13)	10 (28)
Respiratory, Thoracic, and Mediastinal Disorders	29 (97)	36 (100)
Acquired Bronchomalacia	6 (20)	6 (17)
Acute Respiratory Failure	0 (0)	4 (11)
Atelectasis	7 (23)	8 (22)
Bronchospasm NOS	2 (7)	6 (17)
Bronchostenosis	5 (17)	6 (17)
Cough	8 (27)	18 (50)
Crackles Lung	3 (10)	7 (19)
Dyspnea Exacerbated	4 (13)	11 (31)
Dyspnea NOS	18 (60)	19 (53)
Emphysema	3 (10)	2 (6)
Hemoptysis	0 (0)	5 (14)
Hoarseness	4 (13)	2 (6)
Hypoxia	1 (3)	5 (14)
Lung Consolidation	4 (13)	11 (31)
Lung Disorder NOS	4 (13)	5 (14)
Lung Infiltration NOS	5 (17)	8 (22)

Appendix 8.2. Adverse Events Reported by ≥ 10% of ACS001 Patients		
System Organ Class **Adverse Event**	**Placebo, n (%)** **(n = 30)**	**CyIS, n (%)** **(n = 36)**
Nasal Congestion	4 (13)	4 (11)
Pharyngitis	5 (17)	14 (39)
Pleural Effusion	15 (50)	17 (47)
Pneumothorax NOS	7 (23)	7 (19)
Postnasal Drip	11 (37)	10 (28)
Productive Cough	1 (3)	5 (14)
Pulmonary Congestion	2 (7)	4 (11)
Pulmonary Embolism	5 (17)	3 (8)
Pulmonary Fibrosis	3 (10)	6 (17)
Pulmonary Hemosiderosis	19 (63)	23 (64)
Pulmonary Hypertension NOS	5 (17)	2 (6)
Rales	1 (3)	4 (11)
Respiratory Disorder NOS	0 (0)	7 (19)
Respiratory Failure	5 (17)	4 (11)
Respiratory Tract Irritation	1 (3)	6 (17)
Sinus Congestion	3 (10)	3 (8)
Sinus Pain	7 (23)	7 (19)
Snoring	5 (17)	4 (11)
Wheezing	6 (20)	12 (33)
Skin and Subcutaneous Disorders	18 (60)	19 (53)
Contusion	4 (13)	1 (3)
Skin Lesion NOS	1(3)	5(14)
Sweating Increased	3 (10)	2 (6)
Vascular Disorders	27 (90)	27 (75)
Hypertension Aggravated	8 (27)	4 (11)
Hypertension NOS	8 (27)	13 (36)
Hypotension NOS	8 (27)	10 (28)
Orthostatic Hypotension	1 (3)	4 (11)
Venous Thrombosis Deep Limb	8 (27)	5 (14)

Appendix 8.3. Adverse Events Reported by ≥ 10% of ACS002 Patients	
Adverse Event	**CyIS Patients (N = 70)**
Pyrexia	38 (54%)
Nausea	35 (50%)
Diarrhea NOS	30 (43%)
Dyspnea NOS	29 (41%)
Dyspnea Exacerbated	28 (40%)
Vomiting NOS	28 (40%)
Anaemia NOS Aggravated	27 (39%)
Edema Peripheral	27 (39%)
Bronchitis NOS	25 (36%)
Cough	25 (36%)
Headache NOS	25 (36%)
Renal Impairment NOS	25 (36%)
Dizziness	21 (30%)
Pneumonia NOS	21 (30%)
Wheezing	19 (27%)
Fatigue Aggravated	18 (26%)
Hypertension NOS	18 (26%)
Tremor	18 (26%)
Vision Blurred	18 (26%)
Abdominal Pain NOS	17 (24%)
Cough Aggravated	17 (24%)
Fatigue	17 (24%)
Hyperkalemia	17 (24%)
Respiratory Failure	15 (21%)
Anemia NOS	14 (20%)
Bronchospasm NOS	14 (20%)
Chest Pain	14 (20%)
Pleural Effusion	14 (20%)
Myalgia	13 (19%)
Oral Candidiasis	13 (19%)

Appendix 8.3. Adverse Events Reported by ≥ 10% of ACS002 Patients	
Adverse Event	**CyIS Patients (N = 70)**
Tachycardia NOS	13 (19%)
Back Pain	12 (17%)
Hypotension NOS	12 (17%)
Postnasal Drip	12 (17%)
Rigors	12 (17%)
Abdominal Distension	11 (16%)
Arthralgia	11 (16%)
Chest Tightness	11 (16%)
Constipation	11 (16%)
Hypoxia	11 (16%)
Osteoporosis NOS	11 (16%)
Upper Respiratory Tract Infection NOS	11 (16%)
Insomnia	10 (14%)
Lung Infiltration NOS	10 (14%)
Muscle Cramps	10 (14%)
Pharyngitis	10 (14%)
Renal Failure NOS	10 (14%)
Weakness	10 (14%)
Anxiety	9 (13%)
Dyspepsia	9 (13%)
Lung Infection Pseudomonal	9 (13%)
Malaise	9 (13%)
Pulmonary Hemosiderosis	9 (13%)
Sweating Increased	9 (13%)
Thrombocytopenia	9 (13%)
Appetite Decreased NOS	8 (11%)
Dyspnea Exertional	8 (11%)
Memory Impairment	8 (11%)
Pain In Limb	8 (11%)
Pharyngolaryngeal Pain	8 (11%)

Appendix 8.3. Adverse Events Reported by ≥ 10% of ACS002 Patients	
Adverse Event	**CyIS Patients (N = 70)**
Respiratory Distress	8 (11%)
Sinusitis NOS	8 (11%)
Acquired Bronchomalacia	7 (10%)
Ascites	7 (10%)
Aspergillosis	7 (10%)
Bronchitis Bacterial NOS	7 (10%)
Bronchostenosis	7 (10%)
Confusion	7 (10%)
Dehydration	7 (10%)
Gastroesophageal Reflux Disease	7 (10%)
Hemoptysis	7 (10%)
Hyperglycemia NOS	7 (10%)
Hypertension Aggravated	7 (10%)
Leukocytosis	7 (10%)
Malnutrition NOS	7 (10%)
Nasal Congestion	7 (10%)
Pneumonia Cytomegaloviral	7 (10%)
Pneumothorax NOS	7 (10%)
Productive Cough	7 (10%)
Sleep Disorder NOS	7 (10%)
Snoring	7 (10%)

Appendix 8.4. SAEs Reported by ≥ 10% of Patients in ACS001		
System Organ Class Serious Adverse Event	**Placebo, n (%) (n = 30)**	**All CyIS, n (%) (n = 36)**
Blood and Lymphatic Disorders	7 (23)	5 (14)
Anemia NOS	5 (17)	2 (6)
Leukopenia NOS	3 (10)	1 (3)
Neutropenia	3 (10)	0 (0)
Cardiac Disorders	5 (17)	6 (17)
Gastrointestinal Disorders	8 (27)	8 (22)
Abdominal Pain NOS	3 (10)	2 (6)
Nausea	3 (10)	1 (3)
Vomiting NOS	3 (10)	1 (3)
Gen. Disorders and Admin. Site Cond.	6 (20)	10 (28)
Pyrexia	3 (10)	5 (14)
Immune System Disorders	3 (10)	1 (3)
Infections and Infestations	19 (63)	17 (47)
Pneumonia Cytomegaloviral	6 (20)	1 (3)
Pneumonia NOS	6 (20)	4 (11)
Injury and Poisoning	3 (10)	2 (6)
Investigations	3 (10)	5 (14)
Metabolism and Nutrition Disorders	9 (30)	10 (28)
Neoplasm, Benign/Malignant	3 (10)	1 (3)
Nervous System Disorders	4 (13)	2 (6)
Psychiatric Disorders	1 (3)	5 (14)
Renal and Urinary Disorders	6 (20)	10 (28)
Renal Failure NOS	1 (3)	4 (11)
Renal Failure Acute	3 (10)	3 (8)
Respiratory, Mediastinal, Thoracic Disorders	16 (53)	17 (47)
Dyspnea Exacerbated	1 (3)	5 (14)
Dyspnea NOS	6 (20)	4 (11)
Pleural Effusion	3 (10)	1 (3)

Appendix 8.4. SAEs Reported by ≥ 10% of Patients in ACS001		
System Organ Class Serious Adverse Event	**Placebo, n (%) (n = 30)**	**All CyIS, n (%) (n = 36)**
Pneumothorax NOS	3 (10)	0 (0)
Pulmonary Embolism	5 (17)	2 (6)
Respiratory Failure	4 (13)	4 (11)
Vascular Disorders	7 (23)	13 (36)
Hypotension	1 (3)	6 (17)
Venous Thrombosis Deep Limb	3 (10)	5 (14)

Appendix 8.5. Deaths Occurring in ACS001 Patients.

ACS001 Patient No.	Age, yr	Sex	Duration of Exposure	Study Arm	Cause of Death	Diagnosis (Associated Morbidity Condition)
S0501	47	M	211	CyIS Open	Cytomegalovirus Pneumonitis	Multisystem Organ Failure, Diffuse Alveolar Damage
S0506	66	M	250	CyIS Open	Disseminated CMV Infection/Staphylococcal Sepsis	Thrombotic Thrombocytopenic Purpura, Acute Renal Failure
S0507	67	M	1105	CyIS Open	Bronchiolitis Obliterans	Parainfluenza, Virus Infection Pulmonary Infarct, Pulmonary Embolus
S0508	57	M	923	CyIS Open	Undifferentiated Carcinoma	Unknown
S0510	23	F	1935	CyIS Open	Bronchiolitis Obliterans	Acute Rejection
S101B	40	F	230	CyIS Random	Pneumonia	Acute Rejection, Renal Failure
S102A	65	M	945	Placebo	Unknown	Bronchiolitis Obliterans Syndrome
S109D	47	M	826	Placebo	Sepsis	Peritonitis, Bronchiolitis Obliterans
S112B	64	M	605	CyIS Random	Unknown	Bronchiolitis Obliterans, Acute Rejection
S119A	55	M	28	Placebo	Pneumonia	Acute Rejection, Diffuse Alveolar Damage
S123D	53	M	327	Placebo	Pneumonia	Pulmonary Embolus, Acute Rejection
S201A	60	M	1484	Placebo	Congestive Heart Failure	Bronchiolitis Obliterans, Renal Failure
S205D	51	M	91	Placebo	Pneumonia	Sepsis Pulmonary Embolus
S206A	64	M	583	Placebo	Bronchiolitis Obliterans	Pneumonia

Appendix 8.5. Deaths Occurring in ACS001 Patients.

ACS001 Patient No.	Age, yr	Sex	Duration of Exposure	Study Arm	Cause of Death	Diagnosis (Associated Morbidity Condition)
S303D	55	F	1217	Placebo	Bronchiolitis Obliterans	Acute Rejection
S304A	46	F	878	Placebo	Pulmonary Embolus	Bronchiolitis Obliterans
S305B	58	F	569	CyIS Random	Pneumonia	Bronchiolitis Obliterans, Acute Rejection
S306A	42	F	938	Placebo	Sepsis	Pneumonia, Pulmonary Hypertension
S313D	48	M	369	Placebo	Sepsis	Pneumonia
S317A	55	M	67	Placebo	Sepsis	Pneumonia, Graft Versus Host Disease
S402D	19	F	1020	Placebo	Unknown	Bronchiolitis Obliterans Syndrome, Pneumonia
S408D	51	M	344	Placebo	Unknown	Pneumonia Renal Failure

Appendix 8.6. Deaths Occurring in ACS002 Patients

ACS002 Patient No.	Age, yr	Sex	Duration of Exposure	Study Arm	Cause of Death	Diagnosis (Associated Morbidity Condition)
C005	59	M	63	CyIS	Fulminant Hepatitis C	Hepatorenal Failure
CH09	13	F	499	CyIS	Pneumonia	Unknown
R031	37	M	9	CyIS	Cerebellar Herniation	Cerebral Edema, Status Epilepticus, Acute Cellular Rejection
R037	29	F	2000	CyIS	Respiratory Failure	Renal Failure, Ventilator Dependence
R039	59	F	1056	CyIS	Cerebrovascular Hemorrhage	Unknown
R042	49	F	217	CyIS	Bronchiolitis Obliterans	Unknown
R047	54	F	498	CyIS	Shock and Respiratory Failure	Post-Transplant Lymphoproliferative Disorder, Sinusitis, Stomatitis
R053	30	F	1644	CyIS	Bronchiolitis Obliterans	Renal Failure
R057	57	M	1900	CyIS	Respiratory Failure Pseudomonas Infection	Unknown
R060	50	M	2211	CyIS	Carcinoma of the Lung	Unknown
R065	37	F	598	CyIS	Viral CMV Infection	Unknown
R071	38	F	122	CyIS	Bronchiolitis Obliterans Syndrome	Gastroparesis
R073	49	M	532	CyIS	Bacterial Septicemia	Rejection of Transplanted Lung, Alpha-1 Antitrypsin Deficiency, CMV Pneumonia
R082	24	F	626	CyIS	Pulmonary Embolism	Lung Abscesses, Respiratory Failure

Appendix 8.6. Deaths Occurring in ACS002 Patients

ACS002 Patient No.	Age, yr	Sex	Duration of Exposure	Study Arm	Cause of Death	Diagnosis (Associated Morbidity Condition)
R083	25	F	179	CyIS	Respiratory Failure	Aspiration Pneumonia, Aspergillus Tracheobronchitis
R085	31	F	2122	CyIS	Unknown - Lost to Follow up	Unknown
R087	32	M	1737	CyIS	Bacterial Septicemia	Unknown
R088	25	F	704	CyIS	Allograft Failure Secondary to Chronic Rejection	Unknown
R089	65	M	173	CyIS	Allograft Failure Secondary to Chronic Rejection	Unknown
R093	43	F	1290	CyIS	Graft Failure, Chronic Rejection	Unknown
R094	20	F	309	CyIS	Recurrent Lung Infection, Chronic Lung Rejection (Allograft) Recurrent	Cystic Fibrosis, Gastroparesis, Bilateral Pulmonary Fibrosis
R095	31	F	222	CyIS	Acute Cellular Rejection, Bacterial Pneumonia	Cystic Fibrosis, Pulmonary Hemorrhage, CMV Pneumonia
R097	60	M	37	CyIS	Acute Cellular Rejection Allograft Failure	Clostridium Difficile Colitis, Emphysema
R100	57	F	553	CyIS	Pulmonary Embolus	Pulmonary Hemorrhage, Crest Syndrome
R102	62	M	1928	CyIS	Graft Failure due to Chronic Rejection	Unknown
R105	66	M	939	CyIS	CMV Hemorrhagic Cholecystitis with Intraperitoneal Hemorrhage	Acute Respiratory Distress Syndrome, Bacterial Septicemia
R106	58	F	352	CyIS	Unknown	Unknown
R107	59	M	781	CyIS	Multi Organ Failure	Unknown

Appendix 8.6. Deaths Occurring in ACS002 Patients

ACS002 Patient No.	Age, yr	Sex	Duration of Exposure	Study Arm	Cause of Death	Diagnosis (Associated Morbidity Condition)
R115	52	M	253	CyIS	Bacterial Pneumonia, Allograft Failure	Unknown
R118	34	F	251	CyIS	Non-Specific Graft Failure	Unknown
R121	40	M	269	CyIS	Post Transplant Lymphoproliferative Disorder	Peritonitis, Acute Rejection, Renal Failure
R123	17	F	842	CyIS	Septic Shock	Respiratory Failure, Disseminated Intravascular Coagulation, Acute Renal Failure, Severe Metabolic Acidosis
R124	46	M	1004	CyIS	Bacterial Pneumonia	Cor Pulmonale, Renal Insufficiency
R126	29	F	138	CyIS	Sepsis	Pneumonia
X120	23	F	992	CyIS	Bronchiolitis Obliterans	Bronchopneumonia, Cor Pulmonale