Inhaled Cyclosporine and Pulmonary Function in Lung Transplant Recipients

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Abstract

Background: Chronic rejection, manifesting as bronchiolitis obliterans, is the leading cause of death in lung transplant recipients. In our previously reported double-blinded, placebo-controlled trial comparing inhaled cyclosporine (ACsA) to aerosol placebo, the rate of bronchiolitis-free survival improved. However, an independent analysis of pulmonary function, a secondary endpoint of the trial, was not performed. We sought to determine the effect of ACsA, in addition to systemic immunosuppression, on pulmonary function.

Methods: From 1998–2001, 58 patients were randomly assigned to inhale either 300 mg of ACsA (28 patients) or placebo aerosol (30 patients) 3 days a week for the first 2 years after transplantation. Longitudinal changes in pulmonary function of ACsA patients were compared to aerosol placebo patients. In another analysis, the rate of decline from 6-month maximum FEV₁ in randomized patients was compared to the rate of decline in patients receiving conventional immunosuppression from the Novartis transplant database (644 patients, 12 centers worldwide, transplanted from 1990–1995).

Results: The average duration of ACsA and aerosol placebo was 400 days $\pm$ 306 and 433 $\pm$ 256, respectively. The change in FEV₁ of ACsA patients (adjusted for Cytomegalovirus (CMV) mismatch and transplant type, followed for a maximum duration of 4.6 years) was superior to the aerosol placebo controls (9.0 $\pm$ 71.4 mL/year vs. -107.9 ± 55.3 , $p = 0.007$). The FEF_{25–75} decreased by -220.3 ± 117.7 L/(second $\times$ year) vs. -412.2 ± 139.2 , $p = 0.07$, respectively. Similarly, percent FEV₁ decline from maximal values was improved in ACsA patients compared to aerosol placebo and Novartis controls (ACsA $-0.43 \pm 1.12\%$ /year vs. aerosol placebo -4.08 ± 1.4 , $p = 0.04$; ACsA vs. Novartis -4.7 ± 0.31 , $p = 0.007$). Single-lung recipients receiving ACsA showed improvement in FEV₁ compared to Novartis controls (FEV₁ $-0.8 \pm 1.8\%$ /year vs. -4.94 ± 0.4 , $p = 0.03$) but double-lung recipients showed improvement compared to aerosol placebo controls only (FEV₁ $-0.28 \pm 1.22\%$ /year vs. -8.53 ± 5.95 , $p = 0.048$).

Conclusions: In this single center trial, ACsA appears to ameliorate important pulmonary function parameters in lung transplant recipients compared to aerosol placebo and historical control patients. Single- and double-lung transplant recipients may not respond uniformly to treatment, and ongoing randomized trials in lung transplant recipients using ACsA may help elucidate our findings.

Key words: inhaled cyclosporine, pulmonary function, lung transplant, chronic rejection, bronchiolitis obliterans

Introduction

OUTCOMES FOLLOWING LUNG TRANSPLANTATION are poor compared to the transplantation of other solid organs.⁽¹⁾ Death is commonly due to chronic rejection, a process that occurs in more than half of all lung recipients.⁽²⁾ Pre-

vention of this process remains the greatest challenge to this field. Chronic rejection presents histologically as bronchiolitis obliterans and manifests physiologically as a progressive decline in FEV₁. Bronchiolitis obliterans syndrome, the physiologic manifestation of chronic rejection, is defined as a loss of FEV₁ of 20% or greater from the individual patient's

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maximum post-transplant value.⁽³⁾ Preventative and therapeutic strategies for this process have been largely unsuccessful.⁽²⁾ Obliterative bronchiolitis most likely represents an immune process having multiple risk factors and related to the abnormal proliferation of T cells.⁽⁴⁻⁶⁾ Because the immunosuppressive effect of cyclosporine is dose dependent, targeted delivery of this drug to the inflamed airways by inhalation may improve efficacy by increasing local cyclosporine concentrations.^(7,8)

Research in animal and human lung transplant models using inhaled cyclosporine (ACsA) shows that the drug deposits in high concentrations in lung tissue and it reduces the inflammatory response associated with rejection without toxicity.⁽⁹⁻¹¹⁾ Moreover, a recently reported double-blinded, placebo-controlled trial using ACsA demonstrated significant improvement of survival and freedom from chronic rejection in patients randomized to the drug arm versus aerosol placebo.⁽¹²⁾ Although an important secondary outcome parameter, longitudinal changes of lung function of all patients in the aforementioned study was not analyzed. Thus, as an additional analysis to our initial report, we sought to assess the effect of prophylactic ACsA on pulmonary function by comparing ACsA patients to aerosol placebo patients and, in order to further evaluate these results, to historical control patients from the Novartis lung transplant database.

Methods

Study design

A randomized, double-blinded, placebo-controlled trial of ACsA, given in addition to conventional immunosuppression, was conducted at the University of Pittsburgh Medical Center with institutional review board approval. Fifty-eight recipients were enrolled from 1998 to 2001. Recipients were eligible for the trial if they signed informed consent, were at least 18 years of age, and underwent single or bilateral lung transplantation. Study criteria had to be met within 42 days after transplantation and drug or placebo was started no later than 55 days after transplantation. Patients were excluded from this study if they had an active pulmonary infection, unresolved diffuse alveolar damage, untreated bronchial stenosis, or were receiving mechanical ventilation. Because peak lung function is typically achieved more than 6 months after transplantation, three patients who did not survive beyond 6 months were excluded from this analysis.⁽¹²⁾

Inhaled cyclosporine administration

After receiving 2% lidocaine inhalation (3 ml) and 2.5 mg of albuterol, patients were given inhaled cyclosporine mixed in propylene glycol (62.5 mg/ml) or placebo (propylene glycol alone) administered initially for ten consecutive days, then thrice weekly using a commercially available jet nebulizer (AeroTech II, CIS-US, Bedford, MA). ACsA was initiated at a dose of 100 mg and increased by incremental doses of 100 mg up to 300 mg or to a maximally tolerated dose. After documented patient competence, aerosols were self-administered by patients and temporarily discontinued if the treating physician reported an infection that persisted after antibiotic therapy. ACsA and aerosol placebo compliance was assessed by weekly phone calls by a nurse coordinator and scheduled follow-up clinic visits, at which time drug

vials were quantified and patients were interviewed in reference to their use of study drug or placebo.

Post-transplant monitoring

Because bronchiolitis obliterans is not uniformly detectable by biopsy, spirometry is used as a surrogate marker to diagnose this process.⁽³⁾ In randomized patients, spirometry was routinely performed at approximately 3-month intervals for the first 2 post-operative years and then at 4- to 6-month intervals thereafter. Although not part of the randomized trial, similar interval spirometric measurements were obtained among patients in the Novartis control database (ACsA 10.8 ± 1.0 PFTs/patients/year, aerosol placebo 13.1 ± 1.1 , Novartis 13.0 ± 0.4 , $p = \text{NS}$). In all randomized study subjects, spirometry was performed in accordance with American Thoracic Society standards and expressed as the percentage of predicted values.⁽¹³⁾ Airflow measurements were evaluated for bronchiolitis obliterans syndrome criteria, defined as a sustained decrease in FEV₁ of at least 20% from the individual patient's maximum values in the absence of other causes for functional loss.^(1,3)

Clinical management

Both ACsA and aerosol placebo groups received conventional immunosuppression including tacrolimus (0.06 mg/kg/day), azathioprine (2 mg/kg/day), and prednisone (20 mg/day) in addition to their aerosol treatments. Subsequent adjustment was at the discretion of the treating clinician. Enhanced immunosuppression for treatment of acute rejection ($\geq$ grade 2) or active bronchiolitis obliterans consisted of pulse corticosteroids (intravenous methylprednisolone, 1 g/day $\times$ 3 days or oral prednisone, 100 mg tapered to 10 mg over 14 days) or rabbit antithymocyte globulin (Thymoglobulin, 1.5 mg/kg/day $\times$ 5-7 days) (Sangstat, Fremont, CA).

End points

The primary end point of the randomized trial was frequency of histologic acute rejection as reported in the previous trial.⁽¹²⁾ Prespecified secondary end points of the randomized trial included an analysis of mean pulmonary function testing, assessment of longitudinal post-transplant changes of function and rate of decline from maximum lung function values. The rate of decline from maximum FEV₁ in ACsA and aerosol placebo patients was also compared to Novartis lung transplant patients.

Novartis lung transplant database

This database includes 12 international lung transplant centers with a total of 700 patients (331 single-lung and 369 double-lung and heart-lung recipients transplanted between 1/1/1990 and 12/31/1995). Exclusion criteria included patients younger than age 14 or patients who received lobar transplants or retransplants. Participating centers included Royal Prince Alfred Hospital, Sydney, Australia; Saint Vincent's Hospital, Sydney, Australia; Hannover Medical School, Hannover, Germany; Papworth Hospital, Cambridge, United Kingdom; Cedars Sinai Medical Center, Los Angeles, California; Cleveland Clinic, Cleveland, Ohio; New York-Presbyterian, The University Hospital of Columbia

and Cornell, New York, New York; Duke University Hospital, Durham, North Carolina; University of Pittsburgh Medical Center, Pittsburgh, Pennsylvania; Loyola Hospital, Chicago, Illinois; Stanford Hospital, Stanford, California; and University of California San Francisco Medical Center, San Francisco, California.

Statistics

We sought to determine the effect of ACsA on pulmonary function by comparing ACsA to aerosol placebo and Novartis historical control patients. Randomized patients who received either ACsA or aerosol placebo were analyzed by intention to treat and no patients were lost to follow-up. All randomized patients were followed for a minimum and maximum of 2 to 4.6 years, until study closure in August 2003, and all lung function values were included. To determine whether functional changes in aerosol placebo patients were consistent with general lung transplant patients receiving systemic immunosuppression, Novartis controls were used. Demographic data and calculated means were recorded as mean $\pm$ standard error and are shown excluding

patients who did not survive beyond 6 months. Continuous variables were assessed using two-sided Student's *t*-test or analysis of variance (ANOVA). Categorical values were compared using the chi-square test.

Mean lung function values in randomized patients were compared using three-way ANOVA adjusting for aerosol type, Cytomegalovirus (CMV) donor/recipient matching and type of transplant. Longitudinal changes in FVC, FEV₁, and FEF₂₅₋₇₅ in the randomized study subjects were further assessed by least-squares linear regression utilizing data from the randomized trial. In this analysis, the slopes or rates of decline were compared using three-way ANOVA adjusting for CMV donor/recipient matching and type of transplant. Patients not surviving past 6 months post-transplantation in all three groups (ACsA, aerosol placebo, and Novartis controls) were excluded from this analysis because peak FEV₁ values typically occurred following a 6-month interval from transplantation. FEV₁ values were standardized to each patient's respective maximum using the following formula: (FEV₁ – maximum FEV₁)/maximum FEV₁ $\times$ 100%. FVC and FEF₂₅₋₇₅ were standardized in a similar manner. Functional values were assessed at 6-month intervals $\pm$ 2 months from the date of

TABLE 1. DEMOGRAPHICS AND IMMUNOSUPPRESSIVE REGIMENS OF PATIENTS SURVIVING BEYOND 6 MONTHS

Patient characteristics		ACsA (N = 28)	Aerosol placebo (N = 27)	Novartis controls (N = 644)
Age (years $\pm$ SEM)		50.9 $\pm$ 2.3 ^a	51.9 $\pm$ 2.3 ^b	42.4 $\pm$ 0.5 ^{a,b}
Sex	Male (% patients)	17 (61)	15 (56)	299 (46)
	Female	11 (39)	12 (44)	344 (53)
Diagnosis	COPD/Emphysema	13 (46)	15 (56)	293 (46)
	Cystic fibrosis	5 (18)	4 (15)	126 (20)
	IPF	6 (21) ^{c,d}	2 (7) ^c	55 (9) ^d
	Pulmonary hypertension	1 (4) ^e	0 (0)	132 (21) ^e
	Connective tissue Disease	1 (4)	2 (7)	29 (5)
	Other	2 (7) ^f	4 (15) ^g	9 (1) ^{f,g}
Transplant type	Single	17 (61) ^h	23 (85) ^{h,i}	299 (46) ^l
	Double	11 (39) ^j	4 (15) ^{j,k}	345 (54) ^k
PFT data	Total PFTs	575	551	18278
	PFTs/patient/year	10.8 $\pm$ 1.0	13.1 $\pm$ 1.1	13.0 $\pm$ 0.4
	Time to maximum FEV ₁ (years)	1.19 $\pm$ 0.18	0.86 $\pm$ 0.1	0.87 $\pm$ 0.03
	Mean follow-up (years)	2.73 $\pm$ 0.19	2.48 $\pm$ 0.22	2.66 $\pm$ 0.06
	Mean maximum FEV ₁	2.51 $\pm$ 0.17	2.09 $\pm$ 0.12 ^l	2.57 $\pm$ 0.04 ^l
	Mean last FEV ₁	2.10 $\pm$ 0.22	1.57 $\pm$ 0.11	1.81 $\pm$ 0.04
Immunosuppressive therapy	Prednisone	25 (89)	25 (93)	643 (100)
	Azathioprine	6 (21) ^m	8 (30) ⁿ	618 (96) ^{m,n}
	Cyclosporine	4 (14) ^{o,q}	1 (4) ^{p,q}	629 (98) ^{o,p}
	Tacrolimus	24 (86) ^r	26 (96) ^s	26 (1) ^{r,s}
	Mycophenolate mofetil	5 (18) ^t	7 (26) ^u	22 (1) ^{t,u}
Rescue treatments	Methylprednisolone pulses (patient/year)	1.22 $\pm$ 0.24	1.32 $\pm$ 0.27	1.98 $\pm$ 0.19
	Antithymocyte globulin treatments (patient/year)	0.24 $\pm$ 0.12	0.22 $\pm$ 0.09	0.11 $\pm$ 0.02
CMV status	CMV mismatch	5 (18)	7 (26)	N/A
	CMV other	23 (82)	20 (74)	N/A
Biopsies (patient/year)		5.90 $\pm$ 0.49	5.71 $\pm$ 0.47	4.09 $\pm$ 0.32
Acute rejection events (patient/year)		1.24 $\pm$ 0.11	1.30 $\pm$ 0.11	1.83 $\pm$ 0.14

^a*p* = 0.002, ^b*p* = 0.001, ^c*p* = 0.008, ^d*p* = 0.03, ^e*p* = 0.001, ^f*p* = 0.03, ^g*p* < 0.001, ^h*p* = 0.05, ⁱ*p* < 0.001, ^j*p* = 0.001, ^k*p* < 0.001, ^l*p* = 0.03, ^m*p* < 0.001, ⁿ*p* < 0.001, ^o*p* < 0.001, ^p*p* < 0.001, ^q*p* = 0.02, ^r*p* < 0.001, ^s*p* < 0.001, ^t*p* < 0.001, ^u*p* < 0.001.

N/A = not available; COPD = chronic obstructive pulmonary disease; ACsA = inhaled cyclosporine; CMV = cytomegalovirus; IPF = idiopathic pulmonary fibrosis; PFT = pulmonary function test.

TABLE 2. MEAN LUNG FUNCTION IN PATIENTS RANDOMIZED TO ACsA AND AEROSOL PLACEBO

Patient groups	Treatment	FVC (L)	FEV ₁ (L)	FEF ₂₅₋₇₅ (L/sec)
All (N = 55)	ACsA (N = 28)	2.92 ± 0.05 ^a (72.0 ± 0.8)	2.09 ± 0.04 ^b (67.8 ± 0.9)	1.78 ± 0.06 ^c (53.2 ± 1.5)
	Aerosol placebo (N = 27)	2.39 ± 0.03 ^a (62.8 ± 0.6)	1.67 ± 0.02 ^b (58.1 ± 0.6)	1.27 ± 0.04 ^c (41.1 ± 1.0)
Single Lung (N = 40)	ACsA (N = 17)	2.40 ± 0.03 ^d (64.3 ± 0.8)	1.59 ± 0.02 (57.8 ± 0.9)	1.12 ± 0.04 (39.4 ± 1.5)
	Aerosol placebo (N = 23)	2.29 ± 0.03 ^d (62.3 ± 0.6)	1.60 ± 0.02 (58.5 ± 0.6)	1.15 ± 0.03 (39.6 ± 0.9)
Double Lung (N = 15)	ACsA (N = 11)	3.67 ± 0.08 ^e (83.6 ± 1.2)	2.82 ± 0.06 ^f (82.6 ± 1.3)	2.73 ± 0.09 ^g (74.0 ± 2.3)
	Aerosol placebo (N = 4)	2.99 ± 0.09 ^e (65.6 ± 1.6)	2.07 ± 0.10 ^f (56.5 ± 2.5)	2.01 ± 0.16 ^g (50.0 ± 4.1)

Percent predicted in parentheses after absolute value, $p \leq 0.05$ corresponds with significance in absolute spirometric values.

^a $p < 0.001$, ^b $p < 0.001$, ^c $p < 0.001$, ^d $p = 0.01$, ^e $p < 0.001$, ^f $p < 0.001$, ^g $p < 0.001$.

Data presented as mean values for the entire group ± standard error and are adjusted for type of transplant and CMV status.

transplantation for up to 48 months. Slopes may be positive or negative depending on the time point of the maximum post-transplant lung function value. Least-squares regression was used and slopes were obtained for ACsA, aerosol placebo, and Novartis patients. These slopes were compared using the two-sided Student's *t*-test and *p*-values are significant if ≤ 0.05 . All analyses were conducted with the use of SPSS 15.0 software.

Results

Patient characteristics

In the randomized trial, 28 patients received ACsA and 30 patients received aerosol placebo. Three patients were excluded because they did not survive past 6 months (duration of ACsA and placebo, respectively 400 days ± 306, range 0–735 and 433 ± 256, range 42–742). The duration of follow-up in ACsA, aerosol placebo, and Novartis patients was 2.7 years ± 0.19, 2.5 ± 0.22 and 2.7 ± 0.06, respectively ($p = \text{NS}$). All 1126 pulmonary function tests (PFTs) from the randomized study were analyzed. No patients were lost to follow-up. There were six patients in the ACsA group and four in the aerosol placebo group who were noncompliant and withdrew from the study but were followed to study completion. Table 1 shows the demographic and immunosuppressive information for the ACsA, aerosol placebo, and Novartis patients. There were more single-lung transplant recipients in the aerosol placebo group (ACsA vs. aerosol placebo: 17 vs. 23, $p = 0.05$) and more double lungs in the ACsA group (ACsA vs. aerosol placebo: 11 vs. 4, $p = 0.001$). There were more patients with idiopathic pulmonary fibrosis in the ACsA group than aerosol placebo ($p = 0.008$) and Novartis controls ($p = 0.03$).

The Novartis control group included 644 patients following the exclusion of patients who did not live beyond 6 months post-transplant. All 18,278 PFTs from this cohort were analyzed. The Novartis controls were similar to the ACsA group in sex, transplant type, rejection events, lung biopsies, and

pulses of augmented immunosuppression (Table 1). These groups did differ with respect to age, Novartis recipients being younger compared to ACsA ($p = 0.002$). ACsA patients received more tacrolimus and mycophenolate mofetil than Novartis controls ($p < 0.001$).

Mean lung function parameters in randomized patients

ACsA patients maintained greater spirometric values than aerosol placebo controls; (FVC 2.92 ± 0.05 L vs. 2.39 ± 0.03, $p < 0.001$; FEV₁ 2.09 ± 0.04 L vs. 1.67 ± 0.02, $p < 0.001$; FEF₂₅₋₇₅ 1.78 ± 0.06 L/sec vs. 1.27 ± 0.04, $p < 0.001$) (Table 2). Single-lung recipients had significant differences in FVC exclusively (ACsA 2.40 ± 0.03 L vs. aerosol placebo 2.29 ± 0.03, $p = 0.01$), whereas double-lung recipients demonstrated differences in all functional parameters (FVC 3.67 ± 0.08 L vs. 2.99 ± 0.09, $p < 0.001$; FEV₁ 2.82 ± 0.06 L vs. 2.07 ± 0.1, $p < 0.001$; FEF₂₅₋₇₅ 2.73 ± 0.09 L/sec vs. 2.01 ± 0.16, $p < 0.001$).

Longitudinal pulmonary function changes in randomized patients

Using all lung function values in 6-month survivors and linear regression to calculate individual patient slopes, ACsA resulted in continuing improvement of FEV₁ compared to a negative slope in patients receiving aerosol placebo (FEV₁ 9.0 ± 71.4 mL/year vs. −107.9 ± 55.3, $p = 0.007$; FEF₂₅₋₇₅ −220.3 ± 117.7 L/(second × year) vs. −412.2 ± 139.2, $p = 0.07$) (Table 3). Single-lung recipients showed no difference in pulmonary function when compared to aerosol placebo (FEV₁ 12.2 ± 80.5 mL/year vs. −74.3 ± 39.8, $p = 0.50$; FEF₂₅₋₇₅ −94.4 ± 104.4 L/(second × year) vs. −383.5 ± 148.0, $p = 0.70$). In contrast, double-lung recipients using ACsA had improved pulmonary function (FVC 276.0 ± 74.0 mL/year vs. −35.8 ± 149.3, $p = 0.002$; FEV₁ 4.2 ± 137.7 mL/year vs. −301.4 ± 311.6, $p = 0.01$; FEF₂₅₋₇₅ −414.9 ± 249.2 L/(second × year) vs. −576.7 ± 445.4, $p = 0.05$).

TABLE 3. LONGITUDINAL CHANGES IN PULMONARY FUNCTION IN THE ACsA AND AEROSOL PLACEBO PATIENTS

Patient groups	Treatment group	FVC (mL/year)	FEV ₁ (mL/year)	FEF ₂₅₋₇₅ [(mL)/(sec × year)]
All (N = 55)	ACsA (N = 28)	181.6 ± 62.46	9.0 ± 71.4 ^a	−220.3 ± 117.7 ^b
	Aerosol placebo (N = 27)	71.6 ± 64.3	−107.9 ± 55.3 ^a	−412.2 ± 139.2 ^b
Single lung (N = 40)	ACsA (N = 17)	120.6 ± 89.7	12.2 ± 80.5	−94.4 ± 104.4
	Aerosol placebo (N = 23)	90.3 ± 71.4	−74.3 ± 39.8	−383.5 ± 148.0
Double lung (N = 15)	ACsA (N = 11)	276.0 ± 74.0 ^c	4.2 ± 137.7 ^d	−414.9 ± 249.2 ^e
	Aerosol placebo (N = 4)	−35.8 ± 149.3 ^c	−301.4 ± 311.6 ^d	−576.7 ± 445.4 ^e

^a $p = 0.007$, ^b $p = 0.07$, ^c $p = 0.002$, ^d $p = 0.01$, ^e $p = 0.05$.

Data presented as mean values for the entire group ± standard error.

Changes from maximum 6-month post-transplant forced expiratory volume in one second in inhaled cyclosporine, aerosol placebo, and Novartis controls

The decline from maximal 6-month FEV₁ values during a 48-month follow-up was more than eightfold less in ACsA patients compared to the aerosol placebo and the

Novartis controls (ACsA vs. aerosol placebo: FEV₁ $-0.43 \pm 1.12\%$ /year vs. -4.08 ± 1.4 , $p=0.04$; FVC $0.56 \pm 0.74\%$ /year vs. -3.24 ± 1.13 , $p=0.005$) (Table 4A, Figure 1A). A similar pattern in FEV₁ decline from maximum was observed with ACsA compared to the Novartis controls (FEV₁ $-0.43 \pm 1.12\%$ /year vs. -4.70 ± 0.31 , $p=0.007$) (Fig. 1B).

TABLE 4. PERCENT CHANGE PER YEAR FROM MAXIMUM LUNG FUNCTION VALUES IN ACsA COMPARED TO AEROSOL PLACEBO AND NOVARTIS CONTROL PATIENTS

A: All Patients		6–18 Months	6–24 Months	6–30 Months	6–36 Months	6–42 Months	6–48 Months
FVC (percent change)	ACsA	2.04 ± 2.65	2.20 ± 1.67^a	0.79 ± 1.32^b	-0.096 ± 1.03^c	0.36 ± 0.86^d	0.56 ± 0.74^e
	Aerosol placebo	-2.62 ± 3.16	-4.8 ± 2.2^a	-6.35 ± 1.85^b	-4.94 ± 1.54^c	-4.07 ± 1.28^d	-3.24 ± 1.13^e
	Novartis	1.75 ± 0.67	0.53 ± 0.44	0.07 ± 0.34	-0.3 ± 0.28	-0.79 ± 0.24	-1.06 ± 0.20
FEV ₁ (percent change)	ACsA	-3.00 ± 3.79	-0.98 ± 2.42^f	-2.42 ± 1.99^g	-1.98 ± 1.55^h	$-1.02 \pm 1.30^{i,k}$	$-0.43 \pm 1.12^{j,l}$
	Aerosol placebo	-6.61 ± 3.46	-7.85 ± 2.6^f	-9.08 ± 2.29^g	-6.29 ± 1.91^h	-5.14 ± 1.61^i	-4.08 ± 1.4^j
	Novartis	-3.31 ± 0.95	-4.30 ± 0.65	-4.20 ± 0.50	-4.43 ± 0.41	-4.65 ± 0.36^k	-4.70 ± 0.31^l
FEF _{25–75} (percent change)	ACsA	-11.23 ± 5.75	-6.97 ± 9.84	-7.5 ± 2.93	-5.76 ± 2.35	-5.02 ± 1.98	-3.8 ± 1.76
	Aerosol placebo	-12.86 ± 6.61	-9.85 ± 4.1	-9.62 ± 3.29	-5.48 ± 2.77	-4.2 ± 2.35	-3.8 ± 2.04
	Novartis	-10.81 ± 1.64	-9.98 ± 1.12	-8.53 ± 0.85	-8.38 ± 0.68	-7.93 ± 0.59	-7.44 ± 0.52
B: Single-Lung Recipients		6–18 Months	6–24 Months	6–30 Months	6–36 Months	6–42 Months	6–48 Months
FVC (percent change)	ACsA	-0.9 ± 4.14	-0.38 ± 2.65	-1.87 ± 2.08	-1.84 ± 1.57	-1.26 ± 1.38	-0.4 ± 1.2
	Aerosol placebo	-4.91 ± 3.6	-5.02 ± 2.52	-5.38 ± 2.09	-4.79 ± 1.73	-4.03 ± 1.4	-2.99 ± 1.21
	Novartis	-0.76 ± 0.94	-1.48 ± 0.64	-1.08 ± 0.47	-1.03 ± 0.37	-1.54 ± 0.32	-1.74 ± 0.29
FEV ₁ (percent change)	ACsA	-5.05 ± 5.38	-2.84 ± 3.6	-5.28 ± 3.05	-3.54 ± 2.32	-2.18 ± 2.05	-0.8 ± 1.8^a
	Aerosol placebo	-8.65 ± 4.01	-5.65 ± 2.64	-5.6 ± 2.16	-4.61 ± 1.78	-4.36 ± 1.48	-3.37 ± 1.26
	Novartis	-5.12 ± 1.18	-5.96 ± 0.83	-5.24 ± 0.64	-4.9 ± 0.52	-5.02 ± 0.44	-4.94 ± 0.4^a
FEF _{25–75} (percent change)	ACsA	-10.25 ± 8.04	-6.59 ± 5.56	-9.11 ± 4.19	-6.06 ± 3.3	-5.09 ± 2.92	-3.25 ± 2.59
	Aerosol placebo	-14.92 ± 7.32	-7.43 ± 4.43	-6.98 ± 3.46	-4.16 ± 2.89	-4.09 ± 2.4	-3.61 ± 2.04
	Novartis	-9.76 ± 2.17	-9.65 ± 1.45	-8.02 ± 1.09	-7.75 ± 0.86	-7.42 ± 0.74	-7.00 ± 0.66
C: Double-Lung Recipients		6–18 Months	6–24 Months	6–30 Months	6–36 Months	6–42 Months	6–48 Months
FVC (percent change)	ACsA	6.18 ± 2.41	5.08 ± 1.38^a	3.71 ± 1.03^b	2.02 ± 0.89^c	1.69 ± 0.73^d	1.24 ± 0.66^e
	Aerosol Placebo	5.53 ± 3.36	-4.88 ± 3.62^a	-11.08 ± 3.85^b	-6.34 ± 3.4^c	-4.69 ± 3.04^d	-4.69 ± 3.04^e
	Novartis	4.04 ± 0.94	2.34 ± 0.62	1.16 ± 0.48	0.46 ± 0.4	-0.06 ± 0.34	-0.4 ± 0.3
FEV ₁ (percent change)	ACsA	-0.14 ± 5.28	1.1 ± 3.08^f	0.78 ± 2.22^g	-0.06 ± 1.8^h	-0.08 ± 1.42^i	-0.28 ± 1.22^j
	Aerosol Placebo	0.55 ± 3.65	-17.9 ± 7.98^f	-23.1 ± 7.16^g	-12.6 ± 6.54^h	-8.53 ± 5.95^i	-8.53 ± 5.95^j
	Novartis	-1.7 ± 1.44	-2.82 ± 0.98	-3.25 ± 0.77	-3.98 ± 0.64	-4.3 ± 0.55	-4.46 ± 0.48
FEF _{25–75} (percent change)	ACsA	-12.53 ± 8.33	-7.48 ± 5.3	-5.68 ± 4.08	-5.42 ± 3.38	-5.05 ± 2.66	-4.48 ± 2.4
	Aerosol Placebo	-6.28 ± 15.4	-21.24 ± 10.86	-20.77 ± 9.12	-10.87 ± 7.96	-4.93 ± 7.38	-4.93 ± 7.38
	Novartis	-11.66 ± 2.41	-10.18 ± 1.63	-8.78 ± 1.26	-8.62 ± 1.04	-8.11 ± 0.9	-7.64 ± 0.79

^a $p=0.01$, ^b $p=0.002$, ^c $p=0.008$, ^d $p=0.004$, ^e $p=0.005$, ^f $p=0.06$, ^g $p=0.03$, ^h $p=0.08$, ⁱ $p=0.05$, ^j $p=0.04$, ^k $p=0.04$, ^l $p=0.007$.

^a $p=0.03$.

^a $p=0.003$, ^b $p=0.000$, ^c $p=0.002$, ^d $p=0.005$, ^e $p=0.007$, ^f $p=0.009$, ^g $p=0.000$, ^h $p=0.01$, ⁱ $p=0.05$, ^j $p=0.048$.

FIG. 1. Percent change from maximum FEV₁ values within 6 months of transplantation are shown at 6-month intervals in ACsA, aerosol placebo (A) and Novartis control patients (B). Each point represents a mean percent FEV₁ change from maximum calculated at 6-month intervals. Individual lines are calculated using least-squares linear regression using all points from six to 48 months. Stabilization of lung function is noted in ACsA patients by slope analysis between 6 and 48 months compared to aerosol placebo (A) and Novartis controls (B) ($p=0.04$, $p=0.007$, respectively). N represents the number of pulmonary function tests used in the linear regression analysis of each group.

Analyzing lung function changes by transplant type, single-lung recipients receiving ACsA had approximately five-fold improvement in FEV₁ compared to Novartis controls (FEV₁ $-0.8 \pm 1.8\%/year$ vs. -4.94 ± 0.4 , $p=0.03$) (Table 4B, Figure 2A); significance was achieved only at the 48 months. In contrast, double-lung recipients showed consistent improvement in FEV₁ and FVC compared to aerosol placebo (FEV₁ $-0.28 \pm 1.22\%/year$ vs. -8.53 ± 5.95 , $p=0.048$; FVC $1.24 \pm 0.66\%/year$ vs. -4.69 ± 3.04 , $p=0.007$) (Table 4C, Fig. 2B). No difference was observed between ACsA and Novartis double-lung patients.

Discussion

Chronic rejection, presenting as bronchiolitis obliterans syndrome, is the principal cause of late mortality after lung transplantation.^(14,15) This process accounts for the poor prognosis of recipients of a lung allograft compared to other solid organ transplant transplants. Unfortunately, no therapeutic advancements have been made over the previous 2 decades in either the prevention or treatment of chronic rejection.^(16,17) Because the therapeutic index of systemically delivered cyclosporine is low but its effect on lymphocyte suppression and allograft rejection is dose dependent, we hypothesized that targeted delivery of cyclosporine directly to bronchioles via inhalation would ameliorate allograft function.⁷

In our previous double-blinded, placebo-controlled trial comparing ACsA to aerosol placebo controls published in 2006, a substantial survival advantage as well as an improvement in the rate of bronchiolitis-free survival was demonstrated but a detailed analysis of lung function was not performed. In order to assess the effect of ACsA on pulmonary function, ACsA patients were compared to two

control groups. One group consisted of aerosol placebo subjects who were part of the double-blinded randomized trial. Because 14 of the 30 aerosol placebo subjects expired during the study, a significant survival bias could account for the observed difference in bronchiolitis obliterans syndrome in the previously published manuscript. Therefore, study patients were also compared to historical control patients from a large, international database comprising 644 patients who underwent lung transplantation from 1990 to 1995 in 12 centers worldwide. Based on the current analysis of lung function, a secondary end point of the aforementioned trial, patients who were randomized to ACsA soon after lung transplantation in addition to conventional immunosuppression appear to demonstrate improvement in important lung function parameters compared to both control groups receiving conventional immunosuppression exclusively. Since those patients who received ACsA represent a relatively small number of study subjects, these findings will need to be corroborated in a larger trial.

There are other obvious limitations to our study. One discrepancy is in the transplantation era between the randomized subjects (1998 to 2001) compared to the Novartis controls (1990 to 1995). Although a modest but significant improvement in overall survival is noted over the past decade of lung transplant experience, this difference is predominately related to posttransplant survival in the first year. According to the 2008 International Society of Heart and Lung Transplantation registry, survival among 1-year survivors has not appreciably changed among the time periods of 1988 to 1994 and 2000 to 2006 (6.9 years vs. 7.1 years respectively).⁽¹⁶⁾ Because our analysis evaluates only those patients who have survived greater than 6 months after transplantation, the chance of a survival bias affecting lung

FIG. 2. Percent change from maximum FEV_1 values within 6 months of transplantation for single- and double-lung recipients are shown at 6-month intervals in ACsA, aerosol placebo, and Novartis control patients. Each point represents a mean value of FEV_1 from each individual patient's maximum calculated at 6-month intervals. Individual lines are calculated using least-squares linear regression using all points from 6 to 48 months. Stabilization of lung function in single-lung recipients is noted in ACsA patients by slope analysis at 48 months compared to Novartis controls (**B**; $p = 0.03$) but not to aerosol placebo (**A**; $p = 0.23$). A similar pattern is seen in double-lung ACsA recipients compared to aerosol placebo (**C**; $p = 0.048$) but not to Novartis controls (**D**; $p = 0.10$). N represents the number of pulmonary function tests used in the linear regression analysis of each group.

function parameters may be reduced. In support of this concept, longitudinal lung function was found to be similar between the aerosol placebo and the Novartis control patients who both received conventional immunosuppression regimens. Another limitation includes baseline immunosuppression variation among the Novartis controls and the study group. The impact of immunosuppressive variation remains inconclusive. Some studies show a trend toward fewer acute and chronic rejection events with tacrolimus and

mycophenolate mofetil but results in reference to pulmonary function were not statistically significant or were not evaluated.^(18,19) Other studies have shown no difference between tacrolimus and cyclosporine in the rate of acute rejection or survival,^(20,21) and there have been no immunosuppressive drug regimens in any transplant era that has been shown to decrease the rate of decline in FEV_1 .

The type of transplant may also limit efficacy of the drug. Double-lung recipients demonstrated a greater degree of

benefit in all values compared to aerosol placebo but the number of study subjects was small, and results need to be confirmed in a larger study. In contrast, no differences in longitudinal functional values were observed among randomized single lung patients. Significant variability of the cyclosporine deposited in the transplanted lung has been previously demonstrated, with drug deposition per allograft ranging from 6.5 to 26.9 mg.^(22,23) There also appears to be a threshold drug dose of cyclosporine that is necessary to provide an improvement in lung function. In a subset of 15 ACsA recipients from the randomized trial who underwent aerosol deposition testing, higher peripheral allograft cyclosporine concentrations were found to offer a therapeutic advantage in longitudinal FEV₁ improvement in a dose-dependent fashion with higher peripheral lung doses resulting in the greatest augmentation of FEV₁ over a 6-month interval.⁽²⁴⁾ Thus, variability of allograft dosing may explain absence of a functional difference in single-lung recipients because native lung deposition could potentially account for an inconsistent portion of the inhaled dose.

Chronic rejection, the Achilles' heel of lung transplantation, is a devastating process limiting the utility of this intervention resulting in an inexorable loss of lung function and excessive patient disability and mortality. Although the pathogenesis of chronic rejection may be multifactorial, preservation of lung function noted in this analysis further supports the clinically important survival advantage, the reduction in bronchiolitis obliterans syndrome, and the histological bronchiolitis obliterans-free survival demonstrated in the previously reported randomized controlled trial and other clinical trials supporting the use of ACsA in lung recipients. Should the results of ongoing clinical studies using ACsA in greater patient numbers demonstrate a similar functional benefit, ACsA would become a useful adjunct for lung transplant immunosuppression.

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Author Disclosure Statement

Drs. Iacono, Corcoran, and Johnson act as consultants for APT, the company that is performing the multicenter lung transplant trial using inhaled cyclosporine. Dr. Iacono has a patent for inhaled cyclosporine through the University of Pittsburgh School of Medicine. Dr. Smaldone also assisted in the development of inhaled cyclosporine at the State University of New York at Stony Brook. Royalties would be distributed to SUNY and the University of Pittsburgh if the drug is commercialized.

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