Title: EXTRACORPOREAL PHOTOPHERESIS TO ATTENUATE DECLINE IN LUNG FUNCTION DUE TO REFRACTORY OBSTRUCTIVE ALLOGRAFT DYSFUNCTION.

Authors: THE EPI STUDY GROUP: Chadi A. Hage,[1] Julia Klesney-Tait,[2] Keith Wille,[3] Selim Arcasoy,[4] Gordon Yung,[5] Marshall Hertz,[6] Kevin M. Chan,[7] Matt Morrell,[8] Hilary Goldberg,[9] Suresh Vedantham,[10] Mary Clare Derfler,[10] Paul Commean,[11] Keith Berman,[12] Ed Spitznagel,[13] Jeff Atkinson,[14] George Despotis[15]

Affiliations: [1]Indiana University Department of Medicine, Division of Pulmonology,

[2]University of Iowa Department of Medicine, Division of Pulmonology,

[3]University of Alabama, Department of Medicine, Division of Pulmonology,

[4]Columbia University of Alabama, Department of Medicine, Division of Pulmonology,

[5]University of California San Diego, Department of Medicine, Division of Pulmonology,

[6]University of Minnesota, Department of Medicine, Division of Pulmonology,

[7]University of Michigan, Department of Medicine, Division of Pulmonology,

[8]University of Pittsburgh, Department of Medicine, Division of Pulmonology,

[9]Brigham and Women's Hospital, Harvard Medical School, Department of Medicine, Division of Pulmonology,

[10]Clinical Coordinating Center, Washington University School of Medicine, Mallinckrodt Institute of Radiology,

[11]Data Coordinating Center, Washington University School of Medicine, Mallinckrodt Institute of Radiology,

[12]Health Research Associates,

[13]Washington University, Department of Mathematics.

[14]Washington University School of Medicine, Department of Internal Medicine, Division of Pulmonary Medicine

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[15]Washington University School of Medicine, Department of Pathology & Immunology, Division of Laboratory & Genomic Medicine, Department of Anesthesiology, Division of Cardiothoracic Anesthesiology

Attribution, Correspondence and Requests for Reprints should be sent to: Dr. George Despotis
Department of Pathology & Immunology, Division of Laboratory & Genomic Medicine, Washington University School of Medicine
425 S. Euclid Avenue, Campus Box 8118, Saint Louis, MO 63110
gjdespotis@wustl.edu, Office Phone: 314 362 6586

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ABSTRACT:

Background: This study was designed to prospectively evaluate the efficacy of extracorporeal photopheresis (ECP) to attenuate the rate of decline of FEV_1 in lung transplant recipients with refractory Bronchiolitis Obliterans. Due to an observed higher than expected early mortality, a preliminary analysis was performed.

Study Design and Methods: Subjects from 10 lung transplant centers were assigned to ECP treatment or to observation based on spirometric criteria, with potential crossover for those under observation. The primary endpoint of this study was to assess response to ECP (i.e., greater than a 50% decrease in the rate of FEV_1 decline) before and 6 months after initiation of ECP . Mortality was also evaluated 6 and 12 months after enrollment as a secondary endpoint.

Results: Of 44 enrolled subjects, 31 were assigned to ECP treatment while 13 were initially assigned to observation on a non-random basis using specific spirometric inclusion criteria (7 of the observation patients subsequently crossed over to receive ECP). Of evaluable patients, 95% of patients initially assigned to treatment responded to ECP with rates of FEV_1 decline that were reduced by 93% in evaluable ECP-treated patients. Mortality at 6 and 12 months after enrollment was 32% and 41%, respectively. The most common (92%) primary cause of death was respiratory or graft failure. Significantly ($p=0.002$) higher rates of FEV_1 decline were observed in the non-survivors (-212 ± 177 mL/month) when compared to the survivors (-95 ± 117 mL/month) 12 months after enrollment. In addition, 18 patients with BOS diagnosis within six months of enrollment had lost 38% of their baseline lung function at BOS diagnosis and 50% of their lung function at enrollment.

Conclusions: These analyses suggest that earlier detection and treatment of BOS should be considered to appreciate improved outcomes with ECP.

Key Words: Bronchiolitis obliterans syndrome (BOS), extracorporeal photopheresis (ECP), forced expiratory volume in 1 second (FEV_1), Lung Transplantation

INTRODUCTION

Chronic lung allograft dysfunction (CLAD), predominantly related to bronchiolitis obliterans syndrome (BOS) represents the leading cause of morbidity and mortality in recipients of lung allografts beyond the first year[1] with an annual incidence that exceeds 7-8% in the first 10 years after transplantation.[2] BOS is an irreversible fibro-proliferative immune process that results in progressive narrowing of bronchial lumens, ultimately resulting in complete airway occlusion.[3] Despite current clinical use of one or more on or off-label treatment options, no immunosuppressive regimen has been shown to consistently prevent BOS.[4,5]

Extracorporeal photopheresis (ECP), a pheresis-based therapeutic immunomodulatory intervention, was approved by the FDA in 1987 for the management of cutaneous T-cell lymphoma.[6] ECP is additionally covered by Medicare for two off-label uses: management of GVHD after bone marrow transplantation[7,8] and for cellular rejection of orthotopic heart transplants.[9]

Since the early 1990s, ECP has also been used on an off-label basis for treatment of BOS refractory to the currently available armamentarium of immunosuppressive agents in lung transplant recipients.[10–16] Three retrospective analyses have demonstrated a reduction in the rate of decline of lung function in approximately 80% of lung transplant recipients with BOS.[14,15,16] Based on these findings, we submitted a formal request to the U.S. Centers for Medicare and Medicaid (CMS) to revise its ECP National Coverage Determination (NCD) to cover this treatment for patients with refractory BOS.

Pursuant to concerns raised by CMS regarding the study design for a prospective trial, we pursued a follow-up analysis of our previously published 60-patient database[16] to address these issues. In this re-analysis, FEV_1 was the only parameter that correlated with outcome (e.g. 50% survival at 1 year for patients with an $FEV_1 < 1.25$ L when compared to 85% survival at 1 year for patients with an $FEV_1 > 1.25$ L, $p<0.0001$).[17] In addition, two parameters associated with response to ECP were identified in this

analysis: patients with an FEV_1 rate of decline that exceeded 40 mL/month were 12 times more likely to respond to ECP and patients who had a statistically significant ($p<0.05$) rate of FEV_1 decline over time (via linear regression analysis) were 10 times more likely to respond to ECP when compared to those patients whose rate of FEV_1 decline over time was not statistically significant ($p>0.05$).

Under its Medicare Coverage and Evidence Development (CED) authority, CMS published a Decision Memo[18] authorizing use of ECP for treatment of Medicare patients with BOS in the setting of an approved research protocol. In September 2012, CMS approved our prospective, multi-center registry study with a target enrollment of 160 patients over 5 years to attain our primary spirometric rate of decline endpoint (50% decrease in the rate of decline in FEV_1 between a six month period before and after enrollment).[19] After one year of enrollment (n=44), despite improvement in rate of decline in FEV1 observed in the entire cohort, a higher than expected mortality rate was also observed within the first year after enrollment. Therefore, a preliminary analysis was performed with the primary aim of assessing the factors associated with early mortality before completion of the 6-month ECP regimen. Since the protocol construct did not allow patients to receive ECP treatment unless they met defined spirometric criteria, we also wanted to compare the survival between patients who were non-randomly assigned to the either the ECP treatment vs Observation cohorts.

MATERIALS AND METHODS

Subjects

Subjects were enrolled in an ongoing, multicenter, study involving Medicare lung transplant recipients diagnosed with BOS that was refractory to conventional therapy, and therefore eligible to be treated with ECP. Subjects were recruited from nine centers from April 2015 to July 2016.[19] This study protocol (NCT 02181257) was initially approved by the Washington University Human Research Protection Office and subsequently by all local IRBs at enrolling centers.

Refractory BOS diagnosis and treatment regimens

All subjects enrolled in this study received prophylactic standard immunosuppressive therapy pursuant to local practices at enrolling institutions. Pulmonary function was monitored by serial spirometry following lung transplant in accordance with guidelines issued by the American Thoracic Society (ATS).[20] BOS diagnoses were rendered using clinical criteria predicated by FEV_1 values as defined by the International Society for Heart and Lung Transplantation.[21] Enrolling sites had full discretion to administer any new therapy and/or augment the current immunosuppressive regimen after diagnosis of BOS. Refractory BOS was defined as a progressive decline in FEV_1 unresponsive to all interventions as determined by the enrolling investigator.

Registry Study Design

Subject Assignment and Crossover

Enrolled subjects were initially assigned to one of two (ECP vs Observation) cohort on a non-randomized basis predicated on the spirometric criteria previously described;[17] subjects who met the spirometric criteria (i.e., a statistically significant rate of FEV_1 decline that exceeded 10 ml/month if the most recent pre-ECP FEV_1 was < 1200 mL or 30 mL/month if FEV1 > 1200 mL) were assigned to the ECP Treatment

cohort. Subjects who were enrolled in the study but who did not initially meet these spirometric criteria were assigned to the Observation cohort. For subjects in the Observation cohort who continued to have FEV_1 values regularly monitored after enrollment, ECP treatment could be initiated when the subject's FEV1 values subsequently met the aforementioned spirometric criteria; these Observation cohort subjects were designated post hoc as a "Crossover".

ECP regimen and instruments utilized

Subjects that met the enrollment spirometric rate of decline criteria either initially (ECP Treatment cohort) or with Crossover (Observation cohort) were scheduled to receive 24 ECP treatments over a period of 6 months, using a regimen previously described.[9] In summary, treatment centers performed ECP using the intravenous formulation of 8 methoxy psolarin (UVADEX™) with either the UVAR XTS or CELLEX instruments (Therakos, Exton, PA) predicated on instrument availability, patient specific indications or operator experience.

Spirometry Data (FEV_1) between cohorts

FEV_1

Spirometry was performed in clinical laboratories at each enrolling site according to ATS guidelines. FEV_1 data for enrolled subjects were summarized for assigned cohorts at several time points: baseline (as defined by ISHLT guidelines), 1st screen (the first of at least 5 FEV_1 values measured six months prior to ECP), enrollment and monthly (when available) up to 12 months after ECP initiation.

FEV_1 rate of Decline

To assess the relative efficacy of ECP to arrest the rate of decline in lung function, the rate of decline in FEV_1 was calculated via linear regression using five FEV_1 values obtained six months prior to enrollment, and using at least four FEV1 values obtained at six and twelve months after ECP initiation predicated on

availability of FEV_1 values two months after first ECP procedure. Only patients who had at least 3 monthly FEV_1 values after the first ECP treatment were included in the comparative analysis. The change in rate of decline between the pre and post-ECP periods was calculated as the difference between the slope of FEV_1 decline post-ECP (e.g., 3, 6 or 12 months) and the rate of decline just prior to ECP initiation ($slope_{post\text{-}ECP}$ - $slope_{pre\text{-}ECP}$).

Primary Efficacy Outcome

The primary endpoint of the Registry study was the change in rate of FEV_1 decline with "response" defined as a 50% or greater decrease in the rate of FEV_1 decline between pre-ECP and 6 months post-ECP treatment.

Secondary Outcomes

Relationship between initial rate of FEV1 decline and response to ECP

To assess the ability of our spirometric enrollment criteria to identify subjects who respond to ECP, "response" at 3 or 6 months after ECP was summarized for ECP and Observation Crossover Cohort subjects; predictive indices with respect to the ability of spirometric enrollment criteria to identify response were derived using Bayes' Theorem.

Mortality Assessment

As part of our DSMB safety assessment functions, mortality was adjudicated with respect to relatedness to ECP by our DSMB while causality was assessed by local managing physicians. Time to mortality was determined for all subjects who expired within 12 months of enrollment and early mortality was defined as death prior to completion of the six-month ECP regimen (i.e., 24 ECP procedures). The following factors were assessed with respect to a potential association with early mortality: demographics, indication and type of transplant, the rate and degree of decline of pulmonary function at enrollment. In

addition, response to ECP and study design related factors (i.e., spirometric enrollment criteria) were also evaluated.

Requisite time for BOS Diagnosis on enrollment FEV_1 values

To characterize the impact of the requisite time for BOS on the magnitude of decline in FEV_1 at enrollment, % change from baseline FEV_1 values were summarized at various times point in a subset (n=18) of patients who were initially assigned to ECP treatment whose BOS diagnosis was made within six months prior to enrollment.

Statistical Methods

Chi square and Fischer's exact test were used to compare categorical variables. Either two-sample Wilcoxon rank-sum or one-way analysis of variance (ANOVA) were used for comparison of continuous variables at one or more time periods. In circumstances where there were missing data (in part related to early mortality), specific data points were displayed in distribution plots rather than mean values and when data were expressed as mean values in tables, the number of data points for each condition was included.

Univariate linear regression was used to evaluate the decline in lung function via generation of slope values using time (independent variable) and FEV_1 values (dependent variable) at multiple different time periods. Univariate and multivariate linear regression analyses were performed to identify potential covariates that may be associated with mortality at either 6 or 12 months after enrollment and with response to ECP therapy; only variables that had a p-value < 0.1 with univariate analyses were included in multivariate models. A p-value of less than 0.05 was considered statistically significant. Statistical analyses were performed using STATA14 software (StataCorp, College Station, TX).

RESULTS

Subject Enrollment and Assignment

Of 44 patients enrolled in the ECP Registry Study, 31 were initially assigned to the ECP cohort while 13 were assigned to the Observation cohort in a non-randomized fashion based on spirometric enrollment criteria. Thirty-seven subjects received ECP as follows: 30 of 31 ECP Treatment cohort patients received ECP (one excluded due to venous access issues) while 6 of 7 Observation cohort subjects who crossed over received ECP therapy (Crossover Cohort) (**see Figure 1**). The average number of days between enrollment and the first ECP treatment was 10 days for ECP cohort subjects while Crossover subjects received ECP on average 28 days after Observation cohort enrollment; this 18 day difference translated into an average loss of 51 mL in FEV_1.

Patient demographics, indication for lung transplant and immunosuppressive regimen

Demographic, primary disease indication for transplant, type of lung transplant (i.e., single vs bilateral) and stage of BOS at enrollment were similar between the two cohorts (**Table 1)**. A substantial percentage of patients were in advanced stages (58 vs 50% in Stage II/III in the ECP and Observation cohorts, respectively).

A similar overall distribution of maintenance immunosuppressive medications was observed between cohorts (Table 2). Accordingly, similar percentages of patients had received either Azithromycin or anti-thymocyte globulin between the two cohorts, with p-values of 0.3 and 1.0, respectively. **(Table 2).**

Spirometric Analyses: FEV_1 and FEV_1 rates of decline

Table 3 summarizes FEV_1 values at four time points in each cohort (ECP vs Observation vs Crossover). When compared to the ECP cohort, the Screening FEV_1 was statistically lower ($p<0.01$) in the Observation subjects who did not cross-over to ECP treatment.

Table 4 summarizes rates of FEV_1 decline and p-values (i.e., values derived from the FEV_1 vs time plots and slopes expressed in mL/month) between cohorts at two time points (before and 6 months after enrollment); the number of data points included was predicated on availability of FEV_1 measurements as detailed at the bottom of the table. As expected based on the spirometric criteria for cohort assignment, the slopes of FEV1 decline pre-enrollment were much steeper (p=0.004) in the ECP cohort (-192 ± 167 mL/month) when compared to the observation cohort (-46 ± 46 mL/month).

Outcomes:

Primary Outcome: Assessment of spirometric "response":

The primary outcome of the registry study with respect to the change in the rate of FEV1 decline could only be assessed in a subset of enrolled patients that had at least 6 monthly FEV1 values after ECP treatment as follows: 63% (19 of 30) of ECP cohort subjects and 71% (5 of 7) Crossover subjects. At 6 months, 19 evaluable ECP cohort subjects demonstrated a 93% decrease (from -136 to -10 mL/month, p=0.0002) in the mean rate of FEV1 decline after ECP (**Table 4, Figure 2**). In contrast, only a trend (p=0.29) in reduced mean rate of FEV1 decline (65%) 6 months after ECP was observed in 5 initially assigned Observation patients who crossed over to ECP treatment (Table 4).

Using a 50% or more decrease in the rate of FEV1 decline as a response criteria, 95% (18 of 19 evaluable subjects) of subjects initially assigned to ECP treatment responded to ECP. A statistically (p=0.001) lower percentage (25% or 2 of 8 evaluable subjects) of Observation subjects responded to ECP. Of 6 Observation subjects who crossed over and received ECP treatment, only 5 were evaluated as one subject did not have enough FEV_1 values after crossover due to early mortality. Of these 5 evaluable Crossover subjects, two demonstrated no change in the rate of FEV1 decline at 6 months after ECP (Table 4, see **Supplemental Figures E2 and E3 for Crossover subjects**). Of 3 evaluable non-crossover Observation cohort subjects, no patient (0%) had a change in their FEV1 rate of decline as they

continued to have a stable FEV_1 pattern for at least 3 months after enrollment (i.e., no change in FEV1 rate of decline – see **Supplemental Figure E4 for all non-Crossover subjects**).

Secondary Outcomes

Assessment of spirometric enrollment criteria to identify patients who responded to ECP

Of 36 patients who received ECP (30 ECP and 6 Crossover), 24 patients (19 ECP Treatment and 5 Observation with crossover) had 6 monthly post enrollment FEV_1 values. Of six Observation patients, three had at least 3 monthly post enrollment FEV_1 values. Data from these 27 patients were used to assess the predictive capacity (using Bayes Theorem derived predictive indices like sensitivity) of the spirometric enrollment criteria to identify response to ECP (the spirometric primary endpoint). Predictive indices for a response to ECP using the initial assignment as directed by the spirometric enrollment criteria were as follows: Sensitivity: 90%, Specificity: 85%, Positive Predictive Value: 94% and Negative Predictive Value: 75% **(see Figure 3)**.

Mortality Analysis

Of 44 subjects, 32% (12 ECP and 2 Crossover) expired within six months of enrollment while 43% (15 ECP and 3 Crossover and one Observation) expired within 12 months of enrollment. There were no treatment related deaths as adjudicated by our DSMB and a complete description of mortality the etiology of mortality between cohorts at various time periods is summarized in Table 5. The most common primary cause of death among all non-survivors (both ECP cohort and Observation cohort subjects) was respiratory or graft failure (90%). There was no difference in mortality between the two cohorts (p=0.2). Although a higher numeric percentage of subjects initially assigned to ECP treatment (n=31) expired at both 6 (ECP: 39% vs Obs: 23%) and 12 (ECP: 48% vs Obs: 31%) months after enrollment when compared to subjects initially assigned to Observation (n=13), these trends were not significant

p=0.49 and p=0.34, respectively. These findings were not unexpected as they were most likely related to subject assignment in a non-random fashion using spirometry-based criteria which assigned subjects to ECP treatment based on higher rates of FEV1 decline.

Comparison of Spirometry between Survivors and non-Survivors

To evaluate the potential effects of reduced lung function on survival, FEV_1 values were compared at several time points between patients who survived for either 6 or 12 months (Survivors) vs those who expired in that period of time (Non-survivors). Specifically, 14 and 19 subjects who expired had higher mean FEV_1 at the first screening FEV1 prior to enrollment when compared lower mean FEV_1 in 40 and 36 subjects who survived (p=0.01) 6 and 12 months after enrollment, respectively. (**Supplemental Table 1**, **Supplemental Figure E1).** However, similar mean FEV_1 values were observed at baseline and at enrollment between Survivors and Non-survivors (Supplemental Table 1). These findings were explained by the comparison of rate of decline in FEV_1 between survival cohorts. Significantly (p=0.009) higher rates of FEV_1 decline were observed in the Non-survivors (-232 $\pm$ 195 mL/month) when compared to the Survivors (-101 $\pm$ 110 mL/month) at 6 months and similarly at 12 months after enrollment (p=0.002)(Supplemental Table 1). When all relevant covariates were included in a multivariate logistic regression analysis to identify potential variables associated with either early mortality or 12 month mortality, only pre-enrollment FEV_1 rate of decline in lung function was associated with both early (6 month) (p=0.005) and 12 month mortality (p=0.005).

<u>The impact of requisite time for BOS Diagnosis on enrollment FEV_1</u>

To assess the impact of the requisite time for BOS diagnosis on lung function at enrollment, mean FEV_1 values obtained during the 6 month period prior to and at enrollment were calculated for 18 patients who had a diagnosis of BOS within the 6 month FEV_1 screening period. **Figure 4** illustrates that patients had

lost an average of 38% of their lung function at the time of BOS diagnosis (on average at two months prior to enrollment), with further reduction to an average of 50% of baseline lung function by enrollment. Accordingly, the mean time for diagnosis on average approximated 3 to 4 months. This information prompted us to send a survey to our enrolling centers requesting the typical institutional frequency for laboratory spirometry for the BOS surveillance population. A review of responses from 6 of 10 enrolling centers revealed a median spirometry monitoring frequency of 3 months (Range: 1-6 months) for enrolled patients after the first year of transplant at our enrolling centers.

DISCUSSION

Although ECP was associated with a 93% reduction in the rate of decline in FEV_1 at 6 months after ECP initiation in our non-randomized Registry study, we also observed a concerning early mortality rate. Safety of the ECP procedure was assessed by our DSMB which adjudicated that none of the fatal outcomes were related to ECP and local investigators characterized that 92% were due to end stage pulmonary dysfunction. Although higher than expected mortality was observed after enrollment in patients non-randomly assigned to ECP Treatment based on spirometry criteria, these findings were related to the more aggressive nature of BOS in patients assigned to ECP treatment (i.e., statistically significant four fold greater rate of FEV1 decline in patients assigned to ECP treatment) which was shown to be the most important and only factor associated with mortality; this artifact was clearly related to the study design since spirometric criteria were used to assign patients to treatment in a non-randomized fashion with those assigned to ECP having a resultant much greater rate of FEV1 decline.

With respect to our study design, our current analysis revealed that our spirometric criteria enabled accurate identification of responders to ECP. In the Observation cohort involving patients with low rates of FEV_1 decline, only two subjects (25%) of 8 evaluable subjects (crossover and non-crossover) had a 50% change in the enrollment FEV_1 rate of decline. Poor treatment response in a slow FEV_1 decline phenotype was originally described by Jackson et al in a large series of patients with BOS[22] and more recently confirmed with another analysis.[17] We also confirmed that the current spirometric enrollment criteria can detect patients who respond to ECP and should not have a substantial clinical impact with respect to delay in ECP treatment since the time for crossover was nominal (18 days) with minimal loss of FEV_1 volume in that period.

The findings of low FEV_1 values at BOS diagnosis (Figure 4) and the higher rates of decline in FEV_1 in non-survivors highlights the potential importance of early detection and expedited management of BOS with ECP as first line therapy rather than use for refractory disease to arrest disease progression before lung function reaches a critically low level. Accordingly, we have modified our CMS approved study to now include a randomized, controlled trial (RCT) arm that involves use of ECP as first line therapy when compared to local standard of care management of BOS.

Delays in detection of BOS may also be an important factor that led to higher early mortality in our series. Generally, FEV_1 measurements are made biweekly or monthly during the first year following lung transplant, with variable extension to every 3, 6, or 12 months between institutions,[23] despite a fairly consistent annual BOS incidence of at least 7-8% per year.[2] Data from our previous publication[16] demonstrates that this approach results in loss of up to 1 liter of FEV_1 volume at the diagnosis of BOS as well as a prolonged delay (mean= 401 days) for ECP initiation at our institution.

Accordingly, earlier detection of BOS via frequent spirometry coupled with earlier use of ECP or other new therapies may result in better functional status and prolonged survival for either primary or refractory BOS. However, use of a frequent monitoring schedule (every 4-8 weeks) for conventional laboratory spirometry over a long surveillance period (i.e., up to 15 years) may not be feasible for many patients, especially for patients who live far from their treatment or diagnostic facilities or who are not compliant with laboratory based spirometry predicated on safety concerns in the setting of the current COVID-19 pandemic. Home Spirometry monitoring systems can lead to early detection of acute rejection and infection in lung transplant recipients,[24–27] and may also be preferable if these systems can automatically transmit spirometric[26] and symptom[26,28] data to facilitate discrimination of variance results between infections vs rejection.

Despite our findings that support use of ECP in lung transplant recipients with BOS, there were several limitations to our registry study. The most notable involved the use of ECP for refractory BOS rather than at initial diagnosis of BOS, but also includes lack of a control comparator cohort to assess important outcomes, premature assessment of efficacy of ECP to attenuate the rate of decline of lung function, lack of uniform prophylactic and BOS treatment anti-rejection regimens, the lack of use of a standardized approach for early detection of BOS and the inclusion of only Medicare patients. Although Medicare patients are typically older aged, age was not identified as a confounder with respect to attenuation of the rate of FEV1 decline by ECP or survival. These limitations and our preliminary analyses prompted us to revise the study to promote early detection (i.e., with use of an automated Home Spirometry Method) and treatment of refractory BOS at early stages and to evaluate all of the outcomes CMS had previously outlined: the impact of ECP on rate of decline in FEV_1, survival and quality of life in a RCT arm using ECP as first line therapy when compared to local Standard of Care.

In summary, these preliminary analyses support earlier detection and treatment of BOS especially in patients who have a rapid decline in lung function. In light of the preliminary suggestion of ECP's efficacy in reducing the rate of decline of lung function, we are modifying our study to add a RCT arm that will enroll patients with newly diagnosed BOS. Based on these findings, we will continue to utilize our spirometric criteria to enroll patients who are more likely to respond to ECP.

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Table 1 Demographics, indications for transplant, type of transplant and BOS staging at the Time of Photopheresis Initiation, data expressed as % or mean (SD)

	ECP arm (n=31)	Observation arm (n=13)	P-value
Age, years	57 (13)	61 (8)	0.36
Gender			
Male	53	42	
Female	47	58	0.73
Pre-transplant diagnosis*			
COPD	34	17	
Cystic fibrosis	10	8	
Interstitial Lung Disease	38	43	
α-Antitrypsin deficiency	7	8	
Primary pulmonary hypertension	0	8	
Sarcoidosis	0	8	
Pulmonary venous occlusive disease	4	0	
Other	7	8	0.67
Type of transplant			
Bilateral lung	83	64	
Single lung	17	36	0.22
BOS stage**			
1	42	50	
2	29	33	
3	29	17	1.0

*Comparison in 41 subjects (ECP=29, Observation=12)

**Comparison in 35 subjects (ECP=28, Crossover=6, ECP (non-treated=1)

Table 2 Current Immunosuppressive Regimens at the Time of Extracorporeal Photopheresis Initiation – data expressed as %

Maintenance immunosuppression*	ECP cohort	Observation cohort
Prednisone, mycophenolate, CSA	4	10
Prednisone, tacrolimus, azathioprine	10	10
Prednisone, tacrolimus, mycophenolate	45	30
Prednisone, tacrolimus, mycophenolate, azathioprine	21	0
Prednisone, tacrolimus, mycophenolate, CSA	7	0
Prednisone, tacrolimus, mycophenolate, everolimus	4	10
Prednisone, tacrolimus, mycophenolate, sirolimus	4	30
Prednisone, tacrolimus, mycophenolate, sirolimus, azathioprine	7	0
Prednisone, tacrolimus, sirolimus	0	10
Azithromycin Use	79	100
Anti-thymocyte globulin Use	41	40

*p=0.11 when comparing treatment regimens between cohorts

Table 3: Spirometry FEV_1 Values prior to and after enrollment between non-randomized cohorts allocated to either ECP treatment or Observation based on spirometric criteria

FEV1 in L and data expressed as Mean ± SD

	All Patients (n=44)	ECP Cohort (n=31)	Observation Cohort (n=13)	
			Crossover (n=7)	No Crossover (n=6)
Baseline FEV1 after Transplant*	2.7 ± 0.9	2.8 ± 0.9	2.7 ± 0.5	1.7 ± 0.7
First Screening FEV1	2.0 ± 0.8	2.2 ± 0.8	1.8 ± 0.8	1.2 ± 0.4**
Enrollment	1.4 ± 0.6	1.4 ± 0.6	1.6 ± 0.6	1.2 ± 0.4
Last FEV1 after enrollment	1.0 ± 0.5	1.0 ± 0.6	1.0 ± 0.4	1.2 ± 0.4

*Baseline values based on data from 40 subjects. **$p<0.01$ when compared to ECP Cohort

Table 4: Rates of FEV_1 decline prior to and after enrollment between non-randomized cohorts allocated to either ECP treatment or Observation based on spirometric criteria. Linear regression derived values (slope and p-values) from FEV_1 vs time relationship.

Slopes (mL/month) and p-values expressed as Mean ± SD

	ECP cohort (n=30)	Observation cohort (n=13)	
Time after Enrollment		Crossover (n=6)¥	No Crossover (n=6)
0 (ECP: n=30, CO: n=7, No CO: n=6)			
FEV1 rate of decline	-148 ± 154	-38 ± 51	-1.2 ± 18*£
P-value	0.009 ± 0.01	0.12 ± 0.08*	0.32 ± 0.15*£
0+ months (ECP: n=19, CO: n=5)¥¥			
FEV1 rate of decline	-136 ± 117	-81 ± 36	
P-value	0.01 ± 0.01	0.08 ± 0.11	
6 months (ECP: n=19, CO: n=5)¥¥			
FEV1 rate of decline	-10 ± 58¥	-28 ± 98	
P-value	0.18 ± 0.13¥	0.1 ± 0.12	

ECP = extracorporeal photopheresis cohort, CO = Observation patients who cross over to ECP treatment

0+ refers to either at enrollment (ECP cohort) or at Crossover (Observation cohort)

¥ only 6 of 7 crossover subjects included since one did not receive ECP

¥¥ only 19 of 30 ECP subjects and 5 of 7 crossover subjects included based on available FEV_1 values at 6 months for calculation of FEV_1 rate of decline

*p<0.05 when compared to ECP arm

£p<0.05 when compared to Crossover

¥p<0.05 when compared to Time 0

Table 5: Mortality after enrollment between non-randomized cohorts allocated to either ECP treatment or Observation based on spirometric criteria.

Months from Enrollment	FEV1 Decline (mL/month)*	Terminal FEV1	Mortality (days after enrollment)	Mortality	ECP cohort (n=31) [£]	Obs cohort (n=13)
6	-246 ± 194	805 ± 182	86 (17 – 182)	Respiratory Failure	11 (36%)[¥]	3 (23%)
				Pneumonia		
				CVC Sepsis	1 (3%)	
				Total	12 (39%)	3 (23%)
6 - 12	-117 ± 58	868 ± 365	235 (213 – 268)	Respiratory Failure	2 (7%)	1 (8%)
				Pneumonia	1 (3%)	
				CVC Sepsis		
				Total	3 (10%)	1 (8%)
12	-212 ± 177	819 ± 223	118 (17 – 268)	Respiratory Failure	13 (42%)[¥]	4 (31%)
				Pneumonia	1 (3%)	
				CVC Sepsis	1 (3%)	
				Total	15 (48%)	4 (31%)

ECP = subjects assigned to extracorporeal photopheresis, Obs = subjects assigned to Observation with potential for crossover to ECP. Mortality was categorized into three etiologic categories as related to Pneumonia, central venous catheter (CVC) related sepsis and/or Respiratory Failure which included either acute or chronic designations by enrolling physicians while [¥]one patient had pneumonia concurrent with respiratory failure – the % values were calculated using the # of patients in the respective cohort as the denominator. The most common primary cause of death among all non-survivors (both ECP cohort and Observation cohort subjects) was respiratory or graft failure (17/19 or 90%). *Rate of FEV1 decline calculated at enrollment. Terminal FEV1 (mL) was defined as last measured FEV1 before expiration in 18 patients (results do not include the FEV1 in the subject who expired from Sepsis who had a 2400 mL FEV1). [£]One subject did not receive ECP and the mean # ECP procedures performed in 30 patients was 12 (range 7-20).

Figure Legends

Figure 1 is a flow diagram that illustrates patient enrollment on a non-random basis using spirometric enrollment criteria, assignment and crossover to ECP treatment.

Figure 2 illustrates a density distribution of FEV_1 rate of decline (slope in mL/month) values along the y axis for the 20 ECP Treatment cohort patients before and after ECP treatment at the following monthly time periods (x-axis): at enrollment prior to ECP and 6 months after Enrollment.

Figure 3 is a flow diagram that illustrates patient enrollment, assignment and crossover to ECP treatment. It also designates how many patients an adequate number of FEV_1 values to enable calculation of rates of decline.

Figure 4 plots mean FEV1 values obtained during the peri-enrollment period for a series of 18 patients ECP cohort subjects who were treated with ECP and who had a diagnosis of BOS within the 6 month FEV1 screening period revealed that patients had lost 38% of their lung function by the time that BOS was diagnosed with even further reduction by the time of ECP initiation.

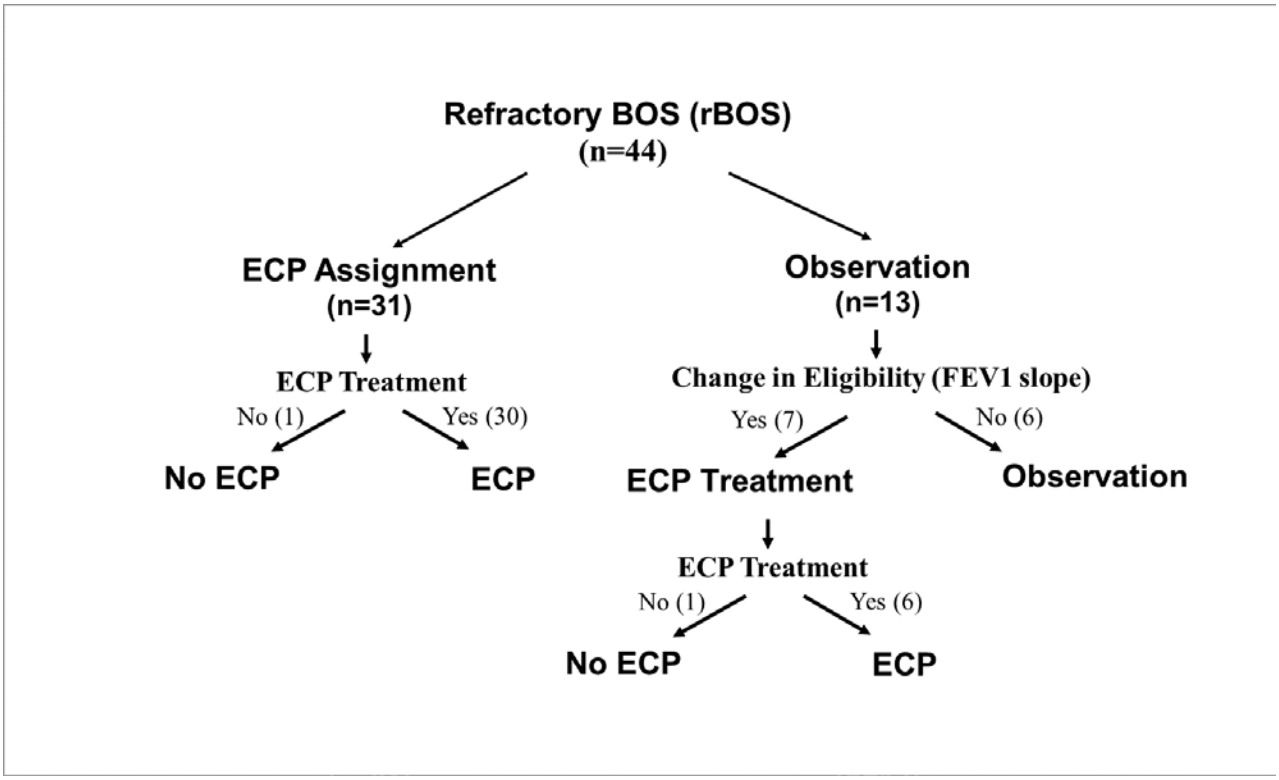

Figure 1 is a flow diagram that illustrates patient enrollment on a non-random basis using spirometric enrollment criteria, assignment and crossover to ECP treatment.

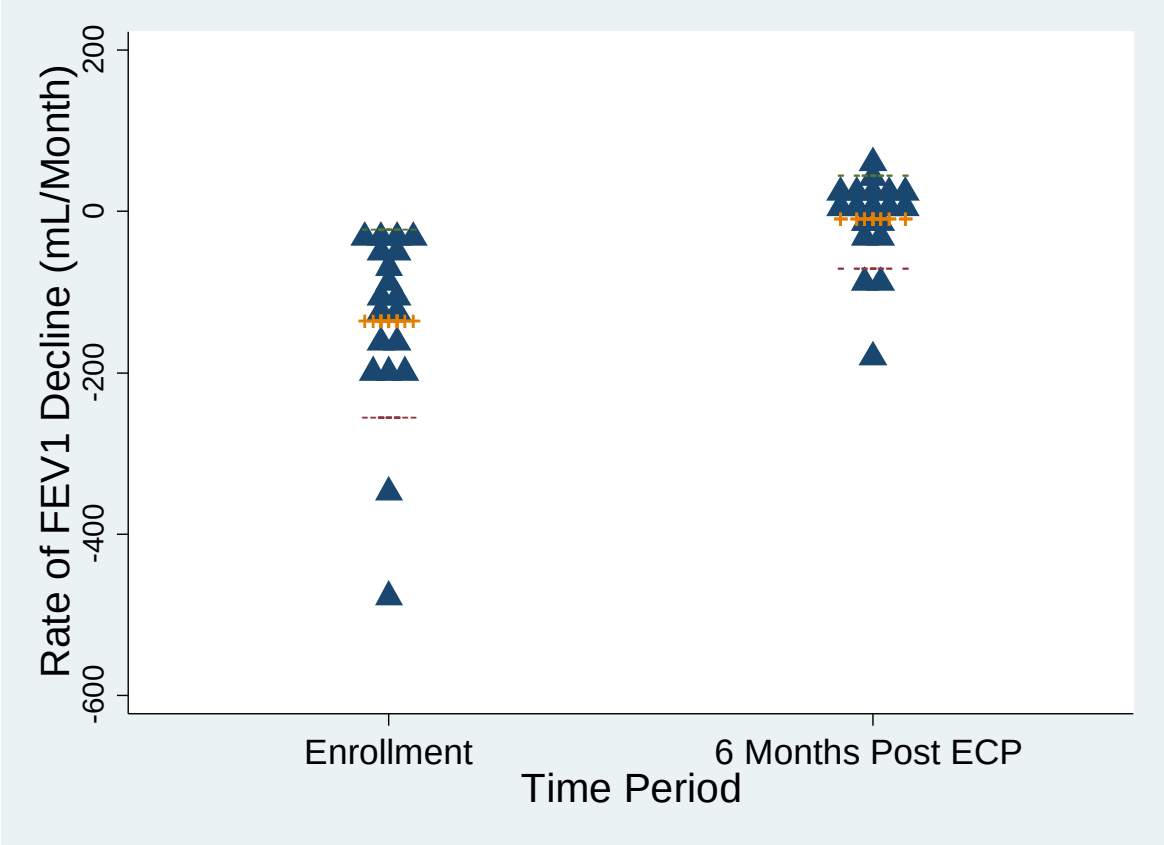

Figure 2 illustrates a density distribution of FEV_1 rate of decline (slope in mL/month) values along the y axis for the 19 ECP cohort subjects before and after ECP treatment at the following monthly time periods (x-axis): at enrollment prior to ECP and 6 months after Enrollment.

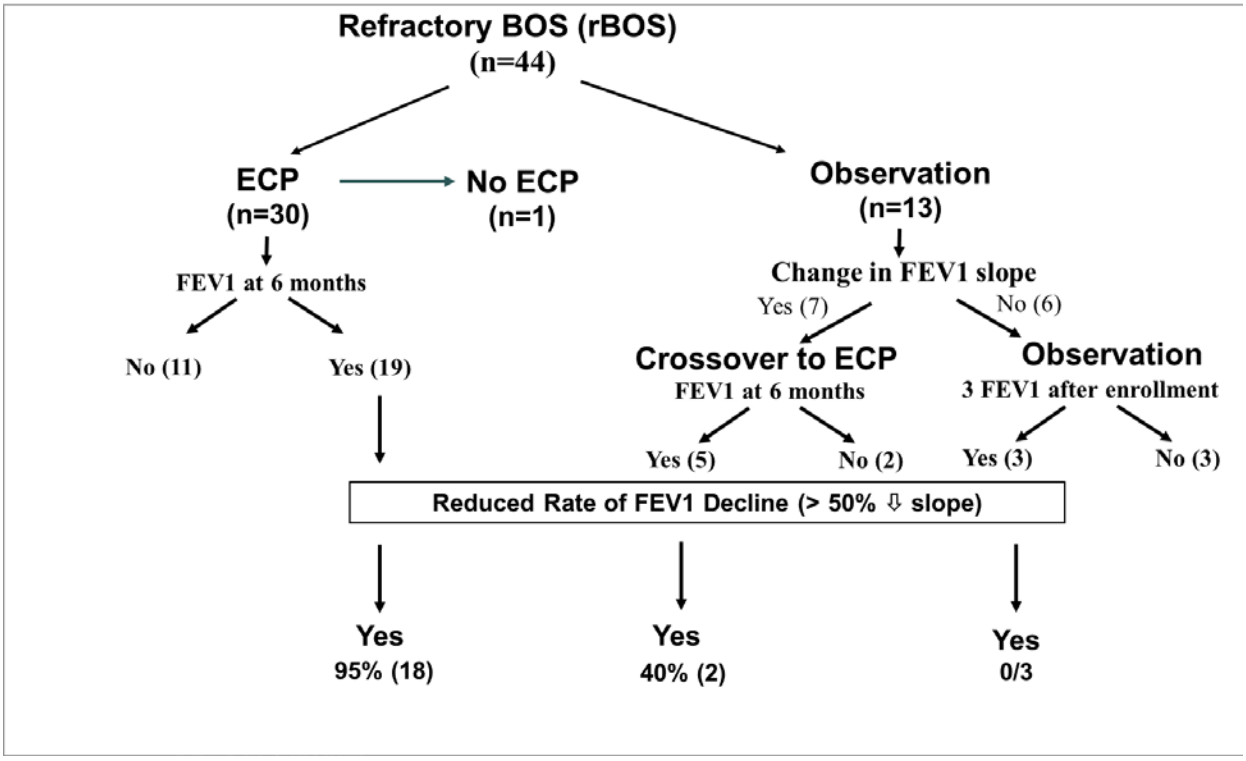

Figure 3 is a flow diagram that illustrates patient enrollment, assignment and crossover to ECP treatment. Three of seven crossover subjects did not have FEV_1 values at six months since one expired prior to receiving ECP and two expired within the first few months after ECP was initiated. It also designates how many patients an adequate number of FEV_1 values to enable calculation of rates of decline.

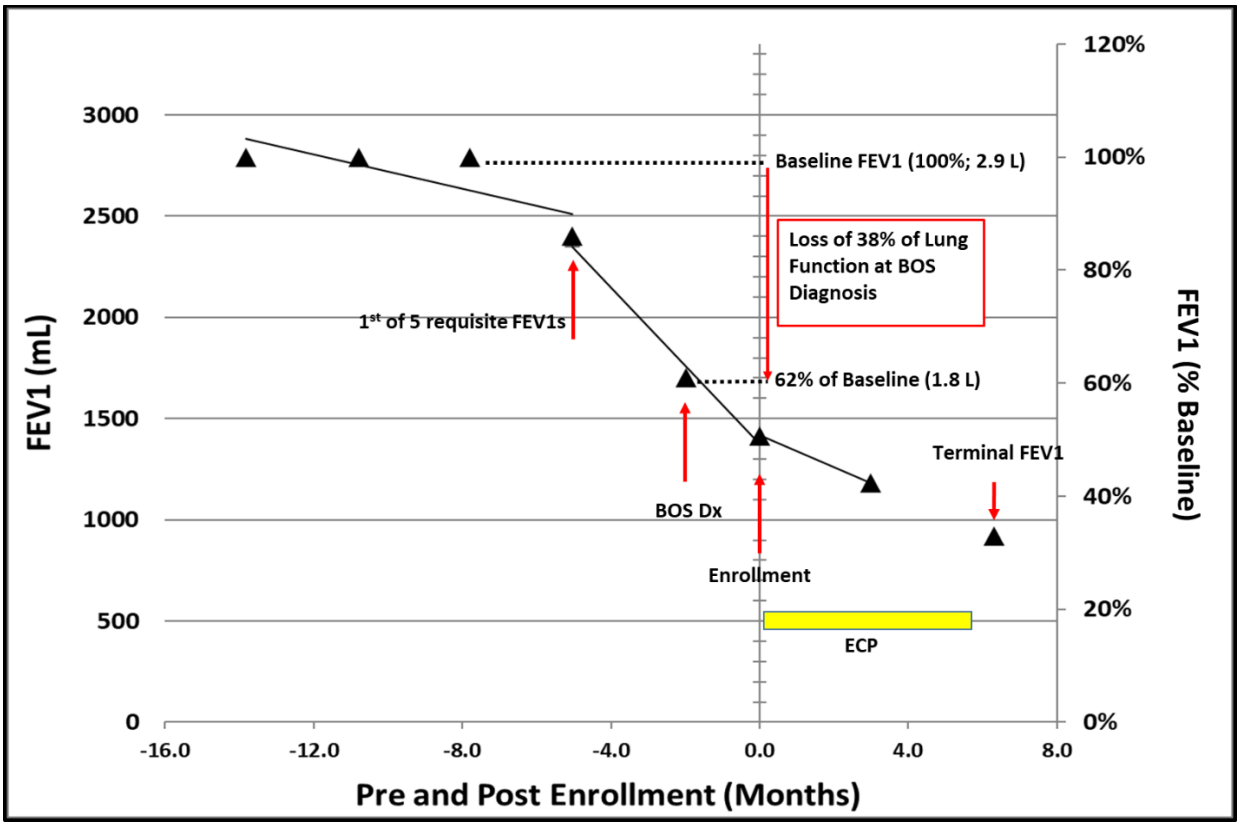

Figure 4 plots mean FEV1 values obtained during the peri-enrollment period for a series of 18 patients in the ECP cohort who were treated with ECP and who had a diagnosis of BOS within the 6 month FEV1 screening period revealed that patients had lost 38% of their lung function by the time that BOS was diagnosed with even further reduction by the time of ECP initiation.