Long-Term Off-Line Extracorporeal Photochemotherapy in Patients with Chronic Lung Allograft Rejection Not Responsive to Conventional Treatment: A 10-Year Single-Centre Analysis

Claudia Del Fante^a Luigia Scudeller^b Tiberio Oggionni^c Gianluca Viarengo^a
Francesca Cemmi^c Monica Morosini^c Alessandro Cascina^c Federica Meloni^{c, d}
Cesare Perotti^a

^aImmunohaematology and Transfusion Service, ^bScientific Direction, Biostatistics Unit, and ^cDepartment of Respiratory Diseases, Fondazione IRCCS Policlinico S. Matteo, and ^dDepartment of Molecular Medicine, University of Pavia, Pavia, Italy

Key Words

Bronchiolitis obliterans syndrome · Chronic lung allograft dysfunction · Extracorporeal photochemotherapy · Lung transplant rejection · Restrictive allograft syndrome · Survival

Abstract

Background: Extracorporeal photochemotherapy (ECP) for chronic lung allograft dysfunction (CLAD) has been reported as beneficial in a few short-term studies. **Objectives:** In this retrospective cohort study on 48 CLAD patients treated by ECP (off-line technique) for a period of >8 years (compared to 58 controls), we explored potential predictors of survival and response. **Methods:** Failures were defined as a decrease in forced expiratory volume in 1 s (FEV₁) of >10% from ECP initiation. **Results:** ECP patients were enrolled between February 2003 and December 2013; 14 (29.2%) with restrictive allograft syndrome (RAS) and 34 with bronchiolitis obliterans syndrome. Grade 1 severity was indicated in 58.3%, grade 2 in 20.8%, and grade 3 in 20.8% of patients. The median follow-up was 65 months (cumulative 2,284.4 person-months). Twenty (41.7%) patients died, including 17 (85%) CLAD-related deaths. Among the controls, there were 42

deaths (72.4%), of which 32 (76.2%) were CLAD related, over a median of 51 months (cumulative 3,066.5 person-months; $p = 0.09$). Among ECP patients, the FEV₁ slope flattened out after a decline in the initial months (slope -19 ml/month in months 0–6, $+4$ in months 36–48 and later; $p = 0.001$). RAS was associated with poorer survival, whereas a ‘rapid decline in the previous 6 months’ was not. No ECP side effects or complications were observed. **Conclusion:** Long-term ECP for CLAD is safe and reduces FEV₁ decline over time; the RAS phenotype might show a poorer response. ECP deserves to be evaluated in a randomized controlled trial.

© 2015 S. Karger AG, Basel

Introduction

Lung transplantation (LTx) represents the only therapeutic option for patients affected by end-stage lung diseases (mainly cystic fibrosis, chronic obstructive pulmonary disease, idiopathic or secondary pulmonary fibrosis, and pulmonary hypertension) unresponsive to other

F.M. and C.P. equally contributed to this paper.

Table 1. Available studies on ECP for CLAD

Reference	Patients, n	Median follow-up from ECP, person-years	Design	Technique	ECP, n/duration, months	Slope before ECP	Slope after ECP	Response by CLAD grade	Mortality within study, %	Comments
Benden et al. [14], 2008	12 CLAD/ 12 recurrent acute rejection	4.9 (range 0.5–8.4)	single-centre, retrospective cohort	on-line	12/6	–112	–12	n.a.	25	short ECP duration, lack of control group, very small sample size, heterogeneity, potential selection biases
Morrel et al. [17], 2010	56	2.6	single-centre, retrospective cohort	on-line	24/6	–116	–29	no	45	short ECP duration, lack of control group, heterogeneity, potential selection biases
Jaksch et al. [16], 2012	51 for 3 months (25 for 12 months); 143 controls	2.3 (extrapolated from graph)	single-centre, retrospective cohort	on-line	18–30/ 6–12	–123	–18	yes	29	short ECP duration, comparisons not adjusted for CLAD grade
Greer et al. [15], 2012	65	1.4 (IQR 1.1–2)	single-centre, retrospective cohort	on-line	12–30/ 3–12	n.a. (21 rapid decliners)	n.a. (35 stabilized/ improved)	no	23	lack of control group, short ECP duration
n.a. = Not available.										

medical or surgical treatments [1]. Notwithstanding the advances in surgical techniques and the introduction of new medications during the last 20 years, chronic lung allograft dysfunction (CLAD) is responsible for the poorest long-term outcome among solid organ transplantations (with a median overall survival of 5.3 years among males and 5.7 years among females according to the most recent International Society for Heart and Lung Transplantation figures) [2, 3].

Until recently, CLAD was identified only with its main phenotype bronchiolitis obliterans syndrome (BOS), i.e. a fibrous obliteration of the bronchiolar lumen corresponding to a progressive loss of graft function, often complicated by airway colonization and infections [4]. A new CLAD phenotype has recently been described, called ‘restrictive allograft syndrome’ (RAS), characterized by a restrictive pulmonary function defect, with a histology showing interstitial fibrosis and sub-pleural and upper lobe fibrosis [5]. Both CLAD phenotypes are characterized by a progressive increase in dyspnea and impairment of blood gas exchanges; however, evolution towards end-stage respiratory failure appears much faster in RAS [6].

Once chronic lung function decline is established, therapeutic options are scarce: only macrolides have been shown to reverse graft dysfunction in a limited number of patients with consistent neutrophilic airway inflammation (neutrophilic reversible allograft dysfunction) [7]. In macrolide-unresponsive patients, a number of treatment approaches have been reported (including anti-leukotrienes,

total lymphoid irradiation, and modulation of/increase in immunosuppression) mainly with immunosuppressive/anti-inflammatory purposes; however, the overall response rate of these strategies remains suboptimal [8].

Besides the above strategies, extracorporeal photopheresis (ECP), successfully used to treat cutaneous T-cell lymphoma [9], is an immunomodulating treatment effective in selected immunomediated diseases like acute and chronic graft-versus-host disease (GvHD) after allogeneic stem cell transplantation [10–12]. In the LTx setting, the first reports on ECP date back from 1995 [13]; more recently, ECP has been shown to induce stabilization/partial reversion of lung function in a relevant subset of CLAD patients (54–61%) in three single-centre retrospective cohort studies with limited follow-up; at present, no randomized controlled trials exist in this setting [14–17] (table 1).

We report our favourable experience with long-term (up to 9 years) ECP on LTx recipients (LTxR) with CLAD, not responsive to conventional therapy, and compare them to a cohort of 58 controls in terms of mortality and failure.

Materials and Methods

Study Design

Overall, 55 adult patients diagnosed with CLAD (BOS or RAS) were treated with ECP at our centre between February 2003 and December 2013. Of these, 1 concurrently had lung cancer (adeno-

carcinoma); patients with ≤ 8 procedures at the time of analysis ($n = 6$) were also excluded, leaving 48 patients in this retrospective cohort study.

A control group of 58 patients who could not undergo ECP due to logistic reasons (e.g. living too far from our centre) or who continued on standard immunosuppressive (IS) regimen was also included.

According to the requirement of our Ethics Committee, all patients provided written consent to the use of anonymized personal and clinical data prior to treatment; of note, most of these patients have been enrolled in a 3-year observational study on ECP feasibility and effectiveness approved by Regione Lombardia in 2009 (DR8024 of 31/07/2009).

Setting and Patients

Our hospital is a 1,200-bed teaching hospital; LTx has been performed since 1991. Since 2003, CLAD patients unresponsive to macrolide therapy and to modulation of the IS regimen have been referred to the Apheresis Unit for compassionate ECP; in 2009, an observational protocol approved by Regione Lombardia offered ECP salvage treatment to all BOS grade I patients after failure of a 3-month azithromycin course.

The standard IS regimen was based on cyclosporine, azathioprine, and low-dose steroids until 2005 and was changed into tacrolimus, mycophenolate mofetil, and low-dose steroids afterwards. All patients undergo surveillance bronchoscopies; biopsy-proven episodes of acute rejection [18] are treated with steroid boluses and, in case of acute rejection recurrence or persistence, with a standard anti-thymoglobulin course and a modulation of the IS regimen (shifting cyclosporin A to tacrolimus, and azathioprine to mycophenolate mofetil). Our surveillance protocol has been reported elsewhere [19]. BOS diagnosis has been made according to published guidelines [4, 6]; the CLAD subtype RAS has been retrospectively re-classified according to radiological (with a CT scan showing a pattern of persistent interstitial/upper lobe fibrosis) and functional criteria [persistent decline in forced expiratory volume in 1 s (FEV_1) of $>20\%$ compared to the best postoperative values and a decline in total lung capacity of $>10\%$ compared to baseline] [5].

In case of a BOS 0p or early RAS diagnosis, patients are usually prescribed a 3-month course of chronic low-dose azithromycin [5, 20]. At the same time, patients undergo oesophagogastroduodenoscopy and, in case of gastro-oesophageal reflux disease with bronchoalveolar lavage findings consistent with microaspiration (high pepsin levels and $>5\%$ alveolar macrophages positive to Red Oil stain), maximization of anti-reflux medical treatment and mini-invasive surgical fundoplication are offered. In case of a further decline consistent with a BOS grade I diagnosis, despite the above treatment strategies, ECP is initiated after informed consent to the procedure. Our cytomegalovirus surveillance protocol has been detailed elsewhere [21].

Enrolled controls have been transplanted from 2001 to 2012 and have been treated according to previously detailed immunosuppressive protocols. These CLAD patients were not offered ECP either because of a CLAD diagnosis before 2003 or because they lived too far from our reference centre.

ECP Procedures

Patients referred for ECP have been treated independently of their CLAD phenotype, BOS severity, oxygen therapy, anaemia, oral anticoagulant treatment, or colonization. ECP was delayed only for those patients with signs of systemic infection (i.e. a fever

$>38^\circ\text{C}$, C-reactive protein elevation, and increased sputum production with or without bacterial, fungal, or viral identification), an absolute mononuclear cell (MNC) count of $<200 \times 10^9/\text{l}$, acute heart failure, severe renal dysfunction, or haemorrhagic diathesis.

All ECP procedures were performed at the Apheresis Unit. Before each procedure, complete and differential blood counts were obtained. ECP was performed using the off-line technique as previously described [12]. Briefly, MNCs were collected from the patient using a third-generation cell separator device, processing 1.5–2 blood volumes. To prevent hypocalcaemia, calcium gluconate was administered intravenously during the procedure, at a mean dosage of 3,000 mg, which was increased in case symptoms of hypocalcaemia appeared (mostly chills and paraesthesias). After collection, cells were immediately irradiated (UV-A at 2 J/cm²; Macogenic, Macopharma, France) in the presence of 8-methoxypsoralen (at a concentration of 200 ng/ml). Finally, the photoactivated MNCs were reinfused into the patient. The vital signs (blood pressure, heart rate, and oxygen saturation) were monitored during the entire procedure. Major reinfusion adverse events were defined as asthma, bronchospasm, and dyspnoea. A sample (3 ml) from the leukapheresis collection bag was always obtained for differential and total blood count to detect MNC purity. Quality controls for bacteria and fungi detection and UV-A irradiation efficacy were performed as previously described [12]. Considering the low extracorporeal volume of the cell separator devices (ranging from 140 to 195 ml) with a consequent low impact on the cardiovascular system, in our protocol, only patients with pre-procedure haemoglobin (Hb) values <7.5 g/dl are transfused. For each patient, after MNC reinfusion, a sample for complete blood count was obtained. Also, the dilution factor was calculated for every procedure as follows: fluid overload/total blood volume $\times 100$. Fluid overload was the sum of total anticoagulant citrate dextrose solution A infused (ml) + bag volume (ml) + calcium gluconate administration diluted in saline solution (ml). Anticoagulant citrate dextrose solution A was set at a higher ratio (1:15 to 1:18) in patients under oral anticoagulant therapy. Patients on O_2 therapy continued O_2 administration during the entire procedure.

To minimize the risk of catheter infections, a double-lumen central venous catheter was inserted and kept in place during the intensive treatment and was then inserted and removed at the end of every cycle when peripheral venous accesses were inadequate to perform the procedure. The ECP treatment schedule was borrowed from the protocol used in our centre for GvHD and modified as follows: 1 cycle (i.e. 2 procedures) per week for 3 weeks, 1 cycle fortnightly 2–3 times, 1 cycle per month if improved/stabilized; then, patients were maintained *chronically* on ECP, progressively lengthening the treatment intervals to 2 months. Even if the patient did not improve/stabilize, ECP was continued upon a case-by-case basis (e.g. re-transplant, logistical reasons, or personal preferences).

ECP was temporarily suspended in case of an onset of fever of $>38^\circ\text{C}$, an absolute MNC count of $<200 \times 10^9/\text{l}$, heart and/or renal failure, and haemorrhagic diathesis.

Statistical Analyses

Descriptive statistics were produced for demographic, clinical, and laboratory characteristics of patients. The mean and standard deviation are presented for normally distributed variables, the median and interquartile range (IQR) for non-normally distributed variables, and numbers and percentages for categorical variables.

Table 2. Patient characteristics at baseline (ECP initiation or, for controls, time of CLAD diagnosis)

Characteristics	ECP patients (n = 48)	Controls (n = 58)	p value
Males	34 (70.8%)	40 (68.9%)	0.37
BMI (mean $\pm$ SD)	23.7 $\pm$ 4.2	22.8 $\pm$ 23.65	0.40
Age at transplant (mean $\pm$ SD), years	44.6 $\pm$ 13.4	46.11 $\pm$ 13.9	0.48
Age at ECP (mean $\pm$ SD), years	51.5 $\pm$ 12.9	–	
Underlying disease			0.99
Pulmonary fibrosis	20 (41.67%)	22 (45.8%)	
Primary pulmonary hypertension	10 (20.83%)	2 (4.2%)	
Emphysema	5 (10.42%)	11 (22.9%)	
Cystic fibrosis	3 (6.25%)	10 (20.8%)	
Chronic rejection (Re-LTx)	3 (6.25%)	0 (0%)	
Bronchiectasis	3 (6.25%)	5 (10.4%)	
Eisenmenger syndrome	1 (2.08%)	3 (6.3%)	
Lymphangioleiomyomatosis	1 (2.08%)	1 (2.1%)	
Other	0 (0%)	4 (8.3%)	
Type of transplant			0.81
DL	28 (58.3%)	30 (51.7%)	
SL	19 (39.6%)	26 (44.8%)	
HL	1 (2.1%)	2 (3.4%)	
Haematocrit (mean $\pm$ SD), %	35.3 $\pm$ 5.4	n.a.	
Haematocrit <30%	8 (16.7%)	n.a.	
Median time from transplant to CLAD (IQR), months	38.5 (23.4–88.5)	45.5 (18.3–75.1)	0.72
Time from CLAD to ECP, months		–	
Median (IQR)	4.1 (1.7–23.5)		
>6 months	21 (43.8%)	–	
Median time from transplant to ECP (IQR), months	65.5 (30.7–125.9)	–	
CLAD phenotype, RAS	14 (29.2%)	15 (25.9%)	0.82
CLAD stage (BOS/RAS)			<0.001
I	28 (20/8) (58.3%)	53 (39/14) (91.3%)	
II	10 (5/5) (20.8%)	1 (1/0) (1.7%)	
III	10 (9/1) (20.8%)	4 (3/1) (6.9%)	
Immunosuppression at baseline			
Cyclosporin	11 (22.9%)	16 (27.6%)	0.658
Mycophenolate	12 (25.0%)	7 (12.1%)	0.126
Tacrolimus	35 (72.9%)	42 (72.4%)	0.99
Prednisolone	48 (100%)	58 (100%)	–
Azathioprine	20 (41.7%)	18 (31.0%)	0.311
Everolimus	5 (10.4%)	2 (3.4%)	0.24
Acute rejections prior to CLAD			0.336
0	17 (35.4%)	18 (31.0%)	
1	11 (22.9%)	21 (36.2%)	
≥ 2	20 (41.6%)	19 (32.8%)	
Grade B ≥ 1	18 (51.4%)	17 (48.6%)	0.41
Rapid decliners in the 6 months prior to ECP	10 (20.8%)	21 (36.2%)	0.09
O ₂ therapy	14 (29.2%)	0	
At least one bacterial infection up to ECP ^a	36 (75%)	45 (77.6%)	0.82
Median number of episodes (IQR)	1.5 (1–4)	2 (1–5)	0.44
CMV-positive patients	47 (97.9%)	55 (94.8%)	0.41
At least one CMV reactivation up to ECP ^a	34 (70.8%)	42 (72.4%)	0.99
Median number of episodes (IQR)	3 (1–5)	2 (0–4)	0.59
Airway colonization by <i>Aspergillus</i> spp. up to ECP ^a	13 (27.1%)	14 (24.1%)	0.82
Median number of episodes (IQR)	0 (0–1)	0 (0–2)	0.37
Airway colonization by <i>Pseudomonas aeruginosa</i> ^a	12 (27%)	16 (27.6%)	0.82
GERD before ECP	15 (31.2%)	17 (29.3%)	0.83
Fundoplication before ECP	5 (10.4%)	0	0.017
Neoplasia before ECP ^b	9 (18.7%)	3 (5.2%)	0.034

DL = Double-lung; SL = single-lung; HL = heart-lung; n.a. = not available; CMV = cytomegalovirus; GERD = gastro-oesophageal reflux disease.

^a These data are from LTx to ECP.

^b Includes: skin cancers (n = 7 in ECP patients, n = 2 in controls), prostate cancer (n = 1 in ECP patients), colon adenocarcinoma (n = 1 in ECP patients, n = 1 in controls).

Groups were compared to parametric or non-parametric tests, according to data distribution, for continuous variables, and with Pearson's χ^2 test (Fisher's exact test where appropriate) for categorical variables. All tests were two-tailed; $p < 0.05$ was considered significant.

Both ECP-treated patients and controls were defined as failures when FEV₁ measurements decreased >10% from ECP initiation or CLAD diagnosis, respectively. Analogously to previous papers, the patients were also categorized as failure/no failure at 6 months.

Survival analysis techniques were used for time to death (for CLAD-related death, whereas death from other causes was considered a competing risk) and for time to failure (defined as a FEV₁ drop of >10% from baseline, i.e. at ECP initiation). In the control group, the date of CLAD (i.e. BOS I) diagnosis was used as baseline.

In these analyses, the log-rank test was used for group comparisons, and univariate Cox models to assess variables associated with death/failure; owing to the small number of events, multivariate analyses were not possible.

The incidence of infections before and during ECP was compared using Poisson models. FEV₁ slopes were modelled using multilevel mixed linear regression models, with random effects for patients and decline over time from ECP start, and fixed effects (i.e. adjustment) for best FEV₁, CLAD grade at ECP initiation, and time from ECP (included as linear splines at months -12, -6, 0, 6, 12, and yearly afterwards) [22].

Pre-ECP fast decliners were defined as a linear slope of >100 ml/month in the 6 months preceding ECP start (with slopes derived from models as specified above).

STATA for Windows (StataCorp. 2009, Stata Statistical Software, release 13; StataCorp LP, College Station, Tex., USA) was used for statistical analyses.

Results

ECP Safety

The demographic and clinical features of 48 ECP patients and 58 controls are reported in table 2.

Overall, 1,756 ECP procedures were performed, i.e. a median of 26 procedures/patient (IQR 16–42, range 10–143; table 3). No complications occurred during ECP. In particular, O₂ demand did not increase during ECP despite the extracorporeal volume and fluid overload (around 18% of blood volume). Only 2 patients required diuretic therapy for moderate peripheral oedema. No relevant alteration in haemodynamic parameters was observed; also, no haemorrhage or local bleeding was observed in patients under anticoagulant therapy. The mean purity of reinfused MNCs was 85% (range 50–98.2). No MNC reinfusion-related adverse events were observed, even in these very compromised patients. Only 4 patients required a central venous catheter, and in 1 patient, a catheter-related thrombosis occurred. No patient dropped out for clinical frailty (table 4), and renal func-

Table 3. ECP in 48 patients with CLAD: description of procedures

Characteristics	
Procedures per patient	
Median (IQR)	26 (16.5–41.5)
Range	10–143
Oral anticoagulant, n (%)	10 (20.8)
Central venous catheter, n (%)	4 (8.3)
Blood transfusion requirement, n (%)	0 (0)
Blood volume processed/procedure, ml	
Median (IQR)	8,640 (7,000–10,000)
Range	4,900–12,500
Procedure duration, min	
Median (IQR)	126 (120–135)
Range	113–140
Volume at collection/bag, ml	
Median (IQR)	102 (96–111)
Range	55–154

tion was stable in all patients. Compared to the pre-ECP period, cytomegalovirus infections [incidence rate ratio 0.47, 95% confidence interval (CI) 0.32–0.70; $p < 0.001$] and bacterial infections [hazard ratio (HR) 0.40, 95% CI 0.25–0.66; $p < 0.001$] were less likely, while the number of fungal infections (incidence rate ratio 1.09, 95% CI 0.41–2.84; $p = 0.87$) did not significantly differ. Five patients underwent laparoscopic anti-reflux surgery for gastro-oesophageal reflux disease prior to ECP start.

Haematological Changes and Transfusion Requirement

The mean pre-/post-ECP procedure change in lymphocyte percentage was -0.27% (95% CI -1.9 to +1.36; $p = 0.74$), 0 (95% CI -217 to +35; $p = 0.15$) in absolute lymphocyte count ($\times 10^9/l$), 0.1% (95% CI -0.62 to 0.80; $p = 0.80$) in monocyte percentage, 0 (95% CI -232 to +22; $p = 0.25$) in absolute monocyte count ($\times 10^9/l$), -11 (95% CI -16 to -6.4; $p < 0.001$) in platelet count ($\times 10^9/l$), and -6.7% (95% CI -1.1 to -0.24%, $p = 0.003$) in haematocrit percentage.

There was no pre-procedure red blood cell (RBC) transfusion or albumin infusion requirements. The mean Hb level before the procedure was 11 g/dl (range 7.6–15). Despite a mean patient fluid overload of 1,090 ml (range 630–1,720) and a mean dilution factor of 18%, the expected decrease in Hb and platelet values did not require RBC or platelet transfusions after the ECP procedure. No patient required erythropoietin administration due to ECP.

Table 4. Outcomes of 48 ECP-treated CLAD patients and 58 controls (from CLAD diagnosis)

	ECP	Controls
Follow-up, months		
Median	65	51.2
IQR	25.8–94.3	11.3–81.5
Range	2.01–152.2	1.25–168.27
Cumulative, person-months	2,284.4	3,066.49
ECP outcome, ECP stopped for logistical/personal choice	4 (8.3%)	not applicable
Mortality		
Deaths*	20 (41.7%)	42 (72.4%)
CLAD	17 (85%)	32 (76.2%)
Cancer	1 (5%)	6 (14.3%)
Liver failure	1 (5%)	–
Subarachnoid haemorrhage	1 (5%)	1 (2.4%)
Sudden death	–	3 (7.1%)
Mortality rate per 100 person-months (95% CI)	1.48 (0.96–2.30)	1.37 (1.01–1.85)
Time to death, months		
Median (IQR)	17.03 (10.4–35.9)	40.17 (9.63–70.04)
Range	1.15–80.14	1.25–118.22
Failure		
Failures over time**	32 (66.7%)	54 (93.1%)
Failure rate per 100 person-months (95% CI)***	5.82 (4.12–8.23)	2.78 (2.13–3.63)

* $p = 0.002$; ** $p = 0.001$; *** $p = 0.012$.

Graft Function Stabilization during ECP

After ECP start, FEV₁ slopes appear to flatten out (i.e. slope = 0), particularly after 12 months of treatment (fig. 1). Average slopes are reported in table 5.

Cumulative failures over time (defined as above) are reported in table 4; in particular, among ECP patients, 32 patients experienced failure, of whom 19 (59.3%) within the first 6 months, while among controls (all enrolled at the time of CLAD diagnosis, therefore with better lung function at baseline), of the overall 54 failures, only 17 (29.3%) occurred in the first 6 months ($p < 0.001$). Variables associated with failure over time are reported in table 6.

Time from transplant to CLAD onset was inversely associated with failure (HR 0.98 per month, 95% CI 0.97–0.99; $p = 0.01$), and being a fast decliner in the 6 months prior to ECP initiation had a 4.9-fold higher rate of failures (95% CI 2.03–11.81; $p < 0.001$). Of note, severity of graft dysfunction at ECP initiation was not associated with higher failure rates.

Survival

Survival between ECP patients and controls is reported in figure 2 and was not statistically different. Among

ECP patients, variables associated with overall mortality are reported in table 6.

The only variables associated with worse survival were O₂ therapy, fungal airway colonization ($n = 4$) after ECP start, and BOS/RAS; survival according to baseline lung function, BOS/RAS, and the rate of decline in lung function in the 6 months prior to ECP initiation are shown in figure 3. In addition, there is some evidence that fast decliners had a worse survival than slow decliners in the control group but not in the ECP group (HR 5.75, 95% CI 1.6–20.4; $p = 0.007$).

The mortality rate according to failure at 6 months from baseline is significantly higher for controls with failure ($p < 0.001$) than for controls with response to first-line therapy, and for ECP patients either failing or responding to ECP (fig. 4).

Discussion

We report the longest-term study on 48 patients undergoing ECP for LTxR; our study demonstrates the efficacy and tolerability of ECP in patients with CLAD maintained on ECP over a long time period (some pa-

Fig. 1. FEV₁ slopes (ml) before and during ECP in 48 patients with CLAD. Red lines: patients died from BOS. Orange lines: patients died from other causes. Green lines: surviving patients. Patients are coded by year of ECP start and progressive number.

Table 5. Average slopes (FEV₁ change per month) and 95% CIs for 48 CLAD patients treated with ECP

Period from ECP start, months	Patients	Average slope ^a , ml	95% CI	p value
-12 to -6	43	-4.58	-8.13 to -1.04	0.011
-6 to 0	48	-48.38	-60.59 to -36.16	<0.001
0 to 6	48	-18.59	-34.65 to -2.53	0.023
6 to 12	24	-17.36	-34.16 to -0.56	0.043
12 to 24	27	-3.96	-15.04 to 7.11	0.483
24 to 36	17	-4.11	-12.48 to 4.25	0.335

Estimates are adjusted for baseline grading, best FEV₁, and time from CLAD to ECP. p values are indicated for comparison with 0 slope. ^a Calculated as FEV₁ change/month.

Fig. 2. Survival from CLAD onset in 48 patients treated with ECP and 58 controls.

Table 6. Potential determinants of survival and failure in 48 LTx patients with CLAD undergoing ECP

Variable	Survival		Failure	
	HR	p value	HR	p value
Gender (male vs. female)	1.75 (0.58–5.29)	0.319	0.92 (0.43–1.95)	0.827
BMI (per unit)	1.1 (0.97–1.24)	0.135	1.02 (0.94–1.11)	0.599
Age at transplant (years)	1.05 (1.01–1.1)	0.017	1 (0.98–1.03)	0.799
Age at ECP (years)	1.05 (1.01–1.1)	0.017	0.99 (0.96–1.01)	0.324
Underlying disease				
Emphysema vs. PPH	2.75 (0.58–13.08)	0.202	1.05 (0.31–3.52)	0.938
Cystic fibrosis	0.54 (0.06–4.9)	0.587	2.6 (0.82–8.2)	0.103
Other	2.86 (0.83–9.82)	0.095	0.89 (0.37–2.1)	0.782
Type of transplant				
Single-lung vs. double-lung	4.44 (0.54–36.71)	0.167	0.76 (0.37–1.59)	0.47
Heart-lung	2.06 (0.78–5.43)	0.143	NC	NC
Haematocrit (>30 vs. ≤30%)	0.75 (0.17–3.28)	0.699	0.98 (0.4–2.41)	0.961
Immunosuppression at ECP				
Cyclosporin	2.99 (1.21–7.41)	0.018	2.37 (0.92–6.11)	0.074
Mycophenolate	0.44 (0.13–1.53)	0.198	0.59 (0.25–1.36)	0.214
Tacrolimus	0.35 (0.14–.87)	0.024	0.49 (0.19–1.28)	0.145
Azathioprine	1 (0.28–3.56)	0.995	2.52 (0.94–6.76)	0.065
Everolimus	0.22 (0.03–1.7)	0.147	1.67 (0.71–3.92)	0.239
Azithromycin	2.01 (0.25–15.93)	0.507	0.97 (0.26–3.64)	0.967
Number of IS drugs (3 vs. 2)	0.32 (0.12–.85)	0.022	1.38 (0.66–2.92)	0.395
CLAD stage at baseline				
II vs. I	1.28 (0.44–3.7)	0.645	0.52 (0.2–1.31)	0.163
III vs. I	1.24 (0.41–3.81)	0.703	1.03 (0.43–2.48)	0.947
CLAD phenotype (RAS vs. BOS)	2.5 (0.95–6.57)	0.062	1.49 (0.67–3.3)	0.33
Time from transplant to CLAD (months)	1 (0.99–1.01)	0.573	0.99 (0.98–1)	0.01
Time from CLAD to ECP				
Per month	1.01 (0.99–1.02)	0.265	0.99 (0.98–1.01)	0.299
>6 vs. ≤6 months	2.05 (0.68–6.11)	0.20	0.62 (0.31–1.27)	0.19
Time from transplant to ECP (months)	1 (0.99–1.01)	0.93	0.99 (0.98–1)	0.003
Acute rejections prior to CLAD				
1 vs. 0	1.88 (0.59–5.99)	0.285	0.86 (0.28–2.58)	0.783
≥2 vs. 0	0.61 (0.19–1.99)	0.417	1.75 (0.78–3.96)	0.177
Rapid decliner (yes vs. no)	0.35 (0.1–1.29)	0.115	4.9 (2.03–11.81)	<0.001
O ₂ therapy (yes vs. no)	3.36 (1.37–8.23)	0.008	0.71 (0.04–11.79)	0.809
Statins (yes vs. no)	1.37 (0.52–3.62)	0.52	0.99 (0.42–2.33)	0.99
At least one bacterial infection up to ECP (yes vs. no)	1.11 (0.92–1.33)	0.269	1.07 (0.84–1.36)	0.603
At least one CMV reactivation up to ECP (yes vs. no)	0.96 (0.8–1.16)	0.701	1 (0.81–1.22)	0.964
At least one airway colonization by <i>Aspergillus</i> spp. up to ECP (yes vs. no)	1.1 (0.53–2.28)	0.799	0.26 (0.04–1.72)	0.162
GERD (yes vs. no)	0.9 (0.32–2.5)	0.836	1.08 (0.51–2.29)	0.845
Fundoplication before ECP (yes vs. no)	0.94 (0.21–4.09)	0.932	1.17 (0.16–8.77)	0.876
At least one bacterial infection during ECP (yes vs. no)	1.61 (0.65–4.01)	0.307	1.25 (0.62–2.51)	0.538
At least one CMV reactivation during ECP (yes vs. no)	0.92 (0.37–2.31)	0.866	1.85 (0.92–3.73)	0.086
At least one airway colonization by <i>Aspergillus</i> spp. during ECP (yes vs. no)	3.55 (1.12–11.21)	0.031	4.84 (1.65–14.18)	0.004

PPH = Primary pulmonary hypertension; NC = not calculated; CMV = cytomegalovirus; GERD = gastro-oesophageal reflux disease.

tients even >10 years) throughout which ECP was well tolerated, with no adverse events, and, in particular, with no increased risk of infectious complications.

All papers published at present report the use of the on-line technique for performing ECP, with different

treatment frequencies. Independently from the intensity of the approach (from 10 procedures in the first month [17] to 1 cycle every 4–6 weeks [14] to 1 cycle fortnightly for the first 3 months [15]), responses do not markedly differ. Of note, in all these cases, patients were treated for

Fig. 3. Survival from ECP initiation in 48 LTxR treated with ECP, according to: severity of graft function impairment at ECP initiation (**a**), rate of FEV₁ decline in the 6 months prior to ECP initiation (**b**), and CLAD phenotype (BOS/RAS) at ECP initiation (**c**). CLAD severity (severity of FEV₁ loss at ECP initiation): I = FEV₁ between 79 and 65% of best post-transplant value; II = FEV₁ between 64 and 50% of best post-transplant value, and III = FEV₁ ≤49% of best post-transplant value. Slow decliners: ≤100 ml/month in the 6 months prior to ECP; fast decliner: >100 ml/month in the 6 months prior to ECP. Failure status at 6 months: failure = FEV₁ of <10% compared to baseline.

Fig. 4. Survival according to group (ECP vs. controls) and failure status at 6 months (failure = FEV₁ <10% compared to baseline).

a limited period [14, 15] due to the technical and clinical limitations.

After an initial report on intensive initiation [23], we modified our protocol with a less intensive approach mainly for logistical reasons and taking into account the results of other groups [14, 24]. Unlike in other studies, we report the use of the off-line technique that offers the advantage of a low extracorporeal volume, a short procedure time (about 120 min), and the possibility to perform quality controls on the product collected and reinfused after UV-A irradiation in the presence of 8-methoxypsoralen. The low extracorporeal volume of the cell separator device allowed us to avoid pre-procedure RBC transfusion in anaemic patients (haematocrit level <30%) as well as the infusion of a 5% albumin solution as reported elsewhere [17]. Furthermore, even though patients reached a mean dilution of 18% after MNC reinfusion, we did not observe any major cardiovascular side effects or transfusion requirement, and the O₂ need remained stable. Additionally, haematological changes following ECP procedures were not clinically relevant. In particular, we did not observe significant lymphopenia, and any IS therapy changes (over the long follow-up of our cohort) were driven by a number of other reasons (e.g. response, concomitant infections, renal failure, etc.).

The optimal compliance observed in all patients, especially those in poor clinical conditions, allows for the longest treatment duration reported so far.

Therefore, we have been able to assess efficacy over time: we found a slowing decline or even stabilization in lung function after treatment with ECP. Graft function at 6 months was stable with respect to baseline in 60.4% of treated CLAD patients. These results are in line with previous observations by others [14–17].

The failure to respond to treatment is classically defined as an FEV₁ decline of >10% from baseline and ascertained at 6 months; in our study, we did not limit the analysis to 6 months, but assessed failure over a longer time period. We did not find any association of failure and time to failure with the pattern of FEV₁ decline (slow vs. fast decliners) or specific CLAD phenotype (BOS vs. RAS). Despite not reaching statistical significance (most likely owing to the small sample size and low power), our data point towards a longer survival in certain patient subgroups, such as the BOS phenotype and baseline lung function (grade I vs. II–III); therefore, patient stratification might be useful for the optimal selection of patients that might profit from this therapy [15, 17, 25].

In view of these findings, given that no apparent predictor of response has so far been reliably identified, and

considering the high degree of safety and tolerability and the low cost of the off-line procedure, we think that ECP might be offered to all LTxR patients with CLAD early after diagnosis, as recently advocated [26]. Of note, in our study, the time from transplant to CLAD and the time from transplant to ECP was inversely associated with failure; these findings could be ascribed to a better tolerance of the transplanted organ and, consequently, a slower, less aggressive CLAD.

Our experience in treating patients with chronic GvHD showed a strong correlation between response to ECP and survival [11]. However, in the similar immunologic context of LTxR, we observed that even patients failing, according to the classical definition (a FEV₁ decline of >10% from baseline at 6 months), did not show shorter survival. In fact, our cohort shows higher median survival rates than those reported elsewhere, both in BOS and in RAS patients [5].

Considering the subgroup of patients experiencing failure (i.e. those who experience ≥10% FEV₁ decline from baseline), those treated with ECP have a significantly better survival with respect to controls; therefore (with the limitation of the retrospective nature of the analysis), we speculate that the effect of ECP might be evident over a longer follow-up period and that even those patients who initially fail might stabilize further on. This hypothesis, however, needs to be further addressed with an appropriate randomized controlled trial. Finally, a decline in FEV₁ over time should be evaluated taking into account that it is innate, occurring with age also in non-transplanted patients [27]. In agreement to Greer et al. [15], we found lower survival in the RAS phenotype when compared to BOS, with borderline significance. However, unlike Greer et al. [15], we did not observe survival differences with respect to the rate of lung function decline before ECP initiation; this might be due to the fact that in our cohort, we maintained patients on ECP even if their improvement/stabilization was not apparent in the first 12 months. Also, survival was not influenced by the time from transplant to CLAD nor by the time from transplant to ECP or the time from CLAD to ECP [15].

The strengths of this study are the very long treatment and follow-up periods of one of the largest single-centre cohorts of LTxR patients treated with ECP; importantly, we only considered patients with >8 ECP procedures, since in our opinion, it is reasonable to evaluate the response to ECP only after about 2 months of treatment; also, we did not exclude patients from treatment on clinical grounds. Nevertheless, we did not observe drop-outs for clinical frailty. Finally, we took into account the time

to failure rather than failure at a single time point, thus avoiding the loss of information.

We need to acknowledge a number of severe limitations. The retrospective nature of our study is the most obvious, together with the limited sample size that precludes meaningful subgroup analysis. This entails possible misclassification bias on some variables, particularly in the control group; however, survival and lung function over time was available for all patients. Also, the non-randomized control group is limiting our ability to prove effectiveness; however, it is worth noting that patients in the control group, treated with an immunosuppressive

regimen analogous to the ECP group, had a lower severity of graft dysfunction at baseline, but nevertheless, they did show somewhat lower survival.

In conclusion, ECP in LTxR with CLAD is effective and safe in the very long term; it should be further evaluated in the context of randomized controlled trials, possibly as first-line therapy.

Financial Disclosure and Conflicts of Interest

The authors have no conflicts of interest to disclose.

References

- Christie JD, Edwards LB, Kucheryavaya AY, Benden C, Dobbels F, Kirk R, et al: The Registry of the International Society for Heart and Lung Transplantation: Twenty-Eighth Adult Lung and Heart-Lung Transplant Report – 2011. *J Heart Lung Transplant* 2011;30:1104–1122.
- Benden C, Danziger-Isakov L, Faro A: New developments in treatment after lung transplantation. *Curr Pharm Des* 2012;18:737–746.
- Sato M, Keshavjee S: Bronchiolitis obliterans syndrome: alloimmune-dependent and -independent injury with aberrant tissue remodeling. *Semin Thorac Cardiovasc Surg* 2008;20:173–182.
- Estenne M, Hertz MI: Bronchiolitis obliterans after human lung transplantation. *Am J Respir Crit Care Med* 2002;166:440–444.
- Sato M, Waddell TK, Wagnetz U, Roberts HC, Hwang DM, Haroon A, et al: Restrictive allograft syndrome (RAS): a novel form of chronic lung allograft dysfunction. *J Heart Lung Transplant* 2011;30:735–742.
- Verleden GM, Vos R, Verleden SE, De Wever W, De Vleeschauwer SI, Willems-Widyastuti A, et al: Survival determinants in lung transplant patients with chronic allograft dysfunction. *Transplantation* 2011;92:703–708.
- Verleden SE, Vandermeulen E, Rutten D, Vos R, Vaneylen A, Dupont LJ, et al: Neutrophilic reversible allograft dysfunction (NRAD) and restrictive allograft syndrome (RAS). *Semin Respir Crit Care Med* 2013;34:352–360.
- Weigt SS, DerHovannessian A, Wallace WD, Lynch JP 3rd, Belperio JA: Bronchiolitis obliterans syndrome: the Achilles' heel of lung transplantation. *Semin Respir Crit Care Med* 2013;34:336–351.
- Edelson R, Berger C, Gasparro F, Jegasothy B, Heald P, Wintroub B, et al: Treatment of cutaneous T-cell lymphoma by extracorporeal photochemotherapy. Preliminary results. *N Engl J Med* 1987;316:297–303.
- Flowers ME, Apperley JF, van Besien K, Elmaagacli A, Grigg A, Reddy V, et al: A multicenter prospective phase 2 randomized study of extracorporeal photopheresis for treatment of chronic graft-versus-host disease. *Blood* 2008;112:2667–2674.
- Del Fante C, Scudeller L, Viarengo G, Bernasconi P, Perotti C: Response and survival of patients with chronic graft-versus-host disease treated by extracorporeal photochemotherapy: a retrospective study according to classical and National Institutes of Health classifications. *Transfusion* 2012;52:2007–2015.
- Perotti C, Del Fante C, Tinelli C, Viarengo G, Scudeller L, Zecca M, et al: Extracorporeal photochemotherapy in graft-versus-host disease: a longitudinal study on factors influencing the response and survival in pediatric patients. *Transfusion* 2010;50:1359–1369.
- Slovits BS, Loyd JE, King LE Jr: Photopheresis for chronic rejection of lung allografts. *N Engl J Med* 1995;332:962.
- Benden C, Speich R, Hofbauer GF, Irani S, Eich-Wanger C, Russi EW, et al: Extracorporeal photopheresis after lung transplantation: a 10-year single-center experience. *Transplantation* 2008;86:1625–1627.
- Greer M, Dierich M, De Wall C, Suhling H, Rademacher J, Welte T, et al: Phenotyping established chronic lung allograft dysfunction predicts extracorporeal photopheresis response in lung transplant patients. *Am J Transplant* 2013;13:911–918.
- Jaksch P, Scheed A, Keplinger M, Ernst MB, Dani T, Just U, et al: A prospective interventional study on the use of extracorporeal photopheresis in patients with bronchiolitis obliterans syndrome after lung transplantation. *J Heart Lung Transplant* 2012;31:950–957.
- Morrell MR, Despotis GJ, Lublin DM, Patterson GA, Trulock EP, Hachem RR: The efficacy of photopheresis for bronchiolitis obliterans syndrome after lung transplantation. *J Heart Lung Transplant* 2010;29:424–431.
- Stewart S, Fishbein MC, Snell GI, Berry GJ, Boehler A, Burke MM, et al: Revision of the 1996 working formulation for the standardization of nomenclature in the diagnosis of lung rejection. *J Heart Lung Transplant* 2007;26:1229–1242.
- Meloni F, Solari N, Miserere S, Morosini M, Cascina A, Klersy C, et al: Chemokine redundancy in BOS pathogenesis. A possible role also for the CC chemokines: MIP3-beta, MIP3-alpha, MDC and their specific receptors. *Transpl Immunol* 2008;18:275–280.
- Sato M: Chronic lung allograft dysfunction after lung transplantation: the moving target. *Gen Thorac Cardiovasc Surg* 2013;61:67–78.
- Lillier D, Gerna G, Bruno F, Draghi P, Gabanti E, Fornara C, et al: Systemic and local human cytomegalovirus-specific T-cell response in lung transplant recipients. *New Microbiol* 2013;36:267–277.
- Marchenko Y: Estimating variance components in STATA. *Stata J* 2006;6:1–21.
- Meloni F, Cascina A, Miserere S, Perotti C, Vitulo P, Fietta AM: Peripheral CD4(+) CD25(+) TREG cell counts and the response to extracorporeal photopheresis in lung transplant recipients. *Transplant Proc* 2007;39:213–217.
- Villanueva J, Bhorade SM, Robinson JA, Husain AN, Garrity ER Jr: Extracorporeal photopheresis for the treatment of lung allograft rejection. *Ann Transplant* 2000;5:44–47.
- Hayes D Jr: A review of bronchiolitis obliterans syndrome and therapeutic strategies. *J Cardiothorac Surg* 2011;6:92.
- Barr ML: Call it BOS, call it CLAD – the need for prospective clinical trials and elucidating the mechanism of extracorporeal photopheresis. *Am J Transplant* 2013;13:833–834.
- Oldham PD: Decline of FEV₁. *Thorax* 1987;42:161–164.