Bone Marrow Transplantation (2008) 42, 609–617

www.nature.com/bmt

ORIGINAL ARTICLE

Extracorporeal photopheresis for the treatment of steroid refractory acute GVHD

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Extracorporeal photopheresis (ECP) was given to 23 patients with steroid-refractory acute GVHD (aGVHD, grade II ($n=10$), III ($n=7$) or IV ($n=6$)). The median duration of ECP was 7 months (1–33) and the median number of ECP cycles in each patient was 10. Twelve patients (52%) had complete responses. Eleven patients (48%) survived and 12 died, 10 of GVHD with or without infections and two of leukaemia relapse. The average grade of GVHD was reduced from 2.8 (on the first day of ECP) to 1.4 (on day +90 from ECP) ($P=0.08$), and the average dose of i.v. methylprednisolone from 2.17 to 0.2 mg/kg/d ($P=0.004$). Complete responses were obtained in 70, 42 and 0% of patients, respectively, with grades II, III and IV aGVHD; complete responses in the skin, liver and gut were 66, 27 and 40%. Patients treated within 35 days from onset of aGVHD had higher responses (83 vs 47%; $P=0.1$). A trend for improved survival was seen in grade III–IV aGVHD treated with ECP as compared to matched controls (38 vs 16%; P 0.08). ECP is a treatment option for patients with steroid refractory aGVHD and should be considered early in the course of the disease.

Bone Marrow Transplantation (2008) **42,** 609–617; doi:10.1038/bmt.2008.221; published online 28 July 2008
Keywords: extracorporeal photopheresis; acute GVHD; steroid refractory aGVHD

Introduction

Acute GVHD (aGVHD) is a severe complication of haematopoietic stem cell allograft and the major cause of early transplant-related mortality and long-term complications.[1] Despite improved immunosuppressive prophylaxis, aGVHD occurs even in patients receiving a graft from a matched sibling donor and the risk increases in unrelated or histoincompatible donor transplantations.[2] Corticosteroids alone or combined with other immunosuppressive agents are standard primary therapy for aGVHD and can induce 70% responses;[3] however, only a fraction of these (35%) are durable.[4] Patients who fail to respond to this first-line therapy have a poor prognosis, with high transplant-related mortality[3,5] due to GVHD itself and its treatment complications as opportunistic infections. Several second-line therapies have been proposed for the management of unresponsive GVHD, without obtaining a beneficial effect on patient's outcome or overall long-term survival.[6–8]

In the last decade, extracorporeal photochemotherapy (ECP) has shown some encouraging results in steroid-refractory acute and chronic GVHD (cGVHD).[9–17] Treatment with ECP involves harvesting the peripheral blood white cells from patients receiving 8-methoxypsoralen, exposing cells to UV-A light and reinfusing the cells into the patient after treatment has been completed. This procedure induces an apoptotic cellular cascade in all leukocytes treated within 24–48 h. The mechanism by which ECP exerts its therapeutic effect is still under investigation[18]; it has been reported to induce a state of immune tolerance by modulating APC activation,[19–21] increasing regulatory T-cell production[22–24] and causing a shift from a Th1 to a Th2 cytokine release.[25–27] Compared to conventional immunosuppressive drugs, ECP seems not to interfere with stem cell engraftment and does not suppress T- and B-cell responses to novel or recall antigens,[27] so that common side effects of immunosuppression may be lessened.

Photopheresis has been extensively investigated in the setting of unresponsive cGVHD allowing achievement from 53% to 95–100% of overall responses on the cutaneous and mucosal involvement,[21] whereas minor results have been reported on visceral disease. ECP effects on aGVHD are reported in non-randomized studies, all conducted on a small series of patients from a single centre, reporting best response to treatment in patients with cutaneous grade II–III disease.[15]

In this study, we present our experience with the use of ECP in patients with steroid-unresponsive aGVHD.

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Received 25 May 2007; revised 13 June 2008; accepted 16 June 2008; published online 28 July 2008

Materials and methods

Patients

We retrospectively analysed a series of 23 patients (17 males and 6 females) treated, between 1996 and 2006, with ECP for a steroid-unresponsive aGVHD. Their median age was 41 (range 18–66) years. Clinical data and transplant procedures are shown in Table 1. All patients received an unmanipulated allograft from an HLA-A, -B, -DRB1 identical related ($n=12$) or alternative donor ($n=8$), or from a 1 antigen-mismatched unrelated donor ($n=3$). Ten patients were conditioned with a myeloablative regimen and 13 received a reduced-intensity schedule. The source of stem cells was BM for most of the patients ($n=20$) and peripheral blood in three cases. GVHD prophylaxis consisted of CsA alone or combined with a short-course MTX; pretransplant antithymocyte globulin was used in 10 patients.

Complete haematological recovery was achieved in 20 patients (87%) at a median time of 32 days from SCT (range 15–120).

Table 1 Patients and transplant data

Years	*1996–-2006*
N = age	23
Median	41 (18–66)
Sex	
Male/female	17/6
Disease	
CML	5
AML	4
ALL	4
MM	2
MDS	3
MFI	2
SAA	2
Bechet syndrome	1
Disease status at SCT	
>1 CR/1 CP	13 (56%)
Donor	
Median age	38 (22–69)
Related/unrelated	12/11
Match/mismatch	20/3
Sex mismatch	12 (52%)
Female donor/male recipient	5 (22%)
SC source	
BM/PB	20/3
Conditioning	
MA/RIC	10 /13
aGVHD prophylaxis	
CsA	1 (4%)
CsA–MTX	12 (52%)
CsA–MTX–ATG	10 (43%)

Abbreviations: ATG = antithymocyte globulin; CP = chronic phase; MA = myeloablative; MDS = myelodysplastic syndrome; MFI = myelofibrosis; MM = multiple myeloma; PB = peripheral blood; SAA = severe aplastic anaemia; RIC = reduced intensity conditioning.

Acute GVHD evaluation

Acute GVHD was classified as grade I–IV according to the Glucksberg-Seattle scoring system; no histological examinations were performed. Patients referred to ECP were all diagnosed as steroid-resistant aGVHD, defined as lack of stable clinical improvement after treatment with methylprednisolone at a dosage of 2–5 mg/kg/d for at least 7 days. Four patients were treated beyond day +100, and these were steroid-dependent aGVHD.

Responses to ECP treatment were assessed every month by physical examination, liver function tests and complete chemistry analysis.

We define complete response to ECP as resolution of aGVHD manifestations in all organs involved (GVHD grade 0–I), partial response as stable improvement of global aGVHD Glucksberg score and no response as the lack of any beneficial effect or disease progression.

Clinical GVHD manifestations persisting after day 100 of transplantation are conventionally considered as a progressive cGVHD and reported as none, minimal, moderate and severe, following the National Institute of Health classification.[28,29]

ECP procedure

We performed a clinical evaluation of all patients before every ECP treatment. Complete blood count, chemistry panel and coagulation parameters were controlled before and after each course. Photopheresis was considered only if patients had a WBC count $>1000\times10^9$/l, platelet count $>20\times10^9$/l and Hb level >9.0 g per 100 ml.

The ECP schedule used was the same for all patients: a cycle (= two treatments on consecutive days) every week for the first month, a cycle every 2 weeks for the following 2 months and a cycle every month until complete resolution or stabilization of GVHD. The procedure was conducted using an offline system. Leukapheresis was performed using two different cell separators (Cobe Spectra, Lakewood, CO, USA or Haemonetics UK Ltd, Bothwell, Scotland GB), depending on the availability of our centre, processing 3.5 and 8 l of blood volume, respectively. The mononuclear cell (MNC) concentrate was adjusted to a constant volume of 300 ml by adding normal saline and 3.5–4 ml of 8-methoxypsoralen (Gerot Pharmaceutical, Vienna, Austria), obtaining a final concentration of 200 ng/ml. Cell concentrate was transferred in an EVA plastic bag (Macopharma, Mouvanx, France) and irradiated at 2 J/cm^2 with a Vilbert-Loumart system for about 30 min. Haematocrit of the final product was always below 2.5%. The average number of MNCs collected and reinfused at each procedure was 3.7×10^9 (range 0.4–7.7); a total of 452 ECP treatments were performed and analysed in this series of patients.

Extracorporeal photopheresis was conducted using a single-lumen central venous catheter in 15 patients and both a central and a peripheral vein access in eight. Each ECP treatment lasted about 3–4 h.

Treatment of acute GVHD

The characteristics of aGVHD are outlined in Table 2. When referred to ECP, all the patients received

Table 2 Glucksberg score and immunosuppressive therapy at aGVHD onset and at the start of ECP

aGVHD onset							*ECP Days after aGVHD onset*	*aGVHD day 0 ECP*				*IS*		
Patient no.	*Days after SCT*	*s*	*l*	*g*	*Global*	*Type of IS*		*s*	*l*	*g*	*Global*	*MP*		*Other*
												i.v.	*p.o.*	
1	11	1	0	0	**1**	MP 5–10 + CsA	91	1	4	2	**4**	5.1	0	None
2	8	1	0	1	**2**	MP 5–10 + CsA + ATG	41	1	4	0	**4**	5.0	0	FK 506
3	7	1	0	1	**2**	MP 2 + CsA	63	2	4	1	**4**	5.0	0	CsA + ATG
4	9	1	0	0	**1**	MP 2 + CsA	36	1	3	0	**3**	2.5	0	CsA + ATG
5	16	3	2	0	**3**	MP 5 + CsA + ATG + CTX	42	1	4	2	**4**	3.5	0	CsA
6	7	1	0	0	**1**	MP 2 + CsA	119	2	0	1	**2**	1.0	0	MMF
7	37	1	0	0	**1**	MP 2	113	1	1	2	**3**	2.0	0	CsA
8	43	0	1	2	**2**	MP 2 + CsA	77	1	0	2	**2**	1.5	0	CsA
9	15	2	0	0	**1**	MP 2–5 + CsA	77	3	2	2	**4**	2.0	0	CsA
10	36	1	0	2	**2**	MP 2–5 + CsA	21	2	1	2	**3**	2.1	0	CsA
11	34	1	0	0	**1**	MP 2	95	2	0	1	**2**	1.0	0	CsA
12	22	1	0	0	**1**	MP 2 + CsA + CTX	69	3	1	1	**3**	1.0	0	CsA
13	22	2	0	1	**2**	MP 2 + CsA + CTX	49	2	3	1	**4**	2.5	0	CsA
14	14	2	0	0	**1**	MP 2 + CsA + MMF	91	1	0	1	**2**	1.5	0	CsA
15	17	2	1	1	**2**	MP 2 + CsA	21	1	0	1	**2**	1.0	0	CsA
16	24	1	0	0	**1**	MP 2 + CsA	56	2	0	0	**2**	1.0	0	CsA
17	10	2	0	0	**1**	MP 2 + CsA + ATG	148	2	0	1	**2**	1.5	0	CsA
18	22	2	0	1	**2**	MP 2 + CsA	14	3	0	1	**3**	2.2	0	CsA
19	9	1	0	0	**1**	MP 2 + CsA	35	2	0	1	**2**	2.0	0	CsA
20	9	2	0	1	**2**	MP 2 + CsA	91	1	0	2	**3**	2.0	0	CsA
21	12	1	3	1	**3**	MP 2 + CsA	46	1	1	1	**2**	1.5	0	CsA
22	14	2	0	0	**1**	MP 2 + CsA	21	2	0	2	**3**	2.0	0	CsA + MTX
23	36	2	0	1	**2**	MP 2 + CsA	34	1	0	1	**2**	1.0	0	CsA

Abbreviations: ATG = antithymocyte globulin; s = skin; CTX = cyclophosphamide; ECP = extracorporeal photopheresis; FK 506 = tacrolimus; g = gut; IS = immunosuppression; i.v. = intravenous therapy (mg/kg); l = liver; MMF = mycophenolate mofetil; MP = methylprednisolone; p.o. = oral therapy (total daily dose in mg).
Bold values indicate overall grading of GVHD.

at least one line of therapy, seven of them (30%) had failed two lines of treatment and two (9%) a third line.

Photopheresis was started at median interval of 56 days from aGVHD onset (range 14–148). At the start of ECP, there were no patients with grade Ia GVHD, 10 patients had grade II (43%), seven had grade III (30%) and six had grade IV (27%). ECP was administered for a median of 7 months (range 0–33); all patients received at least one cycle of treatment, 13 completed 3 months of therapy, 12 completed 6 months and six patients were treated for more than 1 year. The median number of ECP cycles performed in each patient was 10 (range 1–25).

All patients were continued on steroids with or without CsA while receiving ECP. The dose of 6 methylprednisolone was recorded at the start of ECP therapy, on days +30, +60 and +90, and considered as an indirect indicator of response.[3]

Statistical analysis

Patient data were analysed with the NCSS package. Comparisons were carried out using the χ^2 test for categorical variables and the non-parametric Mann–Whitney test for continuous variables. Actuarial survival was calculated using death from any cause as an end point. Differences between curves were tested using the log rank test.

Results

Response to treatment and survival

Detailed analysis of aGVHD grading and immunosuppressive therapy at the onset of ECP treatment, on days +30, +60 and +90, are outlined in Table 3.

Figure 1 depicts the average aGVHD score: it was 1.57 at onset of GVHD, 2.83 when ECP was started and then gradually declined to 1.43 on day +90 (Figure 1). The average i.v. methylprednisolone dose declined from 2.17, when ECP was started (day 0), to 0.97 mg/kg (day +30), to 0.43 mg/kg (day +60) and to 0.20 mg/kg (day +90) (Figure 1). In addition, when ECP was started, 23/23 patients were receiving i.v. steroids; after 3 months of ECP treatment, 4/23 patients continued i.v. steroids and eight patients were on oral methylprednisolone (Table 3).

GVHD response on day +90 was evaluated in patients surviving and continuing ECP: 11 patients died within 90 days from ECP of GVHD[9] or leukaemia relapse[2] (Table 3).

The overall complete response rate was 52% (12 out of 23), of which 10 patients responded within 90 days and two responded later; one of the two patients continued ECP for an additional 3 months and the other continued on alternative immunosuppressive treatment.

Complete responses were obtained in 70, 42 and 0% of patients, respectively, with grades II, III and IV aGVHD (Figure 2); complete responses in the skin, liver and gut were 66, 27 and 40% (Figure 2). There was a trend for a higher response rate in patients treated within 35 days from

Table 3 Response rates and survival status after 1, 2 and 3 months of ECP treatment

Patient no.	*ECP day +30*							*ECP day +60*							*ECP day +90*									
	Grade aGVHD				*IS*			*Grade aGVHD*				*IS*			*Grade aGVHD*				*IS*				*Death*	
	s	*l*	*g*	*Global*	*MP*		*Other*	*s*	*l*	*g*	*Global*	*MP*		*Other*	*s*	*l*	*g*	*global*	*MP*		*Other*	*No. of ECP cycles*	*Day after ECP*	*Cause*
					i.v.	*os*						*i.v.*	*os*							*i.v.*	*os*				
1	1	4	0	**4**	1	0	*N*	Death														2	33	aGVHD
2	3	4	0	**4**	2	0	*N*	Death														1	42	aGVHD
3	Death																					1	15	aGVHD
4	Death																					1	4	aGVHD
5	0	4	4	**4**	5	0	CsA + ATG	Death														4	31	aGVHD
6	2	0	1	**2**	1	0	MMF	2	0	0	**1**	0	12	MMF	2	0	0	**1**	0	11	MMF	18	Alive	
7	1	2	1	**3**	0	8	CsA	0	2	1	**2**	0.8	0	CsA	1	2	1	**2**	1	0	CsA	13	Alive	
8	1	0	1	**2**	0.3	0	CsA	1	0	1	**2**	0	8	CsA	1	0	1	**2**	0	8	CsA + azat	12	Alive	
9	2	3	1	**3**	0.7	0	CsA	1	1	0	**2**	0.2	0	N	Death							6	66	ALL relapse
10	1	3	1	**3**	2.1	0	CsA	1	4	1	**4**	2.1	0	CsA	0	2	0	**3**	0.4	0	CsA	10	Alive	
11	2	0	0	**1**	0.3	0	CsA	1	0	0	**1**	0	16	CsA	1	0	0	**1**	0	8	CsA	25	Alive	
12	2	0	1	**2**	0.8	0	CsA	3	1	2	**3**	0.8	0	CsA + ATG	Death							5	90	aGVHD
13	2	4	0	**4**	1	0	CsA + MSC	1	3	0	**3**	0.5	0	CsA	3	3	0	**4**	0.7	0	CsA	9	92	aGVHD
14	Death																					3	17	Infection
15	1	1	1	**2**	1	0	CsA	1	0	0	**1**	0.8	0	CsA + MTX	1	0	0	**1**	0.4	0	CsA	17	Alive	
16	1	0	0	**1**	0	12	CsA	1	0	0	**1**	0	1	CsA	1	0	0	**1**	0	4	CsA	16	Alive	
17	1	0	1	**2**	0.5	0	CsA	1	0	1	**2**	0	12	CsA	1	0	0	**1**	0	5	CsA	14	Alive	
18	1	0	1	**2**	0.6	0	CsA	1	0	0	**1**	1	32	CsA	1	0	0	**1**	0	32	CsA	19	Alive	
19	Death																					1	29	MOF
20	0	1	1	**2**	0.5	0	CsA	1	0	1	**2**	0.3	0	CsA	1	0	0	**1**	0.3	0	CsA	13	Alive	
21	1	0	1	**2**	0.3	0	CsA	1	0	0	**1**	0	24	CsA	1	0	0	**1**	0	24	CsA	14	Alive	
22	1	1	0	**2**	1	0	CsA	1	0	0	**1**	0.3	0	MMF	1	0	0	**1**	0	8	MMF	13	Alive	
23	1	0	0	**1**	0.4	0	CsA	1	0	0	**0**	0	4	CsA	0	0	0	**0**	0	4	CsA	10	Alive	

Abbreviations: ATG = antithymocyte globulin;s = skin; azat = azathioprine; CTX = cyclophosphamide; ECP = extracorporeal photopheresis; FK 506 = tacrolimus; g = gut; IS = immunosuppression; l = liver; MCS = mesenchymal cells; MMF = mycophenolate mofetil; MOF = multiorgan failure. MP = methylprednisolone dosage (mg/kg); os = oral methylprednisolone dosage; i.v. = intravenous methylprednisolone dosage.

Bold values indicate overall grading of GVHD.

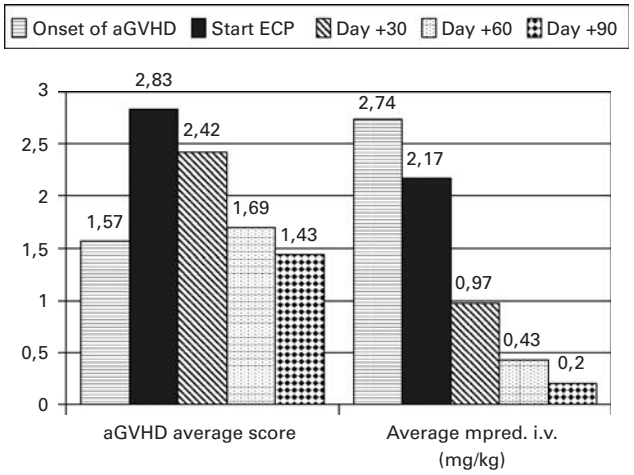

Figure 1 Reduction of aGVHD average score and steroid dose in the first 3 months of ECP treatment. aGVHD, acute GVHD; ECP, extracorporeal photopheresis.

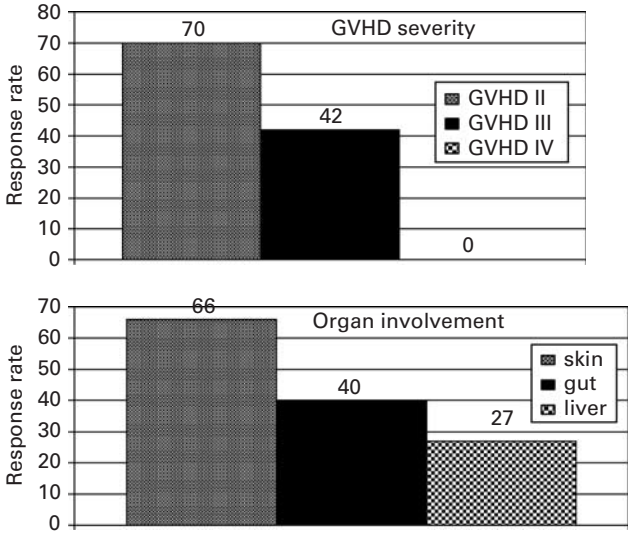

Figure 2 Response rates according to aGVHD severity (upper graph) and organ involvement (lower graph). Best responses in skin/gut GVHD and in patients with grade II–III severity.

the onset of aGVHD (83 vs 47%; $P=0.1$). Eleven of 23 patients (48%) survived, with a median follow-up of 37 months (9–81).

Timing of ECP and response

The 25th percentile interval between aGVHD onset and ECP was 35 days. There was a trend for improved responses in patients treated within 35 days from onset of aGVHD as compared to patients treated later (83 vs 47%; $P=0.1$).

Causes of death

Ten patients died of GVHD and/or infection and/or organ failure (43%): four during the first month of ECP treatment (days 4, 15, 17 and 30); three patients died before the second month of treatment for an unresponsive aGVHD (at days 31, 33 and 42 of ECP); two patients died in the third month of therapy (days 90 and 92) and one patient died 16 months later from complications of cGVHD. Two patients died of recurrence of their original disease (Table 4).

Chronic GVHD

cGVHD was scored as moderate in nine patients (Table 4) and minimal in three patients. At last follow-up, one patient had moderate cGVHD, five had minimal cGVHD and six had no cGVHD. In addition, seven patients were off immunosuppressive therapy.

Acute GVHD-matched controls

We compared this series of patients with 307 GVHD-matched controls transplanted in our department between 1996 and 2006 and not treated with ECP. The two groups were comparable for age, disease state, type of transplantation and aGVHD onset characteristics (Table 5). Controls comprise both patients with steroid-responsive and -unresponsive aGVHD. Overall survival at 4 years is not statistically different (45% in the ECP group vs 44% in controls; $P=$ NS). No survival difference was observed for patients with grade II aGVHD (54 vs 46%) (Figure 3), whereas a trend for improved actuarial survival was seen in those with grade III–IV aGVHD (38% in the ECP group vs 14% in controls; $P=0.08$) (Figure 4).

Adverse events

Citrate toxicity was routinely prevented with calcium gluconate infusion. Few patients experienced mild weakness with hypotension, and low-grade fevers could occur 2–12 h following reinfusion. Two patients reported abdominal pain and diarrhoea episodes soon after ECP procedure. No one stopped photopheresis because of profound pancytopaenia; nine patients kept on needing RBC transfusions and seven required platelet support during all ECP treatments. Their transfusion demand did not increase significantly, compared to the pre-ECP period. Peripheral vein access was preferentially used in this series of patients, although no one developed a life-threatening catheter-related infection.

Discussion

Extracorporeal photochemotherapy was developed in the early 1980s for the treatment of cutaneous T-cell lymphomas[30] and later used with positive results in the management of serious immune-mediated disorders, such as solid organ transplant rejection,[31] autoimmune diseases[32–34] and graft-versus host.[35–39] In 1998, Greinix *et al.*[16] first reported the promising effects of ECP on refractory aGVHD, obtaining best results on skin grade II–III manifestations. Since then, several studies reviewed by Dall'Amico *et al.*[36] showed that ECP is beneficial on both aGVHD ($n=76$) and cGVHD ($n=204$) in patients not responding to standard therapy.

Table 4 Long-term follow-up

No.	*GVHD grade*	*ECP courses*	*Resp*	*Side effects—comments*	*Status at last FU*	*mm FU*	*Cause death*	*Chronic max.gr*	*GVHD last*	*IS therapy*
1	IV	2	No	FUO-TAM-sepsi	Deceased	4	GVHD			
2	IV	1	No	CMV	Deceased	3	GVHD			
3	IV	1	No	CMV-TAM-candidemia-MOF	Deceased	3	GVHD			
4	III	1	No	VOD-MOF	Deceased	2	GVHD			
5	IV	4	No	CMV-TAM-sepsis	Deceased	3	GVHD			
6	II	18	CR	Evolution to cGVHD	Alive	81		Moderate	Minimal	No
7	III	13	CR		Alive	65		Moderate	None	No
8	II	12	Stab	CMV	Deceased	30	Relapse			
9	IV	6	Stab	CMV, SNC toxicity CyA	Deceased	5	Relapse			
10	III	10	CR	*Aspergillus* sinusitis	Alive	61		Moderate	None	No
11	II	25	CR	CMV	Alive	58		Moderate	Minimal	Yes
12	III	5	No	Pneumonia_ CMV	Deceased	6	GVHD			
13	IV	9	No	FUO, CMV, *C. tropicalis*	Deceased	6	GVHD			
14	II	3	No	*Aspergillus* pneumonia, CMV, EBV	Deceased	4	Infection			
15	II	17	CR	FUO	Alive	37		Moderate	Minimal	Yes
16	I	17	CR	EBV, HHV6	Alive	37		Minimal	Minimal	Yes
17	II	14	CR	FUO, CMV, EBV	Alive	36		Moderate	None	No
18	III	19	CR		Alive	36		Moderate	Minimal	Yes
19	II	1	No	CMV, EBV,	Deceased	2	MOF			
20	III	13	No	EBV, CMV	Deceased	16	GVHD	Extensive	Extensive	Yes
21	II	14	CR	Pneumonia, TAM, CMV, hepatic GVHD	Alive	32		Moderate	None	No
22	III	13	CR	EBV	Alive	30		Minimal	None	No
23	II	10	CR	Sepsi, Klebs. pneumoniae, CMV	Alive	9		Minimal	None	No

Abbreviations: FUO = fever unknown origin; HHV6 = herpes virus 6; mm FU = months of follow-up from transplant; MOF = multiorgan failure; TAM = transplant-associated microangiopathy; VOD = veno-occlusive disease.

Table 5 Comparison of 23 ECP patients with 307 GVHD-matched controls

	ECP	*Controls*	P-*value*
No. of patients	23	307	
Tx			
Years	1996–2006	1996–2006	
Age			
Median	41	40	0.3
Range	(23–67)	(18–66)	
Alternative donor	11 (40%)	154 (50%)	0.8
Disease status			
>1 CR/1 CP	13 (56%)	182 (59%)	0.7
Interval Dx–Tx			
Median	432	501	0.5
Range	(189–3628)	(56–7100)	
1°day PMN			
Median	16	18	0.09
Range	(12–25)	(4–41)	
1°day GVH			
Median	14	14	0.9
Range	(3–43)	(3–82)	
aGVHD			
II	10	249	
III–IV	13	58	
Survival			
aGVHD grade II	60%	54%	0.6
aGVHD >grade II	38%	16%	0.08

Abbreviations: CP = chronic phase; Dx-Tx = interval between disease diagnosis and transplantation; ECP = extracorporeal photopheresis.

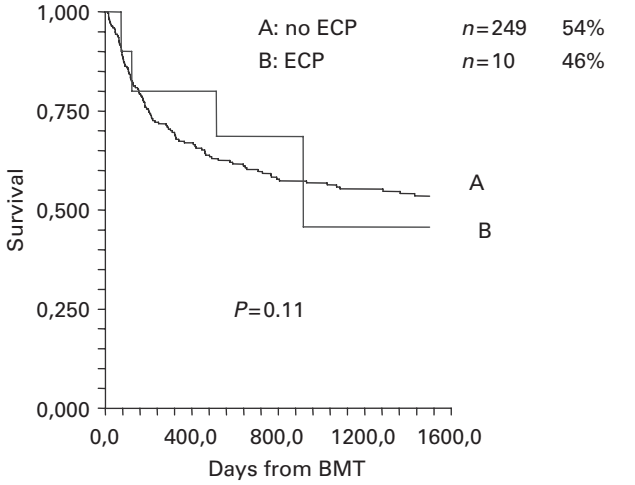

Figure 3 Actuarial survival of patients with acute GVHD grade II treated without (A) or with extracorporeal photopheresis (B). No difference in outcome.

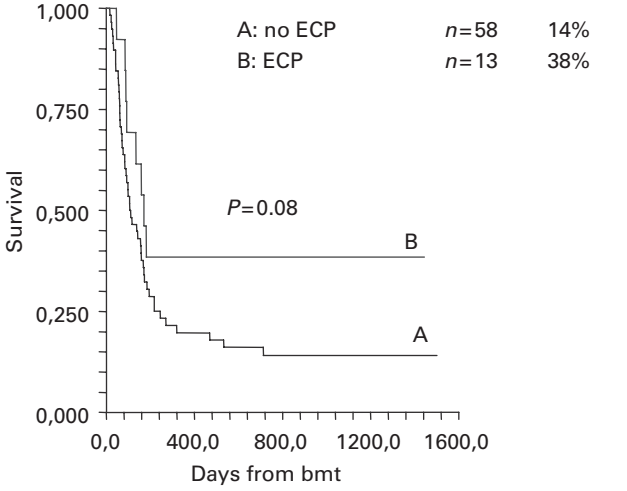

Figure 4 Actuarial survival of patients with acute GVHD grade III–IV treated without (A) or with extracorporeal photopheresis (B). A trend for improved outcome with ECP treatment is noted. aGVHD, acute GVHD; ECP, extracorporeal photopheresis.

We present our experience with the use of ECP for the treatment of unresponsive aGVHD. Our study, with the limits of a retrospective analysis, confirms that ECP is a safe and effective procedure in patients with corticosteroid-resistant aGVHD, with a 52% complete response rate and 48% long-term survival. The low-toxicity profile of ECP allowed the procedure to be performed also in patients with poor performance status. A significant proportion of patients were referred to ECP with advanced GVHD (grade III–IV) after having failed several lines of immunosuppression: these patients are at high risk of opportunistic infections and GVHD progression, as shown by some early GVHD-related deaths.

As reported in the literature,[7,36] we found a trend for improved outcome when starting ECP within 35 days from onset of aGVHD, this time interval being the 25th percentile of our cases: when ECP was started within day 35, the complete response rate was 83 vs 47% for patients starting later. Complete responses were seen in skin GVHD (66%), gut GVHD (40%) and less so in liver GVHD (27%). This is in keeping with another report showing an efficacy of ECP on acute gut GVHD.[39] As expected, complete responses were higher in grade II GVHD (70%) as compared to grade III (42%) and grade IV (0%). Corticosteroids were tapered during treatment, and we have also in the past taken this as an indirect indication of response.[3] Three months post-treatment, the daily dose of prednisolone was reduced, as well as the proportion of patients requiring i.v. therapy.

Treatment of steroid refractory aGVHD has been a challenge over the past 20 years and no single therapy has proven to be superior to conventional steroids; survival at 1 year is usually 30%. A second problem is that causes of death are combined in these patients: it is difficult to assess whether a patient had died of GVHD, infections or organ toxicity. Therefore, the efficacy of any treatment can be suggested by looking at survival and steroid dependence. We therefore attempted to compare this current series of 23 patients with a group of patients matched for maximum aGVHD grade, who had not received ECP. We argued that if we could show a trend for survival advantage in the ECP group, this would have been encouraging, as the control

group would have also included responders to first-line therapy. Actuarial survival was comparable for patients with grade II aGVHD and was superior for ECP patients when GVHD was grade III–IV (38 vs 16%). Therefore, ECP-treated patients with steroid refractory grade II aGVHD do at least as well as patients with grade II GVHD responding or not to first-line steroids, and possibly have a survival advantage when GVHD is grade III–IV.

Chronic GVHD was seen in a significant proportion of patients responding to ECP, from minimal to moderate; however, at last follow-up, 7/12 patients were off immunosuppression, with cGVHD being scored as absent ($n=6$), minimal ($n=5$) and moderate ($n=1$).

To date, no data have been reported concerning an increase of infections or relapse rate associated with ECP.[36] Infections observed in our series of patients, as CMV reactivation or bacterial and mycotic complications, could hardly be referred to photochemotherapy, because these patients were deeply immunosuppressed. Furthermore, immunoglobulin dosage was heavily decreased in both ECP-treated patients and GVHD-matched controls, aGVHD itself being a cause of a delayed immune recovery.

A therapeutic dose of MNCs is still under discussion. No evidence of correlation between the number of lymphocytes collected and clinical efficacy has been found, although Perseghin *et al*.[38] reported a better clinical response and faster improvement in patients receiving higher doses of MNCs. In this series, the composition of products collected, irradiated and infused at each procedure was analysed, and no correlation between the number of cells treated and response was observed.

In conclusion, our analysis confirms a positive impact of ECP as adjunctive salvage treatment of resistant aGVHD, and we have grown more and more confident in using ECP in such patients. The good results in patients receiving ECP, within 1 month from the onset of GVHD and when the severity does not exceed grade II–III may warrant a prospective trial to explore the role of photochemotherapy as upfront treatment of aGVHD.

Acknowledgements

This work was partly supported by Associazione Italiana Ricerca contro il Cancro (AIRC) Milano, CARIGE, and Fondazione Ricerca per Trapianto Midollo Osseo, Genova.

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