Extracorporeal photopheresis for graft-versus-host disease: the role of patient, transplant, and classification criteria and hematologic values on outcome—results from a large single-center study

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BACKGROUND: Extracorporeal photopheresis (ECP) has been shown as active therapy for graft-versus-host disease (GVHD).

STUDY DESIGN AND METHODS: The aim was to ascertain the role of ECP in 71 patients with steroid-refractory or -dependent acute and chronic GVHD (aGVHD and cGVHD) with special focus on hematologic variables and GVHD staging classification. A total of 34 patients were treated for aGVHD and 37 for cGVHD.

RESULTS: The overall response rate (ORR) for aGVHD was 65% and the complete aGVHD-free survival was 50% (95% confidence interval [CI], 36%-70%). The ORR for cGVHD response was 81% while the complete cGVHD-free survival was 50% (95% CI, 34%-73%). The aGVHD-free survival was associated with aGVHD grading (Grade II 81%, Grade III 33%, and Grade IV 0%, p ≤ 0.00) and the absence of visceral involvement (77% vs. 33%, p = 0.03). The cGVHD-free survival was associated with the female sex (67% vs. 25%, p = 0.01) and with the limited form according to the Seattle classification (67% vs. 20%, p = 0.003). No role for hematologic values or apheresis cell count was found, except for the cGVHD ORR (p = 0.037). Transplant-related mortality and overall survival were associated with ECP response 0% versus 54% (p = 0.0001) and 77% versus 45% (p = 0.03) for aGVHD patients and 7% versus 14% (p = 0.02) and 73% versus 20% (p = 0.0003) for cGVHD patients, respectively.

CONCLUSIONS: While confirming a higher probability of GVHD responses for early GVHD, our study shows no role of hematologic values or apheresis cell count on GVHD response.

Graft-versus-host disease (GVHD) remains the most frequent complication after allogeneic hematopoietic stem cell transplantation (HSCT).[1] First described as a "secondary disease" in mice,[2] the syndrome was shown to be triggered by immunocompetent donor cells.[3,4] Despite improvements in posttransplant immunosuppression, up to 30% of HLA-identical graft recipients and up to 90% of recipients of unrelated donor graft still develop significant acute

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TABLE 1. aGVHD and cGVHD patient characteristics*			
Patient characteristics	aGVHD (%)	cGVHD (%)	p value†
Number	34	37	NS
Male/female	16/18	28/9	0.008
Age at HSCT (years), median (range)	12 (2-49)	26 (5-65)	0.001
Disease			
AML	2 (6)	10 (27)	NS
ALL	13 (38)	11 (30)	
CML	6 (18)	4 (11)	
MDS/MPD	2 (6)	2 (5)	
Lymphoma	3 (9)	4 (11)	
Multiple myeloma	0	2 (5)	
Solid tumor	2 (6)	1 (3)	
Nonmalignant	6 (18)	3 (8)	
ABO compatibility			
Major incompatibility	13 (38)	11 (30)	NS
Minor incompatibility	7 (20)	11 (30)	
Identical	14 (41)	15 (40)	
CMV serology			
D–/R–	7 (20)	4 (11)	NS
Other	27 (79)	33 (89)	
Disease status at HSCT			
Early	10 (29)	12 (32)	NS
Advanced	24 (70)	25 (67)	
Donor type			
MFD	8 (23)	25 (67)	0.0008
MUD	26 (76)	12 (32)	
Sex mismatch			
Female donor/male recipient	7 (20)	18 (49)	0.01
Other combination	27 (79)	19 (51)	
Conditioning regimen			
Myeloablative	26 (76)	25 (67)	NS
Nonmyeloablative	8 (23)	12 (32)	
GVHD prophylaxis			
With serotherapy	25 (73)	9 (24)	0.0002
Without serotherapy	9 (26)	28 (76)	
Stem cell source			
BM	22 (65)	17 (46)	0.03
PB	9 (26)	20 (54)	
CB	3 (9)	0	

* Data are reported as number (%) unless otherwise specified.
† The p value was calculated by the chi-square tests or the Fisher exact test.
ALL = acute lymphoblastic leukemia; AML = acute myeloid leukemia; CML = chronic myeloid leukemia; D– = donor CMV serologically negative; MDS = myelodysplastic syndrome; MPD = myeloproliferative disorders; R– = recipient CMV serologically negative. Early disease—CR1 for AML and ALL, CP1 for CML, or other myeloproliferative disorders. MDSs were considered as early or advanced according to blast counts in the peripheral blood, CR2 for lymphomas, or no evidence of disease for solid tumors and all nonmalignant diseases. Advanced diseases were considered any other disease status at HSCT and patients receiving second HSCT. D = donor; MFD = matched family donor; MUD = matched unrelated donor; R = recipient. Myeloablative—any conditioning regimen containing total body irradiation above 9.9 Gy, busulfan above 14 mg/kg, or thiotepa above 10 mg/kg. BM = bone marrow; CB = cord blood; PB = peripheral blood.

Extracorporeal photopheresis (ECP) was introduced nearly 30 years ago to treat cutaneous T-cell lymphoma and autoimmune diseases, such as scleroderma. This procedure has proved effective in the treatment of acute lung, heart, and kidney allograft rejection and in the past 15 years for the treatment of aGVHD and cGVHD, gaining levels of evidence of C for skin aGVHD, B for skin cGVHD, and C for nonskin aGVHD and cGVHD.[24] Although the mechanisms of ECP are not fully understood, recent evidence suggests how the T-, B-, and dendritic cells compartment may be regulated by ECP.[25-27] In this study we report our clinical experience on 71 consecutive patients with GVHD treated with ECP.

MATERIALS AND METHODS

Design of the study and ECP procedures

Seventy-one patients with steroid-resistant or -dependent aGVHD (n = 34) or cGVHD (n = 37, Table 1) were enrolled for ECP treatment in our center from October 2001 to September 2013. Inclusion criteria were as follows: 1) diagnosis of aGVHD or cGVHD based on clinical and laboratory documentation, 2) previous therapy with steroids for at least 7 days plus calcineurin inhibitors for aGVHD or other immunosuppressive treatments but not started 14 days before ECP, and 3) no previous treatment with either antithymocyte globulin or monoclonal antibodies within 1 month before starting ECP. Exclusion criteria were as follows: 1) previous ECP treatment, 2) hemodynamic instability, 3) inadequate compliance to attend the procedures, and 4) no previous corticosteroid treatment.

ECP was performed on both an outpatient and an inpatient basis. Patients were treated with ECP on 2 consecutive days at weekly intervals for the first month, every 2 weeks during the second and third months, and then at monthly intervals for an additional 3 months. Briefly, patients with aGVHD were excluded from the ECP protocol if they had: 1) completed their planned 22 procedures or 2) had aGVHD progression under ECP.

GVHD (aGVHD). Prednisone has been shown to be effective as the first-line therapy in the treatment of established aGVHD, resulting in complete response (CR) rates of 25% to 54%.[5,6] However, patients not responding to steroids are at a high risk of death.[7,8] Furthermore, despite better donor selection, GVHD prophylaxis, and treatment, chronic GVHD (cGVHD) affects 50% of long-term transplant survivors and is lethal in 20% to 40% of affected patients.[9] Primary therapy for extensive cGVHD usually includes steroids and calcineurin inhibitors,[10-18] but the probability of cGVHD response is highly variable.[19-23]

Any concomitant immunosuppressive medication was initially maintained and then modified or discontinued according to the clinical response.

Depending on the characteristics of the patient (pediatric or adult), our center has two different systems for ECP. We use a photopheresis instrument (UVAR, Therakos, Exton, PA) for adults or for patients weighing more than 40 kg because it requires a considerable extracorporeal circulation. After 240 mL of mononuclear cells was collected, 300 mL of plasma was added to 200 mL of normal saline and 8-methoxypsoralen (100 mg, Gerot Pharmaceutical, Vienna, Austria) in aqueous solution and, finally, the buffy coat and plasma were passed in as a thin film through a disposable plastic device, exposed to UVA light (2 J/cm^2) for 90 minutes, and then returned to the patients. For pediatric patients (weighing under 40 kg) lymphocytapheresis procedures were performed by means of a continuous-flow cell separator (Fresenius COM.TECH [before this cell separator, for some patients, we used the CS3000 cell separator, Fenwal, Baxter, Deerfield, IL]) and then, via a closed system, cells were transferred to a 3000-mL thin plastic bag. Thereafter, 100 mg of 8-methoxypsoralen (Gerot Pharmaceutical) was added to a final concentration of 200 ng/mL. Before 2012 the product was photoinactivated (2 J/cm^2) with a dermatologic-use device (PUVA Combi light, PCL Division Overkade, Hands Unit, Leuven, Belgium) and after 2012 by another UVA device (Macogenic G2, Macopharma, Tourcoing, France). Considering that in patients weighing less than 40 kg the Therakos device could not be used due to the high extracorporeal circulation, the company gave us the systems to bypass the collection phase and allowed us to use only the phase of photoinactivation. At least two blood volumes were processed.

During the ECP procedures all patients were monitored for blood pressure, heart rate, and body temperature. Full blood count, liver and kidney function tests, and coagulation variables were obtained before and after each procedure. An aliquot of each collection before reinfusion was analyzed for the cell count.

This study was approved by the local institute review board or ethics committee; all patients or parents or legal guardians gave their consent for ECP procedures. A specific informed consent was given for adolescents.

aGVHD patients

A total of 34 patients underwent ECP for steroid-refractory aGVHD from June 2001 to September 2013. A median of 11 ECP procedures per patient were performed (range, 8-25). The median follow-up for surviving patients was 4 years (range, 2 months-12 years), while it was 7 months for deceased patients (range, 35 days-11 months). The median (range) patient weight was 39 (10-98) kg and aGVHD was graded II for 16 patients (47%), III for 12 (35%), and IV for six patients (18%). The median (range)

white blood cell (WBC) count at ECP start was 5.6×10^9 (1.2×10^9-18×10^9)/L and the first 80 ECP procedures gave a median (range) of 4×10^8 (1×10^8-13×10^8) total nucleated cells (TNCs)/kg. The median (range) patient age at HSCT was 12 (2-42) years, while for donors it was 28 (0-49) years. The median (range) age at ECP start was 12 (2-51) years. The median (range) interval between HSCT and ECP start was 38 (15-97) days (details of aGVHD patients and ECP are outlined in Tables 1 and 2).

cGVHD patients

A total of 37 patients underwent ECP for cGVHD from April 2001 to March 2013. A median of 20 ECP procedures per patient was performed (range, 8-77). The median (range) follow-up for surviving patients was 4 years (1 month-12 years), while it was 2 years for deceased patients (30 days-4 years). The median (range) age at HSCT was 22 (4-64) years, while for donors it was 35 (5-63) years. The median (range) age at ECP start was 26 (5-64) years. The median (range) patient weight at ECP start was 55 (14-100) kg and the median (range) WBC count at ECP start was 6.5×10^9 (2.1×10^9-17.9×10^9)/L, the first eight ECP procedures gave a total of 5.9×10^8 (0.07×10^8-65.3×10^8) TNCs/kg. The median (range) interval between HSCT and ECP start was 193 (10-5681) days.

cGVHD classification

For patients diagnosed before 2005 for National Institutes of Health (NIH) classification and before 2011 for Center for Bone Marrow Transplant Research (CIBMTR) classification, the patients' medical records were retrospectively reviewed. In brief, 12 patients were diagnosed with limited cGVHD and 25 with extensive cGVHD according to the Seattle classification; 14 patients were classified as mild, 18 with moderate, and five with severe cGVHD according to the NIH classification; and finally nine patients were scored as Risk Group 1 (RG1), 26 patients as RG2, and two patients as ≥RG3 according to the CIBMTR classification (details of cGVHD patients and ECP are outlined in Tables 1 and 3). To retrospectively assess the NIH or CIBMTR classification, all medical records and histologic and radiologic tests were critically reviewed by two skilled BMT attending physicians (MB and FF).

Criteria for defining response to ECP

Response to therapy was assessed clinically at weekly intervals. The clinically relevant time points for response were 1 month for aGVHD patients and 3 months for cGVHD. The criteria for defining responses for aGVHD were as previously reported,[28] briefly:

1. CR—overall GVHD Grade 0 to I;
2. Partial response (PR)—more than 50% of organ involvement (skin, gut, and liver);
3. Minor response—tapering of immunosuppressive agents with stable GVHD;

TABLE 2. Details of aGVHD patients and ECP characteristics*				
GVHD diagnosis (days)		18 (9-92)		
Age at GVHD diagnosis (years)		12 (2-49)		
Patient weight (kg)		39 (10-98)		
Karnofsky/Landsky score at GVHD (%)		80 (60-100)		
Maximum GVHD staging				
Grade II		16 (47)		
Grade III		12 (35)		
Grade IV		6 (18)		
	GVHD 0-I	GVHD II	GVHD III	GVHD III-IV
Skin GVHD	2	2	27	5
Liver GVHD	27	2	2	5
Gut GVHD	23	1	6	6
Number of ECP procedures		11 (8-25)		
At ECP start				
WBC count (×10⁹/L)		5.6 (1.2-18)		
Neutrophil count (×10⁹/L)		4.4 (0.6-13.8)		
Lymphocyte count (×10⁹/L)		0.7 (0.1-2.6)		
Monocyte count (×10⁹/L)		0.5 (0-3.9)		
Hemoglobin (g/dL)		10.8 (8.6-15.3)		
Hematocrit (%)		30.2 (25-3-44.4)		
PLT count (×10⁹/L)		74 (31-312)		
ECP Procedures 1-8, harvested cells/kg (×10⁸)				
WBCs		4 (1-13)		
Lymphocytes		1.5 (0.1-9.3)		
ECP Procedures 9-16, harvested cells/kg (×10⁸)				
WBCs		3.3 (0.2-9.5)		
Lymphocytes		1.8 (0.1-8)		
ECP Procedures 17-22, harvested cells/kg (×10⁸)				
WBCs		2.5 (0.9-5.1)		
Lymphocytes		1.5 (0.5-4.9)		
* Data are reported as median (range) or number (%).				

4. Stable disease—less than 50% response of organ involvement (skin, gut, and liver); or
5. Progressive disease (PD)—worsening of organ involvement or new signs and/or symptoms of GVHD. Patients with a CR or PR in one organ and a simultaneous PD in another were diagnosed as progression of aGVHD.

Criteria for defining responses for cGVHD were as follows:

1. CR—complete regression of any cGVHD manifestation.
2. PR—more than 50% in terms of organ involvement. In this case, due to the complexity inherent to the assessment of response in each organ, we defined PR as follows: skin GVHD, for lichenoid rashes a minimum 50% reduction in the body surface area involved; for sclerodermatous involvement, any improvement in the skin score or range of motion, with an improvement of Zubrod/Eastern Cooperative Oncology Group performance status of 1;[29] ocular GVHD, a subjective improvement and a reduction in the frequency of artificial tears administration by 50%, or an improvement in the Schirmer test for one or both eyes; oral GVHD, a 50% improvement in the mucosal area involved with lichenoid and/or ulcer-

ative changes; gastrointestinal and liver, 50% decrease in the volume of diarrhea, bilirubin, alkaline phosphatase, or γ-glutamyltransferase if abnormal at ECP start; bronchiolitis obliterans, sustained improvement in pulmonary function test (1-sec forced expiratory value) assessed by monthly testing and/or the ability to taper steroids by 50% with no deterioration of pulmonary functions.
3. Minor response—tapering of immunosuppressive agents with stable GVHD.
4. Stable disease—less than 50% of cGVHD organ involvement.
5. PD—worsening of organ involvement or new signs and/or symptoms of GVHD. Patients with a CR or PR in one organ and a simultaneous PD in another were diagnosed as progression of cGVHD.[30]

Endpoints, definitions, and statistical analysis

The primary endpoint of this study was to assess the long-term effectiveness of ECP on steroid-resistant or -dependent GVHD. The secondary endpoints were:

1. The overall response rate (ORR; CR+PR) for aGVHD and cGVHD patients.
2. The role of TNCs and lymphocytes collected during the first eight ECP procedures on response. The ECP

TABLE 3. Details of cGVHD patients and ECP characteristics*

Day of cGVHD diagnosis	166 (100-1023)
Age of cGVHD diagnosis (years)	22 (5-64)
Patient weight (kg)	55 (14-100)
Onset	
De novo	12 (32)
Quiescent	14 (38)
Progressive	11 (30)
Seattle criteria	
Limited	12 (32)
Extensive	25 (68)
NIH[20]	
Mild	14 (38)
Moderate	18 (49)
Severe	5 (13)
CIBMTR[21]	
RG1	9 (24)
RG2	26 (70)
≥RG3	2 (5)
GVHD	
Skin	36 (92)
Mouth	19 (49)
Eye	13 (33)
Gastrointestinal	6 (15)
Liver	10 (26)
Joint	5 (13)
Genital	2 (5)
Lung	4 (10)
Number of ECP procedures per patient	20 (8-77)
Day of ECP start	193 (10-5681)
At ECP start	
WBCs ($\times 10^9$/L)	6.5 (2.1-17.9)
Neutrophils ($\times 10^9$/L)	4.1 (0.3-11.5)
Lymphocytes ($\times 10^9$/L)	1.7 (0.2-7.8)
Monocytes ($\times 10^9$/L)	0.8 (0.1-2)
Hemoglobin (g/dL)	13 (8.6-16.3)
Hematocrit (%)	36 (26.2-45.8)
PLTs ($\times 10^9$/L)	231 (21-438)
ECP Procedures 1-8, harvested cells/kg ($\times 10^8$)	
WBCs	5.9 (0.07-65.3)
Lymphocytes	3.8 (0.03-49)
ECP Procedures 9-16, harvested cells/kg ($\times 10^8$)	
WBCs	4.9 (1.3-19.2)
Lymphocytes	2.1 (0.6-14.8)
ECP Procedures 17-22, harvested cells/kg ($\times 10^8$)	
WBCs	4.7 (0.58-49)
Lymphocytes	1.9 (0.28-32)

* Data are reported as median (range) or number (%).

harvested cells/kg count was computed by the addition of harvested TNCs or lymphocytes of ECP from one to eight (that was chosen because all patients completed the first month of treatment);

3. The correlation of WBCs, lymphocytes, monocytes, and platelet (PLTs) before ECP start on response;
4. The cGVHD response according to the Seattle,[19] NIH,[20] and CIBMTR criteria;[21]
5. Transplant-related mortality (TRM), relapse incidence (RI), and overall survival (OS) for both aGVHD and cGVHD groups. For patients treated before 2005, all the medical records were reviewed to stratify patients according to the NIH criteria and for those

who were treated before 2011 the same approach was used for the CIBMTR criteria. To assess retrospectively the NIH or CIBMTR classification, all medical records and histologic and radiologic tests were critically reviewed by two skilled BMT attending physicians (MB and FF).

Steroid-resistant aGVHD was defined as progressive aGVHD after at least 3 days of methylprednisolone (MP; 2 mg/kg/day) or if unimproving Grades III and IV aGVHD persisted after at least 7 days of MP (2 mg/kg/day). Progression was defined as a change in one organ (skin, gut, or liver) leading to an increase by at least one Glucksberg's stage of aGVHD. Unimproving aGVHD was defined as the absence of a difference in any involved organ sufficient to meet minimal criteria for improvement or deterioration.

cGVHD was considered refractory or resistant to therapy if: 1) patients had stable disease (i.e., no response) after 1 month of treatment, 2) no more than a PR occurred after 2 months of treatment, or 3) PD occurred after 2 weeks of initiation of steroid treatment or during MP taper.[31] Steroid-dependent cGVHD was defined as the patient's need for prednisone or MP of at least 1 mg/kg.

Patient-, disease-, and transplantation-related variables are expressed as medians and ranges, or as percentages, as appropriate. The following patient- or transplant-related variables were analyzed for their potential impact on GVHD response and GVHD-free survival: age, sex, stem cell source, sex mismatch, donor type, first-line therapy for aGVHD; and age, sex, stem cell source, sex mismatch, donor type, cGVHD type of onset, Seattle criteria, NIH criteria, and CIBMTR criteria for cGVHD. For aGVHD and cGVHD groups, the TNCs/kg and lymphocytes/kg during the first eight ECP procedures together the WBCs, lymphocytes, monocytes, and PLT count before ECP start were also evaluated for GVHD response and GVHD-free survival.

For the analysis of the ORR the chi-square test or the Fisher exact test were used. For the statistical analyses the continuous variables were categorized as follows: each variable was first divided into four categories at the 25th, 50th, and 75th percentiles. If the relative event rates (the ratio of the observed number of events to the expected number of events in the category) in two or more adjacent categories (and the median time to events) did not differ, those categories were grouped. If no clear pattern was observed for the primary outcomes, the median was taken as the cutoff point.

aGVHD- or cGVHD-free survival was the probability of being alive with continuous complete GVHD resolution from ECP start. The competitive events for calculating GVHD-free survival were both the TRM and the RI. TRM was defined as the probability of dying without a previous occurrence of a relapse, which was its competing event. The method of TRM estimation was the cumulative

incidence curve, the p value was calculated by the Gray test.[32] RI was defined as the probability of having had a relapse before death or the last follow-up. In case of non-malignant disease, the primary or secondary graft failures were categorized as relapse. Death without experiencing a relapse was the competing event. The RI method of estimation was the cumulative incidence curve; the p value was calculated by the Gray test. OS was calculated as the probability of survival irrespective of disease state at any time. OS was calculated by the Kaplan-Meier statistics,[33] and the p value was calculated by the log-rank test.[34]

All variables having a p value below 0.20 in univariate analyses were included in a multivariate analysis by the Cox regression model; the proportional subdistribution hazard regression model was used to perform multivariate analyses of GVHD-free survival, RI, and TRM.[35] Statistical analysis was performed using computer software (SPSS, SPSS, Inc., Chicago, IL) to calculate the OS, while the cumulative incidence curves were calculated by another computer program (NCSS for Windows, NCSS, LLC, Kaysville, UT). The p values for both univariate and multivariate analyses were computed with a statistical computing program (R packages software, http://www.r-project.org/). For all analyses, the significant p value was 0.05.

RESULTS

aGVHD response

Among the aGVHD patients the ORR was 65%. In the univariate analysis the lower GVHD grading (p = 0.0005) and the steroid alone as the previous therapy (p = 0.01) were significantly associated with better probabilities of response. In particular, the Grade II aGVHD had 87% of ORR, and Grade III had 67%, while no patients with aGVHD Grade IV responded to ECP. The collected TNCs or the hematologic values before ECP were not associated with aGVHD response. Notably, the donor type and the stem cell source had no relevance on aGVHD response. Patients with higher WBC had a statistically significant higher TNC yield (p = 0.02), but this did not translate in higher ORR (Table 4).

The median interval between ECP start and the best response day was 47 days. Steroids were tapered in 25 patients (67%) and the median number of days of steroid tapering was 29 days. No toxicity greater than 1 has been reported in accordance with the Common Toxicity Criteria Adverse Event criteria available over the years.

aGVHD-free survival

For all patients the aGVHD-free survival was 50% (95% confidence interval [CI], 34%-73%; Fig. 1). In univariate analysis the aGVHD grades were significantly associated with different outcomes: Grade II had 81% (95% CI, 64%-

100%), Grade III aGVHD had 33% (95% CI, 15%-74%), and Grade IV aGVHD had 0% (p < 0.0008). When we considered the collected TNCs or lymphocytes or the hematologic values before ECP the probability of aGVHD-free survival did not differ even when the patients were stratified according to quartiles (data not reported). The multivariate analysis showed how only the GVHD grade was the sole independent factor with relative risk (RR) of 3.37 (95% CI, 2.44-5.07, p = 0.0015).

TRM, RI, and OS

The 10-year TRM was 16% (95% CI, 8%-35%). The TRM was 0% for responders versus 54% for nonresponders (95% CI, 32%-93%, p = 0.0001). The 10-year cumulative incidence of relapse was 17% (95% CI, 8%-35%). The RI was 27% (95% CI, 14%-54%) for responders and 0% for nonresponders (p = NS). The 10-year OS was 51% (95% CI, 25%-78%). The OS was 77% (95% CI, 58%-94%) for responders and 45% (95% CI, 16%-75%) for nonresponders (p = 0.03; Table 5). The main cause of death was aGVHD (six of 11, 54%) while the disease progression was the cause of death (five of 11, 45%) for the other patients.

cGVHD response

The ORR for cGVHD patients was 81%. Bone marrow as the stem cell source (p = 0.02), the female sex (p = 0.05), the collected lymphocytes/kg during the first eight ECP procedures (p = 0.037), the unrelated donor (p = 0.006) and the limited form according to the Seattle criteria (p = 0.027) were significantly associated with higher response rates (Table 4). No effect of TNC/kg doses or hematologic values at baseline were observed. Finally, the probability of higher TNC collection did not differ according to WBCs at baseline (p = NS). A total of 31 patients (84%) could taper steroids and the median interval from ECP start to steroid tapering was 59 days. Five patients continued ECP therapy beyond 22 procedures as the sole cGVHD treatment. No toxicity greater than 1 has been reported in accordance with the Common Toxicity Criteria Adverse Event criteria available over the years.

cGVHD-free survival

The 10-year cGVHD-free survival rate was 50% (95% CI, 34%-73%; Fig. 2) and the median lapse from ECP start to best response was 393 days (14-1464 days). As reported in Table 6, two variables had a significant deleterious effect on cGVHD-free survival: the male sex (25% [95% CI, 13%-47%] vs. 67% [95% CI, 42%-100%]; p = 0.01) and the extensive type according to the Seattle criteria (20% [95% CI, 9%-44%] vs. 67% [95% CI, 45%-91%]; p = 0.003). When we focused on collected TNCs or lymphocytes, we found

TABLE 4. ORR for patients with aGVHD or cGVHD				
	aGVHD response (n = 34)		cGVHD response (n = 37)	
Variables	Number (%)	p value*	Number (%)	p value*
Age at ECP				
≥Median	8/16 (50)	0.15	12/18 (67)	0.12
<Median	14/18 (78)		17/19 (89)	
Sex				
Male	10/16 (62)	1	14/28 (50)	0.05
Female	12/18 (67)		8/9 (89)	
Stem cell source				
BM	15/22 (68)	0.22	16/17 (94)	0.02
PB	4/9 (44)		12/20 (60)	
CB	3/3 (100)		NA	
Sex mismatch				
Female > male	5/7 (71)	1	14/18 (78)	0.65
Other	17/27 (63)		15/17 (88)	
GVHD grade				
II	14/16 (87)	0.0005	NA	
III	8/12 (67)		NA	
IV	0/6 (0)		NA	
Donor type				
MFD	6/8 (75)	0.68	13/26 (50)	0.006
MUD	16/26 (61)		11/11 (100)	
First-line therapy				
Steroids	22/30 (73)	0.01	NA	
Steroids plus other	0/4 (0)		NA	
Visceral aGVHD				
Yes	11/13 (85)	0.07	NA	
No	11/21 (52)		NA	
cGVHD onset type				
De novo	NA		10/12 (83)	1
Quiescent	NA		11/14 (78)	
Progressive	NA		9/11 (82)	
Seattle criteria				
Limited	NA		11/12 (92)	0.027
Extensive	NA		13/26 (50)	
NIH criteria				
Mild	NA		12/15 (80)	0.56
Moderate	NA		13/18 (72)	
Severe	NA		5/5 (100)	
CIBMTR criteria				
RG1	NA		8/9 (89)	0.12
RG2	NA		14/26 (54)	
RG3	NA		1/2 (50)	
TNCs/kg				
≥Median	10/14 (71)	0.44	14/16 (87)	0.21
<Median	7/13 (54)		10/15 (67)	
Lymphocytes/kg				
≥Median	10/13 (77)	0.23	15/16 (94)	0.037
<Median	7/14 (50)		9/15 (60)	
WBCs before ECP				
≥Median	11/18 (61)	0.72	17/19 (89)	0.12
<Median	11/16 (69)		12/18 (67)	
Lymphocytes before ECP				
≥Median	12/18 (67)	1	15/19 (79)	1
<Median	10/16 (62)		14/18 (78)	
Monocytes before ECP				
≥Median	12/20 (60)	0.71	15/17 (88)	0.22
<Median	10/14 (71)		12/16 (75)	
PLTs before ECP				
≥Median	12/19 (63)	1	15/19 (79)	1
<Median	10/15 (67)		14/18 (78)	

* The p value was calculated by the chi-square test or the Fisher exact test.
BM = bone marrow; CB = cord blood; female > male = female donor for a male recipient; MFD = matched family donor; MUD = matched unrelated donor; PB = peripheral blood.

	aGVHD patients			cGVHD patients		
Outcome	Responders	Nonresponders	p value	Responders	Nonresponders	p value
TRM	0	54 (32-93)	0.0001	7 (2-25)	14 (2-89)	0.02
RI	27 (14-54)	0	0.07	20 (10-41)	43 (18-100)	0.14
OS	77 (58-94)	45 (16-75)	0.03	73 (54-92)	20 (0-55)	0.0002

TABLE 5. Outcome of patients treated with ECP for aGVHD and cGVHD*

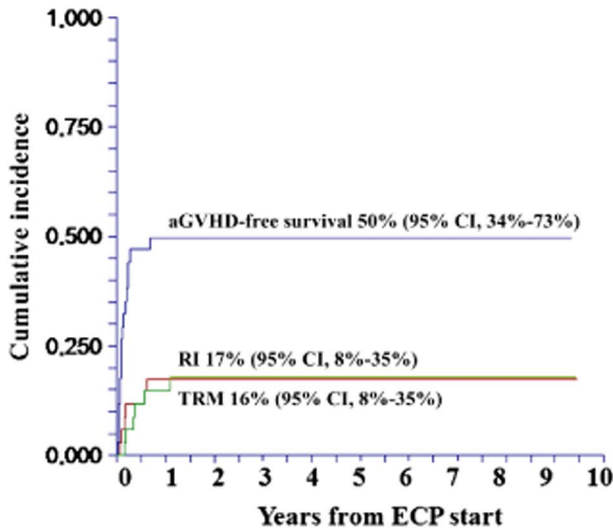* Data are reported as percent (95% CI).

Fig. 1. aGVHD-free survival, TRM, and RI for patients who underwent ECP for aGVHD.
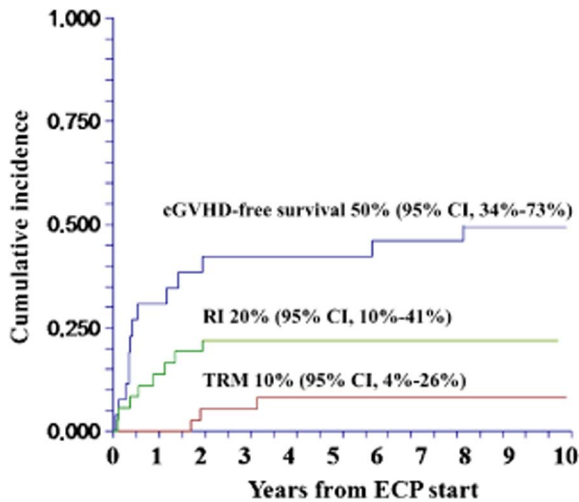

Fig. 2. cGVHD-free survival, TRM, and RI for patients who underwent ECP for cGVHD.

no differences of cGVHD-free survival among the groups, even when the patients were stratified according to quartiles (data not reported). Interestingly, when we compare the cGVHD-free survival for male patients having a female donor to other combinations, no significant differences were observed.

The sex, the extensive form according to Seattle criteria, and the moderate and severe forms according to NIH criteria were independent poor prognostic factors in multivariate analyses: the male sex had a RR of 2.03 (95% CI, 1.78-2.2; p = 0.01), the Seattle extensive form had a RR of 12.31 (95% CI, 7.88-16.6; p < 0.00), the NIH moderate form had a RR of 12.51 (95% CI, 7.22-17.8; p < 0.00), and the NIH severe form had a RR of 10.89 (95% CI, 4.4-16.1; p < 0.00) for poorer cGVHD-free survival.

TRM, RI, and OS

The 10-year TRM for all patients was 10% (95% CI, 4%-26%). The TRMs were 7% (95% CI, 2%-25%) and 14% (95% CI, 2%-89%) for responders and nonresponders, respectively (p = 0.02). The cumulative incidence of RI for the whole patient population was 24% (95% CI, 13%-42%). The RI was 20% (95% CI, 10%-41%) and 43% (95% CI, 18%-100%) for responders and nonresponders (p = NS), respectively. The OS rates were 73% (95% CI, 54%-92%) and 20% (95% CI, 0%-55%) for responders and nonresponders, respectively (p = 0.0002). The main cause of death was disease progression (eight of 10, 80%), and for the others the GVHD (one of 10, 10%) and bacterial sepsis (one of 10, 10%) were the causes of death.

DISCUSSION

Steroids, the first line of GVHD treatment, fail in approximately 50% of patients and are broadly immunosuppressive, increasing the risk of relapse, infections, and other toxicities.[7,18] Strategies to mitigate GVHD while preserving immune functions are important to improve outcomes after HSCT.[36,37] ECP has proven efficacy in treating both aGVHD and cGVHD, even in those patients who are refractory to conventional immunosuppressive therapy, with very few side effects reported.[38] The mechanism of action of ECP in GVHD is not fully understood. It has been proposed that ECP modulates host effector cells, including CD8+ T-lymphocytes, natural killer cells, and circulating antigen-presenting cells, leading to an attenuation of host antigen-presenting activity and thus to the development of tolerance.[39-43] In particular, an elegant study revealed that ECP induces a high percentage of processed monocytes to enter the antigen-presenting dendritic cell

TABLE 6. aGVHD- and cGVHD-free survival				
	aGVHD-free survival (n = 34)		cGVHD-free survival (n = 37)	
Variables	Percent (95% CI)	p value*	Percent (95% CI)	p value*
Age at ECP				
≥Median	37 (20-70)	0.32	22 (9-53)	0.74
<Median	61 (42-88)		47 (29-76)	
Sex				
Male	44 (25-76)	0.43	25 (13-47)	0.01
Female	56 (37-84)		67 (42-100)	
Stem cell source				
BM	54 (37-80)	0.65	47 (28-78)	0.67
PB	33 (13-84)		25 (12-53)	
CB	67 (30-100)		NA	
Sex mismatch				
Female > male	57 (30-100)	0.52	22 (9-53)	0.23
Other	48 (32-71)		53 (34-83)	
GVHD grade				
II	81 (64-100)	0.0008	NA	
III	33 (15-74)		NA	
IV	0		NA	
Donor type				
MFD	42 (27-66)	0.11	27 (14-51)	0.14
MUD	75 (50-100)		54 (32-93)	
First-line therapy				
Steroids	56 (41-77)	0.11	NA	
Steroids plus other	0		NA	
Visceral aGVHD				
Yes	33 (57-100)	0.03	NA	
No	77 (18-61)		NA	
cGVHD onset				
De novo	NA		50 (28-88)	0.30
Quiescent	NA		28 (12-65)	
Progressive	NA		27 (10-71)	
Seattle criteria				
Limited	NA		67 (45-91)	0.003
Extensive	NA		20 (9-44)	
NIH criteria				
Mild	NA		57 (36-90)	0.07
Moderate	NA		28 (13-58)	
Severe	NA		0	
CIBMTR criteria				
RG1	NA		50 (27-94)	0.85
RG2	NA		32 (18-57)	
≥RG3	NA		0	
TNCs/kg				
≥Median	46 (26-83)	0.68	37 (20-70)	0.53
<Median	43 (23-78)		40 (21-74)	
Lymphocytes/kg				
≥Median	43 (23-78)	0.95	50 (31-82)	0.60
<Median	46 (26-83)		27 (11-62)	
WBCs before ECP				
≥Median	56 (37-84)	0.94	47 (29-76)	0.21
<Median	44 (25-76)		22 (9-53)	
Lymphocytes before ECP				
≥Median	57 (36-90)	0.72	42 (25-71)	0.57
<Median	44 (25-76)		28 (13-58)	
Monocytes before ECP				
≥Median	45 (28-73)	0.43	41 (23-72)	0.45
<Median	57 (36-90)		37 (20-71)	
PLTs before ECP				
≥Median	53 (33-86)	0.50	37 (20-66)	0.70
<Median	47 (29-76)		33 (17-64)	

* The p value for aGVHD-free survival and cGVHD-free survival was calculated according to the Gray test.[35]
BM = bone marrow; CB = cord blood; female > male = female donor for a male recipient; PB = peripheral blood.

(DC) differentiation pathway, within a single day, as determined by enhanced expression of over 1000 genes, independent of disease state, supporting the concept that, in the future, the ECP might represent a source of DC.[44]

In our study, aGVHD patients had an ORR of 65%, which is comparable with data in other reports in the literature.[45-47] In particular, we showed that the ECP response was associated with aGVHD severity and with the first line of aGVHD-therapy. A trend for poorer aGVHD response was observed when the visceral organs were involved (p = 0.07). In contrast to the study by Perotti and colleagues,[45] which showed no association between the grade of aGVHD and the response, in our series, we found a high response rate in aGVHD Grades II and III but not in aGVHD Grade IV, confirming a previous experience of high activity of ECP on early aGVHD.[48] These data were then confirmed as a long-term benefit of ECP on early GVHD. Moreover, when we analyzed the TRM incidence, the ECP responders had not only a significantly lower TRM, but also no significant difference in relapse was observed, suggesting the presence of an immunosuppressive effect without the abrogation or reduction of the anti-leukemic activity (GVL). Finally, when the OS was analyzed among responders and nonresponders, a significant difference was observed, gaining evidence that a possible long-term GVHD remission might also be obtained for patients with steroid-refractory or -dependent aGVHD.

Since very few studies have been addressed to establish hematologic variables predicting responses to ECP in either aGVHD or cGVHD, one of the aims of this study was to test whether the collected TNCs or lymphocytes in the first month from ECP start, together with the pre-ECP hematologic values, were able to discriminate patients' responses to ECP. We observed that neither aGVHD response nor aGVHD-free survival were associated with cell doses even when the hematologic counts or apheresis yield were divided into quartiles, confirming literature reports.[45] A possible explanation is given by studies showing how the cell type (the number of dendritic cells or the number of regulatory T cells generated) can affect the GVHD response;[25-27,39-44] therefore, it appears evident that the biologic response is probably the key affecting the response, something this study indirectly confirms.

The early intervention with ECP, as previously reported, was statistically associated with a higher probability of survival in the aGVHD group, but these data might be biased by the type of aGVHD occurrence, since visceral aGVHD had 1) a lower probability of ECP response, 2) a higher mortality rate, and 3) a need for a quick intervention.

In the cGVHD cohort, the ORR to ECP was significantly associated with the stem cell source, the donor type, the cGVHD extensive form, and the lymphocyte apheresis yield. However, the lymphocyte apheretic yield role was not confirmed when we analyzed the cGVHD-

free survival, whereas the univariate and the multivariate analysis showed how the female sex and the limited form of the Seattle classification or the mild form according to the NIH classification were associated with a better probability of cGVHD-free survival. While the better outcome of females remains an unsolved issue, the different cGVHD-free survival indirectly confirms the findings in the large series reported by the CIBMTR[21] and NIH[20] studies in which the mortality (nonrelapse mortality and OS) was strongly related to cGVHD severity. The CIBMTR and NIH findings were indirectly confirmed by our article in which we were able to show a higher cGVHD-free survival for limited or mild cGVHD. Taken together, we observed that the early-stage Seattle or NIH had a higher probability of response, higher cGVHD-free survival, lower TRM, no higher RI, and finally better OS. More importantly, the intermediate stages (moderate for NIH or RG2 for CIBMTR) showed very low CRs, thus confirming how ECP might have great efficacy in the early-stage cGVHD but not in the intermediate–advanced ones. As for the aGVHD cohort, the TNCs/kg harvest or the pre-ECP hematologic values had no role in cGVHD-free survival, also suggesting how patients with low WBC counts might benefit from ECP treatment, without waiting for ECP start.

In conclusion, to our knowledge, this is the first study aimed at ascertaining whether static or dynamic variables might predict GVHD responses after ECP. Despite lacking biologic studies, no significant cell dose effect was observed, even when the patient cohort was divided into quartiles.

CONFLICT OF INTEREST

The authors have disclosed no conflicts of interest.

REFERENCES

1. Jaglowski SM, Devine SM. Graft-versus-host disease: why have we not made more progress? Curr Opin Hematol 2014; 21:141-7.
2. Lorenz E, Uphoff D, Reid TR, et al. Modification of irradiation injury in mice and guinea pigs by bone marrow injections. J Natl Cancer Inst 1951;12:197-201.
3. Billingham RE. The biology of graft-versus-host reactions. Harvey Lect 1966-67;62:21-78.
4. van Bekkum DW, de Vries MJ. Radiation chimeras. New York: Academic Press; 1967.

5. Martin PJ, Rizzo JD, Wingard JR, et al. First and second-line systemic treatment of acute graft-versus-host disease. Recommendation of the American Society of Blood and Marrow Transplantation. Biol Blood Marrow Transplant 2012;18:1150-63.

6. Ferrara JL, Deeg HJ. Graft-versus-host disease. N Engl J Med 1991;325:357-8.

7. Van Lint MT, Uderzo C, Locasciulli A, et al. Early treatment of acute graft-versus-host disease with high- or low-dose 6-methylprednisolone: a multicenter randomized trial from the Italian Group for Bone Marrow Transplantation. Blood 1998;92:2288-93.

8. Van Lint MT, Milone G, Leotta S, et al. Treatment of acute graft-versus-host disease with prednisolone: significant survival advantage for day +5 responders and no advantage for nonresponders receiving antithymocyte globulin. Blood 2006;107:4177-81.

9. Atkinson K. Chronic graft versus host disease. Bone Marrow Transplant 1990;5:69-82.

10. Sullivan KM, Witherspoon RP, Storb R, et al. Alternating-day cyclosporine and prednisone for treatment of high-risk chronic graft-versus-host disease. Blood 1988;72:555-61.

11. Vogelsang GB. How I treat chronic graft versus host disease. Blood 2001;97:1196-201.

12. Locatelli F, Uderzo C, Dini G, et al. Graft-versus-host disease in children: the AIEOP-BMT Group experience with cyclosporin A. Bone Marrow Transplant 1993;12:627-33.

13. Atkinson K, Horowitz MM, Gale RP, et al. Risk factors for chronic graft versus-host disease after HLA-identical sibling bone marrow transplantation. Blood 1990;75:2459-64.

14. Flowers ME, Parker PM, Johnston LJ, et al. Comparison of chronic graft-versus-host disease after transplantation of peripheral blood stem cells versus bone marrow in allogeneic recipients: long-term follow-up of a randomized trial. Blood 2002;100:415-9.

15. Arora M, Burns LJ, Davies SM, et al. Chronic graft-versus-host disease: a prospective cohort study. Biol Blood Marrow Transplant 2003;9:38-45.

16. Arora M, Nagaraj S, Wagner JE, et al. Chronic graft-versus-host disease (cGVHD) after unrelated donor hematopoietic stem cell transplantation (HSCT): higher response rate in recipients of unrelated donor (URD) umbilical cord blood (UCB). Biol Blood Marrow Transplant 2007;13:1145-52.

17. Lee SJ, Klein JP, Barrett AJ, et al. Severity of chronic graft-versus-host disease: association with treatment-related mortality and relapse. Blood 2002;100:406-14.

18. Martin PJ, Storer BE, Carpenter PA, et al. Comparison of short-term response and long-term outcomes after initial systemic treatment of chronic graft-versus-host disease. Biol Blood Marrow Transplant 2011;17:124-32.

19. Shulman HM, Sullivan KM, Weiden PL, et al. Chronic graft-versus-host syndrome in man. A long-term clinico-pathologic study of 20 Seattle patients. Am J Med 1980;69:204-17.

20. Filipovich AH, Weisdorf D, Pavletic S, et al. National Institutes of Health consensus development project on criteria for clinical trials in chronic graft-versus-host disease: I. Diagnosis and staging working group report. Biol Blood Marrow Transplant 2005;11:945-56.

21. Arora M, Klein JP, Weisdorf DJ, et al. Chronic GVHD risk score: a Center for International Blood and Marrow Transplant Research analysis. Blood 2011;117:6714-20.

22. Vigorito AC, Campregher PV, Storer BE, et al.; National Institutes of Health. Evaluation of NIH consensus criteria for classification of late acute and chronic GVHD. Blood 2009;114:702-8.

23. Weisdorf D, Spellman S, Haagenson M, et al. Classification of HLA-matching for retrospective analysis of unrelated donor transplantation: revised definitions to predict survival. Biol Blood Marrow Transplant 2008;14:748-58.

24. Schwartz J, Winters JL, Padmanabhan A, et al. Guidelines on the use of therapeutic apheresis in clinical practice—evidence-based approach from the Writing Committee of the American Society for Apheresis: the sixth special issue. J Clin Apher 2013;28:145-284.

25. Gatza E, Rogers CE, Clouthier SG, et al. Extracorporeal photopheresis reverses experimental graft-versus-host disease through regulatory T cells. Blood 2008;112:1515-21.

26. Kuzmina Z, Greinix HT, Knobler R, et al. Proportions of immature CD19+CD21- B lymphocytes predict the response to extracorporeal photopheresis in patients with chronic graft-versus-host disease. Blood 2009;114:744-6.

27. Berger C, Hoffmann K, Vasquez JG, et al. Rapid generation of maturationally synchronized human dendritic cells: contribution to the clinical efficacy of extracorporeal photochemotherapy. Blood 2010;116:4838-47.

28. Messina C, Locatelli F, Lanino E, et al. Extracorporeal photochemotherapy for paediatric patients with graft-versus-host disease after haematopoietic stem cell transplantation. Br J Haematol 2003;122:118-27.

29. Oken MM, Creech RH, Tormey DC, et al. Toxicity and response criteria of the Eastern Cooperative Oncology Group. Am J Clin Oncol 1982;5:649-55.

30. Couriel DR, Hosing C, Saliba R, et al. Extracorporeal photochemotherapy for the treatment of steroid-resistant chronic GVHD. Blood 2006;107:3074-80.

31. Couriel DR, Saliba R, Escalon MP, et al. Sirolimus in combination with tacrolimus and corticosteroids for the treatment of resistant chronic graft-versus-host disease. Br J Haematol 2005;130:409-17.

32. Kim HT. Cumulative incidence in competing risks data and competing risks regression analysis. Clin Cancer Res 2007;13:559-65.

33. Kaplan EL, Meier P. Nonparametric estimation from incomplete observation. J Am Stat Assoc 1958;53:457-81.

34. Bland JM, Altman DG. The log rank test. BMJ 2004;328:1073.

35. Fine JP, Gray RJ. A proportional hazards model for the subdistribution of a competing risk. J Am Stat Assoc 1999; 94:496-509.

36. Koreth J, Antin JH. Current and future approaches for control of graft-versus-host disease. Expert Rev Hematol 2008;1:111.

37. Marshall SR. Technology insight: ECP for the treatment of GVHD—can we offer selective immune control without generalized immunosuppression? Nat Clin Pract Oncol 2006;3:302-14.

38. Holler E. Risk assessment in haematopoietic stem cell transplantation: GVHD prevention and treatment. Best Pract Res Clin Haematol 2007;20:281-94.

39. Maeda A, Schwarz A, Kernebeck K, et al. Intravenous infusion of syngeneic apoptotic cells by photopheresis induces antigen-specific regulatory T cells. J Immunol 2005;174: 5968-76.

40. Gerner M, Hölig K, Wehner R, et al. Extracorporeal photopheresis efficiently impairs the proinflammatory capacity of human 6-sulfo LacNAc dendritic cells. Transplantation 2009;87:1134-9.

41. Biagi E, Di Biaso I, Leoni V, et al. Extracorporeal photochemotherapy is accompanied by increasing levels of circulating CD4+CD25+GITR+Foxp3+CD62L+ functional regulatory T-cells in patients with graft-versus-host disease. Transplantation 2007;84:31-9.

42. Di Renzo M, Rubegni P, Pasqui AL, et al. Extracorporeal photopheresis affects interleukin (IL)-10 and IL-12 production by monocytes in patients with chronic graft-versus-host disease. Br J Dermatol 2005;153:59-65.

43. Shlomchik WD, Couzens MS, Tang CB, et al. Prevention of graft-versus-host disease by inactivation of host antigen-presenting cells. Science 1999;285:412-5.

44. Spisek R, Gasova Z, Bartunkova J. Maturation state of dendritic cells during the extracorporeal photopheresis and its relevance for the treatment of chronic graft-versus-host disease. Transfusion 2006;46:55-65.

45. Perotti C, Del Fante C, Tinelli C, 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: 1359-69.

46. Greinix HT, Worel N, Knobler R. Role of extracorporeal photopheresis (ECP) in treatment of steroid-refractory acute graft-versus-host disease. Biol Blood Marrow Transplant 2010;16:1747-51.

47. Calore E, Calo A, Tridello G, et al. Extracorporeal photochemotherapy may improve outcome in children with acute GVHD. Bone Marrow Transplant 2008;42: 421-5.

48. Berger M, Pessolano R, Albiani R, et al. Extracorporeal photopheresis for steroid resistant graft versus host disease in pediatric patients: a pilot single institution report. J Pediatr Hematol Oncol 2007;29:678-87. ⬧