the reason for second transplantation. Given the currently unknown long-term effects of a repeated exposure to G-CSF, the factors mentioned above should be kept in mind whenever a request for a second PBSC donation is considered.

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Extracorporeal photochemotherapy for steroid-refractory graft-versus-host disease in low-weight pediatric patients. Immunomodulatory effects and clinical outcome

Pediatric patients with refractory graft-versus-host disease (GVHD) have been considered for extracorporeal photochemotherapy (ECP). The main problems with ECP in children, especially in the smaller ones, are technical difficulties of leukaphereses with a large extracorporeal volume. Only a few pediatric centers have experience with ECP, mainly with older children.[1,2] We report the clinical outcome in very low-weight pediatric patients who underwent ECP for refractory GVHD and the effect on the lymphocyte subsets. Between September 2003 and April 2007, 11 children with a body weight lower than 25 kg underwent ECP by refractory GVHD. Patients' characteristics are shown in Table 1. GVHD prophylaxis consisted of cyclosporine and methotrexate. Diagnosis (clinical and histological) and classification of GVHD was made according to published criteria.[3] Steroid refractory was defined as failure to respond to 2 mg/kg/day of 6-methilprednisolone after five days (acute GVHD) and flare-up of disease activity upon tapering (chronic GVHD). All patients received cyclosporine A or mycophenolate mofetil and steroids as first-line therapy. ECP was used as second-line treatment in 4 patients who received etanercept prior to ECP. Written informed consent was obtained from all patient's parents and our institution's ethics committee approved the study. Lymphocytopheresis was performed using a continuous-flow cell separator (Spectra, COBE, Lakewood, CO, USA; version 6.1) processing 2 blood volumes as previously reported.[4] The product was diluted with normal saline to a volume of 300 mL by the addition of 3 mL of 8-methoxypsoralen (8-MOP) aqueous solution (Gerot Pharmaceutika, Vienna, Austria) to achieve a product concentration of 200 ng/mL. This final product was transferred to a UVA-permeable bag (MacoPharma, Tourcoing, France), exposed to UVA irradiation (UV-MATIC irradiator, Vilbert Lourmat, France) at a dose of 2 J/cm^2 and reinfused.[5] The average time of ECP procedure was 180 minutes. The machine was primed with packed red blood cells in patients with body weight <15 kg. No standard prophylaxis of hypocalcemia was used.

ECP sessions were performed twice a week (consecutive days) until clinical improvement for acute GVHD. Patients with chronic GVHD were given EPC on two consecutive days (one cycle) at 2-week intervals. Progressive tapering of immunosuppressive therapy and discontinuation of ECP depended on clinical response which was defined according to previously published crtiteria.[6]

Table 1. Patients' characteristics and outcome (n=11).

Pt N.	Age (years)	Sex	Weight (Kg)	Diagnosis	Source	Type	Indication	Grade	2nd line therapy	N. of cycles	Duration (days)	Clinical response				Status	Cause of death	Follow-up (months)
												Skin	Liver	Gut	Lung			
1	7	F	22	CML	PB	MSD	cGVHD	Ext.		20	128	PR	–	–	SD	Alive		43
2	5	F	18	Blackfan-Diamond	CB	MUD	aGVHD 39	III		5	14	CR	–	CR	–	Alive		
3	5	F	15	ALL	CB	MUD	aGVHD	IV		10	51	CR	–	PR	SD	Dead	Microangiopathy	5
4	5	F	16	ALL	PB	MSD	aGVHD	IV	Etanercept	10	47	CR	CR	CR	–	Dead	Relapse	3
5	1	F	9	ALL	CB	MUD	aGVHD	II		4	9	CR	–	CR	–	Alive		14
6	1	M	9	ID	BM	MUD	cGVHD	Ext.		25	127	CR	CR	–	–	Alive		14
7	6	M	20	ALL	PB	MUD	a,cGVHD	II, ext		19	267	CR	–	PR	SD	Dead	*Aspergillus*	10
8	5	M	21	ALL	BM	MUD	aGVHD	IV	Etanercept	9	34	CR	–	CR	SD	Dead	*Aspergillus*	5
9	8	M	24	ALL	PB	MSD	aGVHD	III	Etanercept	6	14	CR	CR	PR	SD	Dead	*Aspergillus*	2
10	13	M	24	Sickle cell disease	BM	MSD	cGVHD	Ext.	Etanercept	8	50	CR	PR	CR	PR	Alive		5
11	7	F	23	ALL	PB	MUD	aGVHD	II		2	1	CR	–	–	–	Alive		8

Pt: patient; CML: chronic myeloid leukemia; ALL: acute lymphoblastic leukemia; ID: immunodeficiency; PB: peripheral blood; CB: cord blood; BM: bone marrow; MSD: matched sibling donor; MUD: matched unrelated donor; aGVHD: acute; cGVHD: chronic; Ext: extensive; CR: complete remission; PR: partial remission. SD: stable disease.

Flow cytometry analyses (FACSCanto II, Beckton Dickinson) were carried out on peripheral blood samples. We studied the L-selectin (CD62L) and the CD45RA expression on CD4 and CD8 lymphocytes. This combination allowed us to distinguish naive (TN, $CD62L^+$ $CD45RA^+$), central memory (TCM, $CD62L^+CD45RA^-$), effector memory (TEM, CD62L-$CD45RA^-$), and terminal differentiated T cells (TT, CD62L-$CD45RA^+$), as described elsewhere.[7] We compared the proportion of each of these four subpopulations, as well as the L-selectin positive and L-selectin negative ones, in peripheral blood before the first (pre-) and after the last (post-) ECP procedures. We compared the changes related to the ECP with the T-lymphocyte subset reconstitution in children without GVHD to find out whether ECP could mimic a *normalization* of T-lymphocyte subsets. One hundred and eighteen ECP procedures were performed with a median of 9 procedures (range: 2-25) per patient. ECP was started at a median of 191 days (range: 12-1635) after transplant. Eight patients were thrombocytopenic at the time of starting ECP.

CR was achieved in 5 cases and PR in 6. CR of skin, liver and gut involvement was achieved in 10/11, 3/4 and 5/8 cases respectively. The median time to response was three weeks. Clinical outcome is shown in Table 1.

There was a significant increase in the CD4/CD8 ratio. We found that the proportion of L-selectin expressing T lymphocytes significantly diminished after ECP, both in CD4 and CD8 cells (Table 2). No changes were observed in CD4 and CD8 naive subsets. In patients without GVHD, these analyses showed a progressive decrease in the proportion of CD62L positive memory CD8 lymphocytes, but no significant changes in the expression of CD62L by CD4 lymphocytes (*unpublished data*). No patient developed hemodynamic instability requiring inotropic treatment. Only 2 patients (the smaller patients, weight 9 Kg) needed a fluid bolus because of vasovagal effects, but the procedures were not stopped. No symptomatic hypocalcemia was presented. Three episodes of catheter-related infection (staph. species) were seen. No ECP-related severe infections were observed.

Only one patient transplanted in refractory disease relapsed post-trasplant. Four patients died: one of microangiopathy and 3 of fungal infections.

Table 2. CD4 and CD8 subsets pre- and post-extracorporeal photochemotherapy.

	CD4 subsets and ECP		
	Pre-(%)	*Post-(%)*	*p*
Naive	6.38±2.39	6.18±2.96	0.2395
Central memory	57.41±4.51	43.85±4.78	0.0309
Effector memory	35.62±4.56	46.86±4.89	0.054
EMRA	0.65±0.19	3.11±1.46	0.189
$CD62L^{pos}$	63.79±4.45	50.03±5.45	0.0409
$CD62L^{neg}$	36.27±4.47	49.97±5.45	0.0409
	CD8 subsets and ECP		
	Pre-(%)	*Post-(%)*	*p*
Naive	16.95±3.94	12.53±4.05	0.2343
Central memory	28.8±4.95	12.27±2.86	0.0013
Effector memory	46.62±5.79	51.4±4.22	0.1477
EMRA	11.17±3.34	23.52±4.79	0.0105
$CD62L^{pos}$	45.75±6.1	24.8±5.34	0.0174
$CD62L^{neg}$	53.8±6.07	74.92±5.31	0.0174

Several reports have been published about successful treatment of GVHD by ECP in adults using the UVAR XTS system (Therakos, Inc.).[6,8,9] Only a few pediatric centers perform ECP[1,10] and the main problems are related to the ECP technique itself. As small children may not tolerate the fluid shift during ECP using the UVAR XTS machine, an alternative approach by means of a continuous-flow cell separator (COBE Spectra) has been developed.[11] This procedure allows the processing of more mononuclear cells in a smaller volume with a shorter duration than with the UVAR system. Vasovagal effects were the most frequent adverse events but no procedure was interrupted for this reason. There was no increase of severe infections due to the ECP procedure. Our findings show changes in the immune reconstitution of patients who underwent ECP with a decrease in the proportion of T-lymphocytes expressing L-selectin. This change mimics the *normal* reconstitution kinetics (in patients without GVHD) after transplant for CD8 lymphocytes, but not for

CD4. The proportion of L-selectin expressing T lymphocytes significantly diminished after ECP, both in CD4 and in CD8 cells. The reasons for these changes are currently unknown. L-selectin is an important T-cell homing receptor for T-cell entry into lymph nodes via high endothelial venules. Expression of CD62L is rapidly lost following T-cell receptor activation, leading to exit from the lymph node into the periphery and sites of inflammation. $CD62L^{neg}$ and $CD62L^{pos}$ also differ in their functional abilities, such as cytokine secretion and cytolytic potential.[12] Our results suggest that ECP may have an impact on the trafficking patterns of T lymphocytes, redirectioning T cells from lymphoid to extralymphoid organs.

In conclusion, ECP is well tolerated using the described method in small children with minimal side effects during therapy. Clinical response seems to be associated with change in the peripheral blood lymphocyte subsets.

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Retraction

The article entitled "Heterogeneous promoter activity of the telomerase reverse transcriptase gene in individual acute myeloid leukemia cells defined by lentiviral reporter assay" by Seiichiro Kobayashi, Yasushi Soda, Yuansong Bai, and Arinobu Tojo, published ahead-of-print on May 27, 2008 as doi: 10.3324/haematol.12123, and on July 1, 2008 as Haematologica 2008; 93:1103-5,[1] has been retracted on June 27, 2008, by the corresponding author, Dr. Arinobu Tojo. In his email to the editorial office, Dr. Tojo stated that an investigation of the Institutional Review Board (IRB) records showed that the above study had not been approved by the IRB.
doi: 10.3324/haematol.13594.

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