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Extracorporeal photochemotherapy for treatment of drug-resistant graft-vs.-host disease

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ABSTRACT

Extracorporeal photochemotherapy (EP) is a therapeutic approach to the treatment of drug-resistant graft-vs.-host disease (GVHD) that uses the known immunosuppressive and immunomodulatory effects of ultraviolet light. In 1990, we initiated a pilot study to evaluate the efficacy and safety of EP in patients with refractory GVHD. Between 1991 and 1996, six patients with acute grade IV liver GVHD, 12 patients with chronic following acute GVHD, and six patients with *de novo* chronic GVHD were treated with EP. All patients had failed to respond to conventional GVHD immunosuppressive drug therapy of cyclosporine and prednisone. The six patients with acute liver GVHD had also received antithymocyte globulin (ATG); therapy for chronic GVHD included thalidomide in eight patients, psoralen plus ultraviolet A in five patients, and ATG in two patients. All patients with acute liver GVHD had progressive liver failure with short survival despite frequent EP. The response rate with EP treatment was 3 of 6 for patients with *de novo* chronic GVHD and 3 of 12 for patients with chronic following acute GVHD. Three patients with bronchiolitis obliterans had either no response or no documented disease progression while undergoing EP. Side effects of EP were minor and included gastrointestinal upset frequently, catheter-related sepsis in four patients, increased red blood cell and platelet transfusion requirements in one patient, and leukopenia in two patients. EP was discontinued in three patients because of side effects, including GI upset in one patient and bone marrow suppression in two patients. Side effects were reversible with the discontinuation of EP. We were unable to correlate response to EP with the level of methoxypsoralen, number of lymphocytes treated, or pattern of pre- and posttreatment CD4/CD8 ratio. We concluded that EP has some efficacy in the treatment of drug-resistant chronic GVHD, with minor overall toxicity.

KEY WORDS

Photopheresis • Bone marrow transplantation • Psoralen

INTRODUCTION

Graft-vs.-host disease (GVHD) is a frequent cause of morbidity and mortality after bone marrow transplantation (BMT) from related and unrelated histocompatible donors

and is the single most important obstacle to successful allogeneic BMT. Development of GVHD after BMT is mediated by alloreactive donor T lymphocyte subsets, which become activated after recognition of major histocompatibility complex (MHC) antigens or minor histocompatibility antigens of the host, or both, that are disparate from donor antigens. Recent experimental data suggest that direct cell-mediated attack of donor T lymphocytes on host tissues is only one of several mechanisms leading to host tissue damage [1,2]. As

Table 1. *Patient characteristics*

Number of patients	24
Median age (range)	29 years (5.7–53)
Male/female	19/5
Diagnosis	
CML	11
Acute leukemia	8
Aplastic anemia	2
MDS & myeloproliferative diseases	3
Donor status	
HLA-identical sibling	17
5/6 HLA sibling	1
Matched unrelated donor	6
Transplant preparative regimens	
FTBI and etoposide	11
FTBI and CY	5
FTBI, CY, and etoposide	3
CY and busulfan	3
TLI and CY	1
CY	1
GVHD prophylaxis	
CSA and PSE	7
CSA, PSE, and MTX (days 1, 3, 6)	12
CSA, PSE, and MTX (days 1, 3, 6, 11)	3
CSA	1
CSA, PSE, and ATG	1
Acute grade IV liver GVHD	6
De novo chronic GVHD	6
Chronic GVHD after acute GVHD	12

ATG, antithymocyte globulin; CML, chronic myeloid leukemia; CSA, cyclosporine; CY, cyclophosphamide; FTBI, fractionated total-body irradiation; GVHD, graft-vs.-host disease; HLA, human leukocyte antigen; MDS, myelodysplastic syndrome; MTX, methotrexate; PSE, prednisone; TLI, total-lymphoid irradiation.

discussed in several recent reviews, several lines of evidence now implicate a network of cytokines as primary mediators of experimental and clinical GVHD [3–6]. T cell depletion of the marrow graft to limit the cascade of cytokine production that results from the complex interactions of subsets of T cells, antigen presenting cells, and accessory effector cells decreases the incidence of GVHD, but also significantly increases the risk of graft failure and leukemic relapse [7].

Acute GVHD in the first 100 days post-BMT is a pathologic process characterized by cytolytic damage to the host target tissues of skin, liver, and gastrointestinal tract, with accompanying systemic symptoms of fever and increased capillary permeability. Prophylaxis of acute GVHD with combinations of immunosuppressive agents has decreased the incidence of this complication of BMT to 20–25% in recipients of unmodified (i.e., no *ex vivo* T cell depletion) allografts from human leukocyte antigen (HLA)-identical siblings, but it remains a primary or contributory cause of death in 15–20% of marrow transplant patients [8]. Treatment of established acute GVHD with steroids, cyclosporine (CSA), monoclonal antibodies, and antithymocyte globulin (ATG) has been characterized by high failure rates, significant drug toxicities, and poor survival for nonresponders [9–13].

Chronic GVHD (cGVHD) is the primary cause of nonrelapse morbidity and mortality in patients surviving longer than 100 days after BMT. The clinical syndrome resembles an overlap of various autoimmune diseases, such as progressive systemic sclerosis, Sjögren's syndrome, systemic lupus erythematosus, lichen planus, and primary biliary cirrhosis [14,15]. Because the pathophysiology of this disease process includes the production of autoantibodies and the inability to produce protective antibodies against environmental pathogens, it has been suggested that the primary defect in cGVHD is an imbalance between autoreactive and autoregulatory T cells [16]. cGVHD occurs in 20–50% of recipients of unmodified (i.e., no *ex vivo* T cell depletion) sibling marrow transplants, with mortality rates varying from 20 to 70%, depending on the presence of known associated risk factors for poor outcome [17].

Because prevention and treatment of acute and chronic GVHD has been less than ideal, alternative therapeutic approaches are desirable. One novel approach is photochemotherapy, also known as PUVA therapy, which is the use of oral methoxypsoralen (8-MOP) photoactivated by exposure of skin to ultraviolet A (UVA) light. Multiple studies have demonstrated the efficacy and safety of PUVA therapy in the treatment of cutaneous GVHD [18–25]. Extracorporeal photochemotherapy (EP), also referred to as photopheresis, is a variant of PUVA therapy that involves extracorporeal exposure of blood lymphocytes to UVA followed by reinfusion of the treated cells. Bolwell *et al.* first used EP in the treatment of GVHD; in 1990 they reported the successful treatment of cGVHD in two of three patients [26].

To extend the experience with EP, we conducted a pilot study with 20 patients from 1990 to 1994 in which we evaluated the efficacy and safety of photopheresis in the treatment of drug-resistant acute and chronic GVHD. Since the publication of our original abstract [27], we have treated an additional four patients who are included in this report, and here summarize our results from EP treatment of 24 patients with acute and chronic drug-refractory GVHD.

MATERIALS AND METHODS
Patient population
Between February 1991 and February 1996, 24 patients with drug-resistant acute and chronic GVHD were treated with EP at the City of Hope National Medical Center (COH). The initial 20 patients were treated in a non-randomized pilot study conducted from 1990 to 1994 to evaluate the efficacy and safety of EP in the management of refractory GVHD. Subsequent to the pilot study closure in 1994, we treated an additional four patients, whose data are included in this report.

Patient characteristics are shown in Table 1. The median age of marrow recipients was 29 years, with a range of 5.7–53 years. There were 19 male and 5 female patients. The indications for BMT were chronic myelogenous leukemia (n=11); acute leukemia, including acute lymphoblastic leukemia, acute myelogenous leukemia, and acute biphenotypic leukemia (n=8); aplastic anemia (n=2); and myeloproliferative and myelodysplastic disorders (n=3).

Donor-recipient matching
The histocompatible marrow donors were 6/6 HLA-identical siblings (n=17), 5/6 HLA-matched siblings (n=1),

or matched unrelated donors (n=6). Four unrelated donor-recipient pairs were serologic matches for class I antigens and molecular matches for class II antigens; one unrelated donor-recipient pair had a micro-mismatch at a B locus and a molecular match at class II antigens; and one unrelated donor-recipient pair was serologically matched at all loci with a nonreactive mixed leukocyte culture (MLC). (Information regarding molecular matching was unavailable to us because the patient was transplanted at another institution.)

Transplant preparative regimen

Twenty-two patients underwent BMT at COH and two pediatric patients underwent BMT at other institutions and subsequently were referred to COH for treatment of GVHD. Preparative regimens varied according to disease, remission status, and active institutional protocols at the time of each patient's BMT and were previously reported [28–32]. The regimens used for patients with leukemia were 1320 cGy fractionated total-body irradiation (FTBI) plus 60 mg/kg of etoposide in 11 patients; 1320 cGy FTBI plus 120 mg/kg of cyclophosphamide in 5 patients; a triple regimen of 1320 cGy FTBI plus 60 mg/kg of etoposide and cytoxan (20 mg/kg in one patient and 40 mg/kg in another); and 16 mg/kg of busulfan plus 120 mg/kg of cyclophosphamide in one patient. One patient with aplastic anemia received 50 mg/kg/day of cytoxan for 4 days plus 750 cGy total-lymphoid irradiation and another patient with aplastic anemia received cytoxan alone. Patients with myeloproliferative or myelodysplastic disorders received a regimen of 16 mg/kg of busulfan plus 120 mg/kg of cytoxan (n=2 patients) or 1200 cGy FTBI plus 80 mg/kg of cytoxan plus 60 mg/kg of etoposide (n=1 patient). *Ex vivo* marrow T cell depletion was not used.

GVHD prophylaxis

Acute GVHD prophylaxis regimens varied according to the active institutional protocols at the time of each patient's BMT and have been reported elsewhere. The regimens that these patients received were cyclosporine (CSA) and prednisone (PSE) in seven patients [33]; CSA, PSE, and methotrexate (MTX) on days 1, 3, and 6 in 12 patients [34]; CSA, PSE, and MTX on days 1, 3, 6, and 11 in three patients [29]; and CSA alone in one patient [35]. One pediatric patient, who underwent BMT at another transplant center, received a regimen of CSA, PSE, and ATG according to that institution's GVHD prophylaxis protocol for recipients of unrelated donor marrow transplants.

Diagnosis of GVHD

The diagnosis and grading of acute and chronic GVHD was made by COH clinical investigators according to the standard criteria previously described by the transplant team in Seattle [36]. Biopsies of skin and gut were performed to confirm the diagnosis of GVHD in the majority of cases. Biopsies of liver and lung were infrequently performed because, in many cases, the clinician responsible thought that the procedure was unsafe due to aberrant coagulation parameters. Diagnosis of mouth cGVHD was made without confirmatory biopsy. Six patients with acute grade IV liver GVHD who had failed to respond to standard therapy with CSA, PSE, and ATG were treated with EP. When EP was

Table 2. *Characteristics of chronic GVHD at study entry*

Number of patients	18
Chronic GVHD site	
Skin	2
Skin and mouth plus hepatic dysfunction	3
Progressive liver	4
Progressive liver plus mouth or skin	4
GI plus liver (or mouth and skin)	2
Bronchiolitis obliterans	3
Prior acute GVHD	12
Progressive from acute GVHD	8
De novo GVHD	6
Thrombocytopenia	11
Prior treatment for chronic GVHD	
PSE/CSA	18
Thalidomide	8
PUVA	5
ATG for prior acute GVHD	2

GI, gastrointestinal; PUVA, psoralen plus ultraviolet A light.

started, all patients with acute GVHD received 2 mg/kg of PSE for longer than 2 weeks and were continued on that same dose after initiation of EP. Twelve patients with chronic following acute GVHD and six patients with *de novo* cGVHD who failed to respond to standard therapy, which included thalidomide (eight patients) and PUVA (five patients), were treated with EP. Initial standard therapy for cGVHD patients included at least 10 days of therapy with 2 mg/kg/day of PSE plus CSA in doses to achieve therapeutic levels. The cGVHD patients were eligible for EP treatment if their cGVHD flared as steroids were tapered so that they were on varying doses of PSE at the time EP was started, but all were on CSA doses that resulted in therapeutic CSA levels. Two of the cGVHD patients with prior acute GVHD also had received ATG for treatment of acute GVHD. Characteristics of patients' cGVHD before therapy with EP are outlined in Table 2.

Extracorporeal photopheresis

Treatment with EP was accomplished using the UVAR photopheresis system (Therakos, West Chester, PA), which is diagrammed in Figure 1. The photosensitizing agent was 8-MOP, a furocoumarin that forms covalent bonds with the pyrimidine bases of DNA in the presence of UVA light. The drug preparation used in this study was Oxypsoralen Ultra (ICN Pharmaceuticals, Costa Mesa, CA). The initial oral dose of 8-MOP was 0.6–1.0 mg/kg and the dose was adjusted with subsequent treatment cycles in an effort to achieve a therapeutic level of >50 ng/mL. The 8-MOP dose was increased by 25% if the patient had a subtherapeutic level with the previous cycle of EP. If the patient's 8-MOP level increased with this dose titration but was still subtherapeutic, then the dose was sequentially increased in 25% increments with subsequent cycles until a therapeutic dose was achieved. However, if there was no change in the patient's 8-MOP level after doubling the initial dose, then that patient was assumed to have an inability to absorb the drug and further dose adjustments were not made. The therapeutic level
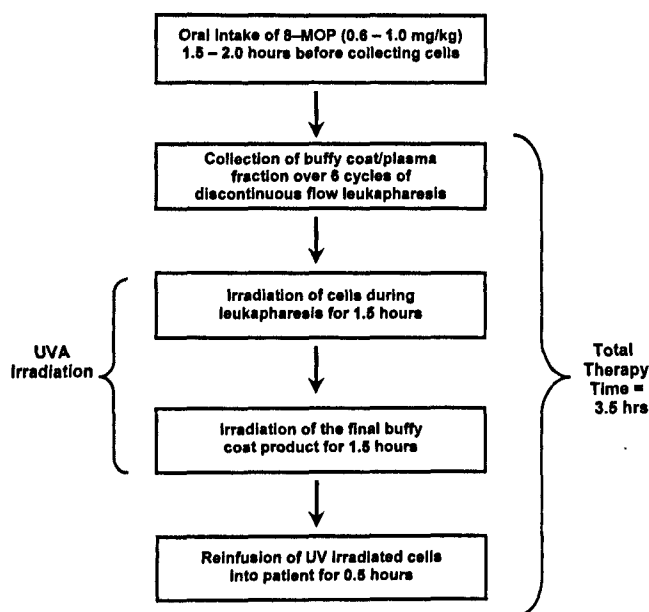
Figure 1. Schema of extracorporeal photochemotherapy using the UVAR photopheresis system for cell collection and irradiation

of 8-MOP was defined as 50 ng/mL based on extrapolation from the photobiology literature regarding the use of EP for the treatment of cutaneous T cell lymphoma [37]. Two hours after ingesting 8-MOP, patients underwent a discontinuous leukapheresis procedure, which resulted in the collection of a buffy coat preparation that contained an estimated 25–50% of the total peripheral blood mononuclear cell compartment. The final buffy coat product was then circulated as a 1-mm film through a sterile cassette surrounded by a UV light source, which yielded an average exposure per lymphocyte of 2 J/cm^2. After photoactivation, the UVA-exposed leukocyte preparation was returned to the patient. The entire procedure lasted 3.5 hours.

8-MOP drug levels

Patient blood samples for 8-MOP levels were collected in heparinized blood collection tubes 2 hours after ingestion of 8-MOP and before the photopheresis procedure. The plasma fraction was then separated by centrifugation and placed in a polypropylene tube. The assay for 8-MOP levels was performed by the high pressure liquid chromatography method at the Yale University Photobiology Laboratory (New Haven, CT).

Treatment plan

The schedule of EP treatments varied during the course of this study. Patients with cGVHD were initially treated on a schedule of 2 consecutive procedures every 3 weeks for the treatment of cutaneous T cell lymphoma, as reported by Edelson *et al.* [37]. Subsequently, the treatment schedule for patients with extensive cGVHD was intensified to 2–3 procedures per week, based on a hypothesis that more intensive EP might yield greater therapeutic benefit. All patients with acute GVHD were treated with an intensive

EP regimen of 2–3 procedures per week. Responding patients continued to receive EP until they achieved maximal response and then intervals between treatment cycles were slowly increased. The time schedule for tapering EP was determined on an individual basis by the patient's primary transplant physician and the transfusion medicine physician. All patients were on CSA and PSE, and some patients were also on thalidomide before entry in this study and were continued on the same doses of their immunosuppressive medications when EP was initiated. In patients who responded to EP, the doses of immunosuppressive medications were progressively reduced as tolerated.

Flow cytometry

Enumeration of CD4 and CD8 cells in peripheral blood samples was performed with flow cytometry. Patient samples were collected before the start and after the completion of the EP course. Samples were stained with fluorochrome-labeled CD4 and CD8 antibodies (Becton Dickinson Immunocytometry Systems, San Jose, CA). LYSIS 2 data acquisition was performed and analyzed using FACScan (Becton Dickinson Immunocytometry Systems).

Statistical methods

The primary objective of this study was to estimate the response rate when EP was used to treat drug-refractory cGVHD. Univariate and multivariate logistic regressions were performed, with outcome being treatment success or failure, to evaluate whether any treatment variables were predictive of treatment outcome. The covariates that were tested to determine if they influenced the outcome of treatment included the following: total number of lymphocytes treated per procedure and per course, the number of EP treatments, days between first and last treatments, and 8-MOP dose. The 8-MOP dose was quantified in the following ways: total dose across all treatments, average dose, highest single dose, dose >50 ng/mL for more than half of the treatments, dose >100 ng/mL for more than half of the treatments, dose >50 ng/mL for at least one treatment, dose >50, 75, and 100 ng/mL for any two treatments, and dose >50, 75, and 100 ng/mL for any three treatments. Another point of interest was overall survival, measuring time from start of EP to death or date of last contact. The survival curve for the patients with cGVHD was drawn using the product-limit method of Kaplan and Meier.

Evaluation of response

Response was defined as a 50% or better improvement in measurable parameters of GVHD, i.e., extent of skin involvement, total bilirubin and alkaline phosphatase values, volume of diarrhea, and pulmonary function tests. Pulmonary function was assessed by spirometry with measure of forced vital capacity, forced expiratory volume in 1 second, maximal midexpiratory flow, maximal expiratory flow rate, and maximal expiratory flow, at 50 and 75% of expired vital capacity.

The primary transplant physician for each patient and the transfusion medicine physician (I.S.) who treated all patients in this study performed serial assessments of the skin's appearance in terms of the amount of induration or softness and the presence or absence of ulcerations and ero-

Table 3. *Schedule of EP treatments for patients with GVHD*

No[a]	UPN	Donor type	Days post-BMT	Number of EP processes	EP schedule	Interval between 1st & last EP (days)	Total number lymphs treated/course of therapy ($\times 10^9$)	Average number lymphs treated/EP ($\times 10^9$)	8-MOP dose (mg)	Level ng/mL (median [range])	Outcome
1	565	MSD	538	10	2 EP q. 3 weeks	85	24.1	2.4	50	150 (120–184)	PR skin; death
2	444	MSD	1414	19	2 EP q. 3 weeks	260	35.1	1.95	50	117 (0–262)	CR skin; alive
3	726	MSD	83	26	3 EP q. week	89	76.1	2.9	70	83 (0–242)	NR; alive
4	001	MSD	457	15	2–3 EP q. week	95	28.3	1.9	50	101	NR; death
5	693	MSD	182	22	2 EP q. 2 weeks	148	42.77	1.94	70	352 (106–507)	R then prog; death
6	631	MSD	602	6	2 EP q. week	19	2.03	0.4	50	288	NR; death
7	525	MSD	784	48	2 EP q. month; then 2 EP q. 3 weeks	539	144.27	3.0	50	104 (0–515)	CR liver; alive
8	2021	URD	326	18	2 EP q. week	59	20.8	1.15	40	73 (0–116)	NR; death
9	732	MSD	153	16	2 EP q. week	52	12.2	0.8	70	50 (40–75)	NR; alive
10	465	MSD	1333	25	2–3 EP q. week	101	49.71	1.99	40	152 (102–230)	CR liver, skin; alive
11	595	MSD	694	46	3 EP q. week	113	47.9	1.1	50	150 (50–270)	NR; death
12	561	MSD	414	22	2 EP q. 3 weeks	175	57.2	2.6	40	0 (0–0)	NR; death
13	650	MSD	282	26	2 EP q. 3 weeks	117	66.04	2.54	40	118 (0–383)	PR liver; alive
14	604	MSD	373	14	2 EP q. 3 weeks; then 2 EP q. 2 weeks	113	15.5	1.14	50	75 (28–122)	NR; death
15	537	MSD	1051	30	2 EP q. 3 weeks; then 2 EP q. week	132	126.9	4.2	70	29 (0–113)	R then prog; death
16	2105	URD	58	28	3 EP q. week	103	17.55	0.65	5.4	0 (0–270)	PR skin; alive
17	844	MSD	821	6	2 EP q. week	30	2.5	0.42	50	35	NR; death
18	2125	URD	154	14	2 EP q. week	58	36.04	2.57	40	12 (0–27)	NR; death
19	002	URD	134	20	2 EP q. week	24	22.3	1.1	20	50 (0–148)	NR; death
20	721	MSD	97	4	3 EP q. week	11	1.7	0.4	70	87 (0–173)	NR; death
21	2022	5/6 MSD	54	11	2 EP q. week	21	5.8	0.53	30	42 (36–49)	NR; death
22	727	MSD	56	24	3 EP q. week	61	12.2	0.5	50	322 (283–361)	NR; death
23	2030	URD	106	23	2 EP q. 3 weeks; then 3 EP q. week	59	49.4	2.1	50	123 (120–126)	NR; death
24	2138	URD	50	16	3 EP q. week	47	6.02	0.40	60	0 (0–0)	NR; death

[a]Patients 1–18 have cGVHD; patients 19–24 have acute GVHD.

CR, complete remission of GVHD; EP, extracorporeal photochemotherapy; MSD, matched sibling donor; NR, no response; PR, partial remission of GVHD; q., every; R then prog, response then progression of GVHD; UPN, unique patient number; URD, unrelated donor.

sions. If both physicians documented resolution of the majority of the skin ulcerations and softening of the skin, then the patient was considered to have had a response. A response for patients with mouth cGVHD consisted of symptomatic improvement in terms of less pain and an ability to eat, as well as a physician assessment of improvement in the appearance of the oral mucosa. Patients who had progression of GVHD while on EP were considered to have had treatment failure.

RESULTS

Six patients with acute GVHD and 18 patients with cGVHD were evaluable for response and toxicity. The group of 18 patients with cGVHD included two patients who were treated with EP before day 100 post-BMT. Unique patient number (UPN) 2105 was treated with EP for acute grade IV skin GVHD from day 58 through day 126 and was restarted on EP on day 140 for progressive cGVHD of skin, mouth, and liver. UPN 726 had acute grade III skin, grade II GI, and grade II liver GVHD, which had progressed to skin and mouth cGVHD by day 83 when he was started on EP. The six patients with acute grade IV liver GVHD were treated with two EP procedures every week (n=1) or three procedures every week (n=5), as shown in Table 3. Despite this schedule of frequent EP, the patients with acute liver GVHD had progressive liver failure with very short survival. The median number of EP procedures that were performed in patients with acute grade IV liver GVHD was 18, with a range of 4–24.

Six patients with *de novo* cGVHD and 12 patients with chronic following acute GVHD were treated with EP on a schedule varying from 2 or 3 procedures every week to 2 procedures every 3 weeks (Table 3). In the patients with

Table 4. *GVHD parameters: pre- and posttreatment in responders*

UPN	Pretreatment	Posttreatment	Outcome
565	De novo scleroderma; extensive ulceration of neck, lower abdominal wall, back, and over sternum;	Healing of all ulcerations started after 2nd EP and continued through 10th EP; 8 months post-EP had 1 superficial erosion; on 20 mg/day of PSE	Noncompliant and lost to follow-up; died 41 months post-BMT
444	De novo scleroderma with leg ulcerations; mild mucositis	Healing of all ulcers increased ROM by 7th EP, permitting thalidomide taper; all but 1 ulcer resolved after 10th EP; PSE gradually tapered during 19 procedures	CR of GVHD on PSE 10 mg every other day at 8.3 years post-BMT
465	De novo mucositis; lichenoid skin changes; AP of 410 IU/L, bilirubin of 3 mg/dL	After 1 month of 3 EP/week, PR of skin and mucositis; AP of 112 IU/L, bilirubin of 0.9 mg/dL; PSE tapered during 25 EP procedures	CR of GVHD on neither PSE nor CSA at 5 years post-BMT
525	Acute progressing into cGVHD of liver; AP 1720 IU/L, bilirubin 6 mg/dL	After 12 months of EP, AP of 994 IU/L, bilirubin of 2.2 mg/dL on 20 mg/d of PSE; EP stopped after 48 procedures	CR of GVHD on neither PSE nor CSA at 7 years post-BMT
650	Acute followed by cGVHD of mouth & liver; AP of 322 IU/L, bilirubin of 3.5 mg/dL	After 26 EP procedures, AP of 964 IU/L, bilirubin of 1.3 mg/dL on 10 mg/d of PSE, 100 mg/d of CSA; sustained PR of liver cGVHD post-EP; CSA and PSE tapered to zero	Onset of skin ulcers on legs at 5 years post-BMT, thus PSE resumed
2105	Acute grade IV skin with bullous lesions of entire body on day 58 while patient on 4 mg/kg/d of PSE; no response to ATG; developed cGVHD of skin, mouth, and liver when EP stopped on day 126	Complete healing of 95% of skin surface, permitting taper of PSE to 3 mg/kg/d by day 128 Restarted EP on day 140 with stabilization of cGVHD	Stable cGVHD of skin and mouth at 13 months post-BMT on PUVA, 2.5 mg/k/d of CSA, and 0.125 mg/kg/d of PSE

AP, alkaline phosphatase; BMT, bone marrow transplant; cGVHD, chronic graft-vs.-host disease; CR, complete remission; EP, extracorporeal photopheresis; PR, partial remission; ROM, range of motion.

cGVHD, the median number of EP procedures per patient was 20.5, with a range of 6–48; the total number of lymphocytes ($\times 10^9$) treated per procedure was a median of 1.9 (range of 0.4–4.2); and the total number of lymphocytes treated per course was 20.5 (range of 2.0–144). The time between the first and the last EP procedure was a median of 107.5 days (range of 29–539) in the 18 patients with cGVHD.

A therapeutic 8-MOP level of >50 ng/mL for more than half the EP treatments was achieved for 67% (n=12) of the cGVHD patients. The 8-MOP level was <50 ng/mL (i.e., never in the therapeutic range) for all EP procedures in 28% (n=5) of the cGVHD patients. Twelve patients had unmeasurable 8-MOP levels at some time during their EP therapy and two patients never achieved detectable levels of 8-MOP. Marked variability in 8-MOP levels were noted in individual patients over time and from patient to patient in this study (Table 3). There was no correlation between the inability to absorb 8-MOP and gut pathology in the cGVHD patients. For example, UPN 561, who never achieved a detectable 8-MOP level, had bronchiolitis obliterans and no known gut GVHD. In patients with acute GVHD, the failure to absorb 8-MOP correlated with the presence of gut GVHD.

The response rate with EP treatment was 3 of 6 for patients with de novo cGVHD and 3 of 12 for patients with cGVHD following acute GVHD. The pretreatment organ system involvement by GVHD in the six EP responders and the changes in the targeted GVHD parameters posttreatment are shown in Table 4. The six responders included two patients (UPN 565 and 444) with de novo skin cGVHD; one patient (UPN 465) with de novo cGVHD of skin and mouth

plus hepatic dysfunction; one patient (UPN 525) with acute progressing into chronic GVHD of the liver, who had previously failed to respond to thalidomide, partially responded to 3 months of PUVA, and had complete remission of liver cGVHD with EP; one patient (UPN 650) with cGVHD of the mouth and liver following acute GVHD; and one patient (UPN 2105) who had improvement of acute grade IV skin GVHD on EP and later was re-treated with EP for progressive cGVHD of skin, mouth, and liver. The highest response rate was 3 of 5 in the group of patients with skin cGVHD (includes patients with skin and with skin and mouth GVHD plus hepatic dysfunction). Two patients (UPN 537 and 693) improved in the cGVHD parameter for which EP was started, but then developed progressive cGVHD in another organ system after 22 and 30 EP procedures, respectively, at which time EP was stopped for treatment failure. Three patients with bronchiolitis obliterans who were treated with 15, 18, and 22 EP procedures had no response or had documented progression on EP.

Toxicities

Overall toxicity was minor with the most common side effect being complaint of GI upset. In one patient (UPN 465), GI upset was significant enough that EP was discontinued despite evidence of therapeutic benefit with the treatment. Four patients with cGVHD (UPN 537, 650, 565, and 604) had catheter-related sepsis while on EP therapy. One patient's (UPN 444) right atrial catheter (RAC) broke and the proximal part of the catheter had to be removed from the right atrium under fluoroscopy. Although these incidents seemed unrelated to the EP procedures, the patient would
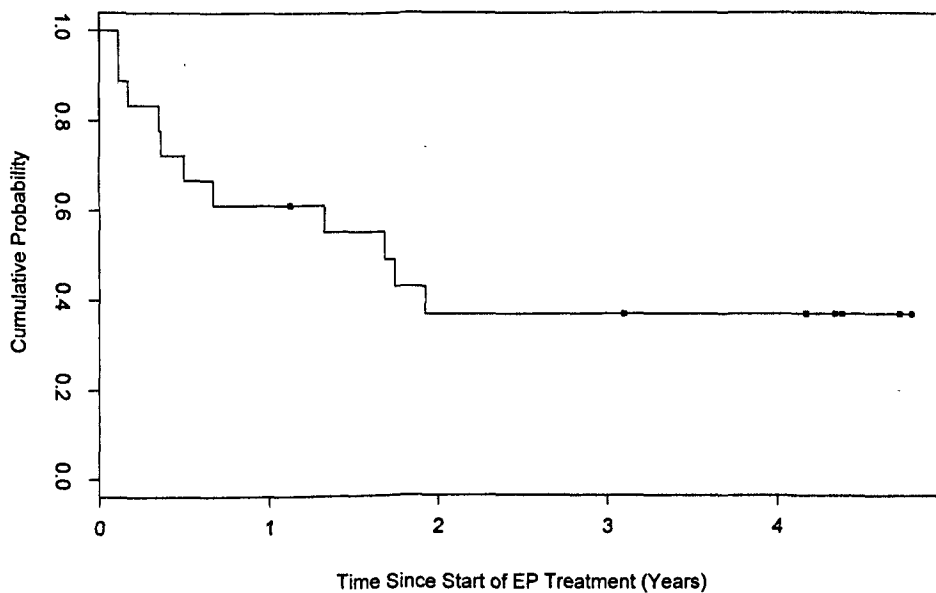
Figure 2. Cumulative probability of overall survival from the start of EP treatment: 16 patients with cGVHD
Marks (■) represent censored data points; ...., 95% confidence interval.

not have required a RAC in the absence of therapy with EP. Another patient (UPN 2021) experienced increased red blood cell and platelet transfusion requirements while on EP. One patient (UPN 727) with acute grade IV liver GVHD who began EP treatments on day 56 after BMT developed severe leukopenia and lymphopenia by day 105, which may have been due to concurrent treatment with ganciclovir. A second patient (UPN 2105) who began EP on day 58 after BMT developed leukopenia after five EP treatments, recovered his white blood cell count when EP was stopped, and did not develop leukopenia when rechallenged with EP on day 85.

With a denominator of 509—procedures performed for the 24 patients in this study (including 411 procedures in 18 patients with cGVHD and 98 procedures in six patients with acute GVHD)—the above described 9 adverse events constitute a complication rate of 1.77%.

Survival and causes of death

The six patients with acute grade IV liver GVHD died of progressive organ failure. Contributing causes of death in those patients included cytomegalovirus pneumonia in two patients and *Klebsiella* sepsis with acute respiratory distress syndrome in one patient.

Two of the six patients with *de novo* cGVHD are alive. Causes of death in the others were relapsed acute myelogenous leukemia and progressive bronchiolitis obliterans; progressive liver cGVHD with renal failure and fungal pneumonia; progressive multiorgan cGVHD and pneumonia; and unknown, but presumed due to complications of cGVHD in a patient with a history of noncompliance and drug abuse, who died at home after being lost to follow-up.

Five of the 12 patients with chronic following acute GVHD are alive. The causes of death in the 7 others were progressive liver failure (two patients), spontaneous bacterial peritonitis complicating liver failure, progressive bronchioli-

tis obliterans with cavitary lung lesions that were probably fungal, hemolytic-uremic syndrome and hepatorenal failure complicating severe progressive multiorgan system cGVHD, progressive bronchiolitis obliterans and *Aspergillus* pneumonia, and bilateral pneumonia.

The survival curve for cumulative probability of overall survival from the start of EP treatment in the 18 patients with cGVHD is shown in Figure 2. Five of the survivors were EP responders; two survivors (UPN 726 and 732) had GVHD progression on EP and then responded to salvage therapy with increased doses of PSE plus thalidomide and CSA.

Predictors of response

We conducted a statistical analysis of EP treatment variables to see if predictors for a response to EP could be identified in the 18 patients with cGVHD. There was a statistically significant difference between responders and nonresponders for the following treatment variables: 8-MOP level of >50 ng/mL for at least one treatment ($p = 0.04$, Fisher's exact test), maximum 8-MOP single dose ($p = 0.02$, Wilcoxon's rank-sum test), and 8-MOP level of >75 ng/mL for two doses ($p = 0.04$, Fisher's exact test). However, the other variables reflecting 8-MOP dose quantification (total dose across all treatments, average dose, 8-MOP level of 50 ng/mL for more than half the treatments, and 8-MOP level above 50 and 100 ng/mL for any two treatments) were not statistically different between the responders and the nonresponders. We therefore concluded that the three 8-MOP treatment variables that had apparent statistical significance in our analysis could not be interpreted as reflective of any meaningful correlation between response and 8-MOP dose or levels in this patient population. The difference between responders and nonresponders approached statistical significance for the number of procedures and the time between first and last

procedures; however, these variables were likely to be surrogate markers for response because nonresponders stopped EP at the time of documented progression and responders continued EP for a longer period of time. No correlation was seen between pre- or posttreatment CD4/CD8 ratio and response to EP.

DISCUSSION

UV irradiation can modify the functional behavior of immunocompetent cells by causing cell membrane alterations, modification of cell surface antigens, and interference with cell–cell interactions, antigen presentation, and cytokine release, as discussed in published reviews of this topic by Deeg [38,39]. UV-irradiated lymphocytes fail to function as stimulator cells in MLC [40]. T lymphocytes, which are known to be involved in initiating and propagating GVHD after BMT, are extremely sensitive to UV irradiation. In animal models, the development of GVHD is prevented by UV exposure of donor lymphocytes before transplantation [41]. Exposure to UV light increases circulating levels of interleukin-1 (IL-1) but decreases macrophage expression of membrane-bound IL-1, increases production of IL-4, and decreases production of IL-2 and interferon γ [39]. UVA irradiation also induces alteration of cell membrane antigenicity (i.e., immunogenicity). In murine models, UVA-induced tumors are not rejected when transplanted into recipients, suggesting that UVA-induced modification of cell membrane antigenicity can prevent sensitization and possibly induce a state of tolerance [42]. Numerous reports in the photobiology literature have demonstrated that UVB light (wavelength of 290–320 nm) and UVA light (wavelength of 320–400 nm), in combination with photosensitizers, can inhibit the expression of surface MHC class II antigens by the epidermal antigen presenting cells (i.e., Langerhans' cells) so that these cells lose their immunologic function in the antigenic stimulation of T lymphocytes [43].

Photochemotherapy, also known as PUVA therapy, is the use of 8-MOP photoactivated by exposure of the skin to UVA light. The photoactivated psoralen covalently binds to the DNA helix between pyrimidine bases, cross-linking DNA. The cross-linked DNA cannot replicate, so the proliferative capacity of cells is suppressed [44]. PUVA therapy suppresses the production of the pro-inflammatory cytokines IL-1, IL-6, IL-8, and tumor necrosis factor (TNF)-α by activated mononuclear cells [45]. PUVA therapy has been extensively used in the treatment of dermatologic diseases, including psoriasis, eczema, lichen planus, atopic dermatitis, and vitiligo. Successful PUVA therapy for cutaneous GVHD was first reported by Glazier *et al.* in 1984 and Hymes *et al.* in 1985 [46,47]. Subsequently, eight published reports confirmed the efficacy and safety of PUVA therapy in the treatment of cutaneous GVHD in larger series of patients [18–25].

EP is the combination of photochemotherapy with leukapheresis so that *ex vivo* exposure of peripheral blood mononuclear cells to UVA light occurs. Edelson *et al.* introduced the use of EP for the treatment of cutaneous T-cell lymphoma in 1987 [37]. They demonstrated that extracorporeal exposure of a lymphocyte-enriched blood fraction of 1–2 J of UVA light per cm^2 in the presence of a plasma 8-

MOP concentration of 50 ng/mL efficiently compromised the viability of the irradiated lymphocytes. They also noted the unpredictable absorption of 8-MOP after oral administration, attributed this to its low aqueous solubility, and emphasized the importance of monitoring 8-MOP plasma levels to guide dose adjustments [37]. Subsequently, EP was used successfully for treatment of several autoimmune diseases, including rheumatoid arthritis, psoriatic arthritis, and scleroderma [48–52]. EP was initially used in solid organ transplantation as salvage therapy for drug-refractory acute cellular rejection of cardiac allografts and subsequently has been used successfully to treat renal and lung allograft rejection [53–57]. Recently, important information has emerged regarding the potential use of EP for the prophylaxis of acute rejection in heart transplant recipients [58,59]. Subsequent to the initial report of successful EP treatment in 2 of 3 patients with cGVHD in 1990 by Bolwell et al. [26], several investigators reported successful EP treatment of refractory GVHD in isolated cases or small series of patients, as outlined in Table 5 [60–66]. Sniecinski has recently reviewed developments in clinical applications and future prospects for EP therapy [67].

This summary of our results of the EP treatment of 24 patients with refractory acute and chronic GVHD represents the largest single institution experience with EP therapy of GVHD. Although this study of EP involved a group of refractory patients with advanced GVHD, it demonstrated some efficacy for EP in the treatment of drug-resistant cGVHD. The high response rate in patients with skin cGVHD was not unexpected because previous reports of PUVA therapy had documented the efficacy of photochemotherapy in the treatment of cutaneous cGVHD. One of the EP responders (UPN 565), however, had received 2 months of PUVA therapy before treatment with EP and, while undergoing PUVA therapy, had documented progression of GVHD with increased skin blistering, ulceration, and thickening. His improvement with EP suggests a role for EP in patients with cutaneous cGVHD who either fail to respond to or cannot tolerate PUVA therapy. The complete resolution of liver cGVHD with EP in a patient (UPN 525) who had previously received PUVA therapy with only stable to slightly improved liver cGVHD also suggests that EP may be used as salvage therapy in patients who do not respond to PUVA therapy. There were three patients with hepatic dysfunction due to cGVHD in our study population (UPN 465, 525, and 650) who had improvement in their liver function test abnormalities with EP, as shown in Table 4. Their response to EP suggests that it may have a role in the therapy of patients with cGVHD who have limited hepatic involvement. Our experience also confirms that the highest response rate for EP occurs in patients with skin cGVHD, as reported in other trials listed in Table 5. Based on our experience, EP does not have a role in the treatment of patients with bronchiolitis obliterans.

Because the clinicopathology of cGVHD resembles an amalgam of autoimmune diseases, it is not surprising that EP therapy, which has documented efficacy in the treatment of various connective tissue disorders, is also efficacious in the treatment of cGVHD. The failure of EP to alter the course of grade IV drug-refractory acute liver GVHD in our study suggests that the amplification of pro-inflammato-

Table 5. *EP in treatment of GVHD: other trials*

Investigator	Entry criteria	Treatment protocol	Adjunct immunosuppression	Response rate
Bolwell et al. (1990) [26]	Refractory chronic skin and liver GVHD	2 proc q. 4 weeks for 6 months	CSA + PSE	2/3 (66%)
Bloom et al. (1991) [60]	Refractory chronic skin, mouth, gastrointestinal, and ocular GVHD	2 proc q. 2 weeks for 3 months	CSA, PSE, Imuran	5/5 (100%) skin 0/5 (0%) other organs
Owsianowski et al. (1994) [61]	Refractory chronic skin GVHD	2 proc q. 4 weeks for 3 months	CSA + PSE	1/1 (100%)
Rossetti et al. (1995) [62]	Refractory chronic skin GVHD	2 proc q. 4 weeks	CSA	1/1 (100%)
Schooneman et al. (1995) [63]	Chronic skin and liver GVHD	2 proc q. 2 weeks or 9–14 months	NA	3/3 (100%)
Volc-Platzer et al. (1996) [64]	Refractory chronic skin GVHD	2 proc q. 2 weeks or 3 months and q. 4 weeks for 3 months	CSA + PSE	3/5 (60%)
Crovetti et al. (1996) [65]	Refractory chronic skin GVHD	2 proc q. 2 weeks for 3 months then tapering for 9 months	CSA + PSE	1/1 (100%)
Dall'Amico et al. (1997) [66]	Refractory chronic skin, mouth, liver, and lung GVHD	2 proc q. 3 weeks	CSA + PSE	4/4 (100%)

NA, not available; Proc, procedures.

ry cytokines and tissue injury, which occurs in late stages of acute GVHD, is too advanced to be affected by the immunomodulatory effects of photochemotherapy. Photochemotherapy may have a role in the early therapy of acute GVHD, in combination with standard steroid treatment, but this has yet to be clinically tested. Because several investigators have demonstrated the efficacy of UV irradiation in preventing transplant-induced alloreactivity in animal models (as discussed above), further exploration of the role of photochemotherapy in the prophylaxis of acute GVHD after human BMT may also be warranted.

We were unable to correlate response to EP with 8-MOP levels, number of lymphocytes treated, or patterns of pre- and posttreatment CD4/CD8 ratio. Perhaps more detailed immunological studies of lymphocyte and macrophage function and cytokine production would have allowed us to identify patient characteristics that correlated with response to EP.

We conclude that EP may be useful in the treatment of drug-resistant cGVHD with minor overall toxicity. We hypothesize that the response rate to EP may be improved by the use of Uvadex, an injectable psoralen that is added to the photoactivation bag *ex vivo*, which allows for consistent achievement of therapeutic 8-MOP levels in the buffy coat pheresis product, thereby eliminating the wide variability of 8-MOP levels that is seen with an oral psoralen formulation. A study of EP with Uvadex is already in progress at COH to test this hypothesis. We also suspect that the response rate to EP may be better if it is used earlier in the course of GVHD. An evaluation of the efficacy of EP therapy in cGVHD by a prospective randomized study conducted early in the course of the disease, either in combination with standard CSA and PSE therapy or as first salvage therapy after failure to respond to CSA and PSE, is needed to determine the place of EP in the armamentarium of therapies for cGVHD. It would be interesting if future clinical studies included detailed assays of immunologic function pre- and post-therapy in an effort to elucidate the biologic mechanisms by which EP exerts its immunomodulatory effect and to correlate patient characteristics with response.

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