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REVIEW

Extracorporeal photopheresis (photochemotherapy) in the treatment of acute and chronic graft versus host disease: immunological mechanisms and the results from clinical studies

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Abstract Extracorporeal photopheresis (ECP) is an immunomodulatory alternative for treatment of graft versus host disease (GVHD). The blood is then separated into its various components through apheresis; buffy coat cells are thereafter treated with 8-methoxypsoralen before exposure to ultraviolet light and finally reinfused into the patient. There is a general agreement that this treatment has an anti-GVHD effect, but the mechanisms of action behind this effect are only partly understood. However, altered maturation of dendritic cells (DC) and thereby indirect modulation of T-cell reactivity seems to be one important mechanism together with DC-presentation of antigens derived from apoptotic donor T cells and induction of regulatory T cells. The treatment has been best studied in patients with chronic GVHD (both pediatric and adult patients), but most studies are not randomized and it is difficult to know whether the treatment is more effective than the alternatives. The clinical studies of ECP in adults with acute GVHD are few and not randomized; it is not possible to judge whether this treatment should be a preferred second- or third-line treatment. There is no evidence for increased risk of leukemia relapse or suppression of specific graft versus leukemia reactivity by this treatment, so specific antileukemic immunotherapy may still be possible. Thus, even though the treatment seems effective in patients with GVHD, further clinical (especially randomized) as well as biological studies with careful standardization of the treatment are needed before it is possible to conclude how ECP should be used in acute and chronic GVHD.

Keywords Allogeneic stem cell transplantation · Extracorporeal photopheresis · Photochemotherapy · Graft versus host disease

Abbreviations

ATG	Antithymocyte globulin
CSA	Ciclosporin A
DC	Dendritic cell
ECP	Extracorporeal photopheresis
GILZ	Glucocorticoid-induced leucine zipper
GVHD	Graft versus host disease
GVL	Graft versus leukemia
Hb	Hemoglobin
IFN	Interferon
IL	Interleukin
MFM	Mycofenolate mofetil
8-MOP	8-methoxypsoralen
mTOR	Molecular target of rapamycin
MTX	Methotrexate
MUD	Matched unrelated donor
NK	Natural killer
RIC	Reduced intensity conditioning

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SCT	Stem cell transplantation
Th	T helper
TNF	Tumor necrosis factor
UV-A	Ultraviolet light A

Introduction

Extracorporeal photopheresis (ECP) is used in the treatment of graft versus host disease (GVHD) after allogeneic stem cell transplantation [1, 2]. It has also been tried in other diseases, especially autoimmune disorders [3, 4] and cutaneous T-cell lymphoma [5] and to treat rejection in organ transplantations [6]. According to previously published reviews, the most important immunomodulatory effects of this treatment seem to be altered T-cell functions and modulation of dendritic cell (DC) maturation [2, 7–9]. In the present article, we give a detailed review and discuss the immunological effects and the clinical experience with this treatment.

The general principles of extracorporal photopheresis

As described in detail in previous reviews [10, 11], ECP is an apheresis procedure in which the isolated leukocytes (i.e., buffy coat cells) are treated with the photoactivating drug 8-methoxypsoralen (8-MOP) before being exposed to ultraviolet light and reinfused into the patient [2, 5]. All other remaining blood components are reinfused without treatment [12]. ECP as GVHD therapy has usually been combined with other types of immunomodulatory strategies at the start of ECP, e.g., steroids, calcineurin inhibitors, mycophenolate mophetil or mTOR inhibitors [13–15]. Most available studies are not randomized, and the effect of ECP has usually been assessed as the ability to achieve clinical improvement and/or reduction of additional immunosuppressive therapy [16].

Effects on T cells of extracorporeal photopheresis

Direct effects of ECP on circulating T cells—apoptosis induction in alloreactive T cells

The effects of ECP on T cells have been studied in murine models of contact sensitivity [17]. Briefly, ECP using 8-methoxypsoralen then caused T-cell apoptosis, and reinfusion of these apoptotic cells generated antigen-specific tolerance; this was different from many other structurally related photosensitizers that failed to induce tolerance even though they had proapoptotic effects. This tolerance was lost by depletion of $CD11c^+$ cells, it was cell-mediated and antigen-specific, and it was also lost after depletion of $CD4^+$ and $CD25^+$ T cells [18]. Finally, experimental data in human cells suggest that ECP induces apoptosis of most treated T cells within 48 h, possibly through upregulated Fas and increased Fas-initiated proapoptotic signaling [19]. Thus, ECP seems to be associated with induction of antigen-specific tolerance possibly because activated T cells are most susceptible to its proapoptotic effect. Similar observations have been made in experimental acute GVHD; transfer of ECP-treated T cells reverses established GVHD by increasing donor Treg levels and inhibiting donor effector lymphocytes [20]. Taken together, the data suggest that ECP-induced T-cell tolerance depends on T-cell apoptosis, the presence of $CD11c^+$ monocytes and Treg cell induction.

The study by Hannani et al. [21] further suggests that alloactivated T cells derived from GVHD patients are particularly susceptible to the proapoptotic effects of ECP. Peripheral blood T cells derived from GVHD patients and expressing activation markers (e.g., HLA-DR) probably include in vivo activated alloreactive cells [22, 23] that seem necessary for ECP efficiency [24–26]. Hannani et al. [21] therefore compared the proapoptotic effect for in vivo activated (i.e., probably alloreactive) HLA-DR^+ T cells with the corresponding effect on HLA-DR^- resting T cells derived from chronic GVHD patients after ECP. This was a small study, but their results suggest that ECP-treated activated T cells undergo apoptosis faster than the resting cells because higher percentages of activated T cells were apoptotic after 18 h of post-ECP in vitro incubation. The cells could not be rescued by T-cell survival factors (IL1, IL7, IL15). Additional experimental studies also showed that in vitro activated T cells included a higher fraction of apoptotic cells after ECP compared with resting cells. This nonrandom proapoptotic ECP effect may contribute to the induction of specific tolerance by ECP. Furthermore, it seems unlikely that T-cell depletion alone can explain the effect of ECP because only 5–10 % of circulating mononuclear cells are exposed to UVA irradiation during each ECP procedure [9]. Additional indirect effects of ECP-exposed cells on other immunocompetent cells are probably operative and induction of regulatory T cells seems to be such an effect.

This induction of antigen-specific tolerance may also explain the clinical experience that ECP is not associated with increased frequencies of severe infections due to general immunosuppression or increased risk of posttransplant relapse due to reduced antileukemic immune reactivity. This last observation may have different explanations: (1) the maximal effect of antileukemic immune reactivity may have been reached prior to the period of treatment-requiring GVHD; or (2) the specific graft versus leukemia (GVL) effect may still be maintained even though the nonspecific GVH reactivity is controlled by the treatment.

Effects of ECP on the levels of circulating lymphocyte subsets

A recent study compared the absolute numbers of circulating $CD4^+$, $CD8^+$ (the two major T-cell subsets), $CD20^+$ (B cells) and $CD56^+$ (mainly NK and NK-T cells) lymphocytes before and during ECP in 39 patients with steroid-refractory chronic GVHD [27]. The patients received ECP on two consecutive days weekly for the first month, two consecutive procedures with 2 weeks intervals for the next 2 months and thereafter the treatment was individualized. The cell levels were not significantly altered after 1 month, whereas after 3 and 6 months the responders to ECP had increased numbers of circulating $CD4^+$ as well as $CD8^+$ T cells compared with nonresponders. Another study described increased pretreatment levels of both $CD4^+$ and $CD8^+$ T cells in the responders; these increased CD8 levels were then maintained, whereas CD4 levels decreased during treatment [28]. The levels of $CD20^+$ and $CD56^+$ cells did not differ in any of the studies. In addition, an increased number of Treg cells was detected after 3 months (see below), but the percentages of $CD4^+$ and $CD8^+$ naive, $CD4^+$ and $CD8^+$ central memory and $CD4^+$ and $CD8^+$ effector memory T cells among total $CD4^+/CD8^+$ T cells were not altered [27]. Thus, treatment for at least 3–6 months was often necessary before an effect on circulating T cells could be detected.

Effects on regulatory T cells

Studies on Treg levels in patients with chronic GVHD *before ECP* have shown conflicting results; both normal [29] and decreased [30] Treg levels have been reported. A possible explanation is that patients differ with regard to immunosuppressive treatment as well as GVHD severity and organ affection. Furthermore, the effect of ECP on circulating Treg levels also varies; most studies describe increased levels [30–33] after ECP, but unaltered/normal levels have also been described [29]. Increased Treg levels have been associated with response to treatment [31] and can then be detected early, i.e., after only 3 weeks of treatment [33].

Effects of ECP on dendritic cells

Effects of ECP on various dendritic cell subsets—phagocytosis of apoptotic T cells as a possible mechanism for specific tolerance induction

Dendritic cells are specialized antigen-presenting cells that are important for initiation of immune responses, including alloreactivity [34]. ECP induces differentiation of monocytes toward a DC phenotype; this can be detected within 24 h and up to 30 % of monocytes then show cytoplasmic expression of the DC marker CD83 [35]. These characteristics are seen both for healthy individuals and patients with chronic GVHD. The monocyte-derived DCs remain viable and can induce antigen-specific cytotoxic T-cell responses at least in experimental in vitro models. The cells also show a distinct gene expression signature consistent with DC differentiation.

The $CD1c^+$ myeloid DCs showed a high viability and a molecular pattern consistent with immaturity in ECP products [36], including (1) low levels of costimulatory CD40, CD80 and CD86; (2) negativity for the maturity-markers CD83; (3) low levels of HLA-class I and II; and (4) high expression of CD11c. This seemed to be a stable phenotype even during in vitro culture, but further maturation could be induced by lipopolysaccharide with increased levels of CD40, CD80, CD83 and CD86. The LPS-induced maturation also increased CCR7-mediated migration and the capacity to stimulate proliferation of alloreactive $CD4^+$ cells. These ECP-treated myeloid DCs showed uptake of exogenous antigens, were able to capture apoptotic lymphocytes, and secreted detectable IL10. Another study described decreased proinflammatory cytokine release after ECP (TNFα, IL1β, IL12) by another myeloid DC subset referred to as 6-sulfo LacNAc DCs [37]. These partly maturated DCs with relatively low levels of costimulatory molecules may correspond to or be included among the tolerogenic DCs described below.

The myeloid DC1 subset shows high IL12 release and stimulates Th1 responses, whereas the lymphoid DC2 subset releases less IL12 and induces Th2 responses [38]. The majority of circulating DCs in patients with chronic GVHD are DC1 cells, but ECP causes a shift from proinflammatory DC1 to DC2 cells for most patients [38]. Finally, responders to ECP show higher pretherapy levels of circulating myeloid and plasmocytoid DCs than nonresponders [28].

Taken together, these results suggest that ECP affects both the number and function of various DC subsets. These effects have usually been associated with decreased immune responsiveness, but even potential proinflammatory effects have been suggested in certain in vitro studies. These apparent discrepancies may be explained by the observations by Durazzo et al. and Edelson [39, 40]; they suggest that the exposure to ECP is not uniform (i.e., exposure conforming to a Gaussian distribution), and their hypothesis is that those cells receiving minimal exposure are capable to fully mature into immunostimulatory DCs. However, induction of tolerogenic DCs will dominate and the phagocytosis of apoptotic alloreactive T cells may then be essential for

the induction of allospecific tolerance and be responsible for the final immunomodulatory or immunosuppressive effects of the treatment. These observations also emphasize that careful standardization of experimental in vitro models is essential to ensure their relevance to clinical use of ECP.

Effects of ECP on dendritic cells—altered levels of circulating DCs and induction of a tolerogenic DC phenotype

The effects of ECP on the monocyte-to-dendritic cell maturation [39, 40] were recently investigated by using a miniature ECP chamber as a highly standardized experimental model. Monocyte interactions with the bioactive membranes in this chamber then induced phenotypic and functional properties of specialized antigen-presenting cells together with a distinct gene expression signature [39, 40]. The flow chamber became coated with monomeric plasma fibrinogen, resting platelets adhered to this layer and became activated, and passaged monocytes then transiently adhered to activated platelets [40]. Integrin-mediated binding was involved in this process [41], and the cells also expressed the glucocorticoid-induced leucine zipper (GILZ) gene that seems to be a marker of tolerogenic dendritic cells [42]. The monocyte maturation seemed to start after internalization of antigen (e.g., apoptotic T cells) and seemed to depend on a transient rather than a prolonged interaction between flowing monocytes and platelets [39]. However, the exposure of monocytes to UV-A was probably not uniform and some of the monocytes may even acquire an immunostimulatory phenotype [40]. These observations are consistent with the hypothesis that the basis for induction of antigen-specific immunological tolerance by ECP is predominant induction of monocyte-derived tolerogenic dendritic cells that internalize apoptotic alloreactive T cells and thereby initiate allospecific tolerance.

Additional ECP effects on the interactions between T cells and dendritic cells

Previous studies have also investigated the effect of ECP on Th1 and Th2 T-cell responses. Firstly, Gerner et al. [37] described that ECP inhibited the ability of the myeloid slan-DC subset to induce (1) proliferative $CD4^+$ and $CD8^+$ T-cell responses; and (2) Th1 polarization of naive $CD4^+$ T cells. Secondly, Gorgun et al. [38] described that ECP induced a shift from circulating DC1 (Th1 inducing) to DC2 (Th2 inducing) cells associated with a posttherapy 1-threefold *decrease* in circulating Th1 cells (interferon-γ and IL2 releasing) as well as a 1-fourfold *increase* of Th2 cells (IL4 and IL10 releasing).

Additional immunomodulatory effects of ECP

Effects of ECP on monocyte function and viability

An early study described no signs of monocyte apoptosis after ECP [43], whereas a more recent study described a proapoptotic effect of photochemotherapy on monocytes [21]. These last authors investigated purified monocytes treated with PUVA plus UV-A, and they described (1) altered in vitro migratory capacity; (2) upregulated expression of costimulatory molecules and release of proinflammatory cytokines with maintained ability to activate alloreactive T cells; but (3) increased monocyte apoptosis after 6 days of in vitro culture. However, further studies are needed to clarify whether these experimental observations are relevant for the clinical effects of ECP.

B lymphocytes in GVHD

B lymphocytes can function as antigen-presenting and immunoregulatory cells, and their possible roles in the development of both acute and chronic GVHD have been discussed in recent reviews [44, 45]. B-cell depletion may also be an effective treatment of GVHD [44]. However, no studies have investigated whether ECP has any immunomodulatory effects mediated by B lymphocytes.

Are effects of neutrophils important?

Only one study has investigated the effect of ECP on granulocytes, and a reduction in the release of oxygen-free radicals was then reported [46]. It is not known whether this is important for the clinical effects of ECP.

Effects of photopheresis on plasma molecules

Human plasma contains UVA-absorbing and possibly also 8-MOP binding molecules [47], but the effects of ECP on plasma molecules have been addressed only in one study [48] describing an ECP-induced decrease in folic acid caused by UV-A-induced degradation but with no effect on vitamin B12 and homocystein. Degradation of folic acid leads to photolytic products that may act as photosensitizers that induce DNA photo-oxidation and thereby cause immunomodulation.

Several immunoregulatory soluble mediators are altered by ECP. Firstly, free L-arginine can enhance T-cell responses and L-arginine depletion due to release of arginase will thereby cause immunosuppression; ECP induces arginase expression in leukocytes and this may contribute to immunosuppression [49]. Secondly, experimental studies suggest that ECP reduces the release of proinflammatory cytokines (e.g., IFNγ, TNFα), whereas the levels of

anti-inflammatory cytokines (especially IL10) may increase [49–51]. These altered cytokine levels may be caused by effects of ECP on cytokine release by various immunocompetent cells [34, 52–55], but it is not known whether ECP has direct effects on these immunoregulatory molecules similar to the effects on folic acid.

Can immunotherapy be combined with photochemotherapy?

Posttransplant antileukemic immune reactivity is mediated by nonspecific GVH and specific GVL effects [1, 2, 51, 53, 54]. Leukemia-directed immunotherapy may therefore be considered to maintain specific GVL activity during generally immunosuppressive anti-GVHD treatment as described below.

Vaccination with cancer-specific or cancer-associated antigens (i.e., peptide vaccination) is considered both for myeloid and lymphoid malignancies [56]. An alternative strategy is vaccination with antigen-loaded dendritic cells [57]. The intention with both these approaches is to achieve effective antigen presentation and thereby induce cancer-specific immunity. However, one cannot exclude that ECP will alter the function of antigen-presenting DCs or induce apoptosis of activated cancer-specific T cells and thereby reduce GVL-reactivity or even induce tolerance similar to the anti-GVHD effect.

Antibody therapy directed against cancer-associated antigens is assumed to facilitate cancer cell clearance through antibody-dependent cytotoxicity, presumably mediated by NK cells, or complement activation [58]. It is not known whether ECP has any effects on NK cells, but we would expect the complement function not to be affected. An alternative approach is to use antibodies to block T-cell inhibitory signaling (e.g., targeting of PD-L1/2/3 or CTLA-4 [58]), but this strategy may also increase GVHD-reactivity and counteract the ECP effects. Finally, NK cell targeting through antibody blocking of inhibitory KIR receptors or transfer of allogeneic KIR-mismatched NK cells may be useful, but further studies of ECP effects on NK cells are needed [58, 59].

No previous clinical studies have investigated whether ECP and anticancer immunotherapy can be combined. Only future clinical studies can elucidate whether any of the currently used immunotherapeutic strategies can be combined with ECP. Finally, targeting of monocytes/eosinophils/neutrophils has also been suggested as a strategy for anticancer treatment [59], but the possible role of these cells in anti-cancer immunity has not been characterized in detail and it is not known whether these cells are affected by ECP.

Clinical studies of extracorporeal photopheresis

Acute GVHD

The results from important clinical studies of ECP in acute GVHD are summarized in Table 1 [60–68]. Greinix et al. have published three reports on photopheresis for the treatment of acute GVHD; the initial report included 6 patients [69], the second reported observations for a total of 21 patients including the first 6 patients [70]; and the last report included 38 additional patients making a total of 59 patients [66]. Only their final report is included in Table 1. Similarly, the 9 patients reported by Salvaneschi et al. in 2001 [61] were also included in the final study published by Perotti et al. in 2010 [60], but both these studies are described in Table 1 because more detailed information was given for the 9 initial patients. Finally, the review article by Dall'Amico and Messina [71] described 14 patients from the Padua center that were included in the final report by Messina et al. [62]; only the results from the final complete study are presented in Table 1. The following conclusions can then be made:

- The studies included 125 children and 94 adults with steroid-refractory acute GVHD after 4–7 days of treatment, i.e., the inclusion criteria differed between studies.
- Not all of the studies gave detailed information on the severity of GVHD; for those studies giving detailed information (1) a total of 105 patients had grade II, whereas 100 patients had grade III/IV; and (2) skin involvement was described for 204 patients, liver involvement for 90 and gastrointestinal involvement for 94 patients.
- Responses were more common for patients with grade II than with grade III/IV GVHD; complete responses were seen in up to 100 % of patients with grade II disease, whereas for patients with grade III/IV disease the studies by Berger et al. [68] and Perfetti et al. [65] reported complete remission for 38 and 42 % of patients, respectively. However, one should emphasize that the estimates for grade III/IV patients are based on observations in relatively small groups of patients.
- Responses to ECP were most common for patients having skin involvement compared with gut or liver disease. Complete responses have been described for up to 70–90 % of patients with skin involvement, but one study also described responses in 71 % of patients with gastrointestinal GVHD.
- Steroid reduction or discontinuation is possible for a subset of patients; in one study median time to steroid discontinuation was 55 days (range 17–284 days) [66].

Table 1 Important and representative clinical studies of extracorporal photopheresis in the treatment of acute GVHD (aGVHD)

Study	Patients	Photopheresis	Outcome
Perotti [60] Retrospective Single-center 50 consecutive children	Inclusion: Steroid resistance after 7 days Age: Median 9.9 years, all < 18 years GVHD prophylaxis: CSA for 32/50, MMF 2/50 and other regimens 16/50 Donors: Unrelated 32/50, related 12/50 and haploidentical 6/50 aGVHD *Grade* Grade II 31/50, grade III 14/50 and grade IV 5/50 *Involvement* Skin 47/50, liver 24/50 gut 11/50. The authors state that a minority of their patients had involvement of mucous membranes, skin, eyes and joints	Median interval from GVHD to first apheresis 9 days (range 6–20 days) Apheresis: Each apheresis processed 2 blood volumes by using a cell separator (Spectra, COBE, Lakewood, CO) Frequency and duration: All patients underwent at least 10 aphereses (median 18, range 12–24 procedures). Initially they received 2–3 procedures weekly until improvement, thereafter 2 procedures/week for two times, 2 procedures every other week for three times and finally 2 procedures/month before individualized gradual tapering	Responses: Overall response rate was 34/50 (68 %). Complete responses were seen in 16/50 (32 %). Partial responses were defined as at least 50 % response of organ involvement and were seen in 18/50 (36 %) Complete responses were observed in 83 % with skin involvement, 67 % with liver involvement and 73 % with gut involvement Response to ECP and decreased steroid dose at 30 days of treatment were associated with increased survival
Salvaneschi [61][a] Detailed description of 9 patients from Perotti [60]	Inclusion: Steroid resistance after 7 days of treatment. Age: Median 10.3 years, range 5.5–17.3 years GVHD prophylaxis: CSA for 8 patients, 5 of them also was receiving MTX. T-cell depletion alone for 1 patient Donors: 5 MUD, 4 family donors GVHD *Onset* Median day +14, range 9–19 *Grade* Grade III or IV 8/9 *Involvement* All with skin involvement, 5 patients with gastrointestinal and 3 with liver involvement	Median interval from GVHD to first apheresis 14 days (range 9–47 days) Apheresis: Each apheresis was processing of 2 blood volumes; maximal procedure time was 180 min and final 8-MOP concentration 200 ng/ml Frequency and duration: Initially 3 times weekly until improvement, thereafter 2 consecutive days with 2 weeks intervals for 3 months before individualized gradual tapering	Responses: 7/9 responders including 5 complete responders; 3 of them discontinued immunosuppression. Complete organ responses were seen both for skin (8/9), gut (3/5) and liver (1/3) Maximal response was reached after a median of 3 weeks Survival: 2/9 died from GVHD and 2/9 from leukemia relapse; 5 patients alive after 3.4–16.4 months of follow-up
Berger [68] Retrospective Single-center 15 children	Inclusion: Steroid-resistant aGVHD. Age: Median 10.7 years, range 5.8–17.5 years GVHD prophylaxis: All received CSA, 8 received additional MTX + ATG and two others ATG + steroids Donors: 5 siblings and 10 MUD; bone marrow 11/15, PUBS 2/15, umbilical cord stem cells 2/15 GVHD *Onset* Median day for first apheresis was day +38 (range +15 to +97 *Grade* Grade II 7/15, grade III/IV 8/15 *Involvement* Skin 14/15, liver 7/15, gut 10/15	Median interval from GVHD to first apheresis 25 days (range 13–55 days) Apheresis: <40 kg body weight continuous flow cell separator; >40 kg UVAR photopheresis (Therakos). Median number of aphereses 12 (range 4–21) Frequency and duration: Treated at two consecutive days and with weekly intervals for the first month, two week intervals for months 2–3 and monthly intervals for 3 additional months	Responses: Overall GVHD-free survival 62 %; grade II 100 % and grade III/IV 38 % complete responses For grade III/IV patients responses were 80 % for skin, 50 % for liver and 33 % for gut involvement Increased transplant-related mortality was associated with no response to ECP and several lines of therapy before ECP

Table 1 continued

Study	Patients	Photopheresis	Outcome
Messina [62] Retrospective Single-center 33 children	Inclusion: Steroid-resistant aGVHD Age: Median age 9.6 years (range 1.5–18.3 years) GVHD prophylaxis: Cyclosporine for related donors; for unrelated donors additional short-term methotrexate plus either ATG or steroids Donors: 29/33 bone marrow grafts; 5 HLA-identical siblings, 4 other family donors, 22 matched unrelated and 2 cord blood donors GVHD *Onset* Median time for start of ECT day +45 (range +13 to +98) *Grade* Overall grading showed grade II in 13, grade III in 13 and grade IV in 7 patients *Involvement* Skin 33/33 with grade IV in 8 and grade III in 15 patients; liver 15/33 with grade IV in 1 and grade III in 3 patients; gut 20/33 with grade IV in 5 and grade III in 2 patients	Median interval from GVHD to first apheresis 30 days (range 5–91 days) Apheresis: One center used the UVAR photopheresis instrument (Therakos, Exton, PA, USA) and three centers a Spectra Cobe Cell separator (Lakewood, Co, USA) Frequency and duration: ECP on two consecutive days weekly for 1 month, thereafter every second week for 2 months and finally at monthly intervals for at least 3 further months. Median duration of treatment was 74 days (range 8–467 days) with a median number of 8 cycles (range 2–20 cycles)	Overall responses: By the end of treatment 18/33 had GVHD grade 0/I. Among the 18 responders 8 patients had grade III and 2 patients grade IV at start of treatment Immunosuppression was discontinued in 8 patients 8/33 were non responders Complete organ responses: Skin 25/33, liver 9/15, gut 15/20 Survival: 5-years overall survival was 69 % for responders and 12 % for nonresponders; 15 out of 19 patients then had no signs of GVHD
Kanold [63] Prospective 12 children	Inclusion: Steroid-resistant aGVHD for 7 days Age: Median age 13.5 years (range 4–18 years) GVHD *Onset* median day +15 (range +7 to +31) *Grade* At start of apheresis Grade II 3/12, grade III 6/12, grade IV 3/12 *Involvement* Skin 10/12, liver 9/12, gut 6/12	Median interval from GVHD to ECP 19 days (range 6–50 days) Apheresis: Leukapheresis using cell separators (Spectra Cobe) Median number of ECP sessions 24.5 (range 10–56)	Complete responses: Skin 9/10, liver 5/9, gut 5/6 Complete responses in 7/12 and partial responses in 3/12 Reduction in steroids after 10 sessions to median 37 %; steroids were at this time discontinued for 2 patients but could be stopped during the ECP program for 6/12 Survival: Median follow-up 8.5 months (range 1–40 months); 5 alive and well, 3 dead from GVHD

Table 1 continued

Study	Patients	Photopheresis	Outcome
Calore [64] Comparative study: 31 consecutive children with acute GVHD, 15 receiving ECP Single center	Inclusion: Steroid-resistant aGVHD in 15 children compared with 16 children having a good response to steroids Indications for ECP were steroid resistance for 4–8 days 7/15, steroid dependency 4/15 and viral reactivation on steroids 4/15 Age: Median age 9.6 years (range (1.4–18.1) for the ECP group GVHD prophylaxis: CSA +MTX SCT: MUD, myeloablative conditioning GVHD *Grade* At start of apheresis Grade II 7/15, grade III 4/15, grade IV 4/15 *Involvement* Skin 13/15, liver 1/15, gut 14/15. One organ involvement 3/15, two organs 11/15 and three organs 1/15	Apheresis: Leukapheresis using cell separators (Spectra Cobe) Frequency and duration: ECP on 2 consecutive days at 1 week intervals for the first month, then every 2nd week for 2 additional months and finally monthly for at least 3 months. Median time on treatment was 171 days (range 35–311 days)	Response: 73 % of patients achieved complete remissions and 27 % partial remissions Complete organ responses: Skin 92 %, liver 1/1, gut 71 % Discontinued steroids: 10/15 by the end of ECP 2-years progression-free survival: 87 % with a confidence interval of 70–100 %.
Perfetti [65] Retrospective Two centers 23 adults	Inclusion: Steroid-resistant aGVHD Age: Median age 41 years, range 18–66 years GVHD prophylaxis: CSA alone 1/23, CSA + MTX 12/23, CSA + MTX + ATG 10/23 Donors: 12 related, 11 unrelated GVHD *Onset* median day +16 (range +7 to +43) *Grade* At start of apheresis Grade II 10/23, grade III 7/23, grade IV 6/23 *Involvement* Skin 23/23, liver 11/23, gut 20/23 *Previous treatment* steroids 23/23 with steroids alone 1/23, CSA 20/23 alone, CSA + ATG 2/23, CSA + Mtx 1/23	Median interval from start of GVHD to ECP 60 days (range 14–119 days) Apheresis: Leukapheresis and photochemotherapy were performed by an open system Frequency and duration: One cycle (i.e., two apheresis) weekly for the first month, a cycle every 2 weeks for 2 months, one cycle monthly until stabilization or complete resolution. Median duration of ECP was 7 months (range 1–33 months); median number of cycles 10	Complete responses: 12/23 (52 %) Average GVHD reduced from grade 2.8 to 1.4; average methylprednisolone reduced from 2.17 to 0.2 mg/kg/day Complete responses and aGVHD grade: Grade II 70 %, grade III 42 %, grade IV 0 % Complete responses and organ: Skin 66 %, liver 27 %, gut 40 % The effect seemed to be best if treated within 35 days after first symptoms
Greinix [66][b] Prospective Single-center 59 adults	Inclusion: Steroid-resistant aGVHD after 4 days of treatment (37 patients), steroid dependent with flare-up during tapering (22 patients) Age: Median age 40 years, range 21–60 years GVHD *Onset:* Median day +17, range 8–42 days *Grade:* 36/59 grade II, 23/59 grade III or IV *Involvement:* Skin alone 31/59; skin + liver 13/59, skin + liver + gut 8/59, others 7/59	Median interval with steroids before ECP 17 days (range 4–49) Apheresis: UVAR photopheresis system (Therakos, West Chester, PA, USA) Frequency and duration: Initial 21 patients: Initially treatment of 2 consecutive days with 1–2 weeks intervals until improvement, thereafter every 2–4 week until maximal response, individualized tapering over 5–25 months Last 38 patients: 2 consecutive days weekly and stopped after achieving maximal response	Complete resolution: Skin 82 %, liver 61 %, gut 62 %. The more intensive protocol used for the last 38 patients was associated with higher complete resolution rates for patients with gut involvement and grade IV disease Probability of complete resolution was associated with: Low grade GVHD at start of ECP; grade II 86 % and IV 30 %; Late onset of steroids after SCT Median time until best response was 1.3 months (range 0.5-6 months) Median time until steroid discontinuation was 55 days (range 17–284 days) Survival: Complete responses 59 % Nonresponders 11 %

Table 1 continued

Study	Patients	Photopheresis	Outcome
Garban [67] Retrospective Single-center 12 adults	Inclusion: Steroid-resistant GVHD after 5 days of treatment Age: Median age 40 years (range 23–63 years) GVHD prophylaxis: CSA + MTX 9/12; CSA + MFM 2/12; CSA alone 1/12 Donors: Sibling 8/12, MUD 4/12 GVHD *Grade:* All patients had aGVHD grade II–IV *Involvement:* Skin 12/12; gut 5/12, liver 2/12	Apheresis: Spectra cell separator (COBE) Frequency and duration: Six cycles during the first 3 weeks, (1) in case of partial response one course weekly until stable response; (2) ECP was stopped if complete or no response after the 6 initial cycles	Responses after 6 initial courses: Progression and death 3/12. Nine patients did not require additional immunosuppression. Steroids stopped after 1–2 months for all responders Complete responses: Skin 8/12, liver 0/2, gut 2/5

ATG antithymocyte globulin, *CsA* ciclosporin A, *MFM* mycophenolate mofetil, *8-MOP* 8-methoxypsoralen, *MTX* methotrexate, *MUD* matched unrelated donor, *SCT* stem cell transplantation

[a] The patients from the study of Salvaneschi et al. [61] were also included in the study by Perotti et al. [60]

[b] The first 6 patients in this study were reported by Greinix HT et al. [69] and the first 21 including the 6 patients from the initial study were reported by Greinix HT et al. [70]

One study has investigated the prophylactic use of ECP [72]; patients were then treated prior to standard myeloablative conditioning therapy, and the results for 31 patients were compared with matched historical controls. Multivariate analysis showed a decreased risk of grade II–IV acute GVHD ($p = 0.04$) and possibly increased 1-year survival (83 vs. 67 %, $p = 0.07$) for patients receiving photopheresis, but both differences reached only borderline significance.

In our opinion, ECP should therefore be regarded as evidence-based second-line treatment in acute GVHD, and the clinical and scientific basis for the use of this treatment is relatively strong compared with the alternative therapeutic strategies (see Supplementary Information).

Chronic GVHD

The effect of ECP in the treatment of chronic GVHD has been studied in one randomized and several nonrandomized studies (Table 2) [73–83]. Taken together, the available data suggest that ECP then is an effective and safe treatment:

- A total of 448 patients were included in these nine studies, almost all of them being adults. Total response rates (partial + complete) up to 60–80 % have been described, but complete responses are uncommon. The treatment can be effective both in moderate, severe and progressive disease.
- Eight of the reports presented detailed information about organ involvement; these studies included 376 patients with involvement of the skin, oral mucosa in 186, eyes in 138, gut in 33, lungs in 43 and liver in 125.
- Complete responses seem to be uncommon among patients with skin disease and have been reported for 10–20 % of these patients; but total response rates up to 50–80 % have been described in several studies with improvement of both lichenoid and sclerodermic forms. However, in one study, later progression was described after a median of 16 days (range 16–188 days) for approximately 1/3 of the patients, but for the other patients responses were maintained for a median of 18 months (range 0.4–65 months). Thus, long-lasting responses may be anticipated.
- Responses are also common for mouth disease; the number of patients in each study is smaller than for the skin disease but response rates as high as 60–80 % have been reported.
- ECP can also improve chronic GVHD involving the eyes, liver, lungs and gut, but it should be emphasized that few patients with such extensive involvement have been reported.
- Only one randomized study has been reported [73] including 95 patients. When using a combined criteria of steroid

Table 2 Important and representative clinical studies of extracorporal photopheresis in the treatment of chronic GVHD (cGVHD) in adult allogeneic stem cell recipients; a summary of 9 clinical studies including at least 25 patients

Study	Patients	Photopheresis	Outcome
Flowers [73] Prospective, randomized, multicenter 95 adults	Inclusion: Corticosteroid dependent (53/95), resistant (12/95) or intolerant (30/95) patients with cutaneous cGVHD GVHD treatment before entry: Median duration of steroids 53 weeks (range 2.7–426), median prednisone dose 18 mg (range 3.3–64 mg). Concurrent MMF 52/95, CsA 52/95, FK-506 17/95 Age: Median 42 years (range 13–67 years) Donors: Related 65/95, bone marrow grafts 32/95 GVHD *Onset:* Median from cGVHD to randomization 599 days (range 1–2,253 days) *Extension and type:* Progressive 53/95. *Involvement:* All had skin involvement, additional affected sites were eyes 55/95, oral 60/95, gut 11/95, liver 28/95, lungs 16/95 and joints 34/95	Apheresis: The study was performed with a Therakos apheresis system, 3 times ECP during the first week and thereafter then twice weekly on consecutive days until week 12; responding patients could continue with two ECP treatments every 4 weeks until week 24 Corticosteroid dose was stable during the first 6 weeks except for patients with unacceptable steroid toxicity requiring reduction, thereafter tapering was allowed Response evaluation: - Blinded assessment; - Additional unblinded assessment done by the clinical investigator	Overall response, cutaneous improvement, steroid reduction: At week 12 the proportion of patients with >50 % steroid reduction plus at least 25 % decrease from baseline total skin score was 8.3 % for ECP and 0 for the controls ($p = 0.04$). For ECP-treated patients there was a continued improvement with reduced steroid doses and improved skin score at the final evaluation after 24 weeks. The Quality of life improved significantly after ECP Cutaneous response: The changes in total skin score after 12 weeks did not differ significantly between ECP-treated patients and controls based on blind assessment After 12 weeks 40 % of ECP patients and 10 % of controls had a complete or partial skin responses assessed by the investigator (unblinded assessment) Extracutaneous manifestations evaluated after 12 weeks: Eye involvement, 30 % resolved after ECP versus 7 % of controls ($p = 0.04$); mouth 53 % versus 27 % ($p = 0.06$)
Greinix [82] Open-label cross-over study 29 adults	Inclusion: 29 patients from the control arm (no ECP treatment) of the study by Flowers et al. (see above) [73]. Patients had either (1) progression of cutaneous chronic GVHD; (2) less than 15 % improvement of their total skin score; or (3) < 25 % reduction of steroid dose at week 12 in the initial study	Apheresis: The study was performed with a Therakos apheresis system, 3 times ECP during the first week and thereafter twice weekly on consecutive days until week 12; followed by 2 treatments monthly until week 24	Overall response: 25/29 patients completed 24 weeks treatment. After 24 weeks the following complete + partial responses were seen: 9/25 skin responses; 8/25 with > 50 reduction of steroids; 14/20 oral mucosa responses; 7/15 ocular responses; 5/12 joint responses; Others: 3/6 liver, 2/4 lung and 2/2 gut responses

Table 2 continued

Study	Patients	Photopheresis	Outcome
Couriel [74] Retrospective 71 adults	Inclusion: cGVHD that was refractory defined as (1) no response after 1 month of treatment; (2) only partial response after 2 months of therapy; (3) progression after 2 weeks of the initial treatment GVHD treatment before entry: All received initial steroids, 59/71 still on steroids at start of ECP and 58/71 also receiving calcineurin inhibitors Age: median 39 years (range 5–70 years) Donors: Matched sibling 43/71, matched unrelated 19/71 GVHD *Involvement:* Skin 56/71 with sclerodermal changes 21/56; liver 21/71, bronchiolitis obliterans 11/71, oral 9/71, eyes 6/71, gut 3/71 *Type:* Progressive 18/71, relapsing 35/71, *de novo* 18/71	Apheresis: UVAR TS apheresis machine (Therakos, Exton, PA) Frequency and duration: Initially 2-4 treatments per week, tapered with 1 treatment per week when partial response was observed. Subsequently placed on maintenance with 2 treatments per every 2 weeks. Individualized discontinuation/duration	Overall response rate 43/71, complete responses 14/71 Organ responses: Skin 43/56 with 14 complete responses and 14/21 in sclerodermal form, liver 15/21, mouth 7/9, eye 4/6, lung 6/11 Median time from onset of ECP to complete response was 27 days (range 13-238 days) Duration of response: 13/43 initial responders progressed after a median of 23 days (range 16–188 days), the remaining 30 patients maintained their response for a median duration of 18 months (range 0.4–65 months) 28/43 had a sustained response after 6 months Survival: 42/71 died after a median follow-up of 34 months for the survivors, the primary cause of death was GVHD plus infection for 28/42. The strongest predictor of nonrelapse mortality was platelets $< 100 \times 10^9$/L at initiation of ECP
Dignan [75] Retrospective 82 adults	Inclusion: Patients with oral or skin involvement from cGVHD and being steroid-refractory, steroid-dependent or steroid-intolerant Age: Median 44.7 years (range 14.1–69.5 years) GVHD treatment at start of ECP: 70/82 received immunosuppressive treatment at the start of ECP, 60/70 receiving at least 2 immunosuppressive drugs and the median steroid dose was prednisolone 25 mg (range 5–135 mg) Donors and conditioning: 78/82 received matched family donors, 43/82 received RIC, 19/82 received myeloablative conditioning and for 20 patients the conditioning was not known GVHD *Onset:* Median time from transplant to ECP was 2.4 years (range 6 months-10 years) *Extension and type:* 75/82 severe disease and 7/82 moderate disease; 24/82 had progressive disease *Involvement:* Skin 75/82 with 40/82 sclerodermic and 34/8 s lichenoid disease, joints 2/10, oral 39/82, liver 11/82, eyes 18/82, gut 3/82, lung 2/82, joints 1/82. 29/82 had multiorgan involvement	Frequency of apheresis: 2 consecutive days every 2 weeks until a partial clinical response (i.e., > 50 % reduction), thereafter reduction to monthly treatment with two consecutive aphereses (UVAR XTS apheresis system, Therakos). Duration of treatment: 52 patients had ended treatment. Median duration of treatment for these patients was 330 days (range 42–987 days), median number of ECP cycles (i.e., two consecutive days) was 15 (1.5–32 cycles) Median time until reduction of treatment 207 days (74–638 days)	Total responses: 69/82 evaluable following 6 months of therapy; 5/69 had complete improvement, 60/69 partial improvement, 4/69 stable disease Skin responses (62/75 evaluable): 7/62 complete improvement. 50/62 partial improvement Oral responses (32/39 evaluable): 1/32 complete improvement 28/32 partial improvement Reduced immunosuppression after 6 months of treatment: 41/53 reduced immunosuppression, 40/50 reduced steroids and 11/50 stopped completely, 12 had > 75 % reduction and 7 had 50–75 % reduction Survival: Overall survival 3 years after start of ECP was 69 %

Table 2 continued

Study	Patients	Photopheresis	Outcome
Tsirigotis [76] Retrospective 58 adults	Inclusion: Patients that had failed at least one line of immunosuppressive treatment, including steroids Age: Median 29 years (range 3–59 years). GVHD prophylaxis: CSA plus MTX GVHD treatment at start of ECP: 39/58 had received at least 2 previous lines of treatment Donors and conditioning: 45/58 had matched family donors, 52/58 peripheral blood stem cell grafts GVHD *Onset:* Median time from transplant to ECP was 16 months (range 3–108 months) *Extension and type:* 23/58 severe disease, 35/58 moderate disease; 32/58 had progressive disease. Platelets < 100×10^9/L 25/58 *Involvement:* Skin 45/58 with 30 sclerodermic and 15 lichenoid disease, joints 5/58, oral 27/58, liver 20/58, eyes 39/58, lung 7/58	Apheresis: The device Therakos-Uvar XTS was used Median interval from GVHD to first apheresis 12.5 months, range 1–110 months Frequency and duration: (1) Two consecutive ECP procedures every week for 4 weeks, thereafter 2 consecutive procedures with 2–4 weeks intervals ($n = 14$); or alternatively (2) two procedures weekly for 4 weeks, thereafter 1 procedure weekly for 8–12 weeks and finally one procedure weekly for 8–12 weeks ($n = 44$) ECP discontinued if progressive GVHD, no response after 3 months, relapse of malignancy or 1–2 months after achievement of maximal response	Total responses: 33/58 showed an objective response Median time to response 10 weeks (range 3–16 weeks). Responses were associated with less severe cGVHD Skin responses: Total responses 27/45, sclerodermic 20/30 and lichenoid 7/15. Oral responses 18/27; eyes 20/39; liver 10/20; joints 1/5; lungs 1/7; Moderate cGVHD 24/35 responses; severe 9/23, progressive 15/32. Increased platelets to > 100×10^9/L 10/25 Permanent discontinuation of immunosuppression in 24 out of 33 responders Survival: Overall survival at 80 months 44 %. Response to treatment associated with significantly decreased nonrelapse mortality
Apisarnthana-rax [77] Retrospective 32 patients (30 adults)	Inclusion: 11 steroid-resistant and 21 steroid-dependent patients with cutaneous disease Age: median age 43 years (range 5–70 years). GVHD treatment at start of ECP: All received steroids, pretreatment with a median of 3 prior cGVHD treatments (range 1–5). 18/32 were on concomitant treatment besides steroids and tacrolimus at initiation of ECP Donors and conditioning: Bone marrow grafts 15 and mobilized stem cells 17. Matched sibling 20/32 GVHD: *Onset:* Median time from cGVHD to ECP 13 months (range 0.3–49.7); median time from transplant to ECP 26.2 months (range 3.6–52.4) *Severity and type:* 28 with extensive and 4 with limited disease; 17 with sclerodermic and 15 with lichenoid cutaneous disease Progressive 15/32, de novo 14/32, quiescent 3/32 *Involvement:* Liver 17/32, gut 11/32, eyes or mouth 11/32. Platelets < 100×10^9/L 16/32	Apheresis: Apheresis performed with the Therakos UVAR or XTS systems; no strict standardization of treatment with regard to frequency and duration Median interval from cGVHD to ECP 13.0 months (range 0.3–49.7). Time from transplant to ECP was 26.2 months (range 3.6–62.5) Frequency and duration: *Number of ECP sessions:* median 34 (range 12–98) *Duration:* Median 5.3 months (range 1–28 months)	Cutaneous responses: Complete 7/32, partial 11/32 Maintained cutaneous responses in all but 2 of the complete responses Responses were similar for (1) patients with and without visceral; (2) *de novo* versus quiescent versus progressive; (3) sibling versus unrelated donor Steroid reduction: 18 out of 28 patients on steroids at start of ECP reduced the dose with at least 50 %

Table 2 continued

Study	Patients	Photopheresis	Outcome
Foss [78] Prospective 25 patients, 23 adults	Inclusion: 25 patients with extensive steroid-refractory cGVHD Age: median age for adults 42 years (range 18–59 years). Previous cGVHD treatment: 15/25 two regimen, 7/25 three regimen and 3/25 four regimen Donors: 17/25 related and 8/25 unrelated donors GVHD *Type:* Progressive type 6/25 *Involvement:* 3/25 one organs, 8/25 two organs, 11/25 three organs and 3/25 four organs	Apheresis: The XTS photopheresis machine (Therakos) was used. No strict standardization of treatment with regard to frequency and duration. ECP was administered until best response or progression Median time from transplant to ECP 790 days (range 196–2,928) Frequency or duration: Every or every second week. Median duration of treatment 9 months (range 3–24 months)	Overall response rate 64 %. Responses showed no significant correlation with donor type, time from transplant to ECP, GVHD severity or type of onset of cGVHD Single organ improvements: 20/25 patients had improvement in cutaneous cGVHD 6/13 oral improvement 3/6 improvement in joint symptoms 1/2 with pulmonary manifestations had improvement and 1/3 with gut symptoms Reduced immunosuppression: 11/25 decreased steroids while on ECP, 12/25 reduced MMF and 5 reduced tacrolimus
Rubegni [79] Prospective 32 adults	Age: median age 35 years (range 18–60 years) GVHD treatment: 27/32 patients received at least two immunosuppressive agents at initiation of ECP GVHD: *Onset:* Median time from transplant to ECP 5 months (range 1–56). *Extension and type:* Skin disease, 7 with sclerodermic and 20 with lichenoid cutaneous disease. Other organ involvements were liver 22/32, eyes 16/32, oral 25/32, lungs 5/32, thrombocytopenia 12/32 Progressive disease in 13/32, de novo 12/32, quiescent 6/32	Apheresis: UVAR photopheresis units (Therakos) were used Median time from transplant to first ECP 5 months (range 1–56 months) Frequency or duration: 1,128 aphereses performed in the 32 patients	Overall effect: ECP was associated with improvement in 24/32 (78 % of patients) Complete responses: Skin 14/27, oral 16/25, eyes 8/16, hepatic 5/22, thrombocytopenia 11/12 Partial responses: Skin 8/27, oral 7/25, eyes 7/25, hepatic 12/22, lungs 2/5
Seaton [80] Prospective 28 adults	Inclusion: Patients refractory to conventional cGVHD therapy Age: median age 34 years, range 18–51 years GVHD treatment before ECP: 26/28 receiving steroids, 24 additional ciclosporin, several patients also received other immunosuppressive agents Donor and conditioning: 19 sibling donors, 9 MUD GVHD *Disease type:* progressive 9/28, de novo 14/28, relapsed 5/28	Apheresis: The UVAR or the UVAR XTS photopheresis system (Therakos) was used Median time to ECP from: allografting 34 months (10–167 months) onset of cGVHD 23 months (2–164 months) Frequency and duration: ECP on two consecutive days fortnightly for the first 4 months, then monthly. At 6 months it was decided whether to continue based on the clinical response	Evaluation of treatment response: Skin: Evaluation possible in 21 patients after at least two cycles, 8/21 were responders after 3 months and 10/21 were responders after 6 months Liver: 25 % improvement in liver enzymes in 8/25 after 3 months Oral: 3/6 with severe disease showed improvement Systemic immunosuppression was stable or improved in 86 % of patients

Table 2 continued

Study	Patients	Photopheresis	Outcome
Perseghin [81] Retrospective 25 patients	Inclusion: Patients refractory to standard immunosuppressive treatment Age: Median 17 years (range 6–55 years) GVHD treatment before ECP: All were on immunosuppressive treatment. 23/25 used steroids and 18/25 ciclosporin; 13/25 used two different agents and 11/25 used 3 immunosuppressive agents Donors: 19/25 related, matched donors cGVHD: *Onset:* Median duration from transplantation to cGVHD 104 days (range 100–720 days) *Extension:* 21/25 with extensive disease *Involvement:* Skin 21/25, mucosal 8/25, gut 2/25, eyes 4/25, liver 6/25	Apheresis: A COBE Spectra cell separator was used (Gambro BCT) Median delay from cGVHG diagnosis to ECP was 2.0 months (range 0.5–28.6 months) Frequency and duration: ECP was administered on consecutive days. Patients underwent 2 treatments weakly for 3 consecutive weeks, thereafter 2 ECPs per fortnight repeated twice and finally 2 ECPs per month until the sixth month for a total of 20 ECPs. Median treatment duration was 177 days (range 28–454 days), median number of ECP treatments 19 (range 8–38)	Overall response: 11/25 complete responses 9/25 partial responses Organ responses: Skin 21/21 responses, mucous 8/8, eye 4/4, liver 4/6, gut 2/2 Immunosuppressive reduction: Out of 20 evaluable patients 8 patients were off treatment, 8 patients on 1 drug, 3 patients on 2 drugs and 1 on 3 drugs after end of treatment
Jagasia [83] Retrospective 43 adults (12 with overlap GVHD and 31 with classic chronic GVHD)	Inclusion: Steroid-refractory, dependent or intolerant patients Age: Adults below 67 years of age GVHD treatment before ECP: Heterogeneous pre-ECP treatment including antithymocyte globulin and infliximab Donors: Sibling and matched unrelated donors cGVHD *Onset:* Mild 4/43, moderate 23/43, severe 16/43	Median delay from cGVHD diagnosis to ECP: For the 31 patients with classic GVHD start of ECP after a median of 786 days; the other patients after 108 days Frequency and duration of aphereses: 2 aphereses weekly for 3–4 weeks, thereafter 2 aphereses with 2-to 3-week interval and finally 4 weeks intervals; individualized overall duration. Median number of aphereses for the 31 patients with classic chronic GVHD 14	Overall response: complete response 5/43, partial response 23/43, stable disease 10/43 and progression 5/43 Survival: Overall 8-years survival for the whole group 50 %. The 2 years nonrelapse mortality was 29 % for the 43 patients, death from relapse approximately 10 % Steroid dose (<1 mg/kg versus >1 mg/kg) at initiation of ECP predicted overall survival

reduction plus skin improvement, there was a difference of borderline significance ($p = 0.04$) between patients with and without ECP treatment, but the change in total skin score between the two groups did not differ after 12 weeks.

Thus, even though we conclude that ECP can be an effective treatment of chronic GVHD especially in patients with skin and mouth affection, additional randomized studies are needed.

The toxicity of extracorporeal photopheresis

Serious side effects during ECP are uncommon. Prophylactic administration of calcium during the procedure can be used if citric acid and not heparin is used for anticoagulation [61], and one study then reported that even for children only 4 % of aphereses had to be interrupted due to side effects [63]. Among, the reported side effects are as follows:

- Hypotension during apheresis can be seen but is usually asymptomatic [61, 64, 68].
- A decrease in hemoglobin (Hb) levels exceeding 2 g/dL is common in children and transfusions may be required [61]; some investigators therefore use preapheresis transfusions for patients with Hb < 9.0 g/dL [68]. In the only randomized study comparing ECP with alternative pharmacological strategies, anemia was more common for the ECP patients (24.5 vs. 6 %, $p = 0.02$) [73]. Transient decreases in platelet and white blood cell counts may occur [68] and in one study 22 % of patients required platelet transfusions [74]. Decreased Hb and platelet levels are associated with lower body weight and large collection volumes [63].
- Abdominal pain during or following the procedure has been reported for 20 % of patients but is usually mild [60]. Fever, chills, nausea, fluid overload and headache are uncommon [60].
- Even though most studies are small, there is no evidence for increased frequencies of bacterial or fungal infections or reactivation of viral infections [63, 64].
- Infections with relation to an indwelling central venous line are seen in <5 % of patients [75] and only exceptional patients experience thrombosis at the catheter site [74, 77].

Conclusion and future perspectives

The limitations of available clinical ECP studies and the importance of randomized studies

There is a general agreement that steroids should be used as the primary treatment of acute and chronic GVHD, and for this reason, ECP was used as salvage therapy in combination with steroids after the initial steroid monotherapy in all the clinical studies (Tables 1, 2). Most studies of ECP in the treatment of chronic GVHD have included patients with steroid resistance or insufficient response to steroids, and most acute GVHD patients have received ECP as a second-line therapy for GVHD grade II or III. The overall results suggest that ECP can be effective in the treatment of GVHD. The evidence is best in chronic GVHD where the treatment can be effective both in children and adults, whereas fewer studies are available in acute GVHD and particularly in adults. The treatment has mainly been tried in combination with pharmacological immunosuppression, and the efficacy measure has mainly been whether ongoing pharmacotherapy (usually steroids but often also other agents) can be stopped or reduced during ECP. Most studies have concluded that a subset of patients respond, i.e., ECP has a steroid-sparing effect. However, it is also difficult to know whether the clinical improvement is caused partly or mostly by the ECP treatment or simply by the prolonged duration of the already ongoing pharmacotherapy.

The ECP regimen needs further standardization. Many studies have used two consecutive treatments per week initially and thereafter two consecutive treatments every second week until further reduction after 2–3 months. However, the duration and criteria for stopping the treatment differ between studies.

The importance of randomized clinical studies is also illustrated by the recently published study by Jagasia et al. [84] that compared ECP (consecutive patients from 3 different centers) with anti-cytokine therapy (imalinomab or etanercept therapy at a forth center) in steroid-refractory acute GVHD. After adjusting for differences between the two groups, the authors described superior survival for patients receiving ECP. However, this study has several weaknesses as discussed in detail by the authors: (1) the cytokine treatment was heterogeneous; (2) the anti-cytokine group included a higher proportion of patients with grade III-IV aGVHD and patients starting treatment with a higher steroid dose of 2 mg/kg; (3) steroid tapering as well as ECP scheduling were heterogeneous; and (4) there was no strict standardization of response evaluation. The final conclusion made by the authors was that their observations require validation in a prospective randomized study. From the ClinicalTrial.gov database (8th of January 2014), it can be seen that two such randomized studies are ongoing together with studies of ECP in combination with immunosuppressive agents (1 study), prophylactic use (2 studies), possible biomarkers to be used for treatment evaluation (2 studies) and ECP used in patients transplanted with umbilical cord stem cells (1 study).

The importance of standardizing the ECP techniques

The technical aspects of ECP have been reviewed recently [10, 11]. There are several differences between the clinical studies summarized in Tables 1, 2 not only with regard to frequency and duration of apheresis but also with regard to (1) peripheral versus central venous access; (2) anticoagulation during treatment; and (3) an open system versus the closed Therakos system used in most recent studies. The immunomodulatory effects of ECP depend on complex interactions between various cells and plasma molecules during the extracorporeal circulation (see above); these observations clearly illustrate the importance of careful standardization with regard to the apheresis equipment and not only duration and frequency of apheresis.

Comparison of ECP to other treatment options

A more detailed discussion of ECP in the treatment of acute and chronic GVHD is given in the Supplementary Information, including a summary of representative clinical studies of alternative therapeutic strategies for salvage therapy in patients with steroid-resistant acute GVHD.

Acute GVHD

Most clinical studies of ECP as well as the pharmacological alternatives for second-line treatment are nonrandomized; many of these studies are relatively small, most of them are retrospective and for each therapeutic alternative relatively few studies are available (Supplementary information, Table) [60, 62–66, 85–107]. The overall experience with ECP is relatively extensive compared with the alternative strategies. The British [108], American [109] and Italian guidelines [110] therefore conclude that the first-line treatment of acute GVHD should be steroids, ECP can be considered for salvage/second-line treatment, and there is at present no support for the choice of any specific agent for second-line treatment of acute GVHD based on 6 months survival data, complete response rates or overall response rates. However, all three guidelines emphasize the excellent safety profile of ECP.

Chronic GVHD

The British guidelines state that steroids should be the first-line treatment eventually combined with a calcineurin inhibitor, as a possible second-line treatment they recommend ECP for skin, oral and liver GVHD [111]. Wolf et al. [14, 15] also recommended ECP as a possible second-line treatment. Both reviews as well as the Italian guidelines [110] concluded that it is not possible to recommend one specific strategy as preferred second-line therapy.

The final conclusion

In our opinion, ECP seems to be a safe and effective treatment of GVHD. Because most of the studied patients received additional pharmacological immunomodulation at the start of ECP, it is in our opinion also justified to conclude that it is safe to combine ECP with other pharmacological strategies. Steroids should still be regarded as the first-line treatment and ECP as second-line or salvage therapy. However, additional randomized ECP studies as salvage treatment are definitely needed, but it may also be important to investigate whether steroids can be combined with ECP as first-line therapy. Furthermore, ECP is an expensive and long-lasting treatment, and even though some studies show a very high response rate the overall results clearly demonstrate that only a subset of patients benefits from this treatment and many patients only achieve partial responses. Future studies should therefore try to find biomarkers that can be used before or during treatment to identify those patients that will benefit from ECP. We would also emphasize that further biological/immunological investigations should be incorporated in future clinical studies. Finally, the beneficial effects of ECP are probably caused by complex and transient ex vivo interactions between serum molecules, platelets and immunocompetent cells [38, 39]; these observations emphasize that a careful standardization of the treatment is needed.

For patients with chronic GVHD, a careful classification of patients according to the standardized National Institute of Health criteria both with regard to organ involvement, severity and response will be important to allow a comparison of different studies [112]. The available data suggest that the treatment may be effective both in patients with severe acute and chronic GVHD and may be useful even in patients with pulmonary affection [113], but a better standardization and the use of generally accepted response criteria will be essential.

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