Extracorporeal photochemotherapy for graft-versus-host disease: Where we are now and where we are going!

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SUMMARY

Graft-versus-host disease remains the leading cause of morbidity, non-relapse mortality and treatment failure after allogeneic haematopoietic stem cell transplantation. So far, steroids are the first line treatment, but around 40% of patients become steroid-resistant or fail to respond at a safe dose. Patients who fail to respond to the initial therapy have a dismal prognosis, and no standard treatment is well established for them to date. Treatments that modulate the immune system rather than directly suppressing its function, although not dampening a potential graft-versus-malignancy effect, would therefore be highly desirable, and extracorporeal photopheresis appeared as being a good candidate to fill in these criteria. Multiple reports of treatments in both paediatric and adult patients with graft-versus-host disease have been published, and the overall favourable profile compared with other available immunosuppressive therapies continues to make extracorporeal photopheresis appealing despite all of the unknowns. In this article, we review the use of extracorporeal photopheresis for the treatment of graft-versus-host disease, including technical aspects, mechanism of action, safety profile and clinical efficacy data.

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INTRODUCTION

Graft-versus-host disease (GVHD) remains the leading cause of morbidity, non-relapse mortality and treatment failure post allogeneic haematopoietic stem cell transplantation (allo-HSCT). It occurs under acute (aGVHD) and chronic (cGVHD) forms. Around 50% of all allo-HSCT patients will develop some type or degree of GVHD.[1-3] GVHD is responsible for one third of the transplant-related deaths but is also the cause of severe morbidities with high impact on autonomy, possibility of self-supporting, chance to resume a professional life and globally on the quality of life of the patient.[4,5] GVHD is responsible for repeated long hospitalisations for infection treatment and organ dysfunctions.[6] So far, steroids and increase or restart of the immunosuppressive treatment that was given for GVHD prevention (cyclosporine, tacrolimus or rapamycin) when it was already tapered or stopped are the widely used first line treatment. Complete response (CR) to first line treatment is reported to occur in 25-40% of patients and clinically relevant improvement is achieved in 40-50%.[7] However, the likelihood of response decreases with increasing severity of the disease.[8,9] Around 40% of patients become steroid-resistant or fail to respond at a safe dose. Patients who fail to respond to the initial therapy have a dismal prognosis and no standard treatment is well established for them to date. A number of phase II trials of secondary regimens for patients with steroid-refractory GVHD have been published, and most of them reported a success rate ranging from 25-60%.[10,11] Second line immunosuppressive treatments are associated with a high mortality from opportunistic infections, malignant relapse, secondary neoplasms and other serious complications. Clinical manifestations of cGVHD can persist for

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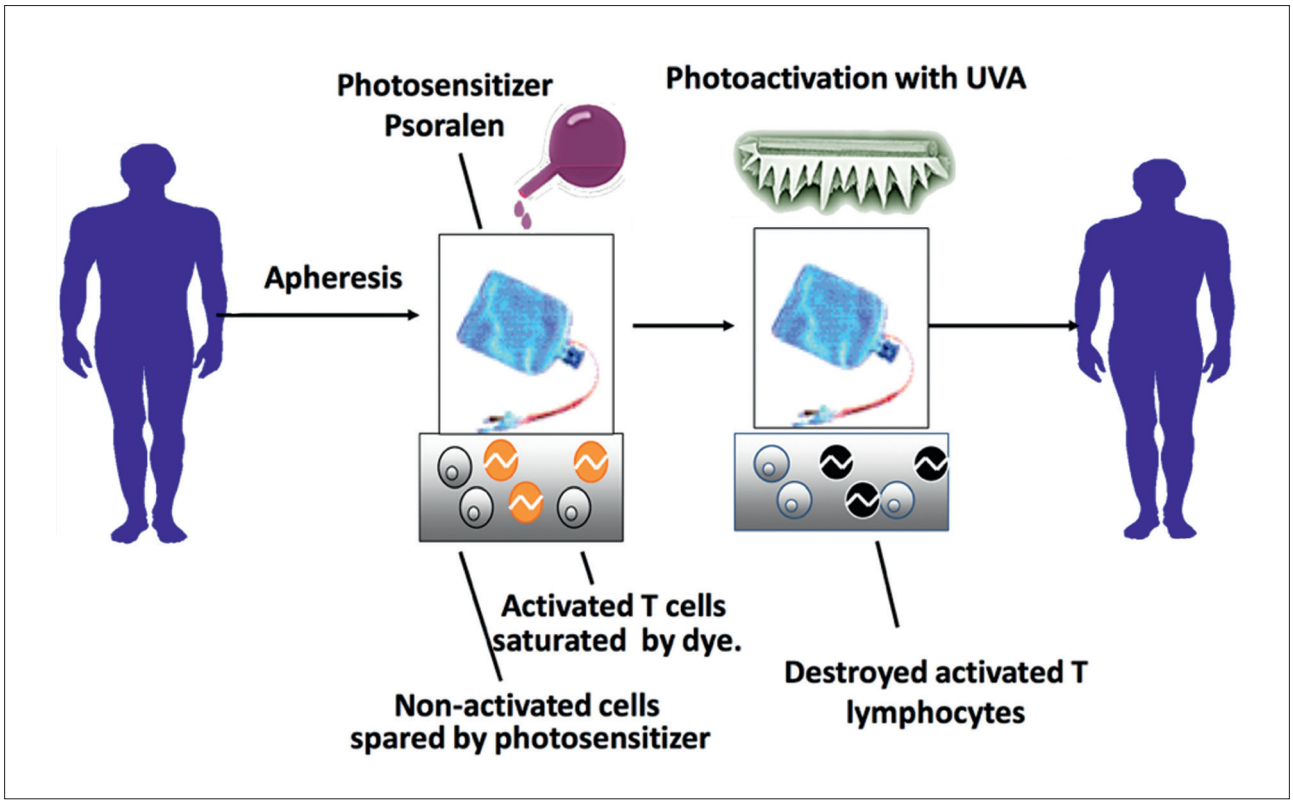

FIGURE 1. The Macopharma open Theraflex ECP system.

prolonged periods of time, and patients require a median of two to three years of therapy. As a result of their chronic immune suppression, about 40% of all patients with cGVHD will die within five years of infection or develop recurrent malignancy.[12,13]

Treatments that modulate the immune system rather than directly suppressing its function, although not dampening a potential graft-versus-malignancy effect, would therefore be highly desirable, and extracorporeal photopheresis (ECP) appeared as being a good candidate to fill in these criteria.[14-16] ECP has indeed emerged as a safe and efficacious non-pharmacologic immunomodulatory approach for the management of patients resistant to the first line treatment of GVHD. Since the Food and Drug Administration (FDA)'s first approval in 1988, ECP is being increasingly used around the world.[17] Despite its frequent usage, the optimal role of ECP in the setting of GVHD still needs to be defined.

TECHNICAL ASPECTS OF EXTRACORPOREAL PHOTOPHERESIS

Each session of ECP is an invasive procedure. Patients should have adequate haemoglobin levels (>10 g/dl) and platelets count (>20 x 10^9/L) as for other apheresis treatments. Patients exhibiting idiosyncratic or hypersensitivity reactions to methoxsalen or other psoralen compounds and patients possessing a specific history of a light-sensitive disease state are contraindicated for such therapies. Diseases associated with photosensitivity include lupus erythematosus, porphyria cutanea tarda, erythropoietic protoporphyria, variegate porphyria, xeroderma pigmentosum, and albinism. There are no adequate studies of methoxsalen in pregnant women, we should therefore consider that it may cause foetal harm when given to a pregnant woman. Psoralen is also contraindicated in patients with aphakia because of the significantly increased risk of retinal damage due to the absence of lenses. Patients should not start photopheresis treatment if they have any contraindications to the apheresis procedure.

There are two methods to perform ECP. The two methods differ in the devices used for the collection of leucocytes as well as for UVA irradiation. They can be classified into 'on-line' and 'off-line' methods based on the type of devices used. The ECP procedure consists of four steps: (a) collection of peripheral blood mononuclear cells by apheresis, (b) *ex vivo* incubation of mononuclear cells with 8-methoxypsoralen (8-MOP; a photoactivating drug), (c) irradiation of cells with UVA, (d) reinfusion of the treated cells into the patient.[23,24] The on-line method allows for a one step procedure, during which the patient remains constantly connected to the system, can be performed on the Therakos CELLEX Photopheresis System (Therakos–Mallinckrodt Pharmaceuticals)

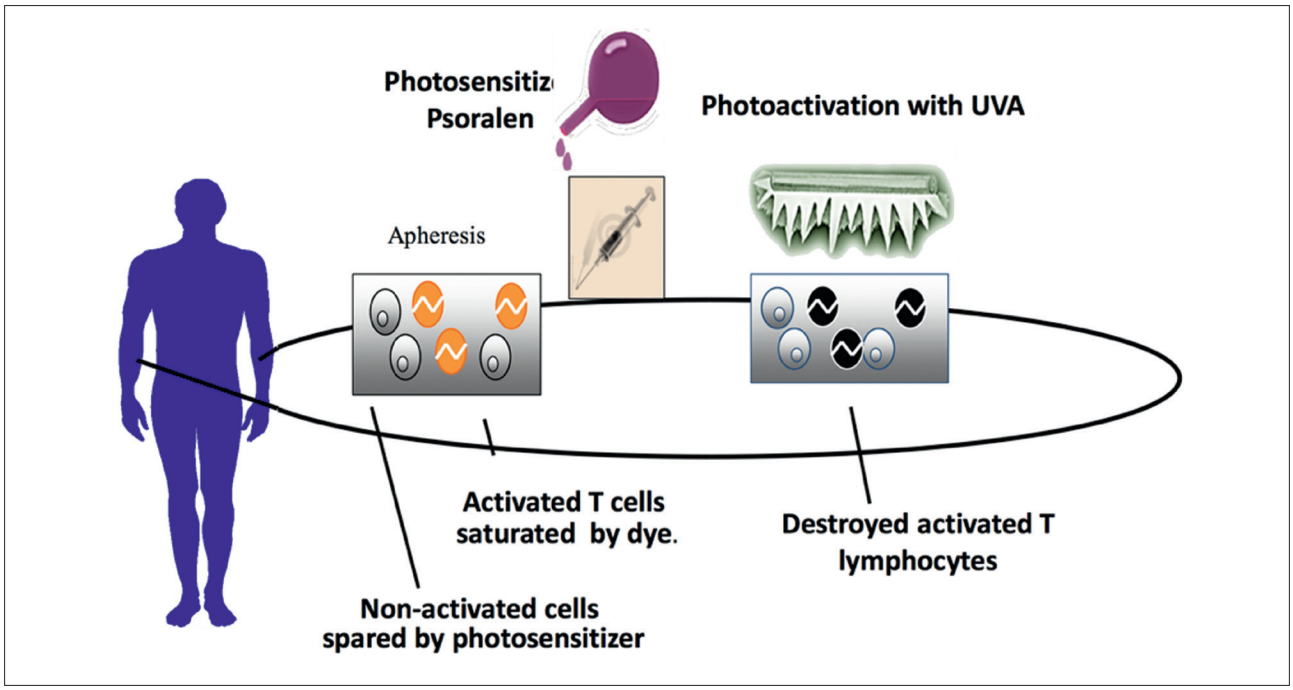

FIGURE 2. The UVAR-XTS and CELLEX photopheresis closed systems.

and is based on an integrated, automated closed loop using a single device integrating the photoactivation chamber. At least 1 x 10^9/L cells in the peripheral blood are recommended before initiating ECP therapy.[25,26] The instrument separates and collects the lymphomonocyte fraction through centrifugal force while the other components are returned back into the patient. The buffy coat fraction remains in the system where it is treated with 8-MOP and subsequently exposed to the UVA.[27] Finally, treated leucocytes are reinfused back into the patients. The Therakos CELLEX instrument can operate in both discontinuous and continuous modes. The continuous, 'double-needle mode' requires separate collection and return vascular sites. If the procedure starts in the double-needle mode and one of the access sites is lost, the mode can be converted to single needle for the completion of the therapy.[28] The closed system approach reduces the risk for bacterial contamination. After each session, the patient should be prescribed high SPF sunscreen (15 or above) and UVA sunglasses (for 24h after each session) to avoid the adverse effect of 8-MOP used. Macopharma has proposed an alternative off-line strategy to perform ECP. The Macopharma (Theraflex ECP) approach is based on a standard mononuclear cell apheresis, injection of the 8-MOP in the apheresis bag, UVA exposure of the bag and reinfusion of the cells into the patients. In off-line methods, new apheresis devices offer a higher collection efficiency of lymphocytes resulting in greater cellular harvest. However, there are no data showing a correlation between a greater number of cells processed and the therapeutic response. A major disadvantage of the off-line method is the need of a cell therapy facility to treat the apheresis bag.

ECP treatment is usually administered in two separate sessions over two consecutive days. Treatment can either be performed on an outpatient (with patients returning home between sessions) or inpatient basis (patients stay overnight with the first treatment in an afternoon and the second treatment on the following morning). It is possible that when a large number of cells are harvested and treated using the off-line system, one session per cycle could be enough.

Several papers have reported on the safety profile of ECP of more than 500,000 ECP treatments performed worldwide since 1987. The incidence of reported adverse events is <0.003%.[29] Adverse reactions can be related to leucapheresis such as reactions to volume shift in the extracorporeal circuit, citrate toxicity due to the anticoagulant used or bleeding from the cannula sites. Reaction related to exposure to psoralen can include increased urinary output, metallic taste and sparkly bits in the eyes. On reinfusion of the ECP products, some patients complain of mild fever, tiredness, headache, nausea and haematuria (due to reinfusion of red blood cells after exposure to 8-MOP).

EXTRACORPOREAL PHOTOPHERESIS' MECHANISM OF ACTION

Although ECP has been in use for 30 years, its immunomodulatory mechanism of action is not yet fully understood.

BOX 1. Key steps through which extracorporeal photopheresis is believed to reduce T-cell-mediated immune responses in patients with graft-versus-host disease.

- apoptosis of white blood cells;
- phagocytosis of these apoptotic lymphocytes by antigen-presenting cells;
- a switch in antigen-presenting cell activity in favour of anti-inflammatory cytokines and away from pro-inflammatory cytokines;
- production of antigen-specific immunosuppressive T-regulatory cells;
- anti-(oligo)clonotypic immune response (anti-, allo- or auto-reactive oligoclonal T cells).

ECP exerts multiple effects on the immune system due to (a) changes induced in the mononuclear cells by the environmental changes of harvested cells, (b) cellular changes due to treatment of cells by psoralen and exposure to UVA rays and, finally, (c) changes in the cytokine environment and immune cell function in the recipient following the reinfusion of the treated cells.

First, ECP induces apoptosis of activated lymphocytes within 24 to 48 hours of treatment, which results from intercalation of DNA when 8-MOP is activated by exposure to UV light.[30] However, it is unlikely that the induction of apoptosis of treated lymphocytes represents the main mechanistic action of ECP, as only 5-10% of circulating leucocytes are treated.[17,31,32] Rather, phagocytosis of the treated apoptotic lymphocytes by antigen-presenting cells (APCs) and the induction of tolerogenic dendritic cells from treated monocytes appear to hold a more pivotal role in the induction of allospecific tolerance.[16,31,33,34] On reinfusion of irradiated cells, the cytokine network shifts with an increase in inhibitory cytokines (interleukin-10, interleukin-4, transforming growth factor beta [TGF-β]) and a decrease in inflammatory cytokines (interleukin-12, interferon-α, tumour necrosis factor-α, interleukin-1) resulting in a shift from T-helper (Th)1 to Th2 response and an increase in antigen-specific circulating T-regulatory cells (Tregs). *In vivo* apoptosis of treated neutrophils could also modulate T-cell proliferation, induce indirect effects on APCs and lead to a decrease of inflammatory activity and tissue damage.[16,35-37] Moreover, Rieber *et al*. demonstrated that ECP treatment in GVHD patients increases neutrophilic myeloid-derived suppressor cells, which modulate Th1 and Th17 responses.[38] It is not known whether this is important for the clinical response to ECP. So far, no study has investigated whether B lymphocytes play a role in ECP immunomodulation.[16]

However, the generation of Tregs and tolerogenic dendritic cells neither explains how ECP selectively targets pathogenic T cells without inducing systemic immunosuppression nor explains how it works in cutaneous T-cell lymphoma.[14,28] How ECP could trigger both an anti-tumour immune response and immune tolerance remains indeed an open question. The pathologies treated by ECP are heterogeneous; however, they are all mediated by a (oligo)clonal T-cell population (tumoral T-cell clones in cutaneous T-cell lymphoma, allo- or auto-reactive oligoclonal T cells in GVHD and autoimmune diseases). Thus, these T cells share unique or a few T-cell receptors (TCR) representing pathogenic T-cell-specific antigens that can be subsequently targeted by ECP-induced immune responses. Importantly, the presence of this pathogenic T-cell population within the treated cells is critical for ECP efficacy.[39] These critical data underlie the necessity of providing dying pathogenic T cells (containing specific antigens) in order to obtain a therapeutic response, evoking an anti-(oligo)clonotypic immune response triggered by the repeated reinfusion of treated pathogenic T cells. ECP-induced immune cell death of pathogenic T cells could reconcile the apparently contradictory modes of action proposed so far.[40,41] By promoting immune tolerance and simultaneously avoiding systemic immunosuppression, ECP could reduce GVHD and enable a reduction in other immunosuppression, allowing thymic recovery, restoration of normal T lymphopoiesis and complete immunoreconstitution.[31]

VENOUS ACCESS

ECP procedures take several hours, and patients undergo these procedures for weeks or months.

A recent international survey of ECP practice found that venous access issues were the number one reason given as a barrier to patients receiving ECP therapy. Peripheral access using venous needles (17-gauge inlet line and 17-/19-gauge return line) is most desirable to minimise any catheter-related infectious risks. In patients who have a long-term central venous access (CVC), this can be used for either inflow or outflow. A double-lumen CVC in subclavian or jugular can also be used (7-10 Fr for children and 12-14 Fr for adults to

TABLE 1. Summary of clinical evidence on extracorporeal photopheresis as second line treatment in adult acute graft-versus-host disease.

Lead author	Year	N	CR, %	OR, %
Abu Dalle[50]	2014	54	Skin 84 GI 65	69
Greinix[48]	2006	59	Skin 82 Liver 61 GI 61	N/A
Perfetti[57]	2008	23	52 Skin 66 Liver 27 GI 40	N/A
Jagasia[58]	2013	57	54 Skin stage I- II 70 stage III-IV 57 Liver stage I-II 72 stage III-IV 50 GI stage I-II 77 stage III-IV 54	66
Malagola[59]	2016	45	Grade II 97 Grade III 67	N/A

CR: complete response, OR: overall response, GI: gastrointestinal.

provide adequate flow rates, i.e., 2-5 mL/kg/min). These temporary central venous catheters are nevertheless not recommended for ECP due to the expected duration of therapy. A preliminarily check to confirm if the patient's venous access is adequate is therefore essential before planning the start of treatment.[42]

The repeated, prolonged venous access required for ECP often necessitates the use of an implantable vascular access device (IVAD), a tunnelled central venous catheter (TCVC) or a tunnelled central venous catheter with port (port-CVC). Although traditional subcutaneous port-CVCs have been used for ECP, these ports are not designed or approved for apheresis therapies. Under the best conditions, it is possible to achieve flow rates of 50-60 mL/min, which is acceptable for ECP but not optimal.[43] In April 2017, the FDA approved the first subcutaneous port-CVC specifically designed for apheresis, the PowerFlow Implantable Apheresis IV Port (Bard Peripheral Vascular). It is designed with a titanium funnel rather than a septum. Unlike traditional port-CVCs that are accessed at 90°, the apheresis port-CVC is accessed at 30° relative to the skin surface. Blood flow rates at normal operating pressures (–100 mm Hg) range from 120 to 150 mL/min depending on the gauge of the IV catheter port.[44]

INDICATIONS OF EXTRACORPOREAL PHOTOPHERESIS IN GRAFT-VERSUS-HOST DISEASE

PREVENTION OF GRAFT-VERSUS-HOST DISEASE

Considering the substantial rates of GVHD despite prophylaxis, novel prevention strategies are highly warranted. ECP, which is immunomodulating rather than immunosuppressive, could therefore be a very good candidate.[45,46] Nowadays, there is no definitive evidence supporting the use of ECP for preventing GVHD occurrence, and it should not be done outside clinical trials.

TREATMENT OF ACUTE GRAFT-VERSUS-HOST DISEASE

ECP is a valuable option in the treatment of either adults or children with steroid-refractory or steroid-dependent aGVHD. Besides anti-thymocyte globulin (ATG), ECP is the second most frequently reported second line treatment of patients with corticosteroid-refractory aGVHD.[18] Some data suggest that early treatment shows better clinical results, but patients with a GVHD are usually qualified for ECP late in the course of the disease, after other therapeutic options are exhausted. At that time, because leukopenia is a typical feature in patients

TABLE 2. Published survival data for adults with acute graft-versus-host disease.

Lead author	Year	N	Years F/Up	OR, %
Malik[60]	2014	595	1	49
Greinix[48]	2006	59	4	59*
Perfetti[57]	2008	23	Up to 81 months**	38

*complete responders only, **Retrospective review 1996-2006, *F/Up: follow-up, OR: overall survival.*

TABLE 3. Published response rates for extracorporeal photopheresis in the treatment of paediatric acute graft-versus-host disease: overall response and steroid tapering

Lead author	Year	N	OR, %	Discontinuation of steroids, %	Tapering of steroids, %
Salvaneschi[61]	2001	9	78	43*	
Messina[62]	2003	33	76	42*	36
Berger[63]	2007	15	Grade II: 100 Grade III: 75 Grade IV: 0		
Kanold[64]	2005	41	73		
Kanold[65]	2007	12	83	30	33
Perseghin[66]	2007	10	70		
Gonzalez-Vicent[67]	2008	8	100		
Calore[68]	2008	15	100	67	
Merlin[69]	2010	12	83		
Gonzalez-Vicent[70]	2010	21	90		
Perotti[71]	2010	50	68	16 at 30 days	

*Responders, *OR: overall response.*

treated for aGVHD, there is a decreased number of UVA irradiated cells, which in turn can limit the efficacy of ECP.[47] It seems therefore reasonable to start the ECP procedure in these patients earlier, when the white blood cell count is still abundant.48 Given its favourable adverse effect profile, ECP could be considered in all patients with aGVHD and certainly for treatment of aGVHD in patients for whom further immunosuppression is contraindicated due to viral reactivation or other infectious complications.[49]

The recommended ECP treatment schedule in aGVHD is not standardised between the different guidelines. In summary, it starts with two or three (intensified regimen) sessions the first to fourth week depending on severity and clinical response, then one ECP cycle (two consecutive ECP sessions) per week from weeks two to five and until week eight to twelve. At eight or twelve weeks: If a CR or partial response (PR) occurs, taper to one cycle every four weeks and stop after six months; if there is mixed response between different

TABLE 4. Published survival data for children with acute graft-versus-host disease on extracorporeal photopheresis.

Lead author	Year	N	Years F/Up	OS, %	PFS, %	DFS, %
Salvaneschi[61]	2001	9	0.75	55		
Messina[62]	2003	33	5	69*		
Berger[63]	2007	15	N/A	100% Grade II; 30% Grade II-IV		
Kanold[65]	2007	12	N/A	67		
Calore[68]	2008	15	2	85	87	
Gonzalez-Vicent[70]	2010	21				43
Perotti[71]	2010	50	5	46		
Merlin[69]	2010	12	5	57		

*Responders, *F/Up: follow-up, OS: overall survival, PFS: progression free survival, DFS: disease free survival.*

GVHD targets, continue with one cycle/two weeks up to maximum six months and taper if a PR or CR is achieved; if steroid-dependent (SD) or progressive disease (PD): stop.[15,29,48,50]

From the data reported in the literature, summarised in *Tables 1-4*, responses were more common for patients with grade II than with grade III/IV aGVHD; CRs were seen in up to 100% of patients with grade II disease, whereas for patients with grade III/IV disease, complete remission was reached in around 40% of cases. Responses to ECP were more common for patients having skin involvement (66-84%) compared with gut (40-65%) or liver disease (27-61%). In general, ECP not only provides higher complete and partial response rates than alternative therapies, it also shows higher survival rates. Nevertheless, ECP superiority over other therapies cannot be firmly stated yet due to the lack of controlled randomised trials. Moving forward, randomised controlled studies are crucial to determine the optimal timing of initiation and treatment schedule for patients with aGVHD. Nowadays, ECP is recommended for the treatment of aGvHD by an increasing number of national and international guidelines and consensus papers.[51-56]

EXTRACORPOREAL PHOTOPHERESIS IN CHRONIC GRAFT-VERSUS-HOST DISEASE

As in aGVHD, no consensus has been reached regarding the optimal second line therapy in cGVHD patients. ECP has been used frequently in patients with steroid-refractory and steroid-dependent disease and is recommended in both adult and paediatric patients, either steroid-resistant or steroid-dependent, irrespective of disease extent and severity.[52] Documented improvements have also occurred in patients who have failed multiple therapies and suffered from GVHD for many months. Much clinical experience in cGVHD is based again on small case series and retrospective reviews. There are very few data available for the use of ECP as the first line therapy of cGVHD. Nevertheless, considering that the graft-versus-lymphoma effect seems to be not impaired by ECP, earlier use of ECP in cGVHD is recommended by some leading experts in the field, especially considering ECP inefficacy after irreversible tissue damage. Objective activity of ECP used as second line therapy and its positive impact on overall quality of life have been documented in all forms of cGVHD.[72,73]

Due to the variety of ECP schedules applied, the impact of dose intensity and length of treatment cannot be assessed accurately based on the currently available literature.[52] The most published treatment scheme for cGVHD is one cycle of ECP (two consecutive sessions) every two weeks up to a minimum of three months. At month three: if a CR or PR occurs, taper to one cycle every four weeks and stop after six months; if there is a mixed response, continue with one cycle/two weeks up to six months; if SD or PD: stop. Then, every three months revaluate the response; if there is a CR, taper to one cycle/four weeks for three months and stop if

TABLE 5. Published response rates for extracorporeal photopheresis in the treatment of chronic graft-versus-host disease in adults.

Lead author	Type	Year	No. studies	N	CR, %	OR, %	
McKenna[88]	Meta-analysis	2006	23	521		68	
Abu Dalle[50]	Meta-analysis	2014	5	87	26	**ORR 64** Skin 71 Mucosa 63	Liver 58 GI 62 Lung 15
Malik[60]	Meta-analysis	2014	18	595	29 (19-42)	**ORR 64 (65-82)** Skin 74 Mucosa 72 Liver 68	GI 53 Ocular 60 Lung 48
Flowers[75]	Randomised multicentric prospective phase II study	2008	N/A	48 ECP *vs* 47 immuno-suppressive drugs alone	N/A	Skin 40 *vs* 10 Mucosa 53 *vs* 27 Liver 29 *vs* NA	Ocular 30 *vs* 7 Joint 22 *vs* 12
Seaton[90]	Prospective				N/A	Skin 53	Mucosa 50
Berger[63]	Single arm prospective	2007	N/A	10	30	**ORR 40**	
Greinix[77]	Crossover prospective	2011	N/A	29	N/A	**ORR 31** Skin 31 Mucosa 70 Liver 50	GI 60 Ocular 27 Lung 57
Foss[76]	Single arm prospective	2005	N/A	25	64	**ORR 64** Skin 80	Mucosa 24 GI 46
Couriel[91]	Retrospective	2006	N/A	71	20	ORR 61 Skin 57 Mucosa 78	Liver 71 Ocular 67 Lung 54
Dignan[53]	Retrospective	2012	N/A	82	7	**ORR 79**	Skin 100 Mucosa 91
Del Fante[92]	Retrospective	2012	N/A	102	16	**ORR 81**	
Malagola[59]	Retrospective	2016	N/A	49	45	**ORR 80**	
Pierelli[85]	Consensus statement	2013	23	735		Skin 64 Mucosa 47-57	Liver 27 GI 57
Scarisbrick[93]	Consensus statement	2008	23	521		Skin 68 Mucosa 63	Liver 63
Arun Alfred[52]	Consensus statement	2017	27	725		**ORR 68** Skin 74 Mucosa 62 Liver 62	GI 46 Ocular 60 Lung 46

*Included children and adults. No statistical difference in CR an OR between children and adults. Adults CR 26%; OR 78%. Paediatric CR 39%; OR 69%. *ECP: extracorporeal photopheresis, OR: overall response, CR: complete response, ORR: overall response rate, GI: gastrointestinal.*

TABLE 6. Published response rates for extracorporeal photopheresis in the treatment of paediatric chronic graft-versus-host disease.

Lead author	Year	N	OR, %	CR, %	Discontinuation of steroids, %	Tapering of steroids, %
Kanold[64]	2005	63	63			
Perseghin[66]	2007	12	75			
Salvaneschi[61]	2001	14	78	64	67	
Halle[94]	2002	8			62	38
Messina[62]	2003	44	73	34	44	73*
Kanold (prospective)[65]	2007	15	73	27	27	Median tapering 30% after 10 sessions of ECP in responders
Berger[63]	2007	10	100% limited GVHD 28% extensive GVHD	40		
Perotti[71]	2010	23		22	26% @ 30 days	
Gonzalez Vicent[70]	2010	6	90	50		

*Included discontinuation, *OR: overall response, CR: complete response, ECP: extracorporeal photopheresis, GVHD: graft-versus-host disease.*

there is a PR, continue with one cycle/four weeks to maximum response, taper and stop; if minor disease (MiD), SD or PD: stop.[4,53,74-77]

ECP has the highest specific response rate in cutaneous and oral mucosa cGVHD (50-85%) with improvement of both lichenoid and sclerodermic forms, followed by ocular (37-78%), liver (33-77%), lung (11-63%), musculoskeletal (18-94%) and gut (9-83%) cGVHD, with conflicting information existing for bronchiolitis obliterans.[78-81] In general, CRs are uncommon; as among patients with skin disease, CR has been reported in only 10-20%.

The survival advantage of ECP in cGVHD has been well documented and is mainly attributed to steroid tapering or discontinuation. Patients benefit directly from steroid tapering, but this effect cannot be achieved without offering them an alternative protection from debilitating complications of non-controlled cGVHD.[82-84] In a study reported by Messina *et al.*, the five-year overall survival was 58% for non-responders *vs* 96% for responders.[62] There is also a suggestion that, in addition to clinical responses, ECP may also lead to an improvement in quality of life in cGvHD.[73,85] The same statements as for cGVHD hold true for patients with overlap syndrome, although based on scant evidence.[84] Available data from the literature are summarised in *Tables 6-8*. The standard use of ECP as a therapeutic option to treat steroid-refractory and -resistant cGvHD is also endorsed by national and international guidelines and consensus papers.[51,52,54,85-89]

APHERESIS CRYOPRESERVATION

So far, few treatment centres that use the off-line method have frozen collected cells in aliquots to be thawed, treated and reinfused at a later time. This practice allows patients who travel long distances, lack appropriate intravenous access or cannot tolerate multiple apheresis procedures like children or because of their level of illness, to receive treatment.[69,95,96]

More interestingly, Radwanski *et al.* reported that cryopreservation did not impair the apoptotic or anti-proliferative responses of ECP-treated lymphocytes from healthy volunteers, which could allow cryopreservation of treated cells.[97] While this method promises important logistical improvements in patient treatment, additional studies are needed

TABLE 7. Published survival data for adults with chronic graft-versus-host disease.

Lead author	Year	N	Years F/Up	OR, %
Messina[62]	2003	44	5	96
Couriel[91]	2006	71	5	41

* Responders only, *F/Up: follow-up, OS: overall survival.*

TABLE 8. Published survival data for children with chronic graft-versus-host disease.

Lead author	Year	N	Years F/Up	OR, %
Salvaneschi[61]	2001	14	3	79
Halle[94]	2002	8	3.6	75
Berger[63]	2007	10	2.6	40
Kanold[65]	2007	15	4.3	67
Perotti[71]	2010	23	5	83
Messina[62]	2003	44	5	77 (96 in responders)

F/Up: follow-up, OS: overall survival.

to determine if these results from healthy subjects are reproducible with patient lymphocytes and if the *in vivo* effectiveness of the cryopreserved ECP-treated cells are maintained. We also need to improve our knowledge on the optimal cell dose to infuse per treatment.

HOW SHOULD EXTRACORPOREAL PHOTOPHERESIS QUALITY BE MONITORED?

According to European guidelines for minimal cell manipulation (Directive 2006/86/EC; Regulation 1394/2007/EC), off-line procedures should be performed in a Class A laminar-air-flow cabinet located in a Class D laboratory. During off-line procedures, cultures of the product for aerobic and anaerobic bacteria and fungi should be done immediately before reinfusion into the patient. Sterility controls before the introduction of 8-MOP are encouraged at least in two non-consecutive off-line procedures of each therapeutic course.

The number of lymphomononuclear cells treated with each ECP cycle is one of the major challenges in standardisation of this treatment modality. There is still no recommendation of a minimum number of cells to be processed per ECP session or an amount of blood volume to be processed for collection of cells.[47] Collected cells from as low as 3.3 x 10^8 (mini ECP) to up to 2.8 x 10^9 have been reported with adequate clinical response. Some studies suggested that CD3+ T-cell dose harvested during the early treatment phase has an impact on subsequent clinical response.[98] This 'cell dose effect' could nevertheless be the reflect of a minimum threshold needed to trigger a therapeutic response rather than a true correlation between cell dose and therapeutic response, or it could be a surrogate marker of the presence of a large number of alloreactive lymphocyte clones in the patient blood. Other studies underline a role for myeloid and plasmocytoid dendritic cell precursors or immature peripheral blood circulating B cells at baseline. Finally, an increase in the Treg population, early during treatment course, has also been correlated to response.[99,100]

The highest cell numbers are collected when using conventional off-line apheresis compared to the on-line system. Most of the clinical data come from the on-line system and from two consecutive days of treatment per cycle. It is, nevertheless, possible that one day of treatment per cycle instead of two could be sufficient if enough mononuclear cells can be collected in a single apheresis procedure. If

KEY MESSAGES FOR CLINICAL PRACTICE

1. **Around 50% of all allogeneic haematopoietic stem cell transplantation patients develop graft-versus-host disease.**
2. **Graft-versus-host disease is responsible for one third of transplant-related deaths.**
3. **Around 40% of patients become steroid-resistant or fail to respond at a safe dose.**
4. **Extracorporeal photopheresis has emerged as a safe and efficacious non-pharmacologic immunomodulatory approach for the management of the resistant graft-versus-host disease.**
5. **Extracorporeal photopheresis access is part of the new standards for JACIE accreditation but is, however, still not reimbursed by RIZIV/INAMI. The reimbursement issue drastically limits extracorporeal photopheresis access for patients in Belgium and creates a major difficulty for the Belgium transplant centres to comply with JACIE standards for accreditation.**

this could be confirmed in a large trial, it could decrease the cost of ECP and make ECP more acceptable for patient quality of live.

As there is no consensus on cell number, critical cell subtypes and the central mechanism of action, there are no accepted standard, valid procedures to qualify the ECP product in a way that is predictable of its *in vivo* efficacy. Some teams have proposed a functional test to show the reduction in lymphocyte proliferative capacity on mitogen stimulation by carboxyfluorescein succinimidyl ester (CFSE) labelling.[101,102] Two mitogens, PHA and CD3-CD28, could be used in parallel. Alternatively, measuring early and late 8-MOP-induced apoptosis by simultaneous staining with annexin V-FITC and propidium iodide could also be used to confirm the technical efficacy of the procedure on mononuclear cells. Taverna *et al*. recommended assessing apoptosis at 24h with a goal of a minimum differential apoptosis rate of 15% between the ECP product and the control sample of the untreated apheresis product. Analysing apoptosis is less time-consuming (24h) than proliferation assays (3-5 days of culture), and easier too.[103] Nevertheless, independently of the tests used, the question on how to define a threshold for considering an ECP procedure 'unsuitable' still warrants further investigations and, currently, stays an open question.

COST EFFECTIVENESS AND REIMBURSEMENT ISSUE

The cost of ECP could be covered by the money saved from the decrease of GVHD or GVHD treatments-related morbidities.[18] It is clearly demonstrated that ECP reduces the rate and duration of hospitalisations associated with serious infections due to immunosuppressive treatments.[19,20] Cost-effectiveness data from Spain and another analyses, conducted in Poland, showed that ECP is the most cost-effective alternative in the management of patients affected by cGVHD.[21,22] ECP is registered as standard therapy covered by social security in most of the European community countries. It is, nevertheless, highly paradoxical that ECP access is currently part of the new standards for the accreditation of the Joint Accreditation Committee for the International Society for Cellular Therapy and European Group for Blood and Marrow Transplantation (JACIE), which is mandatory to be authorised to perform allogeneic transplantation in Belgium, because it is still not reimbursed by RIZIV/INAMI (Federal Institute for Health Insurance). The reimbursement issue drastically limits ECP access for patients in Belgium and creates a major difficulty for the Belgium transplant centres to comply with JACIE standards for accreditation.

DISCUSSION

Although numerous studies on ECP, including those with open-label randomised designs, are available, the quality of evidence on ECP as a treatment option for GVHD is somewhat limited in part due to the absence of blind studies of ECP. Many of the studies quoted in the recommendations are also retrospective in nature. The predominant indication for ECP is the second line management of GVHD, and, as such, the delivery of an ECP service has been included in the Foundation for the Accreditation of Cellular Therapy (FACT)-JACIE quality standard recommendations for allogeneic hematopoietic stem cell transplant units. The standardisation of ECP treatment may be important in delivering

consistent therapy and produce reliable outcomes.[52] Despite the number of proposed biomarkers, there is currently insufficient evidence to recommend the routine use of biomarkers for the diagnosis, risk stratification or assessment of therapy response of GVHD. Studies, including biobanking of samples, attempting to identify biomarkers that could predict response and strict response criteria are being conducted and will help to advance the field significantly.[52] Research will fill the current gaps in the knowledge on how exactly ECP influences the functional integration of various immune components with dissimilar activities. With emerging GVHD therapies modulating the JAK-STAT and BTK pathways, the treatment options for GVHD patients are growing. Recently, ruxolitinib has shown very promising activity as rescue therapy for aGVHD and cGVHD refractory to standard therapy. Clinical trials comparing JAK-STAT and BTK inhibitors with ECP as second line for steroid-refractory GVHD are necessary to generate accurate treatment algorythms.[104] Clinical trials are essential to define the optimal use of ECP in the field of allogeneic HSCT; studies to evaluate its prophylactic use and as combination up-front therapy should be pursued.

CONCLUSION

Clinicians should consider ECP early on as a promising effective, safe and cost-effective therapeutic modality for those patients who do not have a fast and satisfactory response to corticosteroids for the treatment of GVHD irrespective of disease extent and severity. Multiple reports of treatments in both paediatric and adult patients with GVHD have been published, and the overall favourable profile compared with other available immunosuppressive therapies continues to make ECP appealing despite all of the unknowns.

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For the complete list of references, we refer to the electronic version of this article, which can be downloaded from ariez.com.

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