Extracorporeal photochemotheray 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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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 10⁹/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).
and is based on an integrated, automated closed loop using a single device integrating the photoactivation chamber. At least $1 \times 10^9$ cells in the peripheral blood are recommended before initiating ECP therapy.\textsuperscript{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.\textsuperscript{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.\textsuperscript{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%.\textsuperscript{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.
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. 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. 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. On reinfusion of irradiated cells, the cytokine network shifts with an increase in inhibitory cytokines (interleukin-10, interleukin-4, transforming growth factor β [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. Moreover, Rieber et al. demonstrated that ECP treatment in GVHD patients increases neutrophilic myeloid-derived suppressor cells, which modulate Th1 and Th17 responses. 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. 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. 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. These critical data underline 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. 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.

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...
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 preliminary check to confirm if the patient’s venous access is adequate is therefore essential before planning the start of treatment. The repeated, prolonged venous access required for ECP often necessitates the use of an implantable vascular access device (IVAD), a tunneled central venous catheter (TCVC) or a tunneled 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. 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.

**INDICATIONS OF EXTRACORPOREAL PHOTOPHERESIS IN GRAYT-VERSUS-HOST DISEASE**

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

<table>
<thead>
<tr>
<th>Lead author</th>
<th>Year</th>
<th>N</th>
<th>CR, %</th>
<th>OR, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abu Dalle</td>
<td>2014</td>
<td>54</td>
<td>Skin 84</td>
<td>Gl 65</td>
</tr>
<tr>
<td>Greinix</td>
<td>2006</td>
<td>59</td>
<td>Skin 82</td>
<td>Liver 61 Gl 61</td>
</tr>
<tr>
<td>Perfetti</td>
<td>2008</td>
<td>23</td>
<td>52</td>
<td></td>
</tr>
<tr>
<td>Jagasia</td>
<td>2013</td>
<td>57</td>
<td>54</td>
<td></td>
</tr>
<tr>
<td>Malagola</td>
<td>2016</td>
<td>45</td>
<td>Grade II 97</td>
<td></td>
</tr>
</tbody>
</table>

treated for aGVHD, there is a decreased number of UVA irradiated cells, which in turn can limit the efficacy of ECP. 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.

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

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

<table>
<thead>
<tr>
<th>Lead author</th>
<th>Year</th>
<th>N</th>
<th>Years F/Up</th>
<th>OR, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Malik</td>
<td>2014</td>
<td>595</td>
<td>1</td>
<td>49</td>
</tr>
<tr>
<td>Greinix</td>
<td>2006</td>
<td>59</td>
<td>4</td>
<td>59*</td>
</tr>
<tr>
<td>Perfetti</td>
<td>2008</td>
<td>23</td>
<td>Up to 81 months**</td>
<td>38</td>
</tr>
</tbody>
</table>

*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

<table>
<thead>
<tr>
<th>Lead author</th>
<th>Year</th>
<th>N</th>
<th>OR, %</th>
<th>Discontinuation of steroids, %</th>
<th>Tapering of steroids, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Salvaneschi</td>
<td>2001</td>
<td>9</td>
<td>78</td>
<td>43*</td>
<td></td>
</tr>
<tr>
<td>Messina</td>
<td>2003</td>
<td>33</td>
<td>76</td>
<td>42*</td>
<td>36</td>
</tr>
<tr>
<td>Berger</td>
<td>2007</td>
<td>15</td>
<td>Grade II: 100 Grade III: 75 Grade IV: 0</td>
<td>Grade II: 100 Grade III: 75 Grade IV: 0</td>
<td></td>
</tr>
<tr>
<td>Kanold</td>
<td>2005</td>
<td>41</td>
<td>73</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kanold</td>
<td>2007</td>
<td>12</td>
<td>83</td>
<td>30</td>
<td>33</td>
</tr>
<tr>
<td>Perseghin</td>
<td>2007</td>
<td>10</td>
<td>70</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gonzalez-Vicent</td>
<td>2008</td>
<td>8</td>
<td>100</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Calore</td>
<td>2008</td>
<td>15</td>
<td>100</td>
<td>67</td>
<td></td>
</tr>
<tr>
<td>Merlin</td>
<td>2010</td>
<td>12</td>
<td>83</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gonzalez-Vicent</td>
<td>2010</td>
<td>21</td>
<td>90</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Perotti</td>
<td>2010</td>
<td>50</td>
<td>68</td>
<td>16 at 30 days</td>
<td></td>
</tr>
</tbody>
</table>

*Responders, OR: overall response.
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 reevaluate the response; if there is a CR, taper to one cycle/four weeks for three months and stop if

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

<table>
<thead>
<tr>
<th>Lead author</th>
<th>Year</th>
<th>N</th>
<th>Years F/Up</th>
<th>OS, %</th>
<th>PFS, %</th>
<th>DFS, %</th>
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<tr>
<td>Salvaneschi93</td>
<td>2001</td>
<td>9</td>
<td>0.75</td>
<td>55</td>
<td></td>
<td></td>
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<tr>
<td>Messina62</td>
<td>2003</td>
<td>33</td>
<td>5</td>
<td>69*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Berger63</td>
<td>2007</td>
<td>15</td>
<td>N/A</td>
<td>100% Grade II; 30% Grade II-IV</td>
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<td></td>
</tr>
<tr>
<td>Kanold65</td>
<td>2007</td>
<td>12</td>
<td>N/A</td>
<td>67</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Calore68</td>
<td>2008</td>
<td>15</td>
<td>2</td>
<td>85</td>
<td>87</td>
<td></td>
</tr>
<tr>
<td>Gonzalez-Vicent70</td>
<td>2010</td>
<td>21</td>
<td>4</td>
<td>43</td>
<td></td>
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<tr>
<td>Perotti71</td>
<td>2010</td>
<td>50</td>
<td>5</td>
<td>46</td>
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<tr>
<td>Merlin69</td>
<td>2010</td>
<td>12</td>
<td>5</td>
<td>57</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Responders, F/Up: follow-up, OS: overall survival, PFS: progression free survival, DFS: disease free survival.
<table>
<thead>
<tr>
<th>Lead author</th>
<th>Type</th>
<th>Year</th>
<th>No. studies</th>
<th>N</th>
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<th>OR, %</th>
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</thead>
<tbody>
<tr>
<td>McKenna&lt;sup&gt;58&lt;/sup&gt;</td>
<td>Meta-analysis</td>
<td>2006</td>
<td>23</td>
<td>521</td>
<td></td>
<td>68</td>
</tr>
<tr>
<td>Abu Dalle&lt;sup&gt;50&lt;/sup&gt;</td>
<td>Meta-analysis</td>
<td>2014</td>
<td>5</td>
<td>87</td>
<td></td>
<td><strong>ORR 64</strong>&lt;br&gt; Skin 71 Mucosa 63 Liver 58 Gl 62 Lung 15</td>
</tr>
<tr>
<td>Malik&lt;sup&gt;63&lt;/sup&gt;</td>
<td>Meta-analysis</td>
<td>2014</td>
<td>18</td>
<td>595</td>
<td>26</td>
<td><strong>ORR 64 (65-82)</strong>&lt;br&gt; Skin 74 Mucosa 72 Liver 68 Gl 53 Ocular 60 Lung 48</td>
</tr>
<tr>
<td>Flowers&lt;sup&gt;25&lt;/sup&gt;</td>
<td>Randomised multicentric prospective phase II study</td>
<td>2008</td>
<td>N/A</td>
<td>48 ECP vs 47 immuno-suppressive drugs alone</td>
<td>N/A</td>
<td>Skin 40 vs 10 Mucosa 53 vs 27 Liver 29 vs NA Ocular 30 vs 7 Joint 22 vs 12</td>
</tr>
<tr>
<td>Seaton&lt;sup&gt;90&lt;/sup&gt;</td>
<td>Prospective</td>
<td></td>
<td></td>
<td>N/A</td>
<td></td>
<td>Skin 53 Mucosa 50</td>
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<tr>
<td>Berger&lt;sup&gt;83&lt;/sup&gt;</td>
<td>Single arm prospective</td>
<td>2007</td>
<td>N/A</td>
<td>10</td>
<td></td>
<td><strong>ORR 40</strong></td>
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<tr>
<td>Greinix&lt;sup&gt;27&lt;/sup&gt;</td>
<td>Crossover prospective</td>
<td>2011</td>
<td>N/A</td>
<td>29</td>
<td>N/A</td>
<td><strong>ORR 31</strong>&lt;br&gt; Skin 31 Mucosa 70 Liver 50 Gl 60 Ocular 27 Lung 57</td>
</tr>
<tr>
<td>Foss&lt;sup&gt;76&lt;/sup&gt;</td>
<td>Single arm prospective</td>
<td>2005</td>
<td>N/A</td>
<td>25</td>
<td>64</td>
<td><strong>ORR 64</strong>&lt;br&gt; Skin 80 Mucosa 24 Gl 46</td>
</tr>
<tr>
<td>Couriel&lt;sup&gt;91&lt;/sup&gt;</td>
<td>Retrospective</td>
<td>2006</td>
<td>N/A</td>
<td>71</td>
<td>20</td>
<td><strong>ORR 61</strong>&lt;br&gt; Skin 57 Mucosa 78 Liver 71 Ocular 67 Lung 54</td>
</tr>
<tr>
<td>Dignan&lt;sup&gt;53&lt;/sup&gt;</td>
<td>Retrospective</td>
<td>2012</td>
<td>N/A</td>
<td>82</td>
<td>7</td>
<td><strong>ORR 79</strong>&lt;br&gt; Skin 100 Mucosa 91</td>
</tr>
<tr>
<td>Del Fante&lt;sup&gt;22&lt;/sup&gt;</td>
<td>Retrospective</td>
<td>2012</td>
<td>N/A</td>
<td>102</td>
<td>16</td>
<td><strong>ORR 81</strong></td>
</tr>
<tr>
<td>Malagola&lt;sup&gt;59&lt;/sup&gt;</td>
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<td>2016</td>
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<td>45</td>
<td><strong>ORR 80</strong></td>
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<tr>
<td>Pierelli&lt;sup&gt;85&lt;/sup&gt;</td>
<td>Consensus statement</td>
<td>2013</td>
<td>23</td>
<td>735</td>
<td></td>
<td>Skin 64 Mucosa 47-57    Liver 27 Gl 57</td>
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<td>Scarsbrick&lt;sup&gt;23&lt;/sup&gt;</td>
<td>Consensus statement</td>
<td>2008</td>
<td>23</td>
<td>521</td>
<td></td>
<td>Skin 68 Mucosa 63       Liver 63</td>
</tr>
<tr>
<td>Arun Alfred&lt;sup&gt;62&lt;/sup&gt;</td>
<td>Consensus statement</td>
<td>2017</td>
<td>27</td>
<td>725</td>
<td></td>
<td><strong>ORR 68</strong>&lt;br&gt; Skin 74 Mucosa 62 Liver 62 Gl 46 Ocular 60 Lung 46</td>
</tr>
</tbody>
</table>

there is a PR, continue with one cycle/four weeks to maximum response, taper and stop; if minor disease (MiD), SD or PD: stop.4,5,3,7-74
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.5,82,84 In a study reported by Messina et al., the five-year overall survival was 58% for non-responders vs 96% for responders.82 There is also a suggestion that, in addition to clinical responses, ECP may also lead to an improvement in quality of life in cGVHD.71,85

**APHERESIS CRYOPRESERVATION**

So far, few treatment centres that use the off-line method have frozen collected cells in aliquots to be thawed, treated and rein infused 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.86,95,96

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.
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. Collected cells from as low as 3.3 x 10⁸ (mini ECP) to up to 2.8 x 10⁹ 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. 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 plasmacytoid 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. 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

| TABLE 7. Published survival data for adults with chronic graft-versus-host disease. |
|-----------------|-----------------|-------|-------------|-------|
| Lead author     | Year | N    | Years F/Up | OR, % |
| Messina⁵²       | 2003 | 44   | 5           | 96    |
| Couriel⁸¹       | 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, % |
| Salvaneschi⁶¹   | 2001 | 14   | 3           | 79    |
| Halle⁹⁴        | 2002 | 8    | 3.6         | 75    |
| Berger⁶³       | 2007 | 10   | 2.6         | 40    |
| Kanold⁵⁵      | 2007 | 15   | 4.3         | 67    |
| Perotti⁷¹      | 2010 | 23   | 5           | 83    |
| Messina⁶²      | 2003 | 44   | 5           | 77 (96 in responders) |

F/Up: follow-up, OS: overall survival.
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.\(^\text{101,102}\) 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.\(^\text{103}\) 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.\(^\text{19,20}\) It is clearly demonstrated that ECP reduces the rate and duration of hospitalisations associated with serious infections due to immunosuppressive treatments.\(^\text{19,20}\) 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.\(^\text{21,22}\) 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. 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.

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 algorithms.

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.

REFERENCES
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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