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Potential Biomarkers of Therapeutic Response to ECP in Solid Organ Transplantation

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Abstract. One of the major hurdles in solid organ transplantation is graft rejection, which must be prevented with lifelong general immunosuppression. However, modern maintenance immunosuppression is accompanied by serious side effects, such as an increased risk of infection and malignancies. The search for alternative therapies specifically controlling allogeneic responses is fueling renewed interest in extracorporeal photopheresis (ECP). Despite guideline indications for ECP in cardiothoracic transplantation, potential applications in liver and kidney transplantation have not been adequately investigated. Presently, limited understanding of the pharmacodynamic effects of ECP and lack of consensus biomarkers are hindering the development of standardized multiparametric assays to assess patient responses. This review explores current knowledge about immune responses after ECP in transplant recipients and collates a set of biomarkers associated with favorable treatment responses.

(*Transplantation Direct* 2025;11: e1817; doi: 10.1097/TXD.0000000000001817.)

Extracorporeal photopheresis (ECP) is an ex vivo immunomodulatory therapy in which leukocytes are treated with a photosensitizing agent and then exposed to UV A (UVA) irradiation to induce apoptosis before reinfusion into patients.[1] ECP was first approved by the US States Food and Drug Administration in 1988 for treating Sezary syndrome, an advanced type of cutaneous T-cell lymphoma.[2] Originally, ECP was introduced as a procedure to debulk circulating malignant T cells, thereby reducing cutaneous lymphoid infiltrates. This concept was naturally extended to increasing turnover of pathogenic T cells and B cells responsible for allo- and autoimmune disease.

Received 26 November 2024. Revision received 26 March 2025.

Accepted 14 April 2025.

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This work was funded by the European Union through the exTra doctoral network (grant 101119855).

The authors declare no conflicts of interest.

H.V. participated in writing the original draft. M.I.-E. and E.M.-C. participated in writing, reviewing, and editing.

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ISSN: 2373-8731

DOI: 10.1097/TXD.0000000000001817

During ECP treatment, whole blood is collected from the patient through a peripheral venous catheter. White blood cells (WBCs) are then separated from red blood cells and platelets by centrifugation. Red blood cells, platelets, and plasma are immediately returned to the patient, and a concentrated WBC fraction is retained (Figure 1). To these WBCs, 8-methoxypsoralen (8-MOP) is then added, which binds intercalates into DNA, sensitizing cells to UVA light. On UVA irradiation, 8-MOP forms covalent DNA adducts that result in double-stranded DNA breaks, which trigger apoptosis in treated cells. The resulting mixture of living and dying leukocytes, as well as the conditioned plasma in which they are suspended, is called a "photopheresate." This immunologically complex product is then reinfused into patients.[3] ECP regimens vary widely depending upon clinical indications, local practice, and the particular devices being used. However, historical precedent means that most protocols consist of cycles of 2 consecutive days of ECP treatment, which are repeated with intervals of several weeks.[1]

Major advances have been made in treating both acute and chronic graft-versus-host disease (GvHD) and rejection of heart and lung transplants.[4] Over many decades, ECP has proven to be safe, with only limited procedurally related side effects, the most common being mild hypotension.[5,6] This lies in stark contrast with the severe side effects associated with immunosuppressant (IS) regimens used to prevent transplant rejection. These treatments heighten the risk of infections and malignancies and, in the case of calcineurin inhibitors, are nephrotoxic.[6,7]

ECP is only sparsely applied and currently not approved for clinical use in solid organ transplantation (SOT), despite some trials showing promising results in preventing and reducing rejection events, increasing graft lifespan, and allowing for IS sparing.[8] This possibly reflects our current lack of mechanistic knowledge, absence of consensus guidelines about ECP in abdominal organ transplant recipients, and inability to

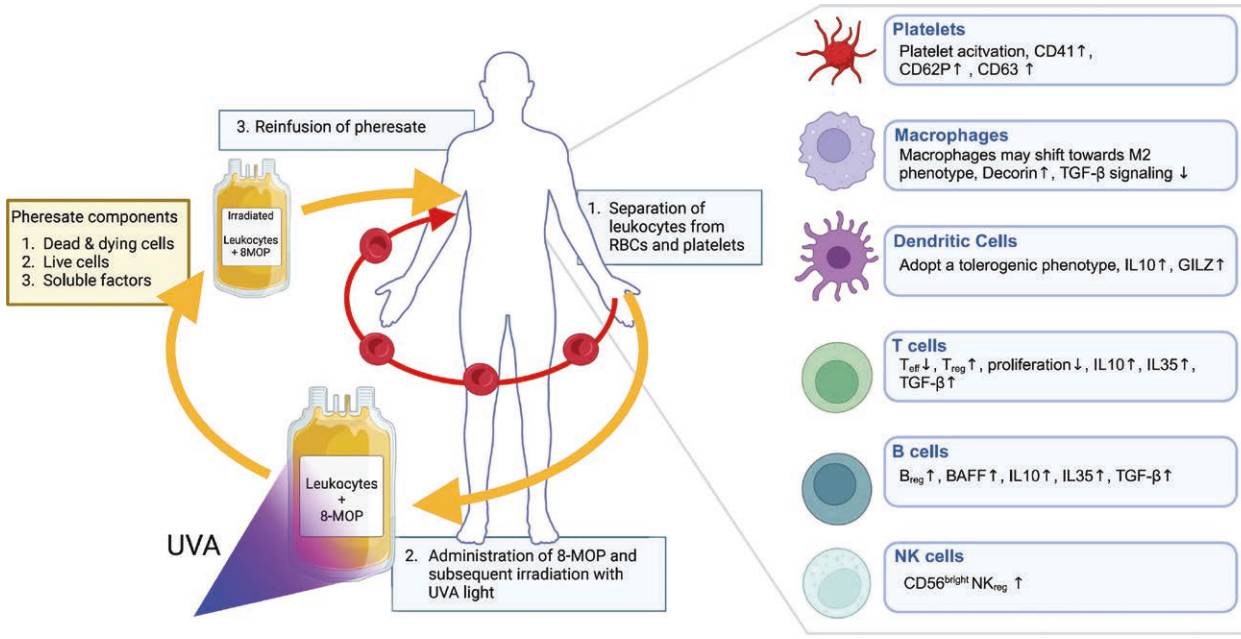

FIGURE 1. A standard extracorporeal photopheresis procedure. Blood is drawn from the patient continuously and leukocytes are separated from RBCs and platelets. The concentrated leukocyte fraction is exposed to 8-MOP, which binds covalently to the DNA. Subsequent irradiation with UVA light causes activation of 8-MOP resulting in extensive DNA damage. The treated leukocyte fraction called pheresate consists of soluble factors, dead and dying cells, and live cells. Subsequent intravenous administration of the pheresate leads to immunomodulation. Created in BioRender. Veltman, H. (2025; https://BioRender.com/1qaq2hv). 8-MOP, 8-methoxypsoralen; RBC, red blood cell; UVA light, UV A light.

measure the therapeutic effects of ECP in patients. To address these deficiencies, our group is participating in exTra, an EU-funded collaborative network investigating applications of ECP and standardized clinical analysis in SOT.[9-12] This review aims to discuss potential biomarkers of therapeutic responses to ECP in SOT recipients.

COMPOSITION OF PHERESATES AND ITS SYSTEMIC EFFECTS

The composition of pheresates can vary significantly depending on the modality used. The 2 primary systems are inline and offline ECP. The inline method is a closed system in which leukocyte separation and treatment occur simultaneously, whereas the offline method separates these procedures. A third approach, mini-ECP, is designed for patients with contraindications to conventional ECP, such as low body weight or limited venous access. In a single procedure, the inline, offline, and mini-ECP methods collect approximately ~5%–10%, ~12%–20%, and <10% of total circulating leukocytes, respectively.[13–15] Significant differences in platelet counts between modalities are reported too, which could affect monocyte activation.[14,16] Although differences in pheresate composition have been described, its effects on treatment efficacy have not been fully explored.[3,14,17]

While the immediate effects of ECP remain unclear, several events are known to occur. First, platelet interaction with plastic surfaces activates monocytes via P-selectin glycoprotein ligand-1 ligation. [13,18] Second, 8-MOP and UVA further activate monocytes, inducing differentiation into monocyte-derived dendritic cells (DCs). Third, injured leukocytes release damage-associated molecular patterns recognized by myeloid cells.[19] Fourth, DNA damage initiates apoptosis, especially in proliferating lymphocytes. In vitro ECP studies show most lymphocytes undergo apoptosis within 48 h in 2 waves: immediate apoptosis through mitochondrial dysfunction and caspase activation after 24 h.[20,21] Stepien et al described ECP-related cell death in further detail.[21]

Clinically, photopheresates are infused continuously or shortly after treatment, receiving a complex suspension of secondarily necrotic, apoptotic, preapoptotic, and living cells, as well as plasma fraction containing soluble mediators, metabolites, subcellular debris, and altered plasma proteins.[22,23] Altered cytokine profiles in both monocytes and lymphocytes have been reported.[24-29] Notably, interleukin (IL)-1β levels increase in both pheresate and peripheral blood after ECP therapy.[24-29] The systemic effects may extend beyond cytokines. MicroRNAs such as miR-23 b-3p and miR-155 are upregulated in lung transplant patients.[29,30] Additionally, ECP significantly increases neutrophil extracellular traps formation immediately after treatment in chronic GvHD, further highlighting its systemic effects.[24–31]

The effectiveness of ECP in treating a wide range of inflammatory and T cell–mediated diseases suggests its benefits stem from different photopheresate components. Some studies link increased apoptosis to clinical success, whereas others propose that surviving leukocytes mediate therapeutic effects. Standardized proliferation and cell death assays are routinely used to characterize and validate the pheresate.[32] The tetrazolium salt assay, combined with 7-aminoactinomycin D (7AAD) and carboxyfluorescein succinimidyl ester, quantifies metabolically active cells and indicates clinical potency of pheresates.[33] However, the effect of ECP may not exclusively be mediated by leukocytes. ECP strongly induces platelet activation, as evidenced by increased expression of CD41, CD62P, and CD63.[34] Activated platelets are known contributors to monocyte activation and modulate lymphocyte function.[13,35]

A major limitation is the poor documentation of photopheresate composition in clinical studies. Our research aims to identify key markers for photopheresates applicable in clinical and research settings. We hypothesize that standardized analysis will improve clinical response predictions and highlight the most immunologically relevant components.

IMMUNE MODULATORY EFFECTS OF ECP IN SOT

Dendritic Cells

Under homeostatic conditions, tissue resident immature DCs take up apoptotic cells and present self-antigens in the peripheral lymph nodes to naive T cells, inducing peripheral tolerance. It is hypothesized that ECP uses this mechanism to induce immune tolerance.[35] ECP has shown to modulate the immune landscape in both animal and human studies by increasing the frequency of regulatory T cells (Treg) in circulation, both $CD4^+$ $FoxP3^+$ Treg and IL-10 producing $CD4^+$ Tr1 cells; a process mediated by tolerogenic DCs (TolDCs).[36-39] ECP-induced preapoptotic leukocytes, administered intravenously, migrate to the spleen and secondary lymphoid tissues, where they release apoptotic-associated molecular patterns, which in turn lead to phagocytosis by resident DCs.[40] Removal of apoptotic leukocytes in the absence of inflammatory signals induces peripheral immune tolerance.[41,42] TolDCs show reduced CD80/86 and increased programmed death-ligand 1 expression, along with higher IL-10 and transforming growth factor beta (TGF-β) secretion.[43] Glucocorticoid-induced leucine zipper, a key regulator of this phenotype, is elevated in monocytes after ECP and is proposed as a marker for TolDCs[44]

T Cells

T cells play a central role in cell-mediated rejection by recognizing alloantigens and orchestrating the subsequent immune response. ECP increases Treg frequency and reduces T-cell proliferation and effector function through cell contact–dependent interactions and inhibitory cytokines, most importantly IL-10, IL-35, and TGF-β.[45-47] Quality control testing after ECP using a simple CD71 staining in the peripheral blood of healthy volunteers showed strong inhibition of T-cell proliferation, which may be used to assess response.[48] Interactions between $FoxP3^+$ Treg and DCs through these same mechanisms inhibit DC maturation and antigen presentation, further reducing T-cell activation.[45,49] This is a crucial first step in the response to ECP treatment, as T cells play a central role in cell-mediated rejection.

The effect of ECP in Treg subsets is extensively studied in GvHD. Increased and decreased $CD4^+$ and $CD8^+$ T cells, respectively, and increased $CD4^+CD25^+CD127^-$ Treg populations are associated with response in steroid-resistant patients.[50] This Treg subset can be further divided into $HLA\text{-}DR^-$ and $HLA\text{-}DR^+$ populations, with higher levels of the latter associated with a reduced risk of developing GvHD.[51] Elevated expression levels of CCR7 in central memory $CXCR3^+$ T cells are also associated with response, suggesting that lymph node homing is essential.[52] In heart transplant recipients, a highly suppressive FoxP3 Treg subset called FoxP3-E2 (exon 2) with elevated CD39 expression levels was found to be increased after ECP treatment.[53] Dieterlen et al[54] also reported increased levels of $CD39^+$ Treg, and increased and decreased expressions of CD62L and CD120b, respectively, were ascertained.[55]

Macrophages

After transplantation, recipient macrophages play a key role in tissue regeneration by removing cellular debris and promoting revascularization.[56] However, during rejection, macrophages shift phenotypically into a proinflammatory state, secreting tumor necrosis factor alpha, IL-6, and IL-1β. In addition, excessive expression of TGF-β contributes to tissue fibrosis, which is an important cause of graft failure.[57,58] ECP may counteract this proinflammatory role by promoting an increase in M2 macrophages exhibiting a tolerogenic and tissue-restorative phenotype. Regulatory macrophages have previously been shown to modulate T-cell immunity and induce a $TIGIT^+$ $FoxP3^+$ Treg phenotype.[59] Moreover, enhanced IL-10 production and reduced alloreactivity inhibit effector T (T_{eff}) cells and promote Treg expansion.[58] In a lung transplant mouse model, ECP was shown to decrease fibrosis by reducing alveolar macrophage-TGF-β signaling through expression of its antagonist, decorin, which may serve as a potential biomarker in bronchiolitis obliterans syndrome.[60] Research on the effects of ECP on macrophage function is limited and is currently being investigated by our collaborative partners.[61,62]

Natural Killer Cells

Natural killer (NK) cells are innate lymphocytes that recognize and kill allogeneic cells due to lack of self-major histocompatibility complex or through antibody-mediated cytotoxicity. During acute rejection, NK cells infiltrate the graft, releasing cytotoxic enzymes and expressing interferon-gamma and tumor necrosis factor alpha, which recruit T lymphocytes and myeloid cells.[63] Several studies in patients with GvHD show that ECP modulates NK cells by reducing their immune activity 24 h posttreatment,[64-66] promoting a regulatory phenotype while preserving their antiviral and antitumor functions.[65,66] Regulatory NK cells can differentiate from a $CD56^{bright}$ to a $CD56^{dim}$ population and subsequently maturate, gaining the capacity to kill alloreactive T cells.[66] Moreover, $CD56^{bright}$ NK cells have been associated with a positive response to ECP in patients with GvHD.[67] These data reveal a clear effect of ECP on NK cell function, which may contribute to increased graft acceptance. Thus, the $CD56^{bright}$ NK cell subset may be a suitable candidate to measure the response to ECP in transplant patients.

B Cells

Antibody-mediated rejection is another major contributor to transplant rejection. B lymphocytes play a key role in the humoral response by producing donor-specific antibodies, which activate the complement system, cause inflammation, and eventually cytolysis.[68] However, B cells can also suppress T-cell activity and promote graft tolerance through expression of IL-10, IL-35, and TGF-β. These cytokines together with cell contact–dependent mechanisms reduce antigen presentation in DCs and macrophages. Furthermore, regulatory B cells (Breg) increase programmed death-ligand 1 and FasL expression, inducing T-cell apoptosis.[69] B-cell depletion in transplantation models can either mitigate or exacerbate rejection, depending on the extent of rejection and timing of B-cell depletion, highlighting their dual role in graft rejection and acceptance.[70,71] Chandra et al[72] found increased levels of IL-10-producing Breg and Wang et al[73] observed increased Breg, defined as $CD24^+CD38^{high}$, in GvHD patients after ECP therapy. Furthermore, reduced levels of immature

TABLE 1.

The effect of ECP on immune cells and their role in graft rejection

Cell type	Role in organ rejection	Effect of ECP on cell fuction
DCs	Direct alloantigen-specific rejection through activation of naive T cells, induces expansion of Teff cells and expression of IL-6, IL-12, and TNF-α[89]	Secrete IL-10, inhibit T_{eff} cells and induce expansion of Tregs[38,90]
Macrophages	Increase tissue fibrosis due to excessive expression of TGF-β and expression of TNF-α, IL-6, and IL-1β[57,58]	Promotes M2 phenotype, induces IL-10 expression, inhibits T_{eff} cells, and induces expansion of Treg[60,61]
T_{eff} cells	$CD4^+$ T cells orchestrate immune response through expression of cytokines/ $CD8^+$ T cells express cytolytic enzymes targeting graft cells[91]	Dampenes T_{eff} function and inhibits expansion[38]
Treg	Reduced activity in acute and chronic rejection[92]	Contributes to graft tolerance, increases Treg expansion, induces expression of IL-10, and reduces T_{eff} function[50,53,55,93]
NK cells	Recognize and kill allogeneic cells, antibody-mediated cytotoxicity, release cytotoxic enzymes, express proinflammatory cytokines (INF-γ, TNF-α)[63]	Reduces cytotoxic activity, kills alloreactive T cells, and maintains antiviral and antitumoral activity[66,67]
B cells	Present antigens to T cells, produce DSAs, expression of proinflammatory cytokines (IL-6, TNF-α)[68]	Increases regulatory phenotype, inhibits alloreactive T cells through IL-10 and IL-35 expression, and reduces antigen presentation in DCs and macrophages[68,72,73]
MDSCs	Immunosuppressive regimens affect MDSC numbers and potentially reduce their immunoregulatory capacity[85,86]	Strong suppressive effect on T_{eff} cells[81-83]

DC, dendritic cell; DSA, donor-specific antibody; ECP, extracorporeal photopheresis; IFN, interferon; IL, interleukin; MDSC, myeloid-derived suppressor cell; NK, natural killer; T_{eff} cell, effector T cell; TGF, transforming growth factor; TNF, tumor necrosis factor; Treg, regulatory T cell.

$CD21^{low}$ B cells and decreased serum B-cell activating factor have also been associated with positive response in GvHD,[74,75] which were previously proposed as predictive biomarkers in Mankarious et al.[76] In lung transplant recipients(LTRs), ECP was found to reduce circulating donor-specific antibodies, which was associated with reduced lung function decline.[77] Additionally, quantifying antibody-secreting cells using standardized enzyme-linked immunospot assays may provide a reliable method to assess reduced antibody-mediated rejection after ECP therapy, as discussed in detail by Nogueira et al.[78]

The effect of ECP on B-cell responses in SOT remains unclear and the definition of Breg is still under debate. Rather than a distinct cell type, multiple B-cell subsets can adopt a regulatory phenotype depending on the microenvironment. Different B-cell subsets can initiate and subsequently terminate IL-10 expression, demonstrating the transient characteristics of Breg.[79] Despite these dynamics, multiple Breg subsets have been identified including B10, regulatory B1 (Br1), $GZMB^+$ Breg, and $TIM1^+$ Breg.[80] Further studies regarding the effect of ECP on Breg populations in SOT will be realized by our consortium partners.[78]

Myeloid-derived Suppressor Cells

Myeloid-derived suppressor cells (MDSCs) are a heterogenous group of myeloid cells that suppress T cells by creating a microenvironment rich in free radicals and depleted of L-arginine and cysteine, essential amino acids for T-cell expansion and function.[81-83] Although their precise role in SOT remains unclear, increased MDSC levels have been associated with graft tolerance. Meng et al[84] found that MDSCs frequency correlated with prolonged graft survival and promoted expansion of $CD4^+FoxP3^+$ Treg while reducing IL-17 expression in vitro. Iglesias-Escudero et al[85,86] observed increased monocytic MDSCs in kidney transplant recipients up to 12 mo posttransplantation and elevated polymorphonuclear (PMN)-MDSCs in LTRs, with the latter linked to long-term outcome. Heigl et al[87] also found increased PMN-MDSCs in stable LTRs compared with patients with chronic lung allograft dysfunction.

Despite promising results, limited data are available on the effect of ECP on MDSCs. Rieber et al[88] observed a sustained increase in PMN-MDSCs with strong T-cell inhibitory potential in GvHD patients post-ECP, correlating with remission. Increased monocytic MDSCs in acute GvHD after ECP therapy have been described too.[73] However, no data exist on the impact of ECP on MDSCs in SOT, and a lack of specific markers complicates standardized analysis. Nevertheless, their strong T-cell inhibitory capacity should not be overlooked when assessing transplant tolerance. Table 1 summarizes the role of each immune cell type in graft rejection and how ECP modulates their function.

ADVANCING PREDICTIVE TOOLS FOR ECP RESPONSE IN SOT

ECP effectively prevents rejection and prolongs graft survival in multiple trials,[4,94-97] but predicting patient response remains challenging, highlighting the need for biomarkers. Its effects vary by organ: heart transplant patients often experience prolonged rejection-free periods, whereas LTRs show only reduced functional decline.[4,94,95] No studies have analyzed biomarkers related to outcome in specific organ transplants, despite immune cell subset relevance varying by transplant type. Thus, organ-specific studies should identify markers associated with treatment response.

Currently, outcome assessment relies solely on clinical data (ie, rejection events, organ performance), requiring months of therapy. Standardized multiparametric immune assays with key biomarkers could help clinicians monitor response and predict outcome, potentially enabling the timely tapering of immunosuppressants. Achieving this requires comprehensive immune profiling to quantify and phenotype regulatory/effector cells and analyze the cytokine milieu. Furthermore, markers of allograft injury, such as donor cell-free DNA, can help detect injury at an early stage,[98] and advanced predictive tools like iBOX may enable clinicians to predict and assess superiority of ECP therapy.[99]

Spectral flow cytometry and multiparametric immunoassays are promising tools for standardized immune profiling. Spectral flow offers high resolution and is already used for

immune monitoring,[100-102] but consensus on relevant markers and assay standardization is needed and interassay variability must be addressed. Standardized immune phenotyping strategies exist, which could aid in establishing immune phenotype profiles for ECP.[103,104] Similarly, multiplex immunoassays, already effective in cytokine profiling in disease, may be crucial for understanding cytokine dynamics in ECP-treated transplant patients.[104]

CONCLUSIONS

ECP is a valuable addition to the clinician's toolbox in SOT, increasing organ lifespan and enabling IS sparing. Animal and human studies across various fields, including SOT, have partly elucidated the mechanism(s) of action of ECP. The availability of affordable multiparametric tools enhances our understanding of the immunomodulatory effects of ECP and helps identify key parameters in SOT. However, more randomized controlled trials are needed, particularly in the context of liver, kidney, and lung transplantation. These trials should include detailed immune monitoring to help establish standardized comprehensive immune profiling assays.

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