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# Transimmunization and the evolution of extracorporeal photochemotherapy

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## Abstract

We are now aware that extracorporeal photopheresis (ECP) – in which a patient's leukocytes are isolated, passed through an ultrathin clear plastic plate, and exposed to 8-methoxypsoralen (8-MOP) and ultraviolet A light prior to reinfusion – is a simple and efficient dendritic cell (DC) therapy and the first FDA approved selective immunotherapy for cancer. DCs, as the most effective antigen presenting cells (APCs), are central to many ongoing efforts to stimulate immune responses to cancer cells. Moreover, ECP has not only demonstrated efficacy in the treatment of a T cell malignancy – namely cutaneous T-cell lymphoma (CTCL) – but also in treatment of oligoclonal T-cell-mediated diseases such as graft-versus-host-disease (GVHD) and organ transplant rejection. Recent advances in the understanding of DC/T-cell interactions provide insight into how ECP-induced DCs (EI-DCs) can be utilized to stimulate specific T-cell (i.e. anti-tumor) responses, or down-regulate a pre-existing potent T-cell response. The mechanism of this apparent paradox of EI-DC functionality is likely dependent on several fundamental principles: (1) the status of existing in vivo T-cell reactions, (2) the temporal stage of EI-DC differentiation, and (3) the affinity of the available repertoire of T-cell receptors (TCRs) for the antigen(s) in question. Further investigation into DC/T-cell interactions will help to shape the future of ECP and the ability to optimize this therapy for the desired immune effect. To this end, we are developing and testing *Transimmunization* to replace conventional ECP. © 2002 Elsevier Science Ltd. All rights reserved.

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## 1. Introduction

Extracorporeal photopheresis (ECP) was originally introduced by Edelson et al. in 1987 for the management of patients with cutaneous T-cell lymphoma (CTCL) [1]. Today, ECP remains the *only* FDA approved tumor-targeting selective im-

muno-therapy for the treatment of any cancer, and it is in use in over 150 centers worldwide [2–8].

The reported full and persistent responses of CTCL to ECP are provocative for several reasons. First, spontaneous remissions do not occur in erythrodermic (leukemic) CTCL, indicating that such observed clinical results are due to ECP. Second, since approximately five percent of any particular patient's malignant cells are extracorporeally altered and returned by the ECP, the elimination of the untreated CTCL cells implies an induced anti-cancer immunization. Third, such

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a selective immunotherapeutic response suggests that CTCL cells may display tumor specific antigens. Fourth, if the mechanism underlying the efficacy of ECP can be deciphered, the therapy might be applicable to other forms of cancers, including solid tumors.

In addition to CTCL, ECP has been shown to have significant efficacy in the management of other T-cell mediated diseases, including graft-versus-host disease (GVHD) after allogeneic bone marrow transplantation [9–16], and progressive systemic sclerosis [17–19]. In multiple studies it has also ameliorated, reversed or prevented rejection of transplanted organs [20–26]. For example, a multi-institutional controlled trial, reported in a 1998 article in the *New England Journal of Medicine*, determined that combination of ECP with conventional immunosuppressive medication was substantially more efficacious than conventional therapy in the prevention of heart transplant rejection [20]. A paper in *Blood*, also in 1998, reported that ECP was particularly effective as well in the reversal of acute GVHD [9].

These studies underscore the ability of ECP to treat both T-cell malignancy, (e.g., CTCL), as well as T-cell mediated diseases (e.g., GVHD, and organ transplant rejection). Until recently, as will be discussed later in this review, it remained puzzling as to how a single treatment modality could both activate the immune system in the protection against a dangerous cancer and suppress T-cell activity in autoreactive disorders.

## 2. ECP as dendritic cell immunotherapy

While ECP has been used worldwide in numerous centers for over 20 years, and has shown efficacy for selected patients with CTCL and several T cell-mediated diseases, much additional investigation is necessary before the full potential of this immunotherapy can be harnessed. Several major investigative questions are reasonably posed, including the following: (1) What is the nature of DC induction by ECP, and can this be applied to malignancies other than CTCL? (2) How can the ability of ECP to down-regulate GVHD and lessen dependence on global immunosuppression post-

transplant be utilized in protocols that take full advantage of the graft-versus-tumor effect? (3) How are activated clones of T-cells suppressed by ECP, and how can this effect be improved upon?

Dendritic cells (DCs) are antigen presenting cells (APCs) which can express an array of costimulatory molecules to become potent activators of T-cell immune responses [27]. Several strategies are being assessed which expose DCs to tumor antigens *ex vivo* before returning them to the patient in attempt to stimulate anti-tumor immunity [28]. Recent work by Berger et al., [29] has shown that ECP – in which a patient's leukocytes are isolated, passed through an ultrathin clear plastic plate, and exposed to 8-methoxypsoralen (8-MOP) and ultraviolet A (UV) light prior to reinfusion – is in fact an efficient dendritic cell immunotherapy for cancer. In the treatment of CTCL, ECP simultaneously induces monocyte-to-dendritic cell differentiation and malignant T-cell apoptosis. The ECP-induced DCs (EI-DCs) actively phagocytose, process, and present tumor-specific CTCL antigens. (This work is reviewed in detail in this issue of *Journal of Apheresis and Transfusion Science* in a separate paper by Berger et al.)

Although more than 10,000 ECP treatments have been administered at the Yale Medical Center over the past 12 years, as well as nearly 250,000 worldwide, two major factors have limited its broad application to the treatment of malignancies other than CTCL and prevention/reversal of rejection of transplanted organs. First, the mechanism underlying its capacity to activate the immune system to effectively control aberrant (malignant or autoreactive) cells was not clearly elucidated. This lack of complete understanding precluded further refinement of the method. Second, ECP in its current state was not amenable to further manipulation (i.e., in order to optimize its immunomodulatory effects).

Berger et al. [29] demonstrated that two major effects on peripheral leukocytes occur after passage through the ECP apparatus: the induction of apoptosis within the lymphocyte population, including the malignant CTCL cells, as well as the initiation of a large scale monocyte-to-DC differentiation. (The major component of ECP which drives this monocyte-to-DC differentiation is the

sheering forces and repeated contact with the large plastic surface of the exposure plate. For more details of this phenomenon, see Berger et al., in this issue.) Furthermore, the new DCs readily phagocytose the apoptotic T cells, and begin to express low levels of co-stimulatory molecules such as B7.1 and B7.2 required for the initiation of cellular immunologic reactions. Hence, ECP provides an apparently ideal means to simply stimulate anti-tumor (anti-T-cell) immunity after antigen processing. This is the first plausible explanation of the observed clinical impact of ECP.

In the case of CTCL, the relevant antigens likely include those that are T-cell receptor (TCR) derived [30,31]. Each malignant clone of T-cells expresses a TCR unique to that clone, which therefore represents a potential tumor-specific antigen. Previously, Berger et al., had shown that MHC-I motif-conforming TCR peptides (but not motif-confirming non-TCR peptide controls) from two different CTCL patients selectively stimulated CD8+ T-cells isolated from the same patient when loaded onto autologous immortalized B cells serving as APCs). In the analogous situation of B cell malignancies, peptides derived from the clone-specific immunoglobulin gene sequences have been successful targets of immunotherapy [32].

While TCR-peptides may provide tumor-specific antigens, any peptide distinguishing the malignant clone from normal T-cells may also serve as potential tumor antigens. Thus, a major advantage of ECP-stimulated anti-tumor immunotherapy is that there is potential for any and all CTCL tumor antigens to be processed and presented – as opposed to other tumor vaccine strategies which may use a single or limited number of known peptides. For example, some investigators have demonstrated in CTCL cells the presence of HTLV tax-related sequences, another potential source of tumor antigens [33].

The ECP approach provides a clinically practical means of developing tumor-loaded cells maturing DCs without a requirement for exogenous cytokines, identification and isolation of tumor antigens, or excessive cellular manipulation. This notwithstanding, in its current state, ECP does not allow for manipulation of the key cellular players which could otherwise lead to novel therapeutic

approaches. For example, modification to the ECP procedure might allow for the development of patient-specific vaccines against solid tumors (Fig. 1).

We have initiated a clinical trial to assess the safety and efficacy of *Transimmunization*, which we propose as the replacement technology for ECP (see below). Transimmunization, which incorporates the crucial step of overnight culture of newly forming dendritic cells with apoptotic tumor cells, is being initially tested in CTCL. Development of anti-tumor vaccines via this method of large-scale dendritic cell loading with apoptotic tumor cell antigens is potentially useful in the immunotherapy of other cancers. In addition, this process may facilitate the down-regulation of potent antigen-specific immune reactions. The ultimate goals of

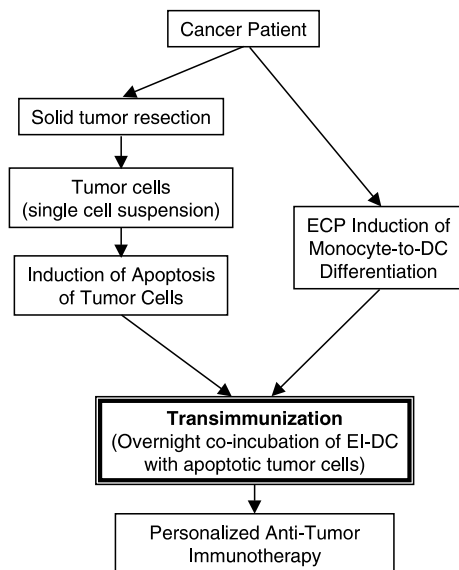


Fig. 1. Modification of ECP for the development of solid tumor vaccines. In this concept, a solid tumor is incompletely resected. The tumor cells are released into a single cell suspension. Apoptosis is induced in the malignant cells (i.e., by exposure to 8-MOP/UVA). ECP is performed on the patient, and the induction of monocyte-to-DC differentiation is stimulated. The apoptotic malignant cells and the newly forming ECP-induced DCs (EI-DCs) are co-incubated overnight. Phagocytosis of the apoptotic malignant cells by the EI-DCs further drives DC differentiation, and leads to processing and presentation of tumor-specific antigens. The tumor-loaded EI-DCs are inoculated into the patient to stimulate anti-tumor immunity.

Transimmunization are to further develop a novel selective immunotherapy for hematologic malignancies, treatment of GVHD, and prevention of rejection of transplanted organs.

### 3. ECP as an immunomodulator: theoretical perspective

The fact that ECP has clearly shown, in the clinical setting, the capacity to stimulate apparently diametrically opposed immune effects – the up-regulation of an anti-tumor immune response (e.g., against CTCL) and the down-regulation of autoimmune disease or allogeneic immune responses (e.g., GVHD, organ transplant rejection) – has major implications for the understanding of the mechanisms of ECP. While it is well known that DCs are critical players in the induction of anti-tumor immune responses, as observed in ECP, more recent studies have revealed that these specialized cells may also play a pivotal role in ECP-mediated immunomodulation.

How is it possible that EI-DCs can stimulate an anti-tumor T-cell response, but in the treatment of GVHD, organ transplant rejection, or autoimmune disorders lead to down-regulation of the disease-mediating T cell response? One hypothesis proposed has invoked the concept of “T cell vaccination” whereby activated disease-mediating T-cells, undergo apoptosis, and are presented by DCs to stimulate an anti-T-cell immune response. This is supported by animal models of autoimmunity and GVHD in which T-cell vaccines have shown efficacy [34–36], and by the demonstration that TCR peptides can act as antigen [37]. However, in human GVHD and organ transplant rejection, despite the fact that a potent immune response is occurring within the host, relatively few

disease-mediating T-cells are accessible from the peripheral circulation. A more likely explanation is that ECP-induced monocyte-to-DC differentiation produces cells with the capacity to both stimulate a new T-cell response or down-regulate an existing T-cell response (Table 1). The major factor determining which effect predominates may be the affinity of the available repertoire of TCRs for the antigen(s) in question.

Existing anti-tumor immune responses within a given patient are likely represented by a relatively few T-cells with relatively weak specificity. For an anti-tumor response to be stimulated by ECP, or by any other “tumor vaccine”, DCs need to engulf tumor cells, process tumor antigens, and present these in the context of MHC molecules on the surface while also displaying co-stimulatory molecules. Those tumor-specific peptides that find their way into MHC class II (i.e. via the endocytic pathway) or MHC class I (i.e., through a process called “cross-priming”) will represent a subset of antigens for which there are T-cells of a limited, low-affinity, TCR repertoire. It is this low-affinity TCR/peptide interaction, in association with co-stimulation, that activates the anti-tumor specific T-cells.

In contrast, in a patient with GVHD or organ transplant rejection, there is already an ongoing, potent T-cell immunity driven by DCs presenting allogeneic antigens and allo-reactive high-affinity CD4 and CD8 recipient T-cells. At the site of tissue damage, monocyte/macrophages are present and actively engulfing host cellular debris. These cells can return to the endoreticular circulation, including the peripheral blood. Once exposed to ECP, monocytes are initiated to undergo DC differentiation and are returned to the peripheral circulation (Fig. 2). In the setting of GVHD or organ transplant rejection, these cells may have

Table 1

Disease state	Example	Relevant antigens	Pre-existing T-cell response	TCR/MHC: peptide affinity	DC effector function
Malignancy	CTCL	Tumor antigens	Weak to non-existent	Low	Stimulation
Graft rejection	Cardiac transplant	Allo-antigens	Yes	High	Inhibition
GVHD	Allogeneic stem cell transplant	Allo-antigens	Yes	High	Inhibition

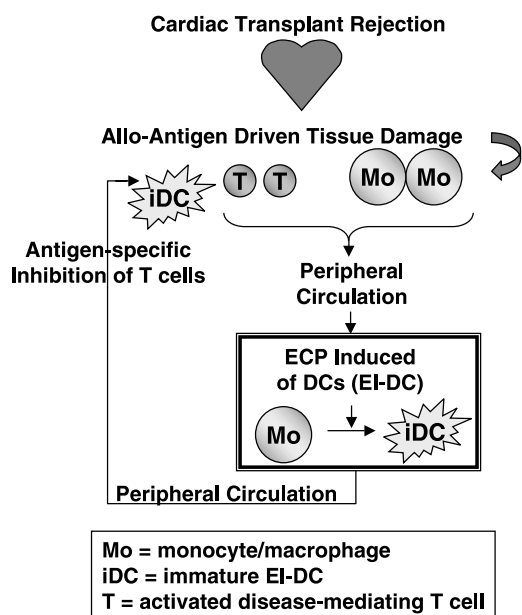


Fig. 2. Potential mechanism of antigen-specific immunosuppression by ECP. During cardiac transplant rejection, for example, T cells are actively mediating allo-antigen driven tissue destruction. Infiltrating monocytes (Mo) readily engulf the damaged tissue, and may return to the peripheral circulation. Monocytes are induced to undergo differentiation to immature DCs, which when returned to the circulation may inhibit the disease mediating T cells.

powerful antigen-specific down-regulatory effects for several reasons.

The EI-DCs are early in their maturation process, and during their first 24–48 h, most express very low levels of co-stimulatory molecules. Several investigators have recently shown that when immature DCs presenting antigens, they can function as antigen-specific inhibitors of an existing effector T-cell response [38–41]. This effect appears to be especially true for antigens presented for which previously activated T cells express high affinity TCRs [40], as are present in GVHD and graft-rejection.

Such DC-mediated down-regulation may, for example, be the result of Fas–Fas ligand killing of the activated T-cells [40], or interleukin-10 immunosuppression [39]. Such down-regulatory cells could return to the site, or draining lymph nodes, of immune destruction to inhibit existing disease-mediating T-cells, including those with the same

antigen specificity, as well as others via a non-antigen-specific “bystander effect”.

As EI-DCs further mature over a five-day period, co-stimulatory molecule surface expression is enhanced, as is thus their ability to stimulate, as opposed to inhibiting, T-cells. However, this maturation appears to be facilitated by the presence of apoptotic tumor cells. (While ECP does induce apoptosis of T-cells in GVHD, there may be a fundamental, yet not understood, DC-maturing effect of apoptotic transformed cells.) In any event, a better understanding of the mechanisms of these diametrically opposed DC effects will likely have tremendous implications for further refinement of Transimmunization to produce the desired outcome.

#### 4. Transimmunization: replacement technology for ECP

To take advantage of our increased understanding of the scientific basis of ECP, we have developed Transimmunization (Fig. 3). The fundamental alteration to conventional ECP is the addition of an overnight incubation step which, in the case of CTCL, brings the newly induced apoptotic malignant T-cells in close proximity with

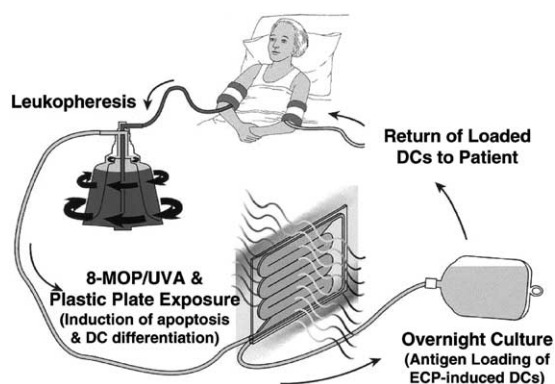


Fig. 3. Transimmunization. The fundamental steps of the Transimmunization procedure are (1) leukopheresis, (2) 8-MOP/UVA-induction of apoptosis of malignant cells, and induction of DC differentiation, (3) co-incubation of DCs with apoptotic tumor cells, and (4) return of the tumor antigen-loaded DCs to the patient.

the newly differentiating DCs. The steps of Transimmunization are as follows:

*Stage 1 Leukapheresis:* Venous blood is centrifuged to enrich the leukocyte fraction. 8-MOP is added to achieve a concentration of 200 ng/ml.

*Stage 2 UVA exposure system for Activation of 8-MOP:* The leukocytes are passed through 1 mm thick flow channels, between the two walls of a plastic plate, and exposed to 2 J/cm of ultraviolet A (UVA) light. The UVA-activation of 8-MOP initiates apoptosis of the leukemic T-cells. Simultaneously passaged monocytes transiently bind to the plastic surfaces, thereby becoming activated and stimulated to differentiate into dendritic cells (DC).

*Stage 3 Overnight cultivation of the ECP-treated cells:* To provide time for the development of both apoptotic T-cells and monocyte-to-DC maturation, and optimize their interactions, the harvested T-cells and monocytes are co-cultivated in a gas-permeable blood storage bag at 37 °C.

*Stage 4 Return of tumor antigen-loaded DC:* The ECP leukocytes are intravenously returned to the patient, to stimulate a CD8 anti-tumor response (see Fig. 3).

In such a protocol, it may be possible to stimulate anti-tumor immunity against other antigenic leukemic malignancies (e.g., multiple myeloma) or even a broad spectrum of solid tumors. As discussed above, the latter may be accomplished through the preparation of single-cell suspensions from surgically removed tumors, whose isolated cells are subsequently induced to undergo apoptosis (i.e., via 8-MOP/UVA exposure) and then co-cultured with the ECP-induced DCs. While ECP for CTCL intravenously delivers the treated cells back to the peripheral circulation, it is as yet unclear whether intradermal inoculation (e.g., after concentrating the cells and freeze-storage as “personalized anti-tumor vaccines”), or any other route of return, may be more advantageous.

As the mechanism of ECP-induced down-regulation of pathogenic T-cells becomes elucidated, the ability to control GVHD, graft-rejection, and

autoimmunity may become more readily achievable. If such responses are indeed achievable via stimulation of anti-T-cell responses, then it may be possible to augment this effect by expanding the relevant clones in vitro, prior to the induction of apoptosis. This could result in a more specific and more potent responses against the disease-mediating T-cells. Lastly, one can imagine a scenario incorporating ECP strategies in a comprehensive anti-tumor immunotherapy: isolation of tumor cells at the time of surgery, PBSCT to initiate a graft-versus-tumor (GVT) response against residual and/or metastatic disease, boosting of anti-tumor immunity via ECP-derived DCs co-incubated overnight with apoptotic tumor cells, and control of any GVHD with ECP. As the T-cell subsets and the specific targets that mediate GVHD and GVT are differentiated and better understood, it may be possible to use Transimmunization to boost anti-tumor immunity without stimulating GVHD.

In summary, ECP’s substantial clinical record, coupled with a rapidly improving understanding of the mechanism underlying its clinical efficacy, opens potentially quite rich new avenues of immunotherapy in the treatment of cancer, GVHD, transplant rejection, and autoimmunity. The very low side effect profile of this therapy has made it a more attractive treatment consideration than many conventional chemotherapeutic and immunosuppressive medications that are presently used. As the mechanism of action of ECP is more completely elucidated and studies investigating different strategies are completed, the role of ECP in modern therapeutics of CTCL and other malignancies, as well as in the treatment of other T-cell mediated diseases, will become clearer. For the past two decades, the increasing use of ECP has been driven by its clinical successes. Now, it will be the scientific principles underlying its positive effects that will propel it forward.

## **5. Future directions: development of Transimmunization for clinical application**

Initially, we are applying Transimmunization to the treatment of advanced CTCL, and GVHD following stem cell transplantation. The responses

of skin lesions of two CTCL patients, previously resistant to conventional ECP and subsequently treated via the intravenous return of tumor-loaded

DC using Transimmunization, can be seen in Fig. 4. Infiltrated cutaneous plaques on the forearm of one patient clinically resolved, as did lesions in all

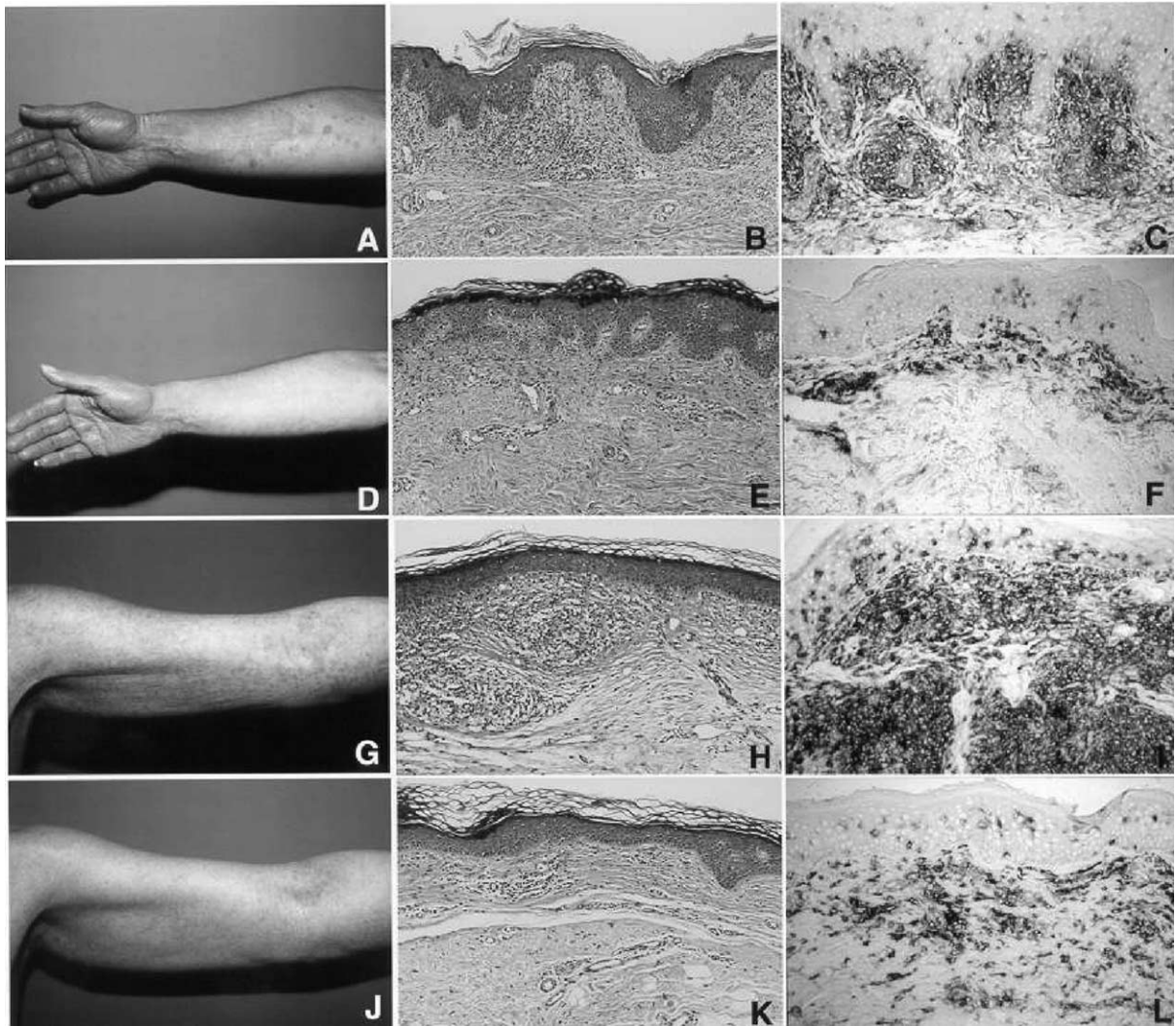


Fig. 4. Responses of skin lesions of two CTCL patients to the intravenous return of tumor-loaded DC. (A, D, G, J) *Clinical lesions pre- and post-treatment.* Infiltrated cutaneous plaques on the forearm of patient 1 (A) clinically resolved (B), as did lesions in all skin regions. Papular lesions that were widely disseminated on patient 2 (G) became reduced in number and induration, but remained subtly evident. (B, E, H, K) *Histopathology of the same skin lesions pre- and post-treatment.* (B) Pre-treatment skin biopsy from forearm of patient 1 reveals dense band-like infiltrate of lymphocytes in the papillary dermis, with epidermotropism typical of CTCL. (E) Post-treatment biopsy from an immediately adjacent site shows marked diminution in the intensity of both intra-epidermal and dermal lymphocytes. (H) Pre-treatment biopsy of a papular lesion from the arm of patient 2 shows dense nodular and band-like infiltrates of atypical lymphocytes. (K) Post-treatment biopsy of the most apparent adjacent papule from patient 2 shows marked diminution of the lymphocytic infiltrate. (C, F, I, L) *Immunostaining of skin biopsies from the same sites.* (C, F) Pre-treatment specimens, respectively, from patients 1 and 2 reveal a marked decrease in both the number of CD3 (red) and CD4 (blue) positive cells compared to the post-treatment (I, L), confirming that the reduction in the infiltrate represents a decrease in the malignant CD4+ T-cell populations.

Table 2

## Clinical considerations of Transimmunization

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*Transimmunization's broad appeal*

- Selective immunotherapy without global immunosuppressive effects
- Without the toxicity of conventional treatments of chemotherapy, radiation therapy, or cytokine therapy
- Delivered to ambulatory patients, in an outpatient Transimmunization center
- Administered by a highly trained, specialized nursing staff dedicated to Transimmunization

*Short-term clinical applications – treatment of leukemialymphoma*

CTCL

- Increased numbers of CTCL patients referred and treated
- Future application to other leukemias/lymphomas and multiple myeloma

GVHD

- Increased number of partially mismatched transplants conducted, and increase the donor pool
- Increased therapeutic effects of stem cell transplants due to graft-versus-leukemia effect

*Long-term clinical applications – other potential applications of Transimmunization*

Extension to solid organ transplantation

- Provide a non-immunosuppressive, steroid-sparing, therapy for solid organ transplant rejection

Extension to solid tumor immunotherapy

- Treatment of numerous solid organ tumors (e.g., melanoma, breast cancer, colon cancer, prostate cancer, etc.)
- Potential use of conveniently delivered, frozen-down, antigen-loaded, stored dendritic cells

Extension to autoimmunity

- Treatment of autoimmune disorders (e.g., pemphigus, pemphigoid, multiple sclerosis, scleroderma, etc.)

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skin regions. Papular lesions that were widely disseminated on a second patient became reduced in number and induration, but remained subtly evident. These clinical changes were confirmed by histological and immunohistologic examination.

Transimmunization is designed as a replacement technology for ECP. By more efficiently bringing dendritic cells in close proximity to the target cells population (i.e., malignant cells, autoreactive T-cells), Transimmunization may prove more efficacious, and require fewer procedures, than ECP. Furthermore, Transimmunization will likely result in the ability to minimize hospital stays due to complications resulting from disease activity or the detrimental effects of more toxic therapies (e.g., chemotherapy used in the treatment of CTCL and other malignancies, and potent immunosuppression used in the treatment of GVHD and organ graft rejection). Such benefits were observed in the randomized, controlled study of ECP in the treatment of cardiac transplantation. ECP-treated patients not only experienced far fewer cardiac rejection events, but moreover they could be tapered to lower doses of immuno-

suppressive medications. In the treatment of GVHD, several studies have demonstrated increased efficacy with the use of ECP as well as the ability of patients to tolerate lowering or discontinuation of global immunosuppression. As a target-specific immunotherapy, Transimmunization, like its ECP-precursor, has a response-to-risk ratio that exceeds that of conventional chemotherapy or broad immunosuppression. The long-term plan for Transimmunization is therapeutic applications beyond CTCL and GVHD. These include solid organ transplant rejection, solid tumor malignancies, and autoimmune disorders (Table 2).

Currently, Transimmunization is not automated. As such, the process requires manual manipulation of cells *ex vivo*, and thus carries a risk of introduction of infection into the culture bag. The overnight culture bag is microporous to allow gas exchange without liquid permeability. Obviously, further refinement is necessary before Transimmunization can be delivered in a standardized fashion at other centers.

In summary, the major advantages of ECP and Transimmunization over conventional therapies

for CTCL (and other leukemia/lymphomas), and GVHD, is selectivity for the pathogenic clones via a relatively non-toxic, steroid-sparing, therapeutic alternative. ECP and Transimmunization are not globally immunosuppressive, but rather are *selectively* immunotherapeutic, targeting malignant or pathogenic T cells in their current forms. Moreover, Transimmunization is a unique and continuously evolving therapy, with potential applications to the treatment of solid tumor malignancies, solid organ transplant rejection, and autoimmune disorders.

## References

- [1] Edelson RL et al. Treatment of leukemic cutaneous T-cell lymphoma with extracorporeally-photoactivated 8-methoxypsoralen. *N Engl J Med* 1987;316:297–303.
- [2] Armus S et al. Photopheresis for the treatment of cutaneous T-cell lymphoma. *J Am Acad Dermatol* 1990;23: 898–902.
- [3] Bisaccia E et al. Extracorporeal photochemotherapy alone or with adjuvant therapy in the treatment of cutaneous T-cell lymphoma: A 9-year retrospective study at a single institution. *J Am Acad Dermatol* 2000;43:263–71.
- [4] Jiang SB et al. Extracorporeal photochemotherapy for cutaneous T-cell lymphoma: A 9.7-year experience. *Photodermatol Photoimmunol Photomed* 1999;15:161–5.
- [5] Heald P et al. Treatment of erythrodermic cutaneous T-cell lymphoma with extracorporeal photochemotherapy. *J Am Acad Dermatol* 1992;27:427–33.
- [6] Zic J et al. Extracorporeal photopheresis for the treatment of cutaneous T-cell lymphoma. *J Am Acad Dermatol* 1992;27:729–36.
- [7] Duvic M et al. Photopheresis therapy for cutaneous T-cell lymphoma. *J Am Acad Dermatol* 1996;35:573–9.
- [8] Zic J et al. Long-term follow-up with cutaneous T-cell lymphoma treated with extracorporeal photochemotherapy. *J Am Acad Dermatol* 1996;35:935–45.
- [9] Greinix HT et al. Successful use of extracorporeal photochemotherapy in the treatment of severe acute and chronic graft-versus-host disease. *Blood* 1998;92:3098–104.
- [10] Richter H, Stege H, Ruzika T, Soehngen D, Heyll A, Krutman J. Extracorporeal photopheresis in the treatment of acute graft-versus-host disease. *J Am Acad Dermatol* 1997;36:787–9.
- [11] Child FJ, Ratnavel R, Watkins P, et al. Extracorporeal photopheresis (ECP) in the treatment of chronic graft-versus-host disease (GVHD). *Bone Marrow Transplant* 1999;23(9):881–7.
- [12] Besnier D, Chabannes D, MahÉ B, Mussini J-M, Baranger T, Muller J, et al. Treatment of graft-versus-host disease by extracorporeal photochemotherapy. *Transplantation* 1997;64:49–54.
- [13] Gerber M et al. Complete remission of lichen-planus-like graft-versus-host disease (GVHD) with extracorporeal photochemotherapy (CP). *Bone Marrow Transplant* 1997; 19:517–9.
- [14] Owsianowski M, Gollnick H, Siegert W, Schwerdtfeger R, Orfanos CE. Successful treatment of chronic graft-versus-host disease with extracorporeal photopheresis. *Bone Marrow Transplant* 1994;14(5):845–8.
- [15] Dall'Amico R, Rossetti F, Zulian F, et al. Photopheresis in paediatric patients with drug-resistant chronic graft-versus-host disease. *Brit J Dermatol* 1997;97:848–54.
- [16] Rossetti F, Zulian F, Dall'Amico R, Messina C, Montini G, Zacchello F. Extracorporeal photochemotherapy as single therapy for extensive cutaneous chronic graft-versus-host disease. *Transplantation* 1995;59(1):49–51.
- [17] Rook AH et al. Treatment of systemic sclerosis with extracorporeal photochemotherapy – results of a multicenter trial. *Arch Dermatol* 1992;128:337–46.
- [18] Zachariae H et al. Photopheresis and systemic sclerosis. *Arch Dermatol* 1992;128:1651–3.
- [19] DiSpaltro F et al. Extracorporeal photochemotherapy in progressive systemic sclerosis. *Int J Dermatol* 1993;32:1–5.
- [20] Barr ML et al. Photopheresis for the prevention of rejection in cardiac transplantation. *N Engl J Med* 1998;339:1744–51.
- [21] Costanzo-Nordin MR et al. Photopheresis versus corticosteroids in the therapy of heart transplant rejection. *Circulation* 1992;86:242–50.
- [22] Rose EA et al. Photochemotherapy in human heart transplant recipients at high risk for fatal rejection. *J Heart Lung Transplant* 1992;11:746–50.
- [23] Sunder-Plassman G et al. Renal allograft rejection controlled by photopheresis. *Lancet* 1995;346:506.
- [24] Wolfe J et al. Reversal of acute renal allograft rejection by extracorporeal photopheresis: A case presentation and review of the literature. *J Clin Apheresis* 1996;11:36–41.
- [25] Slovis BS et al. Photopheresis for chronic rejection of lung allografts. *N Engl J Med* 1995;332:962.
- [26] Andreu G et al. Extracorporeal photochemotherapy treatment for acute lung rejection episode. *J Heart Lung Transplant* 1995;14(4):793–6.
- [27] Mellman I, Steinman RM. Dendritic cells: Specialized and regulated antigen-processing machines. *Cell* 2001;106: 255–8.
- [28] Timmerman JM, Levy R. Dendritic cell vaccines for cancer immunotherapy. *Annu Rev Med* 1999;50:507–29.
- [29] Berger CL et al. Induction of human tumor-loaded dendritic cells. *Int J Cancer* 2001;91:438–47.
- [30] Berger CL et al. The immune response to class I-associated tumor-specific cutaneous T-cell lymphoma antigens. *J Invest Dermatol* 1996;107:392–7.
- [31] Berger CL et al. Tumor-specific peptides in cutaneous T-cell lymphoma: association with class I major histocompatibility complex and possible derivation from the clonotypic T-cell receptor. *Int J Cancer* 1998;76:304–11.
- [32] Timmerman JM, Levy RJ. Linkage of foreign carrier protein to a self-tumor antigen enhances the immunogenicity

- of a pulsed dendritic cell vaccine. *Immunology* 2000;164: 4797–803.
- [33] Zucker-Franklin D. The role of human T-cell lymphotropic virus type I tax in the development of cutaneous T-cell lymphoma. *Ann NY Acad Sci* 2001;941: 86–96.
- [34] Girardi M, Herreid P, Tigelaar R. Specific suppression of lupus-like graft versus host disease using extracorporeal photochemical attenuation of effector lymphocytes. *J Invest Dermatol* 1995;104:177–82.
- [35] Perez M et al. Inhibition of antiskin allograft immunity by infusions with syngeneic photoinactivated effector lymphocytes. *J Invest Dermatol* 1989;92:669–76.
- [36] Berger CL et al. Inhibition of autoimmune disease in a murine model of systemic lupus erythematosus induced by exposure to syngeneic photoinactivated lymphocytes. *J Invest Dermatol* 1990;94:52–7.
- [37] Berger CL, Longley J, Hanlon D, Girardi M, Edelson R. The clonotypic T-cell receptor is a source of tumor-associated antigens in cutaneous T-cell lymphoma. *Ann NY Acad Sci* 2001;941:106–22.
- [38] Albert ML, Sauter B, Bhardwaj N. Dendritic cells acquire antigen from apoptotic cells and induce class I-restricted CTLs. *Nature* 1998;392:86–9.
- [39] Dhodapkar MV, Steinman RM, Krasovsky J, et al. Antigen-specific inhibition of effector T-cell function in humans after injection of immature dendritic cells. *J Exp Med* 2001;193:233–8.
- [40] Kishimoto H, Sprent J. Strong TCR ligation without costimulation causes rapid onset of Fas-dependent apoptosis of T-cells. *J Immunol* 1999;163:1817–26.
- [41] Albert et al. Dendritic cell maturation is required for the cross-tolerization of CD8+ T-cells. *Nature Immunol* 2001; 2(11):1010–7.