Predictors of response to extracorporeal photopheresis in advanced mycosis fungoides and Sézary syndrome

Laura Y. McGirt[1], **Christopher Thoburn**[2,3], **Allan Hess**[2,3], and **Eric C. Vonderheid**[1,2]

[1]Department of Dermatology, Johns Hopkins Medical Institutes, Baltimore, MD, USA

[2]Department of Oncology, Johns Hopkins Medical Institutes, Baltimore, MD, USA

[3]The Sidney Kimmel Comprehensive Cancer Center, Johns Hopkins University, Baltimore, MD, USA

Summary

Background—Extracorporeal photopheresis (ECP) has been utilized for more than 20 years to treat cutaneous T-cell lymphoma (CTCL), but a clinical response can take up to 9 months to manifest. This study was undertaken to determine whether clinical features, laboratory values, cytokine levels, or gene expression levels of tumor markers are useful to predict the subsequent response to ECP in CTCL patients with blood involvement.

Methods—Twenty-one patients with CTCL treated with ECP as monotherapy for at least 6 months were retrospectively identified. Laboratory and clinical data and blood obtained at baseline, 3, and 6 months of treatment were used for analysis.

Results—In pretreatment blood specimens, a lower percentage of Sézary cells and a higher absolute eosinophil count were associated with a favorable clinical response. Clinical evidence of an early response after 3 months of ECP did not reliably predict a favorable response at 6 months or beyond. Comparison of cytokines, gene transcripts, and other laboratory measures of disease did not correlate with the subsequent clinical response, although lactate dehydrogenase levels tended to decrease progressively in ECP-responsive cases and increase progressively in ECP-non-responsive cases. Additionally, serum levels of TNF-α significantly increased from baseline to 6 months of ECP, but was not found to correlate with the clinical response.

Conclusions—Although we found that increased eosinophils and decreased percentage of Sézary cells were associated with a favorable clinical response to ECP, we were not able to identify the predictors of ECP response within the first 3 months of treatment.

Keywords

cutaneous lymphoma; mycosis fungoides; photopheresis; predictors of response; Sézary syndrome

Initially developed by Edelson in the 1970s, extracorporeal photopheresis (ECP) is a process by which peripheral blood mononuclear cells (PBMC) are isolated from the circulation by discontinuous leukapheresis, exposed *ex vivo* to pro-apoptotic doses of 8-methoxypsoralen and ultraviolet A radiation, and then reinfused to the patient (1). ECP was approved by the

Correspondence: Laura Y. McGirt, Department of Dermatology, Johns, Hopkins Outpatient Center, 6th Floor, 601 North, Caroline Street, Baltimore, MD 21287, USA., Tel: +1 410 955 2400, lyoung17@jhmi.edu.

Food and Drug Administration for advanced cutaneous T-cell lymphoma (CTCL) in 1988 (1), and is now utilized for the treatment of various lymphocyte-mediated diseases, including graft-versus-host disease (GvHD) and solid organ transplant rejection (2–4).

Mycosis fungoides (MF) and Sézary syndrome (SS), the major variants of CTCL, are malignancies of clonal T lymphocytes that preferentially infiltrate the skin (5–7). Overt blood involvement is uncommon in clinically early MF, but may be encountered in advanced MF and in SS, which is currently defined as an erythrodermic and leukemic expression of CTCL (8). ECP is often used as a first-line therapy for advanced MF with blood involvement and SS, and the initial pivotal study in which two ECP treatments were administered on consecutive days at 4-week intervals demonstrated that ECP monotherapy induced a substantial improvement in 83% of erythrodermic patients, with 21% of patients eventually achieving a complete response (CR) (1, 9). Of note, in responding patients, the mean time to a positive response was 22.4±9.6 weeks, meaning that ECP must be administered for 32 weeks or more before its effectiveness can be properly assessed. Subsequent studies have shown that the overall response rates to ECP range widely from 31% to 86%, depending on patient selection, treatment frequency, and use of adjunct therapies (10, 11).

Despite its utilization for more than two decades, the precise mechanism(s) of action by which ECP exerts its therapeutic benefit on CTCL is not clearly understood nor have reliable predictors of response been identified (12–14). Possible and not necessarily mutually exclusive mechanisms of action include:

- Induction of an anti-clonotypic immune response against the malignant clone (15).

- Production of immunosuppressive or immunomodulatory cytokines [e.g. TNF-α, interleukin-10 (IL-10)] by treated mononuclear cells with normalization of the Th1/Th2 immune balance (16–20).

- Induction of regulatory T (Treg) cells that inhibit neoplastic T-cell growth directly or indirectly via their effect on dendritic cells (21–24).

Clinical parameters that have been associated with sustained beneficial responses of CTCL to ECP include short disease durations (early treatment) and clinical improvement before 6 months (9, 25, 26). Laboratory parameters that have been reported to be correlated with favorable responses to ECP include (1) near-normal CD4/CD8 ratios or absolute number of CD8+ cells in the peripheral blood (9, 27), (2) the presence of modest numbers of Sézary cells (28), and (3) relatively low percentages of CD4+CD7− T cells, a phenotype that is often expressed by neoplastic T cells (25).

The primary goal of this study was to determine whether the cytokine profile in serum samples obtained at baseline and after 3 months of ECP monotherapy could be used as an early predictor of a clinical response to ECP monotherapy. Previously, we reported that baseline levels of soluble interleukin-2 receptor (sIL-2R) and lactate dehydrogenase (LDH) were not useful in this regard (29). However, sIL-2R and LDH levels did correlate with clinical and other laboratory parameters used to evaluate the response to ECP. A second objective was to evaluate the effect of ECP monotherapy on Th1 and Th2 cytokine levels in the serum. It is widely accepted that the neoplastic T cells of most cases of MF/SS are polarized to secrete Th2 cytokines, and that in advanced CTCL, a Th2 immune profile dominates and contributes to immunosuppression, eosinophilia, and increased IgE levels (30, 31). Our hypothesis was that a shift toward Th1 might occur as an early event in patients responding to ECP as reported for clinically early MF (19). The third goal of this study was to correlate usual clinical and laboratory measures of clinical response to ECP with possible molecular 'tumor-associated markers' (STAT3, MICB, and T-plastin/PLS3) in

patients with blood involvement. Our hypothesis was that response to ECP monotherapy will result in an early decrease in the level of proposed tumor-associated marker message (mRNA) in PBMC samples obtained during the first 3 months of ECP monotherapy. Lastly, we planned to evaluate clinical and laboratory data during the initial 3 months of ECP treatment in order to identify potential predictors of response.

Materials and methods

This retrospective study utilized the CTCL tissue repository at Johns Hopkins School of Medicine and was approved by the Johns Hopkins Institutional Review Board. Twenty-one subjects were selected based on the diagnosis of advanced MF/SS with blood involvement and administration of ECP as the only treatment for at least 6 months.

Patients and treatment protocol

Three patients had the skin manifestations of widespread MF and the remaining 18 patients had generalized erythroderma. The histologic diagnosis of CTCL was confirmed on skin biopsy specimens in all cases, and all had negative serologic testing for human T-cell lymphoma virus, type 1 antibody. Blood studies included quantitative Sézary cell counts, flow cytometry, molecular analysis of the TCR-β chain by Southern blot or the TCR-γ chain by polymerase chain reaction (PCR) for T-cell clonality, and chromosome karyotyping as described previously (29, 32, 33). The blood findings were used to classify the 18 erythrodermic patients into SS (15 cases) and erythrodermic CTCL, not otherwise defined (three cases) subsets, and also for staging as recommended by the International Society for Cutaneous Lymphomas (8). The initial nine patients in this series were reported previously (29).

ECP was administered on 2 consecutive days at 4-week intervals starting between March 1991 and April 2001 (Tables 1 and 2). Before February 1999, methoxsalen was given orally at a dose of 0.6 mg/kg before ECP, but thereafter, methoxsalen solution was added directly to the lymphocyte collection bag. The basic approach was to utilize ECP as the only treatment for the initial 6 months, and thereafter continue ECP monotherapy if there was clinical evidence of improvement or add an adjunct treatment, most often interferon-α (IFN-α), if the response to ECP was deemed to be inadequate. The response to ECP was determined by one of us (E. C. V.) before each ECP cycle, and was based on clinical assessment of skin manifestations and lymphadenopathy relative to baseline status as described previously (29). If improvement on ECP was sustained for at least 4 weeks (two consecutive ECP cycles), the observed global clinical responses were scored as minor responses (MR) if there was an objective improvement but < 50% reduction in skin manifestations (usually diminished intensity of skin inflammation), partial responses (PR) if the skin improvement was > 50% of baseline scores, CR if all clinical manifestations of CTCL disappeared, worsening (usually increased intensity of skin inflammation), or disease progression (DP). Patients were evaluated before each ECP session and blood was obtained for special studies (Sézary cell counts, flow cytometry, LDH, complete blood counts with differential, and serum and lymphocyte storage) at 3-month intervals.

RNA isolation

Immediately before the start of ECP and before the fourth and seventh ECP sessions (3-month intervals), 30 ml of peripheral blood was collected and PBMC were isolated using a Ficoll density gradient. These cells were cryopreserved in liquid nitrogen and were thawed immediately before RNA isolation. 1×10^6 cells were utilized for the trizol-based protocol.

cDNA preparation and real-time PCR

Determination of relative gene expression was performed using quantitative real-time PCR (qPCR) using an ABI 7500 real-time PCR system (Applied Biosystems, Foster City, CA, USA). cDNA was prepared from the isolated RNA using random hexamers to prime reverse transcription (Ready-to-Go You-Prime First-Strand Beads; GE Healthcare, Buckinghamshire, UK) following the manufacturer's protocol. Multiplexed quantitative determination was carried out in triplicate wells using approximately 1 : 30 of the cDNA per well and primer/probe sets for the FAM-labeled target genes and VIC-labeled endogenous reference gene (GAPDH) using the standard ABI chemistry and reagents. Relative transcripts were determined using the formula: $1/2 (CT^{target}–CT^{control})$. Real-time PCR efficiencies of target genes and the reference gene were approximately equal over a concentration of 0.1–200 ng total cDNA. Three possible tumor-associated target genes (T-plastin/PLS3, STAT3, and MICB) were selected for study by qPCR. T-plastin has emerged as a useful molecular marker of neoplastic T cells in CTCL (34–38), and upregulation of STAT3 has been associated with neoplastic cell growth/survival in MF/SS (39–43). MICB might be another molecular marker of CTCL based on our previous study of leukemic CTCL cases (35). Primers for STAT3, T-plastin/PLS3, and MICB were purchased from ABI Corporation (Foster City, CA, USA).

Cytokines

Before the initial ECP and at 3-month intervals, 10 ml of peripheral blood was obtained and centrifuged to separate the serum. The serum was stored at −80 °C, and thawed immediately before use. We utilized the Bio-Plex multiplex assay system based on xMAP technology from Bio-Rad Laboratories (Hercules, CA, USA) to simultaneously measure cytokines possibly implicated in the Th1/Th2 and host immune response, including IL-2, IL-4, IL-5, IL-10, IL-12p70, IL-13, IFN-γ, granulocyte macrophage colony-stimulating factor (GM-CSF), and TNF-α.

Statistical analysis

The results of standard laboratory and cytokine studies were given as mean values and 1 standard deviation (SD) and/or median value with range. The Mann–Whitney (MW) *U*-test was used to test for differences in the laboratory test values for two patient groups. An exact Wilcoxon signed-rank (WSR) test was utilized to test for differences in the direction of cytokine and gene expression levels for paired baseline and later samples on the same patients. Spearman's rank order test was used to test for correlation between two variables. Fisher's (*F*) and Pearson's χ^2 exact tests were used to test categorical data in 2×2 and R×C tables, respectively. The difference in survival curves was tested using the Gehan–Breslow (GB) method in order to lend more weight to deaths at time points closer to ECP usage. The statistical packages used for data analysis were SYSTAT10 and SPSS 13.0 for Windows (SPSS Inc., Chicago, IL, USA).

Results

Clinical response to ECP monotherapy

The overall response to ECP monotherapy was divided into two groups according to the overall assessment of response (Tables 1 and 2). Long-term follow-up indicates that 10 of 12 patients with responsive disease (group 1; Table 1) are currently deceased, with all but one attributed to CTCL. The overall disease-related median survival was 67 months. Conversely, nine patients failed to respond adequately to treatment (group 2; Table 2). At last contact, only one patient is alive with slowly progressing SS. The overall median disease-related survival of this group is 41 months. The difference in survival curves between group 1 and

group 2 patients was significant (GB test, $P = 0.039$). The overall median survival for all patients was 64 months.

The status of patients after 3 and 6 months of ECP monotherapy was categorized as clinically improved (MR, PR, and CR), unchanged (NC), or worse (W, DP) to determine whether early response was a predictor of eventual response to ECP alone. After 3 months of treatment, clinical improvement, which was usually manifested by a reduced intensity of skin inflammation, was noted in nine patients (five MRs, four PRs) compared with baseline. Of these early responders, continued improvement occurred by 6 months for three patients (patient 7: MR to PR; patients 8 and 12: sustained PR), with patient 12 eventually achieving a CR on ECP monotherapy. Of the remaining six early responders, three patients were scored as MR at 6 months (one with PR at 3 months), and three patients had evidence of relapsing disease including patient 16 with PR at 3 months and DP thereafter. Of interest, patient 10, who had an MR at 3 months and an NC at 6 months compared with baseline, eventually had a CR after 13 months. By contrast, 12 patients did not show evidence of clinical improvement at 3 months. However, a delayed response was apparent at 6 months for five patients (NC to MR), with three ultimately achieving a PR (patients 5 and 6) or a CR (patient 11) with continued ECP monotherapy beyond 6 months. This experience indicates that responses to ECP alone are often slow to occur and early improvement after 3 months is not a reliable indicator of a subsequent response to treatment.

A number of clinical parameters available at the start of ECP were compared in patients who responded to ECP monotherapy (group 1) vs. those who did not respond adequately (group 2) (Table 3). Clinical parameters including the intensity of skin erythema (in Caucasian patients only) and T4 vs. T2/T3 skin rating did not correlate with subsequent response to ECP (data not shown).

The effect of ECP on clinical laboratory parameters

Pairwise comparisons of each clinical laboratory parameter were made after 3 and 6 months relative to the pretreatment baseline values. When all 21 cases were analyzed as a single group, the only quantitative change that was statistically significant was a decrease in absolute eosinophils at 3 months (median change, -29 cells/mm^3 or -16%, WSR test, $P = 0.049$) but not at 6 months (median change, $+1$ cells/mm^3 or $+1.5\%$, $P = 0.468$). Additionally, absolute Sézary cell counts, absolute CD4+ counts, and LDH levels decreased a median of -2.6%, -14%, and -7% in responding patients compared with median increases of $+18.8\%$, $+17\%$, and $+3\%$ in non-responding patients, respectively.

The only baseline clinical laboratory parameters that were statistically different between the ECP-responsive and the non-responsive groups were the lower proportion of Sézary cells in the lymphocyte population (median, 32% vs. 54%, MW test, $P = 0.001$) and the higher absolute eosinophil count (median, 388 vs. 87 cells/mm^3 MW test, $P = 0.008$) in responding patients compared with non-responding patients (Table 3).

Baseline cytokine expression

At least one cytokine per growth factor had measurable values in 19 of the 20 pre-ECP serum samples that were studied. At least one Th2-type cytokine (IL-4, IL-5, IL-10, and IL-13) was present in 16 (80%) samples, but IL-4, the prototypic Th2 cytokine, was detected in only one sample (patient 16). Other Th2-type cytokines that were detectable were IL-5 (eight cases, median, 0.125, range, 0.06–0.21 pg/ml), IL-10 (12 cases, median, 0.85, range, 0.14–30.02 pg/ml), and IL-13 (four cases, median, 0.07, range, 0.01–0.54 pg/ml). In addition, at least one Th1-associated cytokine (IL-2, IL-12, TNF-α, and IFN-γ) was detected in 18 (90%) samples. The most commonly detected Th1-associated cytokines were

IL-12 (11 cases, 0.14, range, 0.01–12.69 pg/ml) and TNF-α (15 cases, median, 0.48, range, 0.01–4.78 pg/ml). IFN-γ was present in only three cases (median, 11.95, range, 3.26–12.28 pg/ml) and IL-2 was found in five cases (median, 0.32, range, 0.01–0.63 pg/ml). Finally, GM-CSF was detected in six cases (median, 0.405, range, 0.01–3.53 pg/ml).

Effect of ECP on cytokine expression

Pairwise comparison of pre- and post-ECP samples indicated significant increases in IL-5 and TNF-α cytokine levels and after 6 months compared with baseline (median change, +0.11 and +2.33 pg/ml, respectively; both $P < 0.001$). IL-10 also tended to increase (median change, +0.25 pg/ml, $P=0.091$), whereas IL-12 levels remained unchanged. Changes in these cytokine levels were not apparent in 3-month samples (Data not shown). In 11 cases, the Th2 cytokine IL-5 was below the threshold of detection in pretreatment samples, and became measurable after 6 months of ECP. All four of the positive cases at baseline remained positive in subsequent samples, although the levels increased in only two instances. Similarly, IL-10 transformed from undetectable to detectable in three cases at 6 months or was present in both pre-and post-samples, and none reverted from positive to negative. However, a concurrent inhibitory effect of ECP on Th1 cytokines, IL-12, and TNF-α could not be shown. For example, none of the cases in which TNF-α was detected in pretreatment samples became negative at 6 months, whereas three TNF-α-negative cases at baseline subsequently became positive. Furthermore, TNF-α levels increased significantly after 6 months of ECP.

Correlation of cytokine measurements and clinical laboratory parameters

When correlated with clinical responses after 6 months of ECP, the absolute eosinophil counts tended to decrease in ECP-responsive group 1 cases (median change, −60 cells/mm^3 or −14%) compared with an increase in ECP-non-responsive group 2 cases (median change, +23 cells/mm^3 or +11%, MW test, $P = 0. 0.255$). By contrast, the increase in IL-5 levels in group 1 (median change, +0.21 ng/ml) was significantly higher than group 2 (+0.10 ng/ml, MW test, $P = 0.02$).

LDH levels in pretreatment samples did not correlate significantly with other measures of blood tumor burden, specifically, absolute Sézary cell count ($n= 21$, $\rho= 0.411$, $P= 0.063$) and the CD4/CD8 ratio ($n= 21$, $\rho= 0.375$, $P= 0.092$). Nevertheless, because LDH levels tended to decrease in responsive patients and increase in non-responsive patients, we analyzed the relationship between LDH and IL-10, IL-12, and TNF-α during treatment by categorizing the change relative to baseline values in levels in 3- and 6-month samples and then comparing the results in a two-way contingency table. LDH and TNF-α varied in the opposite direction in samples taken at 3 months (F test, $P= 0.01$). In six of 15 samples, LDH increased while TNF-α deceased at this time point, and conversely, in seven other samples, LDH decreased and TNF-α increased. In addition, a significant correlation was found between the percentage of change from baseline for each variable ($n=15$, $\rho=−0.649$, $P= 0.009$). However, this effect was not observed in samples taken at 6 months.

Correlations with tumor-associated gene expression levels

qPCR was utilized to measure the expression levels of STAT3, T-plastin, and MICB in PBMC collected immediately before and after 3 months of ECP in eight patients with favorable and seven with poor responses to ECP. Overall, RT-PCR demonstrated the presence of the three genes, STAT3, MICB, and T-plastin as compared with the control gene (GAPDH) (Fig. 1). There was no significant difference in expression at time point 0–3-month post-ECP treatment (STAT3, $\Delta\Delta C_t$ 5.30±14.28 vs. 1.01±0.84; MICB, $\Delta\Delta C_t$ 42645.55±159655.41 vs. 658.55±1772.60; T-plastin, $\Delta\Delta C_t$ 0.87±1.62 vs. 1.71±5.55) (Fig. 1, Table 4). We did not have adequate sample numbers to evaluate the 6-month post-ECP

treatment time point. Lastly, the levels of gene expression in baseline samples were not significantly different in ECP-responsive (group 1) vs. non-responsive (group 2) cases (data not shown).

A significant positive correlation was found between T-plastin expression and TNF-α serum levels (n=9, ρ= 0.917, P< 0.001) and serum IL-12 levels (n=9, ρ = 0.730, P=0.02) in the pretreatment samples, and the absolute Sézary cell count was also correlated with the TNF-α serum levels in the pretreatment samples (ρ =0.438, P= 0.067).

Discussion

Our study indicates that baseline and changes in cytokine levels and gene expression levels of 'tumor-associated' molecular markers (T-plastin, STAT3, and MICB) in PBMCs, and clinical response after 3 months of ECP monotherapy in patients with advanced CTCL and blood involvement do not provide useful information about subsequent clinical responses. However, patients with a lower percentage of Sézary cells among lymphocytes and higher absolute eosinophil counts in the pretreatment samples were more likely to respond.

Gottlieb reported that patients with evidence of Sézary cells in the blood were more likely to respond to ECP than patients without these cells, and indeed, this was the best predictor of a good response in their experience (28). However, the percentage of Sézary cells among leukocytes did not correlate well with the observed response. Conversely, Evans found that a higher baseline Sézary cell count as a percentage of the total white cell count, but not absolute counts, predicted a favorable response after 6 months of ECP (14). Therefore, the clinical relevance of Sézary cells in the context of ECP is unclear, but could be related to the well-known subjectivity of Sézary cell counts and that Sézary cells can represent both reactive and neoplastic cells, especially in the setting of erythrodermic CTCL.

To our knowledge, the association of higher eosinophil counts with a better response to ECP has not been reported before, and is somewhat unexpected, considering that a high absolute eosinophil count (> 700 cells/mm^3) has been reported to be indicative of a poor prognosis in CTCL (44, 45). In our cohort, four cases had absolute eosinophil counts exceeding 700/mm^3 and all had a favorable response to ECP.

In GvHD and in type 1 diabetes, an ECP-mediated shift toward Th2 has been reported (46, 47). One potential mechanism that might underlie this observation is that ECP-treated dendritic cells can be primed to increase Th2-producing T cells (47, 48). Conversely, Di Renzo reported that PBMCs from patients with MF at stage Ib (presumably no blood involvement) before ECP produced significantly higher levels of IL-4 and lower levels of IFN-γ and IL-12 (i.e. a Th2 profile), compared with healthy volunteers, and that this reverted to normal after 1 year of ECP (19). A Th1-promoting effect by ECP has also been reported in the setting of GvHD (49, 50). The effect of ECP on the Th1/Th2 balance may depend on the disease setting, and it seems possible that normalization of a skewed Th1/Th2 immune balance in ECP-responsive cases is secondary to the improvement in the disease rather than directly modulating Th1 or Th2 cytokine production. We did not appreciate a clear-cut shift to either Th1 or Th2 when looking at the group as a whole or evaluating ECP-responsive vs. ECP-non-responsive. Overall, we found an increase in TNF-α and IL-5 over the 6 months of ECP treatment, with a predominance of IL-5 in those who responded to ECP.

Many genes have been identified as being up-regulated or altered in expression in CTCL.We highlighted three distinct genes, which we evaluated to look for a change in expression during ECP therapy. STAT3 is an acute-phase response factor within the STAT protein family and has been found to be constitutively phosphorylated in SS neoplastic cells and

may play a role in their growth (51). Additionally, forced expression of a transfected dominant-negative STAT3 in CTCL cell lines led to a marked increase in apoptosis of cells, whereas transfection with normal, wild-type STAT3 did not alter the levels of apoptosis (40). Because of these data, STAT3 has been considered to be a 'malignancy factor' in CTCL (40). Plastins or fimbrins are a family comprised of three isoforms of actin-bundling proteins. Interestingly, although the T isoform (T-plastin) is generally expressed in all tissues, except leukocytes, it was recently demonstrated to be expressed in lymphocytes of SS and leukemic MF (35, 37). Lastly, MICB encodes a heavily glycosylated protein that is a ligand for the NKG2D type II receptor. The study by Capriotti demonstrated MICB transcript expression through RT-PCR in over 90% of 'leukemic CTCL' samples evaluated (35). They also noted 100% T-plastin expression in those same samples.

We did identify measurable transcript expression levels of STAT3, MICB, and T-plastin as compared with the expression of the housekeeping gene, GAPDH. We were not able to find significant changes in the expression of any of these genes between time point 0 and 3 months post-therapy. We feel that this lack of change may be secondary to the wide range of gene expression levels between the individual subjects and the fact that these methods may not be sensitive enough to detect changes in non-purified neoplastic cells in individual patients. We also feel that 3 months of ECP therapy may have simply been too early to detect a change in the expression of these markers. It is also important to note that these genes have not been fully established as tumor markers, and further studies are needed to better address this issue.

The presence of TNF-α in the serum of most of our cases is also worth commenting on because it has been called a sentinel cytokine that can augment host defense mechanisms at low concentrations. The observation that TNF-α, IL-10, and to a lesser degree, IL-12 are present in the sera of patients with advanced CTCL may have relevance to ECP. For example, TNF-α is released by ECP-treated monocytes (16), and if administered to patients with already high endogenous TNF-α levels, this might account for post-treatment flares of erythema and/or fever observed in some patients, but might not affect tumor burden.

It is also worth noting that MF and SS have developed in patients treated with the TNF antagonists (52–58). The commercially available TNF antagonists (infliximab, adalimumab, etenercept, certolizumab pegol, and golimumab) not only remove soluble TNF-α but also bind to its membrane-bound receptors (TNFR1 or TNFR2). This could either block the binding of soluble TNF-α to its receptors or could initiate signaling, resulting in cell activation or apoptosis depending on a complex interplay between the metabolic status and the microenvironment of the cell. Most of the TNF antagonist-associated cases of MF/SS have occurred in patients thought to have psoriasis, and the biologic behavior of the emergent lymphoma was often aggressive. This experience suggests either that soluble TNF-α was inhibiting neoplastic T-cell growth or that TNF antagonists were directly stimulating neoplastic T-cell growth (59). The former concept is supported by one study using a CTCL-derived cell line (SeAx), which had growth retarded through administration of TNF-α (60) and could explain some of the therapeutic effects of ECP (16). Additional *in vitro* studies on the effect of TNF antagonists on neoplastic T-cell growth might be illuminating.

Conversely, TNF-α can also lead to excess inflammation and organ injury at high concentrations. The positive correlation between TNF-α serum levels and T-plastin expression in PBMCs in our cases indicates that neoplastic T cells may actually be a major source of TNF-α. Interestingly, neoplastic cells of SS are capable of producing TNF-α when directly stimulated with bacterial products. Recent studies reveal that TNF-α may act as an

autocrine growth factor for some cell lines as reported for the SS-derived HUT78 cell line (61, 62).

The mechanisms by which ECP exerts its effect are clearly complex and likely involve multiple pathways.We were unable to identify any clinical or laboratory findings after 3 months of ECP therapy that were ultimately associated with a clinical response to ECP at 6 months and beyond. We did document a positive association between a favorable ECP response and elevated eosinophil levels, as well as reduced Sézary cell numbers measured at the initiation of ECP treatment, suggesting that patients with less advanced disease were more likely to benefit. Based on this experience, we would advocate administration of adjunct therapies at the initiation of ECP to hopefully reduce the time to response. Lastly, we also found an increase in TNF-α in the serum of patients after 6 months of ECP treatment. Further investigation will be required to fully appreciate the therapeutic effect of ECP, and to identify early markers that may indicate eventual clinical response to therapy.

Acknowledgments

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STAT3		
AVG	5.30	1.01
MED	1.02	0.71
STDEV	14.28	0.84

T-plastin	
0.87	1.71
0.02	0.01
1.62	5.55

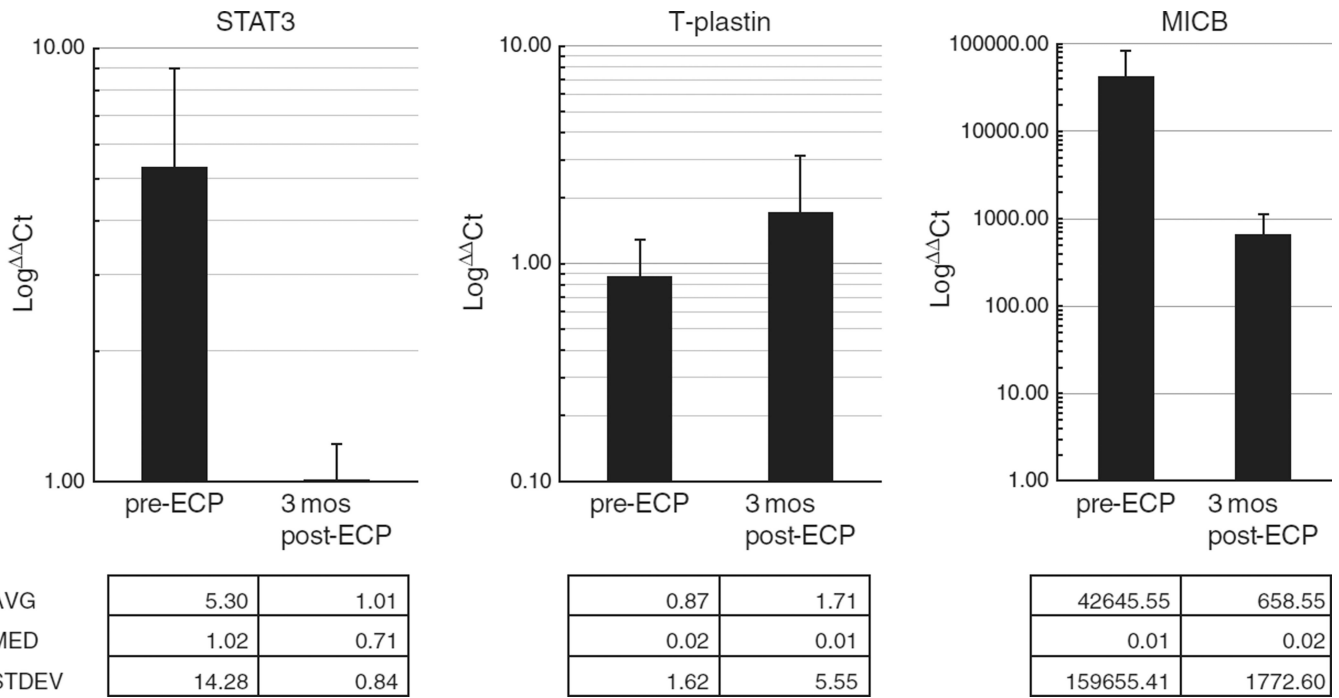| MICB	
42645.55	658.55
0.01	0.02
159655.41	1772.60

Fig. 1.
Real time PCR of the genes STAT3 T-plastin, and MICB demonstrated measurable expression, but significant change was not detected between pre-ECP and 3 months post-ECP.

Table 1

Patients with advance CTCL and favorable short term clinical response on extra corporeal photopheresis momotherapy

Patient/age (years)/sex	DX	TNBM*	Months	Response	%SC	SC/mm³	%CD4/8/7	CD4/8	CD4P7M/mm³†	LDH
1/73/F‡	SS	T4(2)NXB2	0		44	2760	87/3/59	29.0	2070	172
			3	NC	52	2944	76/4/54	19.0	1698	153
			6	MR	58	2127	89/4/72	22.3	734	155
			>6	IFN-α: NR						
2/48/M‡	ECTCL, NOS	T4N1B1	0		17	317	54/24/68	2.3	–	184
			3	MR	15	288	48/18/63	2.7	–	162
			6	MR	13	245	46/18/66	2.6	–	157
			>6	IFN-α: NR						
3/61/M‡	ECTCL, NOS	T4N3B1	0		34	660	84/23/21	3.7	294	
			3	PR	39	452	70/24/35	2.9	200	
			6	MR	32	300	47/27/44	1.7	256	
			>6	IFN-γ: DP						
4/69/M‡	SS	T4N1B2	0		24	2359	86/1/9	86.0	–	193
			3	NR	38	2793	93/1/21	93.0	–	227
			6	MR	30	2297	63/1/20	63.0	–	235
			>6	IFN-α: PR						
5/31/F‡	SS	T4N3B2	0		50	653	82/8/32	10.3	717	214
			3	NR	52	835	84/7	12.0	–	203
			6	MR	54	726	71/6/28	11.8	605	181
			>6	IFN-α: PR						
6/52/M‡	SS	T4N3B2	0		50	1066	89/4/16	22.3	1556	203
			3	NR	39	2048	80/3/16	26.7	1523	224
			6	MR	43	2839	89/3/12	29.7	5282	212
			>6	IFN-α: PR						
7/55/F	MF	T2NXB2	0		24	499	74/9/84	8.2	–	170
			3	MR	14	290	70/12/78	5.8	–	174
			6	PR	22	537	69/8/80	8.6	–	197
			>6	IFN-α: NR						

Patient/age (years)/sex	DX	TNBM[*]	Months	Response	%SC	SC/mm^3	%CD4/8/7	CD4/8	CD4P7M/mm^{3†}	LDH
8/54/F	SS	T4N3B2	0		31	695	86/4/62	21.5	627	263
			3	PR	25	374	83/3/51	27.7	524	260
			6	PR	37	539	81/2/48	40.5	524	264
			>6	IFN-α: NR						
9/72/F	SS	T4N3B2	0		48	5576	98/0.5/5	196.0	10 919	336
			3	MR	43	5315	96/0.5/6	192.0	11 248	364
			6	MR	41	5574	98/2/1	49.0	13 188	325
			>6	IFN-α: NR						
10/79/F	SS	T4N3B2	0		33	340	67/13/66	5.2	–	308
			3	MR	22	172	43/17/61	2.5	–	257
			6	NR	24	133	56/8/57	7.0	–	241
			>6	CR						
11/77/M	ECTCL, NOS	T4N1B1	0		13	121	42/23/80	1.8	–	184
			3	NR	29	339	58/18/84	3.2	–	171
			6	MR	15	171	59/20/83	3.0	–	191
			>6	CR						
12/72/F	SS	T4NXB2	0		0	0	73/5/35	14.6	899	212
			3	PR	0	0	69/5/33	13.8	776	194
			6	PR	0	0	67/4/31	16.8	726	165
			>6	CR						

[*] TNBM, tumor-node-blood-visceral ratings at start of ECP (63); B rating as recommended by the ISCL (8). All cases were at M0.

[†] The absolute number of CD4+CD7– cells for cases in which neoplastic cells have a reduced expression of CD7 estimated by subtracting absolute CD7 from absolute CD3 counts.

[‡] Patients 1–6 previously reported as cases 2, 16, 19, 21, 22, and 29, respectively (29).

DX, diagnosis; SS, Sézary syndrome; ECTCL, NOS, erythrodermic cutaneous T-cell lymphoma, not otherwise defined; MF, mycosis fungoides; IFN-α, interferon-α; CR, complete response; MR, minor response; NR, no response; SC, Sézary cells; LDH, lactate dehydrogenase; CD4P7M, CD4+CD7–, subset of T cells; PR, partial response; W, worse; DP, disease progression; M, male; F, female.

Table 2

Patients with advanced CTCL and lack of short-term clinical responses on extracorporeal photopheresis momotherapgy (Group2)

Patient/age (years)/sex	DX	TNBM	Months	Response	%SC	SC/mm³	%CD4/8/7	CD4/8	CD4P7N/mm³†‡	LDH
13/44/F*	SS	T4N1B2	0		70	2010	79/11/41	7.2	41	251
			3	W	75	2772	77/12/46	6.4	<6	178
			6	NR	55	2884	78/10/28	7.8	28	213
			>6	IFN-α: NR						
14/66/M*	SS	T4N3B2	0		57	614	85/5/87	17.0	–	225
			3	MR	68	799	86/4/85	21.5	–	224
			6	NR	<6	149	–	–	–	213
			>6	IFN-α: MR						
15/69/M*	SS	T4NXB2	0		52	860	88/3/17	29.3	1207	198
			3	NR	67	965	89/3/18	29.7	1051	175
			6	NR	58	438	79/4/28	19.8	416	204
			>6	IFN-α: MR						
16/67/M	SS	T4N3B2	0		52	2677	92/3/94	30.7	–	300
			3	PR	47	2386	94/2/47	47.0	–	385
			6	W	37	2174	95/3/95	31.7	–	347
			>6	IFN-α: NR						
17/50/M	MF	T3(2)N1B1	0		13	496	38/31/74	1.2	–	161
			3	W	36	242	43/27/75	1.6	–	184
			6	DP	16	188	–	–	–	200
18/72/F	SS	T4NXB2	0		54	3558	89/6/12	14.8	5468	216
			3	NR	29	2212	87/7/14	12.4	6101	238
			6	W	52	2777	86/7/11	12.3	4380	293
			>6	IFN-α: NR						
19/61/M	MF	T2NXB2	0		42	511	77/2/69	38.5	–	256
			3	W	56	511	72/2/80	36.0	–	275
			6	NR	43	636	75/2/84	37.5	–	230
			>6	IFN-α: PR						
20/80/M	SS	T4N3B2	0		85	5039	88/1/77	88.0	–	602
			3	NR	80	7410	97/1/80	97.0	–	578

Patient/age (years)/sex	DX	TNBM	Months	Response	%SC	SC/mm³	%CD4/8/7	CD4/8	CD4P7N/mm³†*	LDH
21/73/F	SS	T4NXB2	6	NR	95	5045	98/0.1/79	980.0	–	486
			0		68	3225	79/10/32	7.9	1945	248
			3	NR	76	5194	83/8/27	10.4	4305	250
			6	NR	84	4287	86/9/25	9.6	3471	285

*
Patients 13–15 previously reported as cases 8, 9, and 17, respectively (29).

See footnote of Table 1 for definitions.

Table 3

Comparison of baseline parameters in favorable (Group 1) and poor (Group 2) response patients on extracorporeal photopheresis momotherapgy

Parameters	Group 1 (*n*=12)	Group 2 (*n*=9)	*P*-value
Age (years)	65 (31–79) [*]	67 (44–80)	0.859
Duration of disease (months)	23 (4–240)	54 (8–207)	0.394
WBC/µl	7.8 (5.2–17.6)	8.7 (3.8–12.2)	0.394
Lymphocyte count/mm^3	2062 (928–11 616)	2871 (1078–6588)	0.887
Eosinophils/mm^3	388 (61–1545)	87 (0–351)	0.008
Sézary cells/100 lymphocytes	32 (0–51)	54 (36–85)	0.001
Sézary cells/mm^3	657 (0–5576)	2010 (496–5039)	0.155
%CD4+ cells	83 (42–98)	85 (38–92)	0.499
CD4+ (cells/mm^3)	1585 (390–11 384)	2268 (523–5863)	0.943
%CD8+ cells	6.5 (0.5–24)	5 (1–45)	0.887
CD8+ (cells/mm^3)	118 (58–447)	154 (24–1279)	0.887
CD4/CD8 ratio	12.4 (1.8–196)	17.0 (1.2–88)	0.522
LDH (U/l)	208 (170–336)	248 (161–602)	0.355
IgE (U/l)	92 (2–2716)	35 (5–630)	0.434
IL-5 (pg/ml)	0.06 (0–0.18) [†]	0 (0–0.21)	0.345
IL-10 (pg/ml)	0.14 (0–30.02) [†]	0.63 (0–3.21)	0.504
IL-12 (pg/ml)	0.06 (0–12.69) [†]	0.01 (0–3.20)	0.720
TNF-α (pg/ml)	0.45 (0–1.34) [†]	0.45 (0–4.78)	0.626

[*] Values given as median (range).

[†] Patient 3 not studied.

LDH, lactate dehydrogenase; IL, interleukin.