Extracorporeal photopheresis as a curative treatment strategy in non epidermotropic T-cell lymphoma and large granular lymphocyte leukemia

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Received 9 October 2011; revised 8 January 2012; accepted 10 January 2012

Background: To evaluate the efficacy of extracorporeal photopheresis (ECP) in noncutaneous T-cell lymphoma and large granular lymphocytes leukemia (LGL).

Patients and methods: We have treated 12 refractory/relapsed patients. Six peripheral T-cell lymphoma (PTCL), one T-lymphoblastic lymphoma and five LGL with blood involvement received six biweekly leukapheresis as induction phase, followed by one course a week for 4 weeks as consolidation and one course of maintenance per month for responders until progression/relapse or disappearance of the peripheral clone.

Results: Six patients responded to phototherapy. Two PTCL and two LGL achieved a complete response (CR) and two other PTCL a partial response. The median duration of CR was 117 months (45–150 months) for these four patients. The peripheral clone followed by flow cytometry decreased in all six responders. Two patients with a complete disappearance of the peripheral clone have not relapsed.

Conclusions: As for cutaneous T-cell lymphoma, ECP therefore to be efficient for PTCL and LGL. Early decrease and disappearance of the peripheral clone were the indicators of clinical response and nonrelapse, respectively.

Key words: LGL, peripheral T-cell lymphoma, photopheresis, PTCL

introduction

Extracorporeal chemophototherapy (ECP) has been extensively used in cutaneous T-cell lymphoma (CTCL) since 1983 and is recommended since 2006 as first-line treatment of stage III mycosis fungoides (MF) and Sezary syndrome (SS) by the European Organisation for Research and Treatment of Cancer [1, 2]. Following improved knowledge of its mechanism of action, ECP was explored in other severe diseases such as systemic sclerosis, Crohn’s disease, graft-versus-host disease (GVHD) and for the prevention and treatment of rejection in solid organ transplantation [3, 4].

In CTCL patients, the collection of mononuclear cells by cytapheresis and their irradiation by ultraviolet A (UVA) after sensitization with 8-methoxypsoralen (8-MOP) has been shown to lead to the death by apoptosis of the circulating malignant T-cell clone and to the differentiation of monocytes into efficient antigen-presenting dendritic cells (DC). These two mechanisms lead concomitantly to a decrease of the tumor load and induction of an antitumoral immune response [5]. If the latter is confirmed to be the major therapeutic action of ECP, it should be possible at least in theory to tailor this approach to the treatment of other malignant diseases [5].

Curative treatments for large granular lymphocytic leukemia (LGL) are lacking. Furthermore, there are no standard second-line therapies for either LGL or noncutaneous peripheral T-cell lymphoma (PTCL), which invariably show a very poor prognosis. By assimilation to MF and SS, and to evaluate the sensitivity of LGL and PTCL to ECP, we treated refractory or relapsed patients with a detectable circulating T-cell clone [6–8]. Here, we report the results of this single institution retrospective study.

patients and methods

Between May 1997 and March 2005, 12 refractory/relapsed patients, diagnosed with either PTCL, T lymphoblastic or LGL, were treated in our institution. All patients were selected for ECP because they had a
Annals of Oncology
original articles

Volume 23 | No. 9 | September 2012 doi:10.1093/annonc/mds014 | 2387

Peripheral clone detected by flow cytometry. All provided informed consent to this nonstandard indication of the ECP procedure. The characteristics of the patients’ malignant clones, clinical presentation and response to ECP are listed in Table 1 and Table 2. The therapeutic history and evolution of each patient are described in Figure 1. All samples were reviewed by the local pathologist and described according to the recent World Health Organization 2008 classification [9]. One patient had a lymphoblastic T (LBT) and six patients had a PTCL, respectively, classified as angioimmunoblastic T (AITL, n = 2) and peripheral T cell not otherwise specified (PTCLnos, n = 4) and five had LGL, including one aggressive. Flow cytometry before initiating ECP showed that among the seven lymphomas, four did not express surface CD3 (although CD3 was present in the cytoplasm), six were CD4+ and the LBT was CD3+ but double negative for CD4/CD8. All five LGL clones were CD3+/CD8+ as expected, three were additionally CD16+ and two CD56+. Clonality was clearly identified in eight patients (four lymphomas and four LGL) either by T-cell receptor (TCR) gamma rearrangement analysis (n = 6) and/or a selective Vbeta expansion by flow cytometry (n = 5) [10, 11].

At the time of ECP, the median age of all patients was 49 years old (37–82 years). They were all treated in a refractory phase following at least one previous line of treatment (seven in second line, two in third, one in fourth, one in fifth and one in sixth lines). For all patients, no other treatment was associated with ECP.

All seven lymphomas received at first line a standard regimen either a cyclophosphamide, doxorubicin, vincristine, prednisolone (CHOP) regimen or vepesid, ifosfamide, cisplatin/doxorubicin, bleomycin, vindesine (VIP/rABVD) regimen (for six PTCL) or a CHOP-like regimen (CVDV associating cyclophosphamide, vincristine, doxorubicin, dexamethasone).

Table 1. Characterization of the malignant T-cell clones before extracorporeal chemophototherapy

<table>
<thead>
<tr>
<th>UPN</th>
<th>Type</th>
<th>Immunophenotype</th>
<th>Main Vbeta expansion</th>
<th>TCRγ (DGGE-PCR)</th>
<th>Blood clone (G/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>AIL T</td>
<td>CD3 (−ic+) CD4 + CD7 − CD5 + CD2 + CD10 −</td>
<td>TCR-</td>
<td>No clonal R</td>
<td>0.17</td>
</tr>
<tr>
<td>2</td>
<td>AIL T</td>
<td>CD3 + CD4 + CD7 − CD5 + CD2 + CD10 +</td>
<td>Vbeta17</td>
<td>Clonal R</td>
<td>0.28</td>
</tr>
<tr>
<td>3</td>
<td>PTCLnos</td>
<td>CD3 (−ic+) CD4 + CD7 − CD5 + CD2 + CD10 +</td>
<td>TCR-</td>
<td>Nd</td>
<td>0.31</td>
</tr>
<tr>
<td>4</td>
<td>PTCLnos</td>
<td>CD3 + CD4 + CD7 − CD5 + CD2 + CD10 −</td>
<td>Vbeta13.1</td>
<td>Clonal R</td>
<td>1.97</td>
</tr>
<tr>
<td>5</td>
<td>PTCLnos</td>
<td>CD3 + CD4 + CD7 − CD5 + CD2 + CD10 +</td>
<td>Nd</td>
<td>Clonal R</td>
<td>0.25</td>
</tr>
<tr>
<td>6</td>
<td>PTCLnos</td>
<td>CD3 (−ic+) CD4 + CD7 − CD5 + CD2 + CD10 +</td>
<td>TCR-</td>
<td>Clonal R</td>
<td>0.16</td>
</tr>
<tr>
<td>7</td>
<td>T LB</td>
<td>CD3 (−ic+) CD4 − CD8 − CD7 − CD5 − CD2 + CD10 −</td>
<td>TCR-</td>
<td>Nd</td>
<td>5</td>
</tr>
<tr>
<td>8</td>
<td>LGL T</td>
<td>CD3 + CD8 + CD7 − CD5 + CD2 + CD10 +</td>
<td>Vbeta2</td>
<td>Nd</td>
<td>0.49</td>
</tr>
<tr>
<td>9</td>
<td>LGL T</td>
<td>CD3 + CD8 − CD7 − CD5 + CD2 + CD10 +</td>
<td>Nd</td>
<td>Clonal R</td>
<td>3.6</td>
</tr>
<tr>
<td>10</td>
<td>LGL T</td>
<td>CD3 + CD8 − CD7 − CD5 − CD2 + CD16 + CD56 +</td>
<td>Nd</td>
<td>Nd</td>
<td>1.7</td>
</tr>
<tr>
<td>11</td>
<td>LGL T</td>
<td>CD3 + CD8 + CD7 + CD5 + CD2 + CD16 + CD56 − CD57 −</td>
<td>Vbeta5.1</td>
<td>Nd</td>
<td>4.8</td>
</tr>
<tr>
<td>12</td>
<td>ATC LGL</td>
<td>CD3 + CD8 + CD7 + CD5 + CD2 + CD16 + CD56nd CD57nd</td>
<td>Vbeta2</td>
<td>Clonal R</td>
<td>29</td>
</tr>
</tbody>
</table>

AILT, angioimmunoblastic T; ATC LGL, aggressive T-cell LGL; clonal R, clonal rearrangement; LGL, large granular lymphocytes; Nd, not done; PTCLnos, peripheral T-cell lymphoma without other differentiation; R, rearrangement; T LB, lymphoblastic T.

Table 2. Patient characteristics and clinical responses

<table>
<thead>
<tr>
<th>Characteristics at ECP D1</th>
<th>Previous treatment lines before ECP</th>
<th>ECP treatment</th>
<th>Dottt</th>
</tr>
</thead>
<tbody>
<tr>
<td>UPN</td>
<td>Age, years</td>
<td>WHO</td>
<td>Organ</td>
</tr>
<tr>
<td>-----</td>
<td>--------</td>
<td>-----</td>
<td>------</td>
</tr>
<tr>
<td>1</td>
<td>41</td>
<td>AIL T</td>
<td>LN BM</td>
</tr>
<tr>
<td>2</td>
<td>42</td>
<td>AIL T</td>
<td>LN BM skin</td>
</tr>
<tr>
<td>3</td>
<td>53</td>
<td>PTCLnos</td>
<td>LN Ton</td>
</tr>
<tr>
<td>4</td>
<td>39</td>
<td>PTCLnos</td>
<td>LN BM Spl liv</td>
</tr>
<tr>
<td>5</td>
<td>47</td>
<td>PTCLnos</td>
<td>LN BM Liv</td>
</tr>
<tr>
<td>6</td>
<td>53</td>
<td>PTCLnos</td>
<td>LN BM skin</td>
</tr>
<tr>
<td>7</td>
<td>37</td>
<td>LB T</td>
<td>LN BM</td>
</tr>
<tr>
<td>8</td>
<td>42</td>
<td>LGL T</td>
<td>Blood only</td>
</tr>
<tr>
<td>9</td>
<td>58</td>
<td>LGL T</td>
<td>BM LN</td>
</tr>
<tr>
<td>10</td>
<td>41</td>
<td>LGL T</td>
<td>BM Spl</td>
</tr>
<tr>
<td>11</td>
<td>82</td>
<td>LGL T</td>
<td>BM</td>
</tr>
<tr>
<td>12</td>
<td>63</td>
<td>ATC LGL</td>
<td>BM Liv Int</td>
</tr>
</tbody>
</table>

WHO, World Health Organization; classification according to WHO 2008; AIL T, angioimmunoblastic T; ATC LGL, aggressive T-cell LGL; clonal R, clonal rearrangement; LGL, large granular lymphocytes; PTCLnos, peripheral T-cell lymphoma not otherwise specified. Organs involved: BM, bone marrow (either cytology or histology); Int, intestine; Liv, liver; LN, lymph nodes; Spl, spleen; Ton, tonsils. Organ: organ involvement at the time of ECP. Blood count: for LGL at the time of extracorporeal chemophototherapy (ECP): A, anemia; tr, with transfusion dependance; N, severe neutropenia < 500 cells/μl; T, Previous line: number, number of line before ECP; delay, delay in month between last treatment and ECP; type: chemo, chemotherapy-based regimen; cort, corticotherapy; CR, complete response; DOR, duration of best response in months, with relapse (rel) or not relapse (non rel); gamma, gammaglobulin response; MTX, methotrexate; NR, no response; PD, progressive disease; PR, partial response; N courses, number of ECP courses received; Dottt, duration of treatment in months.
for the T-lymphoblastic lymphoma. Three young patients under 60 years were treated in second line [Unique Patient Number (UPN) UPN 2, 3 and 4]. Despite their refractory disease, we considered ethical to propose ECP because their nonprogressive disease, their preserved performance status, the lack of standard treatment in this circumstance and the good tolerance of the ECP. Patient 6 was treated for the same reasons 3 months after a nonprogressive poor response with a VIP/rABVD regimen followed by 2 months with corticoids. Three patients were treated after second line (UPN 1, 5 and 7). They were refractory to various regimens as 3A (doxorubicin, cytarabine, asparaginase), AVA (cytarabine, vepesid, doxorubicin), CYM (cytophosphamide, methotrexate), MiniBEAM (carmustine, cytarabine, cyclophosphamide, melphalan), MiniBACT (lomustine, cytarabine, cyclophosphamide, teniposide), chlorambucil alone or interferon alone. All the patients began ECP 1 month after their last treatment. All were in Ann Arbor stage IV with nodal involvement and various extranodal sites such as bone marrow (n = 5), liver (n = 2), skin (n = 2), tonsils (n = 1) or spleen (n = 1).

For the LGL patients, four were eligible to receive ECP because they presented either an anemia dependent of blood transfusion (UPN8, 9, 11; with erythroblastopenia on bone marrow analysis for UPN8 and 11) and/or an absolute neutrophil count < 500 cells/μl (UPN 9, 10) refractory to corticoids and for three patients to a second line (gammaglobulins UPN8, danazol UPN9 or methotrexate UPN10). All but one (UPN8) had a bone marrow involvement and two had either splenomegaly (UPN10) or lymph nodes involvement (UPN9) at the time of ECP.

UPN 12 was eligible for PCE for a clinically progressive disease without blood count disorder. At diagnosis, he had a splenomegaly with anemia and B symptoms. After splenectomy, examination of the spleen led to the diagnosis of aggressive T-cell LGL and he recovered a normal blood count and a good performance status. Then a diagnosis of inflammatory bowel disease was established and he was treated with mesalamine, general and local corticoids during 4 years for recurrent diarrheas. PCE was finally indicated for a progressive disease associating hepatomegaly, with increased liver enzymes, increased diarrheas with a proved specific bowel lymphocyte infiltration and B symptoms.

**ECP procedure**

All patients were treated according to the Vilbert Lourma procedure, as previously described [12, 13]. Briefly, leukapheresis was carried out using a Spectra cell separator (COBE Caridian, Lakewood, CO) with treatment of three whole blood masses. Mononuclear cells were transferred to a bag specially adapted for UVA irradiation (MacoPharma, Mouvaux, France) and 8-MOP was added ex vivo. Thereafter, cells were UVA irradiated at 2 J/
cm² (UV-matic irradiator; Vilbert Lourma, France). In cases where the hematocrit was < 5% in the separated cells, irradiation was 2.5 J/cm². The final product was reinfused i.v. to the patient within 3 h after irradiation. Six courses were given during the first 3 weeks, followed by 1 course per week for a total of 10 courses. If at least a partial response (PR) was obtained, the treatment was sustained with one course per month until complete response (CR), progression or disappearance of the peripheral clone for patients in CR.

**evaluation of response**

Responses were evaluated after the 6 courses of induction and then after 10 courses and every 3 months until relapse. For patients with PTCL, the Cheson et al. [14] criteria were used to define CR, PR or nonresponse (NR) including progressive disease. For LGL, the criteria defined by Lamy and Loughran [8] were used to define CR (disappearance of all detectable tumor targets associated to a complete hematological response), PR (reduction of at least 50% of the tumor targets and/or partial hematological response) and NR for patients who fulfilled none of these criteria.

The level of the circulating clone evaluated by flow cytometry was not taken into account for the definition of the response. This monitoring was carried out after the induction phase (M1), after 10 courses (M2) and then every 3–6 months for responders. Assessment of the molecular response was carried out in one LGL patient for whom the blood clone was no longer detectable by flow cytometry.

**results**

Among the 12 patients, 6 were in PR after induction (4 PTCL and 2 LGL) and 6 did not respond [3 progressed during induction (2 AILT, UPN1 and 2 and the LBT, UPN7) and 3 LGL were refractory (UPN 8, 10, 11)]. None of the patients refractory to induction completed their response at 10 courses (Table 2 and Figure 1). Among the six responders, four reached CR after 10 courses [two PTCL (UPN 4, 5) and two LGL (UPN9, 12)] and two PTCL had a sustained PR. The duration of response was 7 and 19 months for the two PR. For the four CR, median duration of response was 117 months (45–150 months).

Among the PTCL patients, all the four PTCLnos responded (two PR and two CR), whereas the other types did not respond or progressed during induction (both AILT and the LBT) (Table 2).

Evolution of the peripheral clone was correlated to the clinical response. Indeed, in the three-evaluated NR patients (UPN 8, 10, 11), the levels of the tumor clone systematically increased in the peripheral blood after induction (Figure 2), whereas the clone of all responders but one (UPN 3) became undetectable at the latest after the consolidation phase. Moreover, circulating tumor levels decreased more rapidly for the four patients fulfilling CR (UPN 5, 7, 9, 12) particularly the two patients who reached negativity by flow cytometry. The latter were one PTCLnos (UPN 5) who showed a rapid decrease in circulating tumor cells and achieved negativity immediately after induction and one LGL (UPN 9) whose tumor clone decreased more slowly to become undetectable after 34 ECP courses (Figure 2). In the case of UPN 9, disappearance of the clone was confirmed by molecular analysis. Only these two patients remain in continuous CR at 136 and 152 months. The two CR patients without

**discussion**

In this original study, we report on the surprising efficacy, in 6 patients, of ECP alone applied to 12 refractory patients with noncutaneous T-lymphoproliferative diseases, 6 PTCL, 1 LBT and 5 T LGL leukemia. Because UVA irradiation-induced apoptosis of the malignant T-cell clone is one of the mechanisms of action of ECP reported in CTCL, these patients were selected on the basis of a detectable peripheral malignant clone [15, 16].

After 10 courses of photopheresis, 4 patients (2 PTCLnos and 2 LGL) fulfilled the criteria of CR and continued ECP with a median response duration of 10 years (6–16 years). Thus, despite the small number of cases, our study proves the efficacy of ECP on other cancers than CTCL, in particular noncutaneous lymphoproliferative mature T cells, as hypothesized recently [5].

All responders were detected very early during the ECP treatment, while two refractory patients progressed during the induction phase and none of the post-induction nonresponders achieved CR. This suggests that tumor cell’s sensitivity to ECP is an intrinsic quality of the tumor clone and that a possible remission by ECP can be detected rapidly. This is similar to the early responses we reported previously in a cohort of patients treated by ECP for GVHD [4]. It might thus be more relevant to consider the clinical response after six courses and to stop in case of poor response.

The second mechanism of action of ECP, proposed in CTCL, is its role in transforming monocytes into DC liable to ingest and process the apoptotic tumor cells [5]. The four patients in CR were CD3+ supporting the hypothesis that specific peptides originating from the CD3/TCR complex, i.e. clone-specific TCR idotype, might indeed be presented by antigen-presenting cells to competent antitumoral T cells. This
modification in antigenic presentation is supported by recent studies demonstrating the impact of ECP in monocytes either in ex vivo models or in GVHD patients [17, 18]. However, because two CD3-negative patients fulfilled a PR, another mode of action could be involved, and apoptosis only might occur in such patients without efficient presentation of tumor-specific antigens and subsequent immunological eradication of remaining cells. It may also be hypothesized that these patients expressed very low levels of TCR/CD3, below the sensitivity of flow cytometry, thus having also less immunogenic peptides to be presented by DC.

Finally, the clinical response was correlated to the evolution of the blood clone and hence sensitivity to ECP as described above. All non responders increased the blood level of the clone, whereas all responders but one showed a decreased tumor burden at least after the 10 first courses. Moreover, the four CR patients had the best rapid regression and the two patients who dropped to negativity have never relapsed. Therefore, the monitoring of blood clone could help us to define clearly the maintenance phase duration.

For the treatment of PTCL patients, pralatrexate, romidepsine or bendamustine have recently been described as promising new drugs. Three phase II studies using these agents alone for patients in relapse reported overall response rates between 29 % and 47 % and CR rate ~20 % with median durations of response between 5 and 10 months [19–21]. In the future, our results with the PCE approach might be confirmed and compared with the new combinations in progress including these new drugs.

In conclusion, we demonstrate with this small series the effectiveness of ECP in the treatment of noncutaneous T-lymphoproliferative diseases such as PTCL and LGL, presenting with a circulating T-cell clone. As limited options are offered to such patients, ECP should therefore be considered for them, closely monitored and sustained for responders who appear to be liable to reach not only CR but possibly full cure. The place of this immune approach remains to be defined in the global management of such patients.

**Acknowledgements**

The authors acknowledge Pr Jean Jacques Sotto who was initiator of this work, Pr Thierry Lamy for his help in clearly defining the large granular lymphocytes patients included in the study, Pr Marie Christine Bene for fruitful discussions and thank all the patients who accepted the extracorporeal chemophototherapy treatment. Martine Chauvet, Isabelle Puteaud and Céline Suchaud are gratefully acknowledged for their contribution to the molecular evaluation. The members of the ARAMIS association are acknowledged for their support over the years.

**Disclosure**

The authors declare no conflicts of interest.

references