Low-dose intermittent alemtuzumab in the treatment of Sézary syndrome: clinical and immunologic findings in 14 patients

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ABSTRACT

Background and Objectives

Alemtuzumab may be effective in Sézary syndrome (SS), an aggressive cutaneous T-cell lymphoma, but is associated with severe hematologic toxicity and infections. This study investigated whether low-dose subcutaneous alemtuzumab can induce hematologic, immunologic, and clinical responses similar to those obtained with the standard regimen, but with less toxicity.

Design and Methods

Fourteen SS patients were enrolled: 11 had relapsed/refractory disease and three had untreated SS with high counts of circulating Sézary cells (SC). Four received 3 mg alemtuzumab on day 1, 10 mg on day 3, then 15 mg on alternating days; circulating SC were evaluated after the fourth 15 mg dose and treatment was interrupted in the presence of counts <1,000/mm^3. A reduced dosage (3 mg on day 1, then 10 mg on alternating days) was administered to the remaining patients, with SC counted before every injection, until a reduction to values of <1,000/mm^3.

Results

The median SC count decreased by 95.5%. Overall, 12/14 patients (85.7%) achieved a clinical response, with three complete responses (21.4%). After a median follow-up of 16 months, the median time-to-treatment failure is 12 months. Infectious complications occurred in 28.6% of patients, all included in the group treated with 15 mg. No patient in the group treated with 10 mg developed hematologic toxicity or infections. An early recovery of circulating NK, B and CD3$^+$CD8$^+$ cells occurred after the first cycle.

Interpretation and Conclusions

Subcutaneous alemtuzumab at very low doses (10 mg maximum per administration), given for a short period based on SC levels, has a good toxicity profile, high response rate and causes durable remissions in SS patients with high tumor burden in the peripheral blood.

Key words: alemtuzumab, Sézary syndrome, intermittent subcutaneous administration, low-dose, flow-cytometry.

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Primary cutaneous T-cell lymphomas (CTCL) encompass a broad spectrum of disease entities with specific clinicopathologic, phenotypic and molecular features, all characterized by the primary cutaneous origin of the lymphoproliferative disorder.[1] The incidence rate of CTCL is 0.45 per 100,000 person-years and the most common subtype is mycosis fungoides (MF).[2-4] Sézary syndrome (SS), which accounts for about 15% of all cases of CTCL,[2] is characterized by a pruritic exfoliative erythroderma, peripheral adenopathies and atypical mononuclear cells with cerebriform nuclei (Sézary cells [SC]) in skin, peripheral blood and lymph nodes. Patients with SS have a poor prognosis with a 5-year survival of 24%. The disease is included in the aggressive CTCL subtypes under the recent WHO-EORTC classification.[5] Most patients die of infectious complications, as a result of the severe disease-related immunosuppression.

Although SS prognostic factors are not well defined, because of the rarity of this disease, the majority of researchers agree that the extent of peripheral blood involvement is one of the most relevant parameters associated with the disease course. Indeed, we observed a 5-year survival rate of 55.8% in patients with slight or moderate blood involvement (SC ≤2,600/mm^3) and only 11.6% in patients with greater involvement (SC >2,600/mm^3).[6] More recently, other researchers have reported a significant increase in the disease-specific death rate with increasing numbers of circulating SC and after correcting for age at diagnosis.[7]

The optimal treatment for SS remains a challenge. Extracorporeal photopheresis, alone or in combination with other agents (such as interferon or chemotherapy), is now considered the first-line option,[8] with response rates ranging between 30% and 80%; however, the response rates are significantly lower in patients with a high SC count and low numbers of circulating CD8$^+$ cells.[9] Single-agent chemotherapies (chlorambucil or purine analogs), as well as combination chemotherapies, have been associated with response rates ranging from 20% to 60%, but complete responses are rare and response duration is usually short (from 3 to 6 months).[10] As yet, no treatment has demonstrated therapeutic activity in patients with a frank leukemic phase.

Recently, studies have shown that the monoclonal antibody alemtuzumab can be effective in the treatment of chronic lymphocytic leukemia, T-cell prolymphocytic leukemia and peripheral T-cell lymphoma.[11-14] Alemtuzumab is a humanized monoclonal antibody directed against CD52, a cell surface antigen expressed on B and T lymphocytes, monocytes and natural killer (NK) cells. The mechanisms through which alemtuzumab exerts its clinical activity are not well known, but likely involve a series of effects, such as apoptosis induction,[15] antibody-dependent cellular cytotoxicity[16,17] and complement-mediated cell lysis.[18,19] In the standard therapeutic schedule, escalating doses from 3 mg to 10 mg and then to 30 mg are administered on alternating days, followed by 30 mg three times a week for at least 12 weeks. Only a few data are available on alemtuzumab treatment in CTCL. In the largest series reported, alemtuzumab administered according to the standard schedule relieved symptoms in 55% of patients with advanced MF/SS (32% achieved complete remission and 23% partial remission) and the median time to treatment failure was 12 months.[20] In another study, a lower response rate was achieved (38%) in a total of eight patients with relapsed or refractory advanced stage CTCL; seven of these had MF/SS.[21] No complete remissions were obtained; moreover, the response duration was short, with all patients developing progressive disease within 4 months of starting alemtuzumab. A preliminary report from Zinzani *et al*. suggested that a reduced-dose alemtuzumab schedule (10 mg three times a week for 1 month) had clinical activity in ten pre-treated T-cell lymphoma patients among whom four had MF.[22]

Treatment with alemtuzumab is, however, accompanied by severe toxicity, particularly hematologic toxicity (grade 3-4 cytopenias in up to 45% of cases) and an increased risk of infectious complications.[23] In a recent review, the risk of infections in nodal lymphoma/leukemia patients ranged between 23% and 79%; the most common complication was represented by viral infections, particularly cytomegalovirus (CMV) infection, occurring in more than 40% of cases, followed by bacterial sepsis and fungal infections.[23] The risk of infection is increased in advanced disease and in patients heavily pretreated with chemotherapy. In the study quoted above involving patients with CTCL, an infectious complication occurred in 50% of cases, with two deaths;[20] Kennedy *et al*. reported an even higher rate of infections (7/8 patients).[21] The development of infectious complications is related to the fact that alemtuzumab induces a profound and long-lasting depletion of mature B- and T-lymphocytes, as well as NK cells and monocytes.[24]

On the basis of these data, we designed a low-dose, patient-tailored schedule of alemtuzumab for the treatment of patients with SS who were refractory to or had relapsed after chemotherapy, and/or had a high peripheral blood tumor burden. We used flow cytometric analysis to monitor the atypical cell subpopulation; measurements determined individual treatment administration and duration. Our objectives were: (i) to ascertain whether low subcutaneous doses of alemtuzumab induce hematologic, immunologic and clinical responses similar to those obtained by the standard regimen; (ii) to verify whether the monitoring of the atypical cell subpopulation in the peripheral blood can help to determine the schedule of treatment; and (iii) to evaluate whether the dose reduction and the intermittent treatment schedule reduce the toxicity and the incidence of infections.

Design and Methods

Patients

This study was conducted from April 2003 to September 2006, and enrolled 14 patients (6 males and 8 females, median age 72 years, range 48-82) with a diagnosis of SS; written informed consent was obtained from each participant before entry into the study. The patients' characteristics are listed in Table 1. The median interval between diagnosis and study entry was 9 months (range, 1 month to 4 years). Eleven patients had rapidly progressive disease, relapsed or refractory to previous treatments; all had previously received one or more systemic chemotherapy regimens. The remaining three patients had newly diagnosed SS with high peripheral blood cell counts. The diagnostic criteria for SS were:[25] (i) erythroderma and peripheral lymphadenopathies; (ii) peripheral blood involvement by circulating SC; (iii) cutaneous biopsy-proven CTCL, confirmed by the finding of a clonal T-cell receptor (TCR)-γ gene rearrangement.[26] Peripheral blood involvement was defined by at least one of the following criteria recently proposed by the International Society for Cutaneous Lymphoma:[25] (i) absolute circulating SC count of ≥1000/mm^3; (ii) CD4/CD8 ratio of ≥10 caused by an increase in circulating T cells; (iii) increased lymphocyte counts with evidence of a T-cell clone in the peripheral blood by polymerase chain reaction (PCR); (iv) circulating $CD4^+CD7^-$ value of ≥40%; (v) aberrant expression of T-cell markers; and (vi) a chromosomally abnormal T-cell clone. In addition, a $CD4^+CD26^-$ value of >30% of peripheral blood lymphocytes was used as a threshold value for the diagnosis and monitoring of peripheral blood involvement.[27] Circulating SC were morphologically identified on the basis of their characteristic cerebriform nuclei,[3] on peripheral blood smears stained with May-Grünwald-Giemsa. Eligibility criteria for inclusion in the study were a diagnosis of SS, with either progressive disease that had relapsed after or was refractory to one or more chemotherapy regimens, or untreated with a high peripheral blood cell count; the presence of CD52 expression on neoplastic cells in both skin and peripheral blood; age >18 years; Eastern Cooperative Oncology Group (ECOG) performance status of ≤2; adequate bone marrow function; normal hepatic, renal and pulmonary function.

Patients were excluded in the presence of an active ongoing infection not adequately controlled by antibiotics or if they were human immunodeficiency (HIV)-positive; other exclusion criteria included a period of <4 weeks from the last systemic treatment and the presence of other severe concurrent diseases or mental disorders.

The evaluation of the patients was based on detailed medical history, physical examination with skin score assessment[28] and color photographs, two-dimensional measurement of enlarged lymph nodes by palpation and ultrasound imaging, complete blood cell count, differential count with quantification of circulating atypical lymphocytes, routine laboratory tests including electrolytes, liver, kidney and thyroid function tests, uric acid and lactate dehydrogenase (LDH) serum levels, and computed tomography (CT) scans of the abdomen, pelvis and lungs. Patients self-assessed the severity of their itching on a visual analog scale (VAS), from 0 for no itching to 10 for worst possible itching. PCR analysis for CMV was performed before treatment in all patients.

Table 1. Patients' characteristics at baseline.

Patient	Age (y)/ Sex	Previous regimens	Interval between diagnosis and entry into the study (months)
1	72/M	CHL+PRED, FAMP, COP, MTX, ExP	17
2	78/F	FC, ExP	10
3	48/F	CHOP	7
4	75/M	CHL+PRED, ExP	12
5	51/F	FC, CHL+PRED, ExP, Bexarotene	28
6	74/M	FAMP, ExP	48
7	82/M	ExP, CHL+PRED	18
8	72/F	None[a]	1
9	60/F	None[a]	1
10	64/F	FAMP, CHL+PRED, MTX	34
11	72/M	CHL, Exp	8
12	69/M	FAMP, CHL, ExP	7
13	82/F	CHL	8
14	74/F	None[a]	1

[a]*These patient had newly diagnosed Sézary syndrome with a high peripheral blood cell count. CHL+PRED: chlorambucil plus prednisone; FAMP: fludarabine monophosphate; COP: cyclophosphamide, vincristine, prednisone; MTX: methotrexate; ExP: extracorporeal photochemotherapy; FC: fludarabine plus cyclophosphamide; CHOP: cyclophosphamide, adriblastine, vincristine, prednisone.*

Treatment plan, clinical response evaluation and toxicity

Alemtuzumab (MabCampath®; Schering AG, Milan, Italy) was administered by subcutaneous injections in an escalating low-dosage regimen, according to two different schedules. In the first four patients (nos.1-4), treatment was administered at 3 mg on day 1, 10 mg on day 3, followed by 15 mg on alternating days for a total of four doses; circulating SC were evaluated only after the fourth 15 mg dose and the treatment was interrupted in the presence of counts <1,000/mm^3.

A reduced dosage (3 mg on day 1 and 10 mg on alternating days thereafter) was administered to nine patients (nos. 5–9 and 11–14) until the number of circulating SC decreased to <1,000/mm^3. The maximum alemtuzumab dose per administration was reduced from 15 mg to 10 mg on the basis of the clinical activity and infectious complications observed in the first four patients. The dosage was further reduced to 1 mg on day 1, 3 mg on days 3 and 5, followed by 6 mg on day 7 in one patient affected by achondroplastic dwarfism (body surface area 1.3 m^2).

Thereafter, patients were clinically and immunologically monitored every 2 weeks for the first 2 months and monthly thereafter. The treatment was repeated for both

schedules only when SC values increased to >2,000/mm^3 and was administered at the dosage of 3 mg on day 1 and 3 or 10 mg (depending on SC values) on alternating days, until the SC counts returned to values <1,000/mm^3. Afterwards, patients resumed clinical and immunological follow-up and were re-treated every time SC values increased to > 2,000/mm^3.

All patients received premedication 30 minutes prior to each alemtuzumab injection. This premedication consisted of oral paracetamol 1g and intravenous (IV) antihistamines (chlorphenamine 10mg); hydrocortisone 100mg was given intravenously as premedication only for the first alemtuzumab administration and thereafter withdrawn. Antibiotic prophylaxis (trimethoprim/sulfamethoxazole twice daily three times a week, valaciclovir 1000 mg/day and itraconazole 100 mg/day) was given throughout the treatment period and continued for 1 month after alemtuzumab discontinuation. All patients also received oral doses of allopurinol 300mg per day.

Response assessment was based on the measurement of clinically apparent disease in the skin, lymph nodes, and peripheral blood, and defined according to ECOG criteria[29] as a complete response (CR), a partial response (PR), stable disease (SD) or progressive disease (PD). A CR was defined as the disappearance of all evidence of clinical disease for ≥4 weeks (clinical complete response, CCR) and a PR as ≥50% tumor regression and ≥50% reduction in the SC counts for a minimum of 4 weeks, without the appearance of new lesions. A patient was defined as a *responder* only if a response was achieved in all of the pretreatment sites of disease involvement. The response was evaluated after 4 weeks, and on a monthly basis thereafter. The cutaneous response was based on the change of the skin score (where 0 = normal; 1 = barely detectable erythema and scaling; 2 = readily detectable erythema, edema and scaling; 3 = marked erythema and exfoliation; 4 = fissuring, maximal erythema, induration and tumors) and surface area percentage.[28] The lymph node response was assessed by physical examination of palpable nodes and ultrasound imaging. Nodal CCR was defined as regression to normal size of all superficial adenopathies (≤1.5 cm in the greatest transverse diameter for nodes ≥1.5 cm before therapy). Previously involved nodes whose greatest transverse diameter at baseline was between 1.1 and 1.5 cm had to regress to ≤1 cm.[30] The response in the blood was determined by comparing the absolute number of circulating $CD4^+CD26^-$ atypical lymphoid cells before and after treatment. Phenotypically identifiable cells in 11 patients confirmed these data according to measurement of an expanded TCRvβ population and a T-cell lineage antigen loss.

Total and differential blood cell counts, as well as SC counts and peripheral blood lymphocyte (PBL) flow-cytometry, were evaluated at baseline, the day before the start of treatment; the same tests were repeated the day after the fourth 15 mg dose in the first four patients (nos.1-4) and the day before every alemtuzumab injection in the remaining cases, until there was a reduction in the atypical subpopulation to values <1,000/mm^3. After alemtuzumab discontinuation, SC counts and PBL flow-cytometry were evaluated every 2 weeks for the first 2 months and then monthly during the follow-up period in both groups. Thyroid function was assessed monthly. Serum electrolytes, liver and kidney tests were performed every 2 weeks for the first month, and every 4 weeks thereafter, as well as PCR for both CMV and Epstein-Barr virus (EBV). In the presence of CMV reactivation in the peripheral blood, patients were treated with IV ganciclovir 5 mg/kg twice daily; alemtuzumab was discontinued and restarted if necessary after the clearance of the viral load. Systemic toxicity from treatment was graded according to WHO criteria.

Peripheral blood lymphocyte flow cytometry

All analyses were conducted on fresh cells. PBL were analyzed according to their immunofluorescence reactivity, using a FACSCalibur flow cytometer (Becton Dickinson, San José, CA, USA). Three or four-color immunofluorescence analyses were performed simultaneously, using fluorescein isothiocyanate (FITC), phycoerythrin (PE), peridinin chlorophyll protein (PerCP) and allophycocyanin (APC) conjugated antibodies. At least 10,000 lymphocytes were collected for each antibody combination. Lymphocyte purity was verified by the usual forward and sideward scattering parameters, by means of a CD45 gating analysis. Isotype-matched negative controls conjugated to each fluorochrome were used to set the location of the cursor for each blood sample. A wide panel of monoclonal antibodies directed against T-cell antigens was tested routinely, and included CD2 (clone S5.5), CD3 (clone SK7), CD4 (clone SK3), CD5 (clone L17F12), CD7 (clone CD7-6B7), CD8 (clone SK1), CD16 (clone B73.1), CD19 (clone SJ25C1), CD25 (clone CD25-3G10), CD26 (clone L272), CD45RA (clone MEM-56), CD45R0 (clone UCHL1) CD52 (clone CF1D12), CD56 (clone MY31), and HLA-DR (clone L243). CD2, CD7, CD25, CD45RA, CD45R0 and CD52 were obtained from Caltag Laboratories (Burlingame, CA, USA), whereas the others were purchased from Becton-Dickinson (Palo Alto, CA, USA). Clonal TCR rearrangement was identified using a panel of 28 monoclonal antibodies directed against the variable regions of the β-chain (Serotec Ltd., Oxford, UK; Immunotech Coulter Company, Marseille, France; Endogen, Cambridge, MA, USA).

TCR-γ gene rearrangement was studied by carrying out a multiplex PCR/heteroduplex (HD) analysis on polyacrylamide gel electrophoresis in both skin specimens and blood samples in all patients. The presence of a clonal TCR-γ gene rearrangement was also evaluated in selected cases using a GeneScan method based on capillary electrophoresis with automated fluorescent DNA.

Statistical methods

The Wilcoxon signed-rank test for paired samples was used for comparisons between the baseline and follow-

Table 2. Alemtuzumab in Sézary syndrome: treatment responses, doses and response duration.

Patient	Overall response[a]	Sézary cells in PB (mm^3)[b]		Response per site			PBL Response induction time (days)	No. of cycles	First cycle alemtuzumab dose (mg)	Cumulative alemtuzumab dose (mg)	Time to treatment failure (months)
				Skin	Lymph nodes						
		Before	After		Size (cm)	Resp.					
1	SD	1,183	8	SD	>5	PR	9	1	73	73	5
2	PR	38,766	312	PR	>5	CCR	8	2	73	86	4
3	PR	11,084	74	PR	>5	PR	11	1	73	73	7
4	PR	7,578	293	PR	2-5	CCR	4	4	88	127	9
5	PR	1,998	105	CCR	2-5	PR	8	7	23	137	35
6	PR	4,189	928	PR	2-5	PR	3	1	13	13	17+
7	PR	2,930	610	PR	≤2	PR	4	7	13	104	17+
8	PR	8,257	421	PR	≤2	PR	4	2	13	29	12
9	CCR	30,863	591	CCR	>5	CCR	13	1	53	53	16+
10	PR[d]	11,053	1,458	PR	2-5	CCR	8	2	13[c]	15	13+
11	PR[d]	3,269	48	PR	2-5	PR	7	2	13	19	7+
12	SD	4,268	603	SD	>5	PR	7	3	23	99	6
13	PR	8,146	764	PR	2-5	PR	4	3	13	99	7+
14	PR	9,324	59	PR	2-5	PR	8	2	23	36	6+

[a]As determined at week 4 of treatment. [b]As determined by morphological and phenotypic analyses. [c]Alemtuzumab dosage was 1mg on day 1, 3 mg on days 3 and 5, followed by 6 mg on day 7. [d]these two patients (no.10 and 11) achieved a CCR (in patient no.10 also confirmed by molecular biology), 6 and 4 months, respectively, after the beginning of treatment. PBL: peripheral blood lymphocyte; PB: peripheral blood; SD: stable disease; PR: partial response; CCR: clinical complete response.

up/end of treatment laboratory values. Fisher's exact probability test was used for cross-table comparisons.

Time-to-treatment-failure (TTF) was measured from the first day of treatment with alemtuzumab to either therapy discontinuation for any reason, disease recurrence requiring a different treatment, or death from any cause. Response duration was counted as the time between the achievement of response and disease recurrence or death. Overall survival was calculated by taking the time elapsed from the beginning of therapy to either last check-up or death. Time-to-treatment-failure, response duration and overall survival were analyzed according to the Kaplan-Meier product-limit method.

Results

Clinical results

Alemtuzumab induced a median reduction of 95.5% (range: 77.8–99.4) in the SC count (Table 2). Values <1,000/mm^3 were obtained from 3 to 13 days after the start of treatment in 13/14 patients. In patient no. 10, affected by dwarfism and heavily pre-treated with fludarabine, alemtuzumab administration was suspended when the SC count decreased from 11,053/mm^3 to 1,458/mm^3; 1 month later, the patient received a 1 mg dose on two alternating days, resulting in a SC decrease to 24/mm^3 (Figure 1A). In patient no. 9, a progressive reduction in both percentage and absolute number of SC (7% and 8/mm^3, respectively), was observed 4 months after discontinuation of alemtuzumab (Figure 1B). No difference in the reduction of SC count was observed between patients treated with up to 15 mg and those who received a maximum dosage of 10 mg. The median dose of alemtuzumab administered in the first course was 73 mg (range: 73-88) in the 15 mg group and 13 mg (range: 13-53) in the 10 mg group. After 4 weeks, 12/14 patients (85.7%)

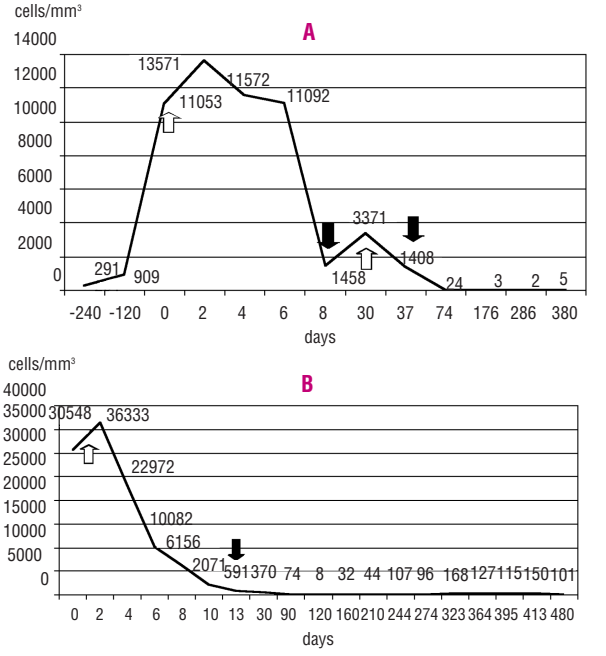

Figure 1. Kinetics of the circulating Sézary cell subpopulation as determined by flow-cytometry, before and during alemtuzumab treatment. A. Patient no. 10: absolute values of clonal circulating $CD4^+CD26^-CD7^-TCRv\beta6.7^+$. B. Patient no. 9: absolute values of clonal circulating $CD4^+CD26^-CD7^-TCRv\beta13.1^+$. The white arrows (⇩) indicate the beginning of the treatment, the black arrows (⇧) the end. Numbers reported in the graph indicate the absolute levels of circulating atypical lymphoid cells. Time *0* on the X axis represents the day of starting treatment.

Table 3. Skin scores at baseline and after 4 weeks of treatment with alemtuzumab in patients with Sézary syndrome.

Patient	Skin	
	Baseline	At Week 4
1	255	228
2	326	48
3	126	41
4	236	67
5	200	0
6	193	90
7	200	100
8	234	115
9	269	0
10	218	109
11	180	90
12	306	236
13	162	72
14	151	69

achieved a clinical response (1 CCR and 11 PR; Figures 2A–F, 3A–F); two cases of SD were observed. According to the site, two patients (nos. 5, 9) achieved a CCR in the skin and four (nos. 2, 4, 9, 10) in the lymph nodes. The reduction in the skin score is shown in Table 3; the median reduction in responding patients was 53.9% (range 50%–100%). Nodal CCR was obtained in two patients with 2-5 cm sized adenopathies (nos. 4 and 10) and in two with > 5 cm sized adenopathies (nos. 2 and 9) (Table 2). The itching score improved from a median of 9 at baseline (range, 4–10) to 3 (range, 0–5) after treatment, and this symptom completely disappeared in two patients.

The treatment was repeated on the basis of the immunological monitoring only when SC values increased to > 2,000/mm^3. Four patients underwent only one cycle of therapy, whereas five were treated with two cycles; the remaining five patients received from three up to seven alemtuzumab courses (Table 2). The median interval between the first and second cycles was 2.9 months (range 1.0–17). The subsequent cycles were performed at a median interval of 3.2 months (range: 1.5-5). The median cumulative alemtuzumab dose administered to each patient was 73 mg (range 13–137).

During the follow-up, two patients with PR at week 4 after the beginning of treatment, developed a CCR (nos.10 and 11). Patient no. 10 obtained CR 5 months after the second course of alemtuzumab; she is still in remission 13 months after inclusion in the study, without any addition-

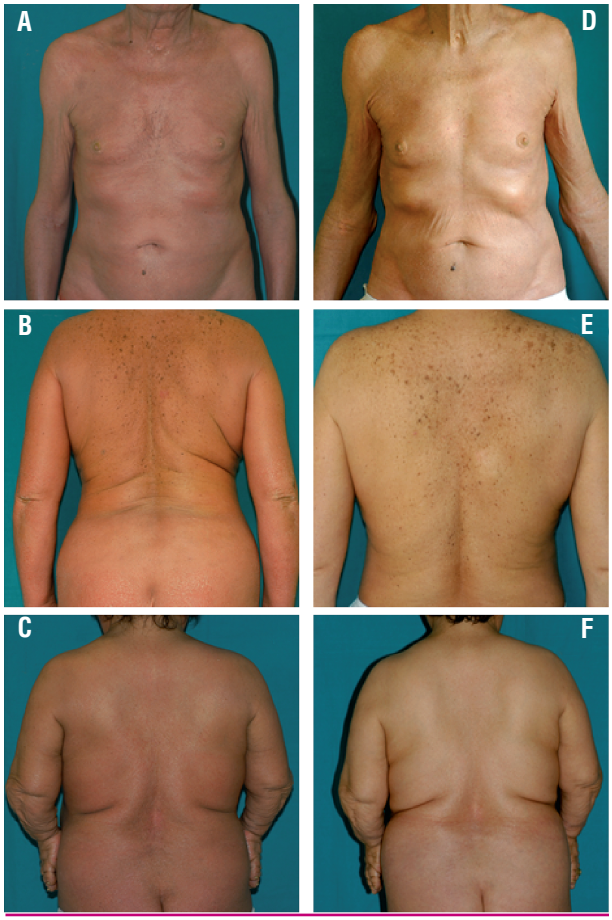

Figure 2. The clinical picture of patients #4 (A), 9 (B) and 10 (C) before and 4 weeks after (D, E, F respectively) starting of treatment.

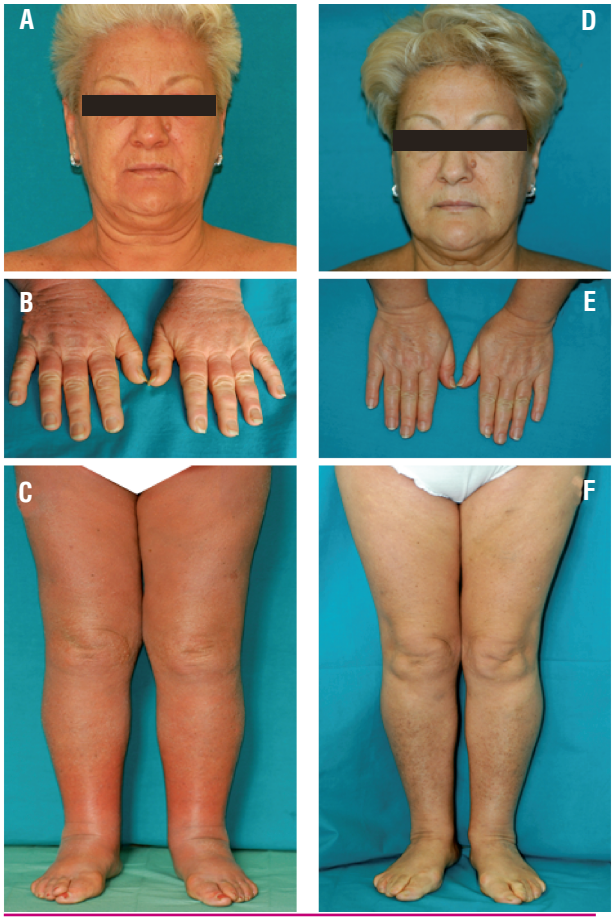

Figure 3. Patient #9 before (A, B, C) and 4 weeks after (D, E, F) starting treatment.

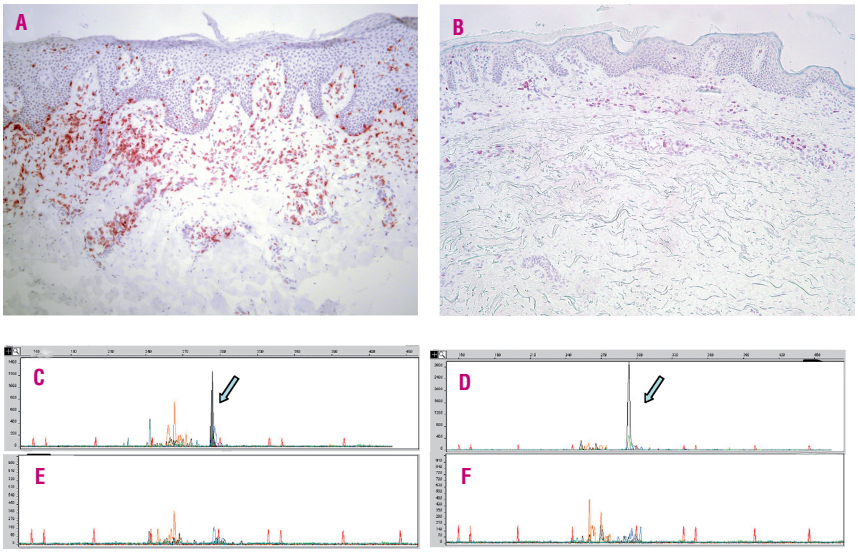

Figure 4. Immunohistochemistry of patient no. 10 before (A) and after (B) treatment, with complete disappearance of the atypical lymphoid infiltrate and persistence of isolated CD3+ normal lymphocytes (CD3 staining, magnification 80×). Gene Scan analyses of patient no. 10 with evidence of the same peak of clonal TCR-γ rearrangement (arrow) in both skin (C) and peripheral blood (D) before treatment; no evidence of clonal rearrangement was detected after treatment (E: skin; F: peripheral blood).

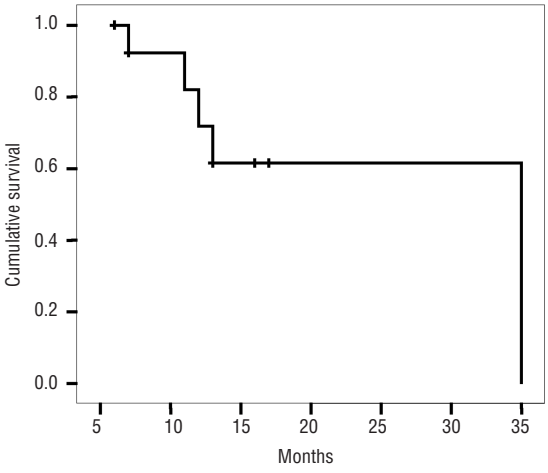

Figure 5. Overall survival curve from the beginning of treatment.

al treatment. The CR was confirmed by the disappearance of the atypical lymphoid infiltrate in the skin (Figure 4A–B) and by the absence of a clonal TCR rearrangement in either the skin or peripheral blood, as determined using GeneScan PCR analysis (Figure 4C–F). Overall, the best responses obtained were three CCR (21.4%) and nine PR. All three patients who achieved CCR belonged to the group treated with a maximum dosage of 10 mg.

The median time-to-treatment failure is now 12 months (range, 4-35). The median response duration has not been reached. Seven out of 12 responders are still in remission (Table 2). In two of them (nos. 6 and 9) the response obtained after the first cycle (PR and CCR, respectively) is still maintained after 17 and 16 months, respectively, without further alemtuzumab administrations. Patient no. 5 developed a colon carcinoma 28 months after the first cycle of alemtuzumab and died of disseminated metastases while maintaining the PR. After a median follow-up of 16 months, treatment was discontinued in four responding patients: two (nos. 3 and 8) developed a high grade T-cell lymphoma and underwent systemic polychemotherapy, one (no. 2) developed disseminated plaques and nodules without further evidence of peripheral blood involvement and was treated with oral bexarotene; the last patient (no. 4) was given oral fludarabine as maintainance treatment after a spinal epidural abscess was found.

The median survival was 35 months (range 5–35) (Figure 5). All the four patients treated with a maximum dose of 15 mg died (two due to CTCL disease progression, one due to infectious complications and one from CTCL-unrelated causes); on the other hand, only one of the ten patients treated with a maximum dose of 10 mg died, due to disseminated colon carcinoma metastases.

Adverse events

Grade 1 erythema and edema occurred in the inoculation site in two patients. Three patients developed fever, headache and grade 1 osteoarthralgia; these events were confined to the first inoculation and disappeared within 12 hours. No patients had rigors, hypotension or urticaria; moreover, no patients developed other skin rashes during the treatment period or follow-up. Hematologic toxicity was mild; only one patient developed grade 3 thrombocytopenia and two experienced grade 1 anemia; all these three patients belonged to the group treated with a 15 mg maximum dosage.

Infectious complications occurred in four patients (28.6%). Three patients (nos. 1, 2, 4) developed staphylococcal sepsis, which was associated with a spinal epidural abscess (no. 4) and with vertebral osteomyelitis (no. 2); patient no. 2 died due to infectious complications, whereas intravenous antibiotics successfully resolved the sepsis in the other two patients. CMV viral load became

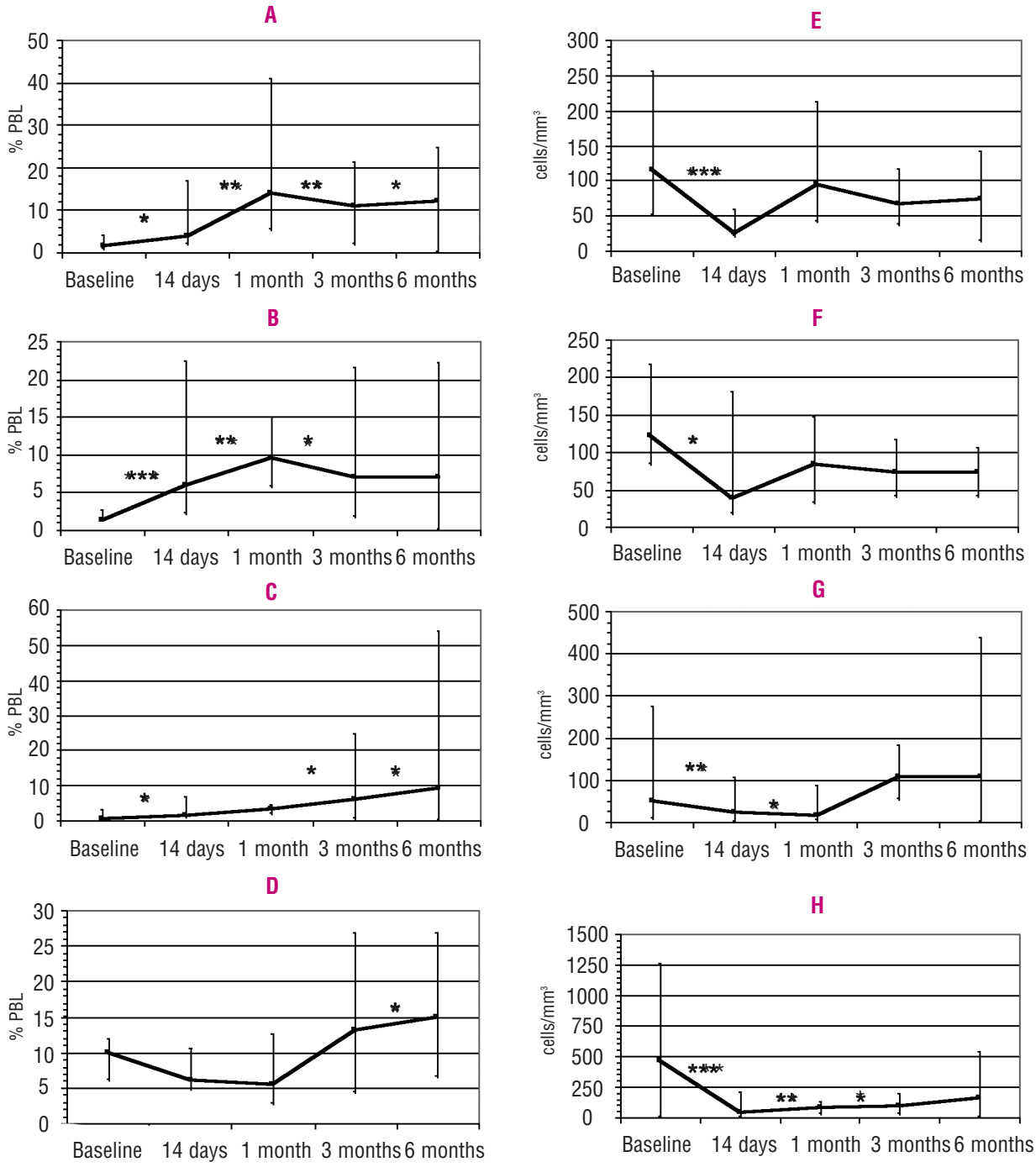

Figure 6. Kinetics of the median percentage (A, B, C, D) and absolute (E, F, G, H) values of the circulating NK (percentage values: A; absolute values: E). $CD3^+CD8^+$ (percentage values: B. absolute values: F). $CD19^+$ (percentage values: C; absolute values: G) and normal $CD3^+CD4^+$ cells (percentage values: D; absolute values: H) during alemtuzumab treatment. The vertical bars represent the upper and lower 95% confidence intervals. *: $p<0.05$; **$p<0.01$: ***$p<0.001$.

detectable in the peripheral blood of three patients (nos. 1, 2, 3) during follow-up; only one developed fever without pneumonitis (no. 1). PCR analysis became negative after IV ganciclovir treatment in all three patients. A close relationship was found between the dose of alemtuzumab administered, the time during which it was given and the occurrence of infectious complications. In fact, infectious complications occurred in all the four patients treated with alemtuzumab at dosages up to 15 mg (median dose in first cycle: 73 mg) and none of patients treated with a maximum dose of 10 mg (median dose of first cycle: 13 mg) (Fisher's exact probability test; $p=0.001$). The cumulative dose was related to the time during which it was administered; the median alemtuzumab dose per day was 0.485 mg in patients with infectious complications and 0.125 mg in those without infections ($p<0.001$).

Immunologic outcomes

The baseline and post-treatment median percentage and absolute values of the normal $CD3^+CD4^+$ subpopulation, as well as $CD3^+CD8^+$, NK ($CD3^-CD16^+/CD56^+$) and $CD19^+$ circulating cells, are shown in Figure 6. The normal $CD3^+CD4^+$ subpopulation was calculated as the difference between the total $CD3^+CD4^+$ cells and the atypical $CD3^+CD4^+CD26^-(TCR\nu\beta^+)$ subset.

The median percentages of NK, $CD3^+CD8^+$ and $CD19^+$ cells increased significantly after the first cycle, with respect to the baseline levels (p=0.011, p=0.002 and p=0.035, respectively); these figures were maintained at the third and sixth month controls in patients who did not receive further courses of alemtuzumab. On the other hand, a reduction of the median percentage values of the normal $CD3^+CD4^+$ subpopulation was observed immediately after the first cycle, followed however by an early recovery; indeed, the median percentages at the third and sixth month were higher than baseline, even if the differences were only of borderline significance. The treatment-related lymphopenia resulted in a decrease in the absolute values of the normal $CD3^+CD4^+$, $CD3^+CD8^+$, NK and $CD19^+$ cells immediately after the treatment. However, an early recovery for the three subpopulations was evident at the first month for $CD3^+CD8^+$ and NK cells, and at the third month for $CD19^+$ cells. These figures were maintained at the sixth month in patients who did not receive further cycles of alemtuzumab. The recovery of the normal $CD3^+CD4^+$ cells occurred later.

Discussion

Our results demonstrate that circulating SC are highly sensitive to alemtuzumab and that low doses administered subcutaneously over a short period are effective in inducing more than 90% clearance of SC in the peripheral blood. A value lower than 1,000/mm^3 was obtained from 3 to 13 days after the start of treatment in 13/14 patients. Moreover, complete remissions were also observed in the skin and lymph nodes, a site considered to be less responsive in patients with chronic lymphocytic leukemia.[31] Overall, 12 out of 14 patients (85.7%) achieved a clinical response, with three CR (21.4%), one confirmed by molecular biology. After a median follow-up of 16 months, seven patients remain in remission; the median time-to-treatment failure is 12 months.

The treatment of SS is unsatisfactory and none of the therapies described so far has been shown to produce a significant impact on the disease outcome. In particular, in patients with high tumor burden or who are refractory to/relapsed after previous treatments, such as those included in the present study, chemotherapy plays only a palliative role and is associated with severe side-effects. The results achieved in our study with low-dose alemtuzumab compare favorably with those obtained by commonly used drugs, such as pentostatin (41% response rate with remission durations not exceeding 8.3 months)[32] or fludarabine (58.3% response rate when associated with extracorporeal photochemotherapy)[33], or by more recently used agents, such as denileukin diftitox (30% response rate in 71 patients with advanced/refractory MF/SS with a median response duration of 6.9 months)[34], oral bexarotene (24% in 17 SS patients)[35] and the histone deacetylase vorinostat (8/33 responses in CTCL patients, with a time to progression of 30.2 weeks)[36]. The clinical activity of alemtuzumab was reported firstly by Lundin *et al.*[20] in a series of 22 MF/SS patients. Other researchers[37-39] have reported positive results in single cases or in series of no more than three patients, whereas Kennedy *et al.*[21] obtained less favorable results in eight patients.

The standard alemtuzumab schedule foresees escalating doses from 3 mg to 10 mg and then to 30 mg administered on alternating days, followed by 30 mg three times a week for at least 12 weeks.

With respect to the standard protocol, our schedule uses the subcutaneous method rather than intravenous route of administration, at lower dosages (10 to 15 mg vs 30 mg) and for shorter periods of time; moreover, the treatment duration is decided for each patient on the basis of the close monitoring of the atypical circulating SC. Our results demonstrate that, despite a significant reduction in the median cumulative dose administered per patient (73 mg in our study vs 753 in the updated report by Lundin *et al.*[40]), this low-dose patient-tailored subcutaneous schedule is associated with clinical activity similar to that of the standard alemtuzumab regimen. Indeed, the overall response rate in our study was 85.7%, with a 21.4% CR rate. Lundin *et al.*[20] obtained a 55% overall response rate, with 32% CR; a 69% response rate was obtained in erythrodermic patients and complete clearance of circulating SC in six out of seven cases. A lower response rate was reported by Kennedy *et al.*[21] (38%), with two out of three SS patients achieving a PR. The median time to treatment failure was 12 months in Lundin's study[20] as well as in ours, whereas Kennedy *et al.*[21] reported a response duration of less than 4 months.

There is, therefore, no need to increase the alemtuzumab dose by increments of up to 30 mg for each administration, or to prolong treatment for up to 12 weeks; in fact, monitoring the circulating SC count indicates at which time treatment can be discontinued or when it is mandatory to recommence treatment. In spite of the relatively low number of patients and the not randomized accrual, the demonstration of similar clinical activity between a 10 mg and a 15 mg maximum dosage suggests that a very low alemtuzumab dose (13-23 mg, maximum dosage per administration 10 mg) during the first cycle is sufficient to induce even durable clinical remissions, and the use of higher doses (73-83 mg; 15 mg maximum dose per administration) does not give additional benefit in terms of clinical response. Indeed, it is noteworthy that all three patients who achieved CCR belonged to the 10 mg-treated group.

The second point on which to focus is that the low-dose intermittent schedule is associated with a more favorable toxicity profile than that seen with the standard alemtuzumab regimen. The subcutaneous route of administration was chosen to avoid the infusion-related reactions occurring when the intravenous route is used.[41] In fact, acute administration-related events such as rigor, rash, urticaria, nausea, hypotension and bronchospasm did not occur in any of the patients. A mild local reaction was observed in only two patients; grade 1 fever, headache and osteoarthralgia occurred in three patients after the first injection and disappeared within 12 hours. In contrast, Lundin *et al.*[20] reported grade 3 rigors in 18% of patients, grade 1–2 fever in 68%, grade 3 fever in 5%, and grade 3 fatigue in 9% of patients. Similarly, hematologic toxicity was mild in our study, with only one case of grade 3 toxicity (thrombocytopenia) in one patient treated with a dose escalated up to 15 mg. In contrast, Lundin *et al.*[20] and Kennedy *et al.*[21] reported the occurrence of grade 4 neutropenia in 18% and 38% of patients, respectively, and thrombocytopenia (5% and 38%, respectively); one patient in the series of Kennedy *et al.*[21] developed grade 4 anemia.

The major concern about the use of alemtuzumab is, however, the risk of infectious complications. Nine out of our 14 patients were older than 70 years and 11 had relapsed after, or were refractory to, standard chemotherapy. The first four patients in our study were treated with an escalating dosage up to 15 mg for a total of at least six alemtuzumab doses. Despite good clinical activity of this regimen, all the patients developed infections; one of these patients had fatal staphylococcal sepsis with diffuse vertebral osteomyelitis. On the basis of these data, the maximum dosage was further reduced to 10 mg and the period of administration was shortened through a closer monitoring of circulating SC, in an attempt to reduce the risk of infections. Alemtuzumab maintained a good clinical activity (responses in nine out ten patients with three CR) and none of these patients had infectious complications (Fisher's exact probability test: p=0.001). For comparison, the incidence of infectious complications was 50% in Lundin's series[20] and 75% in Kennedy's study.[21]

Patients with SS are at particularly high risk of developing opportunistic and other severe infections due to the disease-related suppression of T-cell functions; in fact, in untreated patients, the number of circulating normal CD4, CD8 and NK cells is significantly lower than in healthy subjects.[7] Bacterial sepsis is particularly frequent due to the presence of disseminated ulcerative skin lesions and to the extremely low number of B-lymphocytes.[23] Results from a long-term immunologic analysis of 23 B-CLL patients responsive to alemtuzumab showed a profound depletion of the peripheral $CD4^+$ and $CD8^+$ subsets as well as B and NK cells, lasting beyond 9 months after the end of treatment.[24] Our results show that the percentage values of $CD8^+$, NK and B cells increased immediately after alemtuzumab treatment and continued to increase during follow-up. The absolute values of the three subpopulations, after an initial decrease, recovered from the first to the third month, whereas the recovery of the normal $CD3^+CD4^+$ cells occurred later.

In conclusion, subcutaneous alemtuzumab at very low doses (10 mg maximum dosage per administration) given for a short period of time on the basis of circulating SC levels, shows a good toxicity profile and is associated with a high response rate and durable remissions in SS patients. The improvement of the skin picture, itching, and general conditions with a good quality of life in elderly patients, support the activity of this schedule in SS patients who have relapsed after or are refractory to chemotherapy and/or have a high tumor burden in the peripheral blood. These results suggest the planning of multi-center trials to confirm these data in larger cohorts of patients.

Authors' Contributions

MGB designed the study, examined histo-pathological specimens; MTF and PQ cared for the patients; MGB, PQ, MO controlled and analyzed data; AC, MN and FL performed laboratory research and took the photographic pictures; MGB and PQ wrote the paper; all authors checked the final version of the manuscript.

Conflict of Interest

The authors reported no potential conflicts of interest.

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