Moderate-dose cyclophosphamide in the treatment of relapsed/refractory T-cell large granular lymphocytic leukemia-associated pure red cell aplasia

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Background: T-cell large granular lymphocyte leukemia (T-LGLL) is a rare disorder characterized by clonal proliferation of large granular lymphocytes (commonly CD3+/CD8+/CD57+). However, the available data regarding the optimal treatment for relapsed/refractory T-LGLL patients are limited.

Methods: We retrospectively reviewed 10 patients treated with immunosuppressive therapy consisting of intravenous moderate-dose cyclophosphamide (MD-CTX) together with oral cyclosporine A for relapsed/refractory T-LGLL in our hospital between July 2006 and March 2013.

Results: The overall response rate to MD-CTX was 60% (6/10; hematologic complete remission rate, 50%; hematologic partial remission rate, 10%). The median time to response was 28.5 days (range, 20–118 days). The relapse rate of MD-CTX was 50% (3/6); two of these three patients achieved hematologic complete remission after receiving a second course of MD-CTX. Neutropenia was the major adverse event of the MD-CTX regimen. The median time to neutropenia was 5.5 days (range, 1–10 days) and the median neutropenia duration was 5 days (range, 3–15 days). None of the patients developed severe infection.

Conclusions: The MD-CTX regimen appears efficacious and safe in the treatment of relapsed/refractory T-LGLL patients.

Keywords: T-cell large granular lymphocyte leukemia, Cyclophosphamide, Cyclosporine A, Pure red cell aplasia

Introduction

T-cell large granular lymphocyte leukemia (T-LGLL) is an indolent lymphoproliferative disorder characterized by the clonal proliferation of CD3+ cytotoxic T cells. T-LGLL patients usually present with cytopenia and an increase in the number of large granular lymphocytes (LGLs) in the peripheral blood.1–4 However, one-third of all patients are asymptomatic and require no treatment. In western countries, the major clinical manifestations have been reported to be neutropenia, recurrent infection and mouth ulcers, and autoimmune disorders such as rheumatoid arthritis,5,6 whereas in Asian countries, T-LGLL usually presents as anemia-associated symptoms.7–9 Some, but not all, of these patients are diagnosed as having T-cell large granular lymphocytic leukemia-associated pure red cell aplasia (PRCA) and consequently require treatment. The mechanism of T-LGLL-associated cytopenia remains elusive, but most consider it an immune-mediated phenomenon.10

Currently, there is no standard treatment for patients with T-LGLL, with immunosuppressive therapy (IST) remaining the foundation of treatment for symptomatic T-LGLL patients. First-line agents include methotrexate (MTX), oral cyclophosphamide (CTX), and cyclosporine A (CsA), with or without glucocorticoids.8,11–14 The response rates of all the above regimens have been reported as approximately 40–80%, although the relapse rates remain unknown. Meanwhile, the treatment for relapsed/refractory T-LGLL is especially difficult, with very limited data available regarding the most appropriate therapy.

From July 2006 to March 2013, 10 relapsed/refractory T-LGLL patients were treated with intravenous moderate-dose CTX (MD-CTX) with CsA in our hospital, and this study was conducted to determine the response rate, relapse rate, and toxicity of this regimen for relapsed/refractory T-LGLL.
Materials and methods

Patients
Ten relapsed/refractory T-LGLL-associated PRCA patients (nine men and one woman) treated with MD-CTX from July 2006 to March 2013 in the Institute of Hematology & Blood Diseases Hospital, Peking Union Medical College & Chinese Academy of Medical Sciences (PUMC & CAMS) were analyzed retrospectively. The patients included six cases who initially responded to CsA and glucocorticoid (e.g. prednisone) treatment but relapsed after discontinuation or decrement of drug usage, and four patients who did not respond to CsA with glucocorticoids.

The diagnosis of LGLL was made according to the criteria of Lamy and Loughran as follows: (i) typical LGL morphology observed on the peripheral blood smear and the presence of a persistent (more than 6 months) increase in the number of peripheral blood LGLs (>0.5 × 10^9/L); (ii) T-cell immunophenotype detected by flow cytometric analysis using specific antibodies (CD3, CD8, CD56/CD57-positive cells); (iii) T-cell receptor (TCR)-γ chain gene rearrangement, as determined by polymerase chain reaction, and T-cell clonality, as assessed by TCR-Vβ gene repertoire analysis using flow cytometry; and (iv) an LGL count <0.5 × 10^9/L in the peripheral blood and appropriate clinical context, as determined using bone marrow aspirate and/or biopsy specimen with immunohistochemical staining. Refractory T-LGLL was defined as patients who had no hematologic response 4 months after CsA + Pred, and relapsed T-LGLL was defined as patients who achieved hematologic remission at 4 months after CsA with or without glucocorticoids but required another transfusion after discontinuing or decreasing the drug usage.

Treatment protocol
CTX 20 mg/kg, diluted in 250 mL 0.9% sodium chloride injection, was intravenously infused over 1 h from day 1 to 4. Mesna was administered at a dosage of 10 mg/kg by intravenous infusion 30 min before and 3, 6, and 9 h after each CTX infusion. The oral CsA dosage ranged between 2.0 and 4.0 mg/kg/day, given in divided doses every 12 h. The dose of CsA was adjusted to maintain the trough and peak blood levels at 200–400 ng/mL and 800–1000 ng/mL, respectively. Red blood cells were transfused in cases where the hemoglobin (Hgb) level was <70 g/L.

Response evaluation
The response was evaluated at 4 months after CTX treatment according to the criteria for response to IST in non-severe aplastic anemia. Hematologic complete remission (HCR) was defined as a Hgb level >100 g/L, a neutrophil count >1.5 × 10^9/L, and a platelet count >100 × 10^9/L. Hematologic partial remission (HPR) was defined when either of the following parameters was achieved: the patient became transfusion independent (if previously dependent) or there was an increase in the baseline Hgb >30 g/L (if initially <60 g/L). No response (NR) was defined as worsening of the patient’s conditions or not meeting the above criteria. The response criteria did not include the results of the bone marrow, flow cytometry, TCR-γ chain gene rearrangement, and TCR-Vβ gene repertoire analyses.

Time to response, relapse, and response duration
Time to response (TTR) was defined as the duration from the start of CTX to HPR or HCR. Relapse was defined as the reappearance of transfusion requirement. The response duration was defined as the duration from the start of the first response to the first relapse.

Time to neutropenia and neutropenia duration
Time to neutropenia was defined as the duration from the end of CTX treatment to a neutrophil count <1.0 × 10^9/L. Neutropenia duration was defined as the duration from the start of neutropenia to a neutrophil count >1.0 × 10^9/L.

Theory
CTX is a bi-functional alkylating agent; in addition to its cytotoxic effects, it has strong immunosuppressive effects. Lymphocytes are particularly sensitive to the toxic effects of its metabolite 4-hydroxy-cyclophosphamide, and CTX can thereby be used to efficiently inhibit large granular lymphocyte proliferation. While oral CTX has been previously reported as the preferred treatment of T-LGLL and has been demonstrated to achieve satisfactory results, we hypothesized that intravenous MD-CTX as a second-line regimen would prove beneficial for refractory/relapsed patients.

Results

Clinical characteristics
The median age at diagnosis was 54 years (range, 27–72 years), with men being more commonly affected (male/female ratio, 9:1). All patients were diagnosed with T-LGLL-PRCA. Case 6 had neutropenia, while case 5 had thymoma, which was surgically removed before hospitalization, after which oral CsA with prednisone therapy was administered, although no response was observed after 3 months.

Laboratory results
All baseline laboratory parameters from the second course therapy (CTX therapy) were shown in Tables 1 and 2. The median Hgb level was 58 g/L.
range, 43–79 g/L). All patients were transfusion-dependent. Only one patient had neutropenia (neutrophil count <0.5 × 10⁹/L). The median LGL count in the peripheral blood was 0.61 × 10⁹/L (range, 0.05–1.39 × 10⁹/L). All 10 patients had the predominant LGL phenotype (CD3⁺CD8⁺CD57⁺CD56⁻). Clonal TCR-γ chain rearrangement was analyzed in the peripheral blood mononuclear cells in all patients, all of whom (100%) had positive results. Six patients received TCR-Vβ gene repertoire analysis, out of whom, three showed positive results. Further, all patients were found to have PRCA by bone marrow morphology examination.

Response to moderate-dose cyclophosphamide, relapse, and salvage therapy
The response rate at 6 months after MD-CTX therapy was 60% (6/10; 5 HCR, 1 HPR). Among the six responders, the TTR was 20–118 days (median, 28.5 days) (Table 3). Three of the six patients who responded to MD-CTX treatment relapsed (cases 2, 9, and 10). The relapses occurred within 562–679 days after MD-CTX treatment (median, 580 days). While all three patients relapsed from discontinuation or decrement of CsA usage, cases 2 and 9 increased the dose of CsA to the previous dose and achieved remission again. On the other hand, case 10 was diagnosed with a kidney tumor because of finding the pathological fractures, and thus discontinued the CsA treatment and started transfusion. Two of the four patients who did not respond to MD-CTX with CsA treatment also required transfusion, whereas the other two patients were lost to follow-up.

Toxicity
CTX is a cytotoxic drug known to cause predictable hematopoietic suppression, especially neutropenia. Herein, 8 of the 10 patients developed neutropenia after MD-CTX treatment. The time to neutropenia was 1–10 days (median, 5.5 days), and the neutropenia duration ranged between 3 and 15 days (median, 5 days). Five of the eight neutropenia patients received granulocyte colony-stimulating factor therapy and the treatment duration was 3–8 days (median, 5 days). Fever was present in six of the eight neutropenia patients, and four of these six patients had definite infection. All six patients received antibiotics therapy, at a duration of 3–17 days (median, 10.5 days). On the other hand, the two non-neutropenia patients did not have any infection (Table 4).

Overall survival, event-free survival, and maintenance treatment
The median follow-up time was 913 days (range, 120–2961 days). There were no deaths during the

| Case | LGL (×10⁹/L) | TCR rearrangement | TCR-Vβ gene repertoire | CD3+CD57+LGL (%) |
|------|--------------|--------------------|------------------------|-----------------|
| 1    | 0.35         | +                  | Vb20                   | 31.3            |
| 2    | 1.39         | +                  | ND                     | 81.41           |
| 3    | 0.64         | +                  | Vb3.2                  | 78.9            |
| 4    | 0.77         | +                  | ND                     | 78.9            |
| 5    | 0.45         | +                  | Negative               | 40.72           |
| 6    | 0.06         | +                  | Negative               | 6.6             |
| 7    | 0.89         | +                  | Negative               | 27.5            |
| 8    | 0.05         | +                  | ND                     | 22.7            |
| 9    | 0.57         | +                  | Negative               | 22.7            |
| 10   | 1.16         | +                  | Negative               | 22.7            |

LGL, large granular lymphocyte; TCR, T-cell receptor; ND, not done; the % value indicate from CD3⁺CD8⁺cells.
follow-up period. However, two patients were lost to follow-up at 120 and 153 days. Two lost patients were both classified as NR. By the end of the follow-up, five of the remaining eight patients maintained HCR, while three patients were classified as NR. Three of the five responders received continuous CsA treatment. One of the three NR patients, who achieved CR at 4 months after MD-CTX therapy, developed kidney cancer 24 months after the diagnosis of T-LGLL and MD-CTX treatment, and consequently discontinued the CsA and required transfusion again. The other two patients also discontinued CsA and needed intermittent blood transfusion.

**Discussion**

T-LGLL is a rare indolent lymphoproliferative disorder. Only one prospective clinical trial on the treatment for T-LGLL has been reported to date, and the current treatment strategies are thus based on anecdotal case reports and small retrospective studies. IST remains the foundation of treatment for T-LGLL, including MTX, oral CTX, and CsA. In the previous retrospective studies and case reports, the response rates to CTX, MTX, and CsA ranged between 33 and 75% (median, 61%; n = 52/88, 38 and 100% (median, 58%; n = 56/96), and 21 and 100% (median, 56%; n = 69/123), respectively. Although MTX has been considered as the standard first-line therapy, the CTX response rate compared favorably with that of MTX in one study, and these data suggest that CTX may also be considered as a first-line therapy. Further, many recent studies and reports on the treatment of LGLL have focused on CsA. In our previous study, the overall response rate of CsA was 82.1% (HCR, 57.1%), and most patients tolerated CsA treatment well with few side effects (e.g. renal complications, hypertension). However, long-term maintenance therapy is important for the treatment of LGLL, so CsA with or without glucocorticoids is currently recommended as the first-line therapy for LGLL patients in our hospital.

The relapse rate of treatment reflects the refractory nature of LGLL to many agents. CsA can only suppress, but not eliminate the LGL clone, as compared with CTX; therefore, relapse is common in patients treated with CsA. In this study, most patients relapsed from discontinuation or decrement of CsA usage. Upon administration of CsA at the initial or an increased dosage, most achieved remission again. However, some patients could not achieve hematological remission again. For these refractory/relapsed LGLL patients, the optimal treatment strategy is difficult to assess; purine analogues (e.g. fludarabine, cladribine, and pentostatin), monoclonal antibodies (e.g. alemtuzumab, anti-CD52), and the humanized MiK-beta1 monoclonal antibody), combination chemotherapy (CTX, doxorubicin, vincristine, prednisolone [CHOP]), and splenectomy have all been used in refractory/relapsed and treatment-naïve patients, but the response rates of these agents vary between studies.

Most physicians consider that high-dose combination chemotherapy regimens are relatively ineffective and are associated with more toxicity than benefits for this indolent disorder. Instead, chronic exposure to low-dose therapy seems more efficient than sequential high-dose combination chemotherapy, despite some patients being refractory to single oral MTX or CTX. Of note, because of its adverse events (e.g. neutropenia, secondary tumors), oral CTX is recommended for treating LGLL patients for no longer than 4 months for non-responders and 6–12 months for responders.

CTX is a cytotoxic drug, and lymphocytes are particularly sensitive to its toxic effects. Aplastic anemia is an immune-mediated bone marrow failure disorder, and IST as the first-line regimen is applied to treat aplastic anemia patients who do not have a human leukocyte antigen-matched sibling for allogeneic hematopoietic stem cell transplantation. Currently, the standard first-line IST regimen is horse anti-thymocyte globulin with CsA; however, the cost of this regimen

| Table 3 | Response to MD-CTX therapy |
|---------|-----------------------------|
| Case | Response at 6 months | TTR (days) | RD (days) | Overall response | CsA maintain |
| 1 | NR | — | — | Loss | — |
| 2 | CR | 20 | 562 | CR | Yes |
| 3 | NR | — | — | NR | No |
| 4 | NR | — | — | Loss | — |
| 5 | NR | — | — | NR | No |
| 6 | CR | 35 | 1578 | CR | No |
| 7 | CR | 118 | 1666 | CR | No |
| 8 | PR | 22 | 504 | CR | Yes |
| 9 | CR | 20 | 679 | CR | Yes |
| 10 | CR | 37 | 580 | Relapse* | No |

| Table 4 | Neutropenia in patients after MD-CTX therapy |
|---------|---------------------------------------------|
| Case | TTN (days) | ND (days) | G-CSF (days) | Infection | Antibiotics duration (days) |
| 1 | 7 | 4 | 4 | Gingivitis | 4 |
| 2 | 7 | 8 | — | No | — |
| 3 | 6 | 5 | 3 | Pneumonia | 9 |
| 4 | 10 | 7 | — | No | — |
| 5 | 1 | 15 | 8 | Fever | 13 |
| 6 | — | — | — | No | — |
| 7 | — | — | — | — | — |
| 8 | 4 | 3 | 5 | Pneumonia | 12 |
| 9 | 5 | 5 | — | Gastroenteritis | 3 |
| 10 | 4 | 5 | 7 | Fever | 17 |

TTN, time to neutropenia; ND, neutropenia duration; G-CSF, granulocyte colony-stimulating factor.
is generally too high for patients in developing countries. In a previous US study, high-dose CTX (50 mg/kg daily for four consecutive days) with CsA was used to treat severe aplastic anemia, and 31 of 44 (70.5%) patients achieved a hematologic response.27 In our previous study, we reported that MD-CTX (30 mg/kg daily for four consecutive days) with CsA was an efficacious IST regimen in the treatment of aplastic anemia.28 Nonetheless, IST remains the foundation of treatment for LGLL, and we hence applied modified MD-CTX (20 mg/kg daily for four consecutive days) with CsA to treat refractory/relapsed T-LGLL in this study. The results showed that the response rate to MD-CTX was 60% (6/10; 5 HCR, 1 HPR); the median TTR was 28.5 days, although three of the six responders relapsed (median TTR, 580 days). Two of the three relapsed patients achieved HCR again after a second course of MD-CTX, and by the end of the follow-up period, all seven patients with available follow-up data were alive, including four patients who maintained HCR and three patients classified as NR.

Lastly, we also examined the adverse events associated with MD-CTX therapy. It is known that CTX can cause neutropenia associated with hematopoietic suppression, and our results showed that the median time to neutropenia was 5.5 days, while the median neutropenia duration was 5 days. During the course of neutropenia, eight patients experienced a fever, out of whom six had an infection. All fevers and infections were controlled by antibiotic treatment, and the median antibiotics duration was 10.5 days. The other reported main adverse event of CTX is secondary tumors. In this study, case 10 was diagnosed with a kidney tumor because of finding pathological fractures. However, Viny and Maciejewski recently reported a high rate of hematopoietic and solid tumors associated with LGLL,20 and thus, we cannot conclusively determine whether the kidney tumor of this patient was secondary to the MD-CTX therapy or to the LGLL itself.

Recently, somatic mutations of the signal transducer and activator of transcription 3 (STAT3) gene have been identified to have a high frequency of 33–48% in T-LGLL.29–32 Loughran et al. recently reported that STST3 mutation LGLL patients were more likely to respond to the immunosuppressive therapies.32 But Rajala study suggested that the current IST (included CTX therapy) was not able to eradicate the STAT3-mutated clones.33 However, the STAT3 mutational analyses were not performed in our study because of limited test technology in the past time. Therefore, we will perform further works on STAT3 mutation and large sample size prospective study in the future.

In conclusion, the findings of our retrospective review indicate that MD-CTX therapy for refractory/relapsed LGLL patients is safe and efficacious.

**Disclaimer statements**

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**Ethics approval** Yes.

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