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REFERENCES

1 Allen C, Hills RK, Lamb K, Evans C, Tinsley S, Sellar R *et al.* The importance of relative mutant level for evaluating impact on outcome of KIT, FLT3 and CBL mutations in core-binding factor acute myeloid leukemia. *Leukemia* 2013; **27**: 1891–1901.
2 Schnittger S, Kohl TM, Haferlach T, Kern W, Hiddemann W, Spiekermann K *et al.* KIT-D816 mutations in AML1-ETO-positive AML are associated with impaired event-free and overall survival. *Blood* 2006; **107**: 1791–1799.
3 Paschka P, Du J, Schlenk RF, Gaidzik VI, Bullinger L, Corbacioglu A *et al.* Secondary genetic lesions in acute myeloid leukemia with inv(16) or t(16;16): a study of the German-Austrian AML Study Group (AMLSG). *Blood* 2013; **121**: 170–177.
4 Pollard JA, Alonzo TA, Gerbing RB, Ho PA, Zeng R, Ravindranath Y *et al.* Prevalence and prognostic significance of KIT mutations in pediatric patients with core binding factor AML enrolled on serial pediatric cooperative trials for de novo AML. *Blood* 2010; **115**: 2372–2379.
5 Goemans BF, Zwaan CM, Miller M, Zimmermann M, Harlow A, Meshinchi S *et al.* Mutations in KIT and RAS are frequent events in pediatric core-binding factor acute myeloid leukemia. *Leukemia* 2005; **19**: 1536–1542.
6 Shih LY, Liang DC, Huang CF, Chang YT, Lai CL, Lin TH *et al.* Cooperating mutations of receptor tyrosine kinases and Ras genes in childhood core-binding factor acute myeloid leukemia and a comparative analysis on paired diagnosis and relapse samples. *Leukemia* 2008; **22**: 303–307.
7 Shimada A, Taki T, Tabuchi K, Tawa A, Horibe K, Tsuchida M *et al.* KIT mutations, and not FLT3 internal tandem duplication, are strongly associated with a poor prognosis in pediatric acute myeloid leukemia with t(8;21): a study of the Japanese Childhood AML Cooperative Study Group. *Blood* 2006; **107**: 1806–1809.
8 Pession A, Masetti R, Rizzari C, Putti MC, Casale F, Fagioli F *et al.* Results of the AIEOP AML 2002/01 multicenter prospective trial for the treatment of children with acute myeloid leukemia. *Blood* 2013; **122**: 170–178.
9 Kohl TM, Schnittger S, Ellwart JW, Hiddemann W, Spiekermann K. KIT exon 8 mutations associated with core-binding factor (CBF)-acute myeloid leukemia (AML) cause hyperactivation of the receptor in response to stem cell factor. *Blood* 2005; **105**: 3319–3321.
10 Downing JR. The core-binding factor leukemias: lessons learned from murine models. *Curr Opin Genet Dev* 2003; **13**: 48–54.
11 Peterson LF, Boyapati A, Ahn EY, Biggs JR, Okumura AJ, Lo MC *et al.* Acute myeloid leukemia with the 8q22;21q22 translocation: secondary mutational events and alternative t(8;21) transcripts. *Blood* 2007; **110**: 799–805.
12 Muller AM, Duque J, Shizuru JA, Lubbert M. Complementing mutations in core binding factor leukemias: from mouse models to clinical applications. *Oncogene* 2008; **27**: 5759–5773.
13 Grisolano JL, O'Neal J, Cain J, Tomasson MH. An activated receptor tyrosine kinase, TEL/PDGFbetaR, cooperates with AML1/ETO to induce acute myeloid leukemia in mice. *Proc Natl Acad Sci USA* 2003; **100**: 9506–9511.
14 Creutzig U, Zimmermann M, Bourquin JP, Dworzak MN, Fleischhack G, Graf N *et al.* Randomized trial comparing liposomal daunorubicin with idarubicin as induction for pediatric acute myeloid leukemia: results from Study AML-BFM 2004. *Blood* 2003; **122**: 37–43.
15 Creutzig U, Zimmermann M, Bourquin JP, Dworzak MN, von Neuhoff C, Sander A *et al.* Second induction with high-dose cytarabine and mitoxantrone: different impact on pediatric AML patients with t(8;21) and with inv(16). *Blood* 2011; **118**: 5409–5415.

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Cyclophosphamide as a first-line therapy in LGL leukemia

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Large granular lymphocyte (LGL) leukemia is a T or NK clonal disorder characterized by the tissue invasion of marrow, spleen and liver. Clinical presentation is dominated by recurrent infections associated with neutropenia, anemia, splenomegaly and autoimmune diseases, particularly rheumatoid arthritis.[1–4] Recently, STAT3 and STAT5 mutations have been detected in T-LGL and in NK-LGL leukemias.[5–8] These somatic mutations, coupled to other intrinsic and extrinsic mechanisms, are likely to induce constitutive activation of the JAK/STAT pathway thus contributing to maintenance of leukemic LGL survival.[9] These findings strongly suggest a common specific pathogenic pattern in T-LGL and NK-LGL leukemias and provide justification for consideration of the same treatment options. Indications for treatment are severe or symptomatic neutropenia, symptomatic or transfusion-dependent anemia or associated autoimmune diseases requiring therapy. There is no standard treatment for patients with LGL leukemia. All the largest series published in the literature (collecting data on more than 40 patients) are retrospective. Data are very heterogeneous and treatment outcome per single agent is available for very few patients. Immunosuppressive therapy remains the foundation of treatment including single agents that is, methotrexate, oral cyclophosphamide or cyclosporine. On the basis of an initial study showing very good overall response rate (ORR) using methotrexate, this drug has remained the most recommended option in LGL leukemia.[10] Oral low dose cyclophosphamide was first used in pure red cell aplasia associated with LGL leukemia.[11,12] In a French series, cyclophosphamide was shown to be also efficient in neutropenic patients and for those who failed methotrexate.[13] Those results suggested that cyclophosphamide used as first-line therapy could be an interesting alternative to methotrexate.

In this letter, we describe the encouraging results of cyclophosphamide used in a series of 45 previously untreated LGL leukemia patients. Patients suffering from LGL leukemia and treated with cyclophosphamide as first-line therapy were included in this retrospective study. Patients were screened from the Italian, French and USA Penn State registries. Patients gave their informed consent for data collection. The diagnosis of LGL leukemia was based on a chronic LGL peripheral blood expansion ($>0.5 \times 10^9$/l), usually lasting for more than 6 months. Criteria for T-LGL leukemia included expression of LGL surface markers compatible with a typical T-cell (commonly $\alpha\beta+$ or $\gamma\delta+$/CD3+/CD8+/CD57+ and/or CD16+) phenotype associated with clonal rearrangement of *TCR*γ gene using PCR or clonal Vβ expression

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using flow cytometry. Criteria for NK-LGL lymphocytosis/chronic NK-LGL leukemia included expression of LGL surface markers compatible with a NK-cell (commonly CD3 − /CD8 + /CD16 + and/or CD56 +) phenotype with more than 0.75×10^9/l circulating cells.[14,15] Response to treatment was determined periodically on blood cell count and only best response was taken into account. Hematological complete response (CR) was defined by a normal blood count (hemoglobin (Hb) > 12g/dl, platelets $> 150 \times 10^9$/l, absolute neutrophil count (ANC) $> 1.5 \times 10^9$/l and lymphocytosis $< 4 \times 10^9$/l) and LGL peripheral count in a normal range ($< 0.3 \times 10^9$/l). Molecular CR was based on hematological CR associated with a negative PCR analysis for CD3 + cases. Hematologic partial response was defined as an improvement in blood count specified as follows: ANC increasing more than 50% and reaching more than 0.5 but less than 1.5×10^9/l; Hb level increasing more than 2 g/dl and transfusion independent without reaching 12 g/dl level. Treatment failure was defined as a progressive disease (worsening of cytopenia or organomegaly) or a stable disease (none of the later given criteria met). Some patients received cyclophosphamide because of symptoms not related to cytopenia. For those patients, response criteria included clinical symptom resolution. Patients who received prednisone, granulocyte colony-stimulating factor or erythropoiesis-stimulating agent before or at the same time of cyclophosphamide were included in this retrospective study. For the descriptive analysis, qualitative variables were described using numbers and percentage, whereas medians and extremes were used to describe quantitative analyses. Qualitative variables were compared using χ^2 or Fisher's test.

A total of 45 patients treated with cyclophosphamide as a first-line therapy for LGL leukemia from 1989 to 2012 were retrospectively included in this series. Clinical characteristics are described in Table 1. Starting doses were as follows: 100 mg/day ($n = 36$), 50 to 75 mg/day ($n = 8$) taken orally and 1 g IV/month ($n = 1$). Median time from diagnosis to treatment was 3 months (range, 0–55). Treatment was initiated because of severe isolated neutropenia ($n = 16$, 36%), neutropenia and anemia ($n = 2$, 4%), transfusion-dependent anemia ($n = 15$, 33%) and thrombocytopenia ($n = 5$, 11% including three cases of idiopathic thrombopenic purpura). Seven non-cytopenic patients (15%) were treated for disease-associated LGL leukemia: neuropathy ($n = 3$), vasculitis ($n = 1$) and constitutive symptoms ($n = 3$). ORR was 71% (32/45): there were 21 CR (47%) including three molecular responses (mCR) and 11 (24%) partial responses. ORR was 72% versus 68% for T-LGL and NK-LGL subtype, and 72% versus 67% for neutropenic and anemic patients, respectively ($P =$ not significant). Patients treated for symptoms related to LGL leukemia had a 94% ORR (6/7). Eighteen patients (40%) were initially co-treated with prednisone and no significant impact was found for the response to cyclophosphamide ($P = 0.31$). As well as, concomitant granulocyte colony-stimulating factor administration with cyclophosphamide did not influence the time to response and the ORR. The median time to reach best response was 4 months (range 0.8–21) and patients were treated for a median time of 6.4 months (range 0.5–33). Evidence of clinico-biological improvement was systematically observed within the 4 months following treatment initiation. Therefore, we assume that cyclophosphamide should be given for at least 4 months before changing drug regimen. Median treatment duration was 8.5 months (range 3.4–33) for responders, except one patient who received several courses of cyclophosphamide for 7 years. With a median time follow-up of 35 months (range 3.8–277), four out of the 32 responders relapsed (13%): one of these patients responded again to a short course of cyclophosphamide and was maintained subsequently on cyclosporine decided thereafter. The three others were switched to either methotrexate or cyclosporine. Eight patients experienced grade 1–2 toxicities (18%). Only three patients (7%) stopped treatment: one because of worsened anemia and two because of febrile neutropenia not obviously related to hematological toxicity. One patient experienced temporary worsening of anemia without stopping therapy.

We previously mentioned that cyclophosphamide induces a very good ORR in LGL leukemia in *de novo* or relapsing patients.[14] The ORR of 71% described in our series confirmed and emphasized what has been reported in the literature with a total of 25 responders out of 38 patients treated as first line with cyclophosphamide (66% ORR) (Table 2).[11–13,17,18] We show that cyclophosphamide compares favorably to methotrexate given as a first-line therapy. In 1993, Loughran *et al.*[10] reported a 60% ORR in a prospective series of 10 patients receiving methotrexate at a weekly dose of 10 mg/m^2. These results were less encouraging in two larger series. One comes from the prospective ECOG study showing a 37% ORR in 56 patients and the other is retrospective from the French registry and reported a 44% ORR in 36 patients.[13,19] Furthermore, molecular response is rarely obtained and the incidence of relapse following methotrexate is, at least in

Table 1. Patients characteristics, $n = 45$

Median age	60 (24–90)
Sex ratio (M/F)	24/21
T-LGL	32
NK-LGL	13
Blood counts	
Median lymphocytes (10^9/l)	5.1 (0.5–26.8)
Median LGL ($n = 34$)[a] (10^9/l)	3.34 (0.5–25.2)
LGL $> 4 \times 10^9$/l	21/34 (62%)
ANC $< 0.5 \times 10^9$/l	11/45 (24%)
Hb < 11 g/dl	24/45 (53%)
Hb < 8 g/dl	7/45 (16%)
Platelets $< 100 \times 10^9$/l	10/45 (22%)
LGL marrow infiltration ($n = 40$)[b]	29/40 (73%)
Autoimmune cytopenias[c]	$n = 11$
PRCA	4[d]
AIHA	4
ITP	3
STAT3 mutation	7/20 (35%)
Autoimmune disorders	$n = 11$
Rheumatoid arthritis	2
Hypothyroidism	4
Others	5

Abbreviations: ANC, absolute neutrophil count; F, female; Hb, hemoglobin; LGL, large granular lymphocyte; M, male. [a]Median LGL count was available for 34 patients. [b]Bone marrow infiltration status was only available for 40 patients, assessed on bone marrow aspirate or bone marrow biopsy using the Morice WG criteria (diffuse interstitial pattern with small clusters of CD8 + , TIA-1 + and granzyme B + cells).[16] [c]PRCA, pure red cell aplasia; AIHA, autoimmune hemolytic anemia; ITP, idiopathic thrombopenic purpura. [d]One patient had also Crest syndrome.

Table 2. Summary of results for cyclophosphamide used as a first-line therapy in patients with LGL leukemia

Number of patients	*ORR*	*Complete remission*	*Reference*
16	10 (63%)	6	Dhodapkar[17]
5	4/5 (80%)	4	Go[11]
8	6/8 (75%)	2	Fujishima[12]
4	3/4 (75%)	2	Bareau[13]
5	2/5 (40%)	Unknown	Mohan[18]
45	32/45 (71%)	21	Our series

French experience, estimated at 67%.[13] Our series demonstrates that cyclophosphamide used as first-line therapy is effective in T/NK-LGL leukemia, in both neutropenic and anemic patients. ORR and response duration seem encouraging. We recommend only 9–12 months of treatment. It seems sufficient to induce durable remissions and to avoid the complication of myelodysplastic syndromes/acute myeloid leukemia, which although rare is dependent on cumulative dose and length of exposure. For responding patients, tapering dose to 50 mg per day would be a reasonable option. Although recognizing the limits of a retrospective study, we suggest that cyclophosphamide could be an interesting alternative to methotrexate as first-line therapy in LGL leukemia. A prospective study comparing cyclophosphamide to methotrexate as first-line therapy is currently ongoing in France.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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AUTHOR CONTRIBUTIONS

AM and TL designed the study and performed statistical analysis. AM, ZH, LP, BB, OT, AA, KB, RH TPL, RZ, GS and TL were responsible for data collection, data analysis, data interpretation, manuscript preparation, writing and completion and final approval of manuscript. MR and TF participated in biological analysis. All authors approved the final version of the manuscript and the submission.

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REFERENCES

1 Loughran Jr TP. Clonal diseases of large granular lymphocytes. *Blood* 1993; **82**: 1–14.
2 Loughran Jr TP, Kadin ME, Starkebaum G, Abkowitz JL, Clark EA, Disteche C *et al.* Leukemia of large granular lymphocytes: association with clonal chromosomal abnormalities and autoimmune neutropenia, thrombocytopenia, and hemolytic anemia. *Ann Intern Med* 1985; **102**: 169–175.
3 Sokol L, Loughran Jr TP. Large granular lymphocyte leukemia. *Oncologist* 2006; **11**: 263–273.
4 Lamy T, Loughran Jr TP. Clinical features of large granular lymphocyte leukemia. *Semin Hematol* 2003; **40**: 185–195.
5 Jerez A, Clemente MJ, Makishima H, van Adrichem AJ, Kuusanmäki H, Andersson EI *et al.* STAT3 mutations unify the pathogenesis of chronic lymphoproliferative disorders of NK cells and T-cell large granular lymphocyte leukemia. *Blood* 2012; **120**: 3048–3057.
6 Koskela HL, Eldfors S, Ellonen P, Koskela H, LeBlanc F, Peng NgK *et al.* Somatic STAT3 mutations in large granular lymphocytic leukemia. *N Engl J Med* 2012; **366**: 1905–1913.
7 Fasan A, Kern W, Grossmann V, Haferlach C, Haferlach T, Schnittger S. STAT3 mutations are highly specific for large granular lymphocytic leukemia. *Leukemia* 2012; **27**: 1598–1600.
8 Rajala HL, Eldfors S, Kuusanmaki H, van Adrichem AJ, Olson T, Lagström S *et al.* Discovery of somatic STAT5b mutations in large granular lymphocytic leukemia. *Blood* 2013; **121**: 4541–4550.
9 Teramo A, Gattazzo C, Passeri F, Lico A, Tasca G, Cabrelle A *et al.* Intrinsic and extrinsic mechanisms contribute to maintain the JAK/STAT pathway aberrantly activated in T-type large granular lymphocyte leukemia. *Blood* 2013; **121**: 3843–3854.
10 Loughran Jr TP, Kidd PG, Starkebaum G. Treatment of large granular lymphocyte leukemia with oral low-dose methotrexate. *Blood* 1994; **84**: 2164–2170.
11 Go RS, Li CY, Tefferi A, Phyliky RL. Acquired pure red cell aplasia associated with lymphoproliferative disease of granular T lymphocytes. *Blood* 2001; **98**: 483–485.
12 Fujishima N, Sawada K, Hirokawa M, Oshimi K, Sugimoto K, Matsuda A *et al.* Long-term responses and outcomes following immunosuppressive therapy in large granular lymphocyte leukemia-associated pure red cell aplasia: a Nationwide Cohort Study in Japan for the PRCA Collaborative Study Group. *Haematologica* 2008; **93**: 1555–1559.
13 Bareau B, Rey J, Hamidou M, Donadieu J, Morcet J, Reman O *et al.* Analysis of a French cohort of patients with large granular lymphocyte leukemia: a report on 229 cases. *Haematologica* 2010; **95**: 1534–1541.
14 Lamy T, Loughran Jr TP. How I treat LGL leukemia. *Blood* 2011; **117**: 2764–2774.
15 Semenzato G, Zambello R, Starkebaum G, Oshimi K, Loughran Jr TP. The lymphoproliferative disease of granular lymphocytes: updated criteria for diagnosis. *Blood* 1997; **89**: 256–260.
16 Morice WG, Kurtin PJ, Tefferi A, Hanson CA. Distinct bone marrow findings in T-cell granular lymphocytic leukemia revealed by paraffin section immunoperoxidase stains for CD8, TIA-1, and granzyme B. *Blood* 2002; **99**: 268–274.
17 Dhodapkar MV, Li CY, Lust JA, Tefferi A, Phyliky RL. Clinical spectrum of clonal proliferations of T-large granular lymphocytes: a T-cell clonopathy of undetermined significance? *Blood* 1994; **84**: 1620–1627.
18 Mohan SR, Clemente MJ, Afable M, Cazzolli HN, Bejanyan N, Wlodarski NW *et al.* Therapeutic implications of variable expression of CD52 on clonal cytotoxic T cells in CD8+ large granular lymphocyte leukemia. *Haematologica* 2009; **94**: 1407–1414.
19 Loughran Jr TP, Yao X, Bennett JM, Litzow MR, Evens AM, Tallman MS. Results of a prospective multicenter phase ii study of initial treatment with methotrexate in LGL leukemia (ECOG Protocol E5998). *ASH Annual Meeting Abstracts* 2010; **116**: 702.

Inherited susceptibility to pre B-ALL caused by germline transmission of *PAX5* c.547G > A

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A single-nucleotide polymorphism (SNP) in PAX5 leading to an amino-acid change in the octapeptide domain at position c.547G > A (p.Gly183Ser) has recently been described to confer an inherited susceptibility for childhood pre B-ALL.[1] This susceptibility was transmitted autosomal dominant in two independent families with variable penetrance and aberrations of chromosomal part 9p resulting in loss of the wild-type (wt) PAX5 allele occurruring simultaneously in the leukemic cells.

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