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RESEARCH LETTER

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TO THE EDITOR:

Cyclophosphamide induces durable molecular and clinical responses in patients with relapsed T-LGL leukemia

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T-cell large granular lymphocytic leukemia (T-LGLL) is a clonal proliferation of cytotoxic T-lymphocytes with a terminal effectory memory phenotype ($CD3^+/CD8^+/CD5dim/CD57^+/CD62L/CD45RA^+/CD45RO^-$) that can result in severe cytopenias, including neutropenia, anemia, and pancytopenia/bone marrow failure in severe cases.[1-3] The pathogenesis of T-LGLL is thought to be mediated by increased circulating interleukin-15, and subsequent upregulation of the STAT3 pathway, leading to dysregulation of apoptosis and resultant cellular proliferation and marrow damage. The management of T-LGLL therefore is immune-suppressive therapy, with methotrexate (MTX), cyclophosphamide (Cy), and cyclosporine serving as the standard frontline agents. MTX is the standard frontline therapy based off the prospective ECOG5998 (E5998) study but overall response rate (ORR) was modest at 38%.[4] An initial early report with frontline Cy demonstrated a response rate of 63% in 10/16 patients, including 6 complete response (CR), though at that time, CR was defined as hematologic CR, and response criteria differed from current E5998 criteria.[5] For patients that fail frontline MTX, oral Cy, with a target dose of 100 mg/day is the standard approach. Data from the ECOG5998 trial suggested an increased ORR with Cy in the second-line setting, with 21% CR,[6] and retrospective data from the French cohort study suggested improved response rate, with 63% CR,[7] when Cy was used second line. More intriguingly, anecdotal evidence suggests that because of its alkylating effects, Cy may eradicate the T-LGLL clone, which has not been observed with immune-suppressive-based strategies. However, the degree to which a complete molecular remission (CMR) can be attained, and the lengths of these remissions with Cy remains unknown.

Recently, we published a report on T-LGLL that showed improved clinical responses in patients treated with Cy, especially if this was used as second line after frontline MTX.[8] In that study, 100% of patients (7/7) that were treated with Cy as second-line therapy after frontline MTX had a response to treatment (3 CR and 4 partial response [PR]). Here, we report on additional patients ($n = 22$) treated with Cy for relapsed or refractory T-LGLL, and for the first time report survival outcomes, duration of response, and presence of CMR in the relapsed setting.

We retrospectively evaluated all patients treated for T-LGLL with oral Cy at the Ohio State University (OSU) James Comprehensive Cancer Center from 2000 through 2019. The diagnosis of T-LGLL was made based on 2016 World Health Organization criteria. T-LGLL criteria included a $CD3^+$ $CD8^+$ population on flow cytometry $\geq$500 cells/mm^3 and a positive monoclonal T-cell receptor (TCR). This was considered positive if detected by TCR polymerase chain reaction (PCR) or by restriction of TCR-Vbeta noted on flow cytometry. For patients diagnosed with a clonal TCR by flow cytometry, a panel of 30 TCR-Vbeta rearrangements was used and considered positive if 1 or more clone was detected in $\geq$10% of events. Disease response was defined by the E5998 study criteria and was confirmed retrospectively by the investigators (Z.B. and J.E.B.). Although there is variability in follow-up given the retrospective nature of this study, in general, as standard clinical care, patients were monitored for response on Cy for a minimum of 4 months at the full 100-mg dosage. Standard laboratory values (complete blood count with differential, hepatic function panel, basic chemistry panel) were drawn every 4 to 6 weeks either at OSU or with a local physician. Flow cytometry was

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Requests for data sharing may be submitted to Jonathan E. Brammer (jonathan.brammer@osumc.edu).

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The online version of this article contains a data supplement.

Table 1. Baseline characteristics of patients attaining CR with cyclophosphamide

Age at Dx	Sex	Rheumatologic disease	Prior Tx	Cy line	Cy indication	Max Cy dose	Time on Cy, mo	Time before CR after starting Cy, mo	Did patient have CMR (time to CMR, M)?
66	F	RA	CsA (×2), MTX	4th	Neutropenia	50 mg BID	11	12	No
66	M	None	MTX	2nd	Anemia	50 mg BID	10	5.5	No
70	F	RA	MTX	2nd	Anemia	50 mg daily	12	5	Yes (7 mo)
67	F	None	MTX (×2)	3rd	Anemia and Neutropenia	100 mg daily	13	4	Yes (12 mo)
66	M	None	MTX, BNZ-1	3rd	Neutropenia	100 mg daily	15	5	Yes (11 mo)
65	M	RA	MTX	2nd	Neutropenia	50 mg BID	11	3	No

BID, twice daily; CsA, cyclosporine; Dx, diagnosis; MTX, methotrexate; RA, rheumatoid arthritis; Tx, treatment.

assessed every 2 to 3 months to evaluate for residual disease. Patients who attained an improvement in their affected blood counts (ie, neutropenia or anemia) were continued on Cy, even if full E5998 response criteria were not attained because E5998 criteria were assessed retrospectively only. In this way, patients who attained some response at 4 months could be evaluated months later to assess for a deepening of their clinical response. CMR was defined as: CR by E5998 criteria and clearance of the TCR PCR-based gene rearrangement studies or TCR VBeta flow cytometry assessment for individual clones. The time-to-response (TTR) was measured as time from start of Cy until PR or CR, with patients who failed to respond being censored at the end of their Cy treatment. Leukemia-free survival (LFS) in patients responding to Cy was measured as time from start of Cy until disease progression, with patients without progression being censored at last follow-up. TTR and LFS were compared across variables using Kaplan-Meier curves with median survival and 95% confidence intervals. The OSU's institutional review board approved this study, which was conducted in accordance with the Declaration of Helsinki.

Twenty-two patients with relapsed/refractory T-LGLL, with an average duration of 8 months (range, 1-15) of Cy treatment, and a median follow-up time of 24.4 months, were included in this analysis. Thirteen patients (59%) were treated with Cy as second line and 9 (41%) as third line or greater. Standard dosing included an initial dose of 50 mg daily for 2 weeks with increase to 100 mg if tolerated for 4 months. Cy was dosed at 150 mg (1 patient), 100 mg (17 patients), and 50 mg (3 patients), whereas 1 patient received 1260 mg for multiple myeloma. Assessment for clonal hematopoiesis (CHIP) or associated mutations was not routinely evaluated. As part of our standard panel, mutations were assessed in 11/22 (50%) patients because this panel became available for T-LGLL only in 2018. Four patients had no mutations, and 7 patients were found to have the following mutations (2 patients had 2 separate mutations): STAT3 (×5), CARD11, NOTCH2, SF3B1, and DNMT3A. Two patients were found to have 2 separate mutations, 1 with CARD11/NOTCH2 and another with STAT3 (D661Y)/SF3B1. Of the STAT3 mutations, 3 patients had a D661Y mutation, 1 had a D661V mutation, and 1 had an N647I mutation. Overall, 11 patients were tested for STAT3 mutations because this was only routinely done after 2018. Of those 11 patients, 5 (45%) were found to have a STAT3 mutation (supplemental Table 1). Of these, 4 patients with a STAT3 mutation had no response to Cy and 1 patient had a PR to Cy (this patient also had an SF3B1 mutation) (supplemental Table 1). At the low doses of Cy used for T-LGLL, Cy is generally very well tolerated, with only minor toxicities, including nausea, cytopenias, and fatigue. In our cohort of patients, the most common side effect observed was grade 1 fatigue (n = 2). We also observed grade 1 dizziness (n = 1) and 1 patient with grade 1 leukopenia.

For relapsed/refractory T-LGLL, the ORR was 68% (15/22) with 6 CR (27%) and 9 PR (41%). At the 4-month mark, the ORR was 27% (2 CR, 4 PR). The median time to response was 5.6 months for CR and 5.8 months for PR. Three patients that had a PR at the 4-month mark later converted to a CR. The median time to maximum response TTRMax (CR or PR) was 6 months (95% confidence interval, 4-7), whereas median time to PR and CR were 9 and 7 months, respectively. Baseline characteristics of those achieving CR can be seen in Table 1. Interestingly, of the 6 patients that achieved a CR, 50% had a CMR with durable eradication of their T-cell clone. Among responders, the median follow-up time was 27 months and the median LFS was 24 months. Of note, among those with a CR, median follow-up is slightly longer at 28.5 months. When broken down by type of response, the median LFS for those with PR was 27 months, whereas the median LFS for those with CR was not reached as no patients progressed. Additionally, for patients that achieved a CMR, the LFS has not yet been reached.

Here we report that patients receiving Cy for relapsed T-LGLL can attain durable remissions, including CMR, with a prolonged duration of response with long-term follow-up. Previous reports have focused on response rates of Cy but have not evaluated the duration of response in these patients, and no studies have reported CMR in patients with relapsed/refractory T-LGLL. Of particular interest is that no patients who attained a CR have relapsed, demonstrating durable remissions are attainable with Cy in relapsed T-LGLL. We also demonstrate that a CMR is attainable in refractory patients with Cy, which has previously only been shown in patients that were treated with Cy frontline.[9] Importantly, we demonstrate that there was significant consolidation of response after the initial 4-month treatment period, with an ORR of 27% at 4 months, but an ORR of 64% in long-term follow-up. These novel results suggest that patients should remain on Cy beyond initial response because Cy can deepen the clinical response over time. To assess for response, we recommend checking for the TCR (by PCR of flow cytometry) every 3 months while on treatment to assess for response and CMR. The standard approach at OSU is to treat patients with Cy for a maximum of 12 months because of the increased risk of mutagenesis, myelodysplasia, or marrow injury resulting from prolonged Cy.[10-13] This also seems adequate time to attain a maximum response, as the median TTRMax (CR or PR) in our study was 6 months, and most patients responded by 8 months. However, 4 patients continued treatment beyond 12 months solely at the discretion of their local

physician, outside of OSU recommendations, with 2 patients on Cy for 13 total months, 1 for 14 months, and 1 for 15 months. Our data clearly demonstrate that maximal response is typically obtained by 8 months of treatment, and that these responses are durable, with no long-term relapses in patients with CR or CMR.

We acknowledge certain limitations of this study. No CR patients had pancytopenia, which can result in selection bias in a small population of patients with CR. Further, long-term follow-up is needed to assess the duration of remission, and impact of CMR on patients with relapsed T-LGLL treated with Cy. Clonal hematopoiesis (CHIP) mutations were also not routinely evaluated, and in these patients, caution should be used when initiating treatment with Cy. Further, the clinical and prognostic significance of STAT3 mutations and the response to Cy will need to be evaluated in future prospective studies. Although our results strongly suggest that Cy can produce durable remissions, and CMR, these findings will need to be further validated in a larger cohort in future prospective randomized studies, including the use of CMR as a novel endpoint in T-LGLL. Nevertheless, our results demonstrate that Cy induces durable responses in the setting of relapsed T-LGLL, including CMRs. Pending future studies, we suggest that Cy should be maintained for up to 8 to 12 months to consolidate response, with the goal of attaining a CR or CMR. Further, CMR, as a component of response criteria, should be incorporated in the design of future clinical trials.

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