Cyclophosphamide Induces Durable Molecular and Clinical Responses in Patients with Relapsed T-LGL Leukemia

Tracking no: ADV-2021-006263R1

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Abstract:

Conflict of interest: No COI declared

COI notes:

Preprint server: No;

Author contributions and disclosures: ZB and JEB designed the study. EM performed the statistical analysis. AM assisted with analysis. ZB and JEB were responsible for data collection, data analysis, data interpretation, manuscript preparation, writing and completion and final approval of manuscript. All authors approved the final version of the manuscript and the submission.

Non-author contributions and disclosures: No;

Agreement to Share Publication-Related Data and Data Sharing Statement: The method for data sharing will be emails to the corresponding author

Clinical trial registration information (if any):
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Prior presentation: Presented in abstract form at the 63rd American Society of Hematology Annual Meeting in December 2021.

Data sharing statement

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TO THE EDITOR:

T-cell large granular lymphocytic leukemia (T-LGLL) is a clonal proliferation of cytotoxic T-lymphocytes (CTLs) with a terminal effecory 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. 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 (CsA) serving as the standard frontline agents. MTX is the standard frontline therapy based off the prospective, ECOG5998 study but overall response rate (ORR) was modest at 38%. An initial early report with frontline Cy demonstrated a response rate of 63% in 10/16 patients, including 6 CR, though at that time, CR was defined as hematologic CR, and response criteria differed from current E5998 criteria. For patients that fail frontline MTX, oral cyclophosphamide, with a target dose of 100 mg/day is the standard approach. Data from the ECOG5998 (E5998) trial suggested an increased ORR with Cy in the second line setting, with 21% complete response (CR), and retrospective data from the French cohort study suggested improved response rate, with 63% CR, when Cy was utilized second line. More intriguingly, anecdotal evidence suggests that due to 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. 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 the presence of CMR in the relapsed setting.

We retrospectively evaluated all patients treated for T-LGLL with oral Cy at the Ohio State University James Comprehensive Cancer Center from 2000-2019. 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 ≥500 cells/mm3 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 one or more clone was detected in ≥10% of events. Disease response was defined by the E5998 study criteria and was confirmed retrospectively by the investigators (ZB and JEB). While 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 labs (complete blood count with differential, hepatic function panel, basic chemistry panel) were drawn every 4-6 weeks either at OSU or with a local physician. Flow cytometry was assessed every 2-3 months to evaluate for the presence of residual disease. Patients who attained an improvement in their affected blood counts (i.e., neutropenia or anemia) were continued on Cy, even if full E5998 response criteria were not attained as 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 Ohio State University’s Institutional Review Board approved this study, which and 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. 13 patients (59%) were treated with Cy as 2nd line and 9 (41%) as 3rd 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), while one patient received 1260 mg for multiple myeloma. Assessment for Clonal hematopoiesis or associated (CHIP) mutations was not routinely evaluated. As part of our standard panel, mutations were assessed in 11/22 (50%) patients, as 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 (two patients had 2 separate mutations): STAT3 (x5), CARD11, NOTCH2, SF3B1, and DNMT3A. Two patients were found to have two separate mutations, one with
CARD11/NOTCH2 and another with STAT3 (D661Y)/SF3B1. Of the STAT3 mutations, three patients had a D661Y mutation, one had a D661V mutation, and one had an N647I mutation. Overall, 11 patients were tested for STAT3 mutations, as 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, four patients with a STAT3 mutation had NR to Cy and one patient had a PR to Cy (this patient also had an SF3B1 mutation) (Supplemental Table 1). At the low doses of Cy utilized 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 did also observe grade 1 dizziness (n=1) and one 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% CI: 4-7) while median time to PR and CR were 9 and 7 months respectively. Baseline characteristics of those achieving CR can be seen in the table. Interestingly, of the six patients that achieved a CR, 50% had a CMR with durable eradication of their T-cell clone. Among responders, with median follow-up time was 27 months, the median LFS was 24 months. Of note, amongst 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, while 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. 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, as Cy can deepen the clinical response over time.

To assess for response, we recommend checking for the T-cell receptor (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, due to the increased risk of mutagenesis, myelodysplasia, or marrow injury due to prolonged Cy. 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, it is noted that four patients were continued on 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, one for 14 months, and one for 15 months. Our data clearly demonstrates 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. While 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 8-12 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.
Author Contributions:

ZB and JEB designed the study. EM performed the statistical analysis. AM assisted with analysis. ZB and JEB were responsible for data collection, data analysis, data interpretation, manuscript preparation, writing and completion and final approval of manuscript. All authors approved the final version of the manuscript and the submission.

Conflict-of-Interest Disclosure:

None of the authors have a relevant conflict of interest to disclose
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| Age at Dx | Sex | Rheumatologic Disease | Prior Tx | Cy Line | Cy Indication | Max Cy Dose | Time on Cy | Time Before CR after starting Cy | Did patient have CMR (time to CMR, M)? |
|----------|-----|------------------------|----------|---------|--------------|-------------|------------|--------------------------------|-------------------------------------|
| 66       | F   | RA                     | CsA (x2), MTX | 4th     | Neutropenia  | 50 mg BID   | 11 months  | 12 months                       | No                                  |
| 66       | M   | None                   | MTX      | 2nd     | Anemia       | 50 mg BID   | 10 months  | 5.5 months                      | No                                  |
| 70       | F   | RA                     | MTX      | 2nd     | Anemia       | 50 mg daily | 12 months  | 5 months                        | Yes (7 months)                      |
| 67       | F   | None                   | MTX (x2) | 3rd     | Anemia and Neutropenia | 100 mg daily | 13 months  | 4 months                        | Yes (12 months)                     |
| 66       | M   | None                   | MTX, BNZ-1 | 3rd     | Neutropenia  | 100 mg daily | 15 months  | 5 months                        | Yes (11 months)                     |
| 65       | M   | RA                     | MTX      | 2nd     | Neutropenia  | 50 mg BID   | 11 months  | 3 months                        | No                                  |

Abbreviations: CMR, Complete Molecular Remission; CR, Complete Response; CsA, Cyclosporine; Cy, Cyclophosphamide; Dx, Diagnosis; MTX, Methotrexate; Pt, Patient; RA, Rheumatoid Arthritis