Short-course subcutaneous alemtuzumab induces clinical responses in relapsed T-cell large granular leukemia

T-cell large granular lymphocytic leukemia (T-LGLL) is a clonal lymphoproliferative disorder of mature cytotoxic T-lymphocytes with a terminal effector memory phenotype ($CD3^+/CD8^+/CD5^{dim}/CD57^+/CD62L/CD45RA^+/CD45RO^-$) that can result in severe cytopenias, with resultant transfusion dependence, infections, and bone marrow failure in severe cases.[1-3] Clonal proliferation of T-LGLL cells is mediated by overexpression of interleukin-15, with subsequent dysregulation of STAT3, thereby inhibiting apoptosis and promoting leukemic proliferation.[4-6] The current standard treatments for T-LGLL are oral immunosuppressant agents, including methotrexate, cyclophosphamide, and cyclosporine.[7-10] In the prospective ECOG5998 (E5998) trial, which utilized the only reported standardized response criteria in T-LGLL, the overall response rate (ORR) to frontline methotrexate was noted to be 38% with 2% complete responses (CR).[11] Therapy in the relapsed/refractory setting consists of cycling other immune-suppressive agents (cyclophosphamide/cyclosporine), with response rates ranging from 30-50%, with all patients inevitably relapsing.[9,12] There remains a paucity of data regarding effective therapy for patients with relapsed/refractory T-LGLL once these agents fail, demonstrating the urgent need for the development of novel treatment approaches.

Alemtuzumab is a humanized monoclonal antibody against CD52 that induces antibody- and complement-mediated T-cell lysis.[5,13] Given its targeted T-cell killing, and overall immune-suppressive effect, intravenous (IV) alemtuzumab was evaluated in a phase II trial for patients with relapsed T-LGLL.[13] Patients were hospitalized for 2 weeks, given an initial 1 mg test dose, followed by 10 mg daily for 10 days. Fifty-six percent of the patients achieved a hematologic response, although standardized E5998 response criteria were not utilized. However, significant toxicity was noted. All patients experienced infusion reactions, 88% experienced grade 3/4 lymphopenia, and one patient was hospitalized for febrile neutropenia leading to death (suspected fungal infection). Two additional patients were hospitalized for infections (1 pseudomonal, 1 *Clostridium difficile*). In addition, 64% and 46% of patients experienced cytomegalovirus and Epstein-Barr virus reactivation, respectively, during treatment.[13] While favorable hematologic responses were observed with this regimen, wider use in the clinical setting has been limited due to increased risk of infections and feasibility (prolonged hospitalization, experience in managing alemtuzumab toxicities/administration necessary). Furthermore, since T-LGLL patients are older and often have significant co-morbidities, the toxicity risk of IV alemtuzumab precludes its widespread use, and it is not utilized at our center. Previous studies did not use standardized response criteria, making it difficult to fully assess responses. Nevertheless, given the promising efficacy of alemtuzumab in T-LGLL, we explored the feasibility and efficacy of a short course of subcutaneous alemtuzumab (scAlemtuzumab) in patients with relapsed/refractory T-LGLL using E5998 response criteria and propose an effective, feasible, and safe alternative alemtuzumab regimen for T-LGLL.[12]

Patients were first given a test dose of scAlemtuzumab 3 mg, to ensure that they did not develop allergic/infusion-type reactions. Subsequently, scAlemtuzumab was administered at a dose of 10 mg three times weekly for a minimum of 4 weeks (12 doses) and maximum of 8 weeks (24 doses). The total duration was determined by standard laboratory monitoring (complete blood count, comprehensive metabolic profile, and liver function tests) during treatment. All patients received (val)acyclovir prophylaxis against varicella-zoster virus and prophylaxis with trimethoprim/sulfamethoxazole against *Pneumocystis jiroveci* pneumonia from initiation of scAlemtuzumab until physicians' discretion (typically 3 months after alemtuzumab for *Pneumocystis jiroveci* pneumonia, 1 year for varicella-zoster virus). scAlemtuzumab was withheld in cases of grade 3 infections, or worsening neutropenia (from baseline) until resolution of toxicity and discontinued in cases of persistent neutropenia (>4 weeks).

To evaluate the efficacy of this approach, we reviewed all patients with relapsed/refractory T-LGLL who received scAlemtuzumab between 2009 through 2021 at The Ohio State University James Comprehensive Cancer Center. The research protocol was reviewed and approved by this institution's Institutional Review Board. Kaplan-Meier methods were used to estimate time to response and leukemia-free survival. Response was determined based on the E5998 study response criteria.[11] Eight heavily pre-treated relapsed/refractory T-LGLL patients who had received a median of 2.5 (range, 2-4) prior therapies were treated with scAlemtuzumab. The response rate to the immediate therapy prior to scAlemtuzumab was 38% (all partial responses [PR]), demonstrating the refractory nature of T-LGLL in these patients. Full data, including responses to scAlemtuzumab treatment, are included in Table 1. The median number of scAlemtuzumab injections was 13 (range, 8-24). The median follow-up time was 17 months (range, 9-91 months), and the ORR rate was 75% (6/8 patients) with a PR and CR rate of 38% each. Intriguingly, the three patients who obtained a CR to scAlemtuzumab had never obtained a prior CR to any other therapy. The median time

to response was 2 months (95% confidence interval [95% CI]: 1.2 months-not reached [NR]). One patient achieved a complete molecular response (CMR), defined by clearance of the T-cell receptor clone via polymerase chain reaction analysis, in addition to meeting the criteria for a CR. The median duration of response for all responders was 5.7 months (95% CI: 0.5-NR). The patient with a CMR had a prolonged remission of 21.2 months. We performed an exploratory analysis to determine if there was an impact on type of cytopenia (anemia or neutropenia), or biomarkers predictive of response. There was no association of response to scAlemtuzumab based upon type of cytopenia, *STAT3* mutation, number of prior treatments, or number of doses (*Online Supplementary Table S1*). Furthermore, there was no correlation of T-LGLL count, or pre-treatment hematologic parameters, with response.

scAlemtuzumab was well tolerated overall, with a reduced toxicity profile compared to that of IV alemtuzumab. Grade 2-3 toxicities were observed in four patients. These included one patient each with neutropenia, transaminitis, oral candidiasis, and cytomegalovirus reactivation. No infusion-type reactions or cutaneous skin eruptions were observed. In the patient with neutropenia, the absolute neutrophil count was initially <500 cells/mm^3, then dropped to <50 cells/mm^3, which prompted discontinuation of therapy, but subsequently recovered to the baseline off-therapy value. No patients died because of scAlemtuzumab therapy, and all toxicities were managed in an outpatient setting.

The observed 75% ORR (38% CR, 38% PR) in this heavily pre-treated population of patients with relapsed/refractory T-LGLL suggests that the responses to scAlemtuzumab are similar to those produced by IV alemtuzumab, with significantly lower infectious toxicities. Patients responded rapidly to scAlemtuzumab, typically within 2 months of initiation, with a median duration of response of 5.7 months, using rigorous E5998 response criteria. These results compare to a 56% ORR (36% CR, 20% PR) with IV alemtuzumab. The median duration of response in the prior IV alemtuzumab study was not reported. When comparing responding *versus* non-responding patients treated for their target cytopenia (anemia or neutropenia), an early increase in neutrophil count or hemoglobin concentration was observed after the first month of treatment in responding patients, compared to non-responding patients (*Online Supplementary Figure S1*). No patients proceeded with allogeneic stem cell transplant following treatment with scAlemtuzumab, and there was no clear association of improvement in autoimmune disease symptoms with the administration of scAlemtuzumab in the four patients with autoimmune disease. Importantly, scAlemtuzumab resulted in fewer side effects than those with IV alemtuzumab. We observed no infusion reactions or cutaneous eruptions, and only one case of cytomegalovirus reactivation with no grade 3/4 neutropenia. This compares to a 100% infusion reaction rate, 88% incidence of grade 3/4 lymphopenia, and serious adverse events such as febrile neutropenia leading to death and two hospitalizations for infections in patients treated with IV alemtuzumab. Based on our results, pending future studies, we recommend the following method of administration of scAlemtuzumab for patients with relapsed/refractory T-LGLL. Patients should be given an initial 3 mg scAlemtuzumab dose to evaluate for infusion-type reactions, followed by 10 mg scAlemtuzumab three times weekly for up to a total of 8 weeks with frequent laboratory monitoring, as stated above. For patients who develop recurrent cytopenias off scAlemtuzumab, re-treatment with scAlemtuzumab should be considered, and this should be included in a future clinical trial design and evaluated in retrospective studies. Due to the potential infectious risk, scAlemtuzumab should be used with caution in LGLL patients with persistent neutropenia and recurring infections. To mitigate infectious risk, patients receiving scAlemtuzumab should complete therapy for prior infections, and should receive antifungal, antiviral, and antibacterial prophylaxis per standard institutional leukemia practice. Furthermore, close monitoring for new or recurring infection is warranted, particularly viral (cytomegalovirus) infections.

We acknowledge the limitations of this study. It is a retrospective study of patients treated with standard-of-care scAlemtuzumab without a prospective protocol. However, we believe that there was minimal variability across patients as all were treated with the same approach by JEB and PP. CD52 was not routinely checked, thus we cannot draw any conclusions on CD52 levels and clinical response. Finally, it is difficult to make definitive comparisons between this retrospective study and the larger, prospective trial with IV alemtuzumab. Despite these limitations, since IV alemtuzumab has not been widely utilized due to logistical and toxicity concerns, it is crucial to investigate other alternative methods of administration of this agent, particularly given the lack of available options for patients with relapsed/refractory T-LGLL. Importantly, this study provides the necessary baseline data and approach to inform future trials.

The response rate to scAlemtuzumab was particularly noteworthy when interpreted in the context of the refractory nature of T-LGLL patients treated with this approach. The 75% ORR (38% CR) with scAlemtuzumab was observed in a heavily pre-treated T-LGLL population and was higher than the 37.5% ORR (all PR) to the immediate prior treatment using standard therapies. Intriguingly, scAlemtuzumab also induced CR in three patients who had never attained a CR to any other prior therapy (Table 1). While our results strongly suggest that scAlemtuzumab can be safely and effectively administered with a high overall response rate, these findings will need to be validated in a larger cohort in future, large, prospective studies, including the use of CMR as a novel endpoint.

Table 1. Patients' baseline characteristics and response to subcutaneous alemtuzumab.

Age at diagnosis in years	Sex	Other mutations (VAF)	Associated autoimmune diseases	Prior treatment	ScAl line	ScAl indication	N of ScAl doses	Time on ScAl in days	Response to ScAl	Time to CR on ScAl in months
70	M	None	None	MTX, Cy	3rd	Symptomatic anemia	23	64	PR	PD
52	M	*DNMT3A* (40.8)	Interstitial pneumonitis	MTX, Cy, CsA, MMF	5th	Persistent anemia	12	31	CR	1.18
56	F	None	None	BNZ-1, MTX, Cy, CsA	5th	ANC <500/mm^3	12	24	PR	NA
59	M	*DNMT3A* (9.7); *DNMT3A* (6.7), *STAT3* (19.8)	None	Cy, MTX, CsA	4th	Transfusion-dependent anemia	14	35	PD	NA
53	F	*STAT3* (9.6)	RA	MTX/ Prednisone/ Tofacitinib, Cy, CsA	4th	ANC <500/mm^3	9	19	PR	NA
51	M	*STAT3, TET2, RUNX1*	None	Cy, Splenectomy, MTX, CsA	5th*	Transfusion-dependent anemia	8	21	PD	NA
74	M	Unknown	Acquired factor VIII deficiency	Cy, MTX	3rd	Transfusion-dependent anemia	24	56	CMR	2.3
72	M	Unknown	RA	Cy, MTX	3rd	Symptomatic anemia	24	43	CR	1.7

*Treated concomitantly with romidepsin and subcutaneous alemtuzumab. VAF: variant allele frequency; ScAl: subcutaneous alemtuzumab; N: number; CR: complete response; M: male; MTX: methotrexate; Cy: cyclophosphamide; PR: partial response; PD: progressive disease; CsA: cyclosporine; MMF: mycophenolate mofetil; F: female; ANC: absolute neutrophil count; NA: not available; RA: rheumatoid arthritis; CMR: complete molecular response.

Nevertheless, given the current lack of therapeutic options for relapsed/refractory T-LGLL, and limited clinical trials available for these patients, our data suggest that scAlemtuzumab may be an effective, new therapy for patients with relapsed/refractory T-LGLL, with an improved safety profile compared to that of IV alemtuzumab.

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https://doi.org/10.3324/haematol.2024.286235

Received: 20 July 2024.
Accepted: November 8, 2024.
Early view: November 14, 2024.

Disclosures

No conflicts of interest to disclose.

Contributions

MR and JEB designed this study. EM provided the statistical analysis. PP treated patients and contributed to the study design. MR, AM, ZB, and JEB were responsible for collecting, analyzing and interpreting the data and preparing, writing and completing the manuscript as well as its final approval. All authors approved the final version of the manuscript and the submission.

Funding

This work was supported by the National Cancer Center (NCI), National Institutes of Health grant R03CA286694 (to JEB). The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

Data-sharing statement

Clinical data from this study will not be made available.

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