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

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Data Sharing Statement

Clinical Data will not be made available from this study.

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+/CD5dim/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 IL-15 overexpression, 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 response (CR).[11] Therapy in the relapsed/refractory (r/r) 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 for effective therapy for patients with r/r 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 1mg test dose, followed by 10 mg daily for 10 days. 56% of patients achieved a hematologic response, though 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 (one pseudomonal, one *Clostridium difficile*). 64% and 46% of patients experienced CMV and EBV viral 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 infectious risk and feasibility (prolonged hospitalization, experience managing alemtuzumab toxicities/administration). Further, since T-LGLL patients are older and often have significant co-morbidities, the toxicity risk of IV alemtuzumab precludes widespread use, and is not utilized at our center. Furthermore, previous studies did not use standardized response criteria, making it difficult to fully assess response. Given the promising efficacy of alemtuzumab in T-LGLL, we explored the feasibility and efficacy of short-course subcutaneous alemtuzumab (scAlemtuzumab) in patients with r/r 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 3mg, to ensure no allergic/infusion-type reaction. Subsequently, scAlemtuzumab was administered at 10mg three times weekly for a minimum of 4 weeks (12 doses) and maximum of 8 weeks (24 doses). Total duration was determined by standard laboratory monitoring (CBC, CMP, and LFTs) during treatment. All patients received (val)acyclovir VZV prophylaxis and PJP prophylaxis with trimethoprim/sulfamethoxazole from initiation until physician discretion (typically 3 months post-alemtuzumab for PJP, 1 year for VZV). scAlemtuzumab was held for grade 3 infections, or worsening neutropenia (from baseline) until resolution of toxicity and discontinued for persistent neutropenia (>4 weeks).

To evaluate the efficacy of this approach, we reviewed all patients with r/r 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 (IRB). Kaplan Meier methods were used to estimate time to response and leukemia-free survival (LFS). Response was determined based on the E5998 study response criteria[11]. Eight heavily pre-treated r/r T-LGLL patients with 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 PR); demonstrating the refractory nature of T-LGLL in these patients. Full data, including responses to scAlemtuzumab treatment is included in Table 1. The median number of scAlemtuzumab injections was 13 (range 8-24). Median follow-up time was 17 months (range 9-91 months), and the ORR was 75% (6/8 patients) with a PR and CR rate of 38% each. Intriguingly, three patients obtained a CR to scAlemtuzumab that had never obtained a prior CR to any other therapy. The median time to response was 2 months [95% CI: 1.2 months-Not Reached (NR)]. One patient achieved complete molecular response (CMR) defined by clearance of the TCR clone via PCR, in addition to CR criteria. The median duration of response (DOR) for all responders was 5.7 months (95% CI: 0.5-NR). The patient with 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 predictive biomarkers for response. There was no association of response to scAlemtuzumab based upon type of cytopenia, STAT3 mutation, number of prior treatments, or number of doses (Supplementary Table 1). Further, there was no correlation of T-LGLL count, or pre-treatment hematologic parameters on response.

ScAlemtuzumab was overall well tolerated, with a reduced toxicity profile compared to IV alemtuzumab. Grade 2-3 toxicities were observed in four patients. These included one patient each with neutropenia, transaminitis, oral candidiasis, and CMV reactivation. No infusion-type

reactions or cutaneous skin eruptions were observed. For the patient with neutropenia, ANC was initially <500 cells/mm^3, then dropped to <50 cells/mm^3, which prompted discontinuation of therapy, but ANC subsequently recovered to baseline off therapy. No patients died because of scAlemtuzumab therapy, and all toxicities were managed outpatient.

The observed 75% ORR (38% CR, 38% PR), in this heavily pre-treated r/r T-LGLL population suggests that scAlemtuzumab has a similar response to IV alemtuzumab, with significantly lower infectious toxicities. Patients responded rapidly to scAlemtuzumab, typically within 2 months of initiation, with a median DOR of 5.7 months, using rigorous E5998 response criteria. These results compare to an 56% ORR (36% CR, 20% PR) with IV alemtuzumab. Median DOR in the prior IV alemtuzumab study was not reported. When comparing responding vs non-responding patients treated for their target cytopenia (anemia or neutropenia), an early increase in neutrophil or hemoglobin was observed after the first month of treatment in responding patients, compared to non-responding patients (Supplemental Figure 1). 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 4 patients with autoimmune disease. Importantly, scAlemtuzumab resulted in fewer side effects than IV alemtuzumab. We observed no infusion reactions or cutaneous eruptions, and only one case of CMV reactivation with no grade 3/4 neutropenia. This compares to 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 with IV alemtuzumab. Based upon our results, pending future studies, we recommend the following method of administration of scAlemtuzumab for patients with relapsed/refractory T-LGLL: Patients should receive an initial 3mg scAlemtuzumab

dose to evaluate for infusion-type reaction, followed by 10mg scAlemtuzumab three times weekly for up to 8 weeks total with frequent lab monitoring as stated above. For patients that 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 potential infectious risk, scAlemtuzumab should be used with caution for LGLL patients with persistent neutropenia and recurring infections. To mitigate infectious risk, patients receiving scAlemtuzumab should complete therapy for prior infections, and should receive anti-fungal, anti-viral, and anti-bacterial prophylaxis per standard institutional leukemia practice. Further, close monitoring for new or recurring infection is warranted, particularly with viral (CMV) infection.

We acknowledge the limitations of this study. This is a retrospective study of patients treated with standard-of-care scAlemtuzumab without a prospective protocol. However, we believe there was minimal variability across patients as all were treated with the same approach by J.E.B and P.P. CD52 was not routinely checked, thus we cannot make 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 the logistical and toxicity concerns, it is crucial to investigate other alternative methods of administration of this agent, particularly given the lack of available options in patients with r/r 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 treatment 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 immediate prior therapy using standard therapies. Intriguingly, scAlemtuzumab also induced a CR in 3 patients that *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 ORR, these findings will need to be validated in a larger cohort in future large prospective studies, including using CMR as a novel endpoint. Nevertheless, given the current lack of therapeutic options for r/r T-LGLL, and limited clinical trials available for these patients, our data suggests that scAlemtuzumab may be an effective new therapy for patients with relapsed/refractory T-LGLL, with an improved safety profile compared to IV alemtuzumab.

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Table 1: Patient Baseline Characteristics and Response to Subcutaneous Alemtuzumab

Age at Diagnosis	Sex	Other Mutations (VAF)	Associated Autoimmune Diseases	Prior Treatment	ScAlemtuzumab Line	ScAlemtuzumab Indication	Number of ScAlemtuzumab Doses	Time on ScAlemtuzumab (D)	Response to ScAlemtuzumab	Time To CR on ScAlemtuzumab (M)
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	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	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 months
72	M	Unknown	RA	Cy, MTX	3rd	Symptomatic Anemia	24	43	CR	1.7 months

Abbreviations: ANC, Absolute Neutrophil Count; CMR, Complete Molecular Response; CR, Complete Response; CsA, Cyclosporine; Cy, Cyclophosphamide, MMF, Mycophenolate Mofetil; MTX, Methotrexate; PD, Progressive Disease; PR Partial Response; RA, Rheumatoid Arthritis; ScAlemtuzumab, subcutaneous alemtuzumab

*: Treated concomitantly with Romidepsin and ScAlemtuzumab

Supplementary Table 1: Response by neutropenia and anemia status, Campath dosing, and STAT3/5 mutation

Variable	Group	Response rate (%)	Fisher p-value
All patients	All	6/8 (75%)	-
Neutropenia status	Not neutropenic	3/3 (100%)	0.57
	Neutropenia	1/1 (100%)	
	Severe neutropenia	2/4 (50%)	
Anemia status	No anemia	2/2 (100%)	1.0
	Anemia	4/6 (67%)	
Weeks of Campath	3-4	3/4 (75%)	1.0
	5-8	3/4 (75%)	
Doses of Campath	8-14	3/5 (60%)	0.46
	23-24	3/3 (100%)	
Campath line of treatment	1^{st}-3^{rd}	3/3 (100%)	0.46
	4^{th}-5^{th}	3/5 (60%)	
STAT3/STAT5 mutation	Negative	3/3 (100%)	0.25
	Positive	1/3 (33%)	
	Unknown	2/2 (100%)	

Supplemental Figure 1: Hemoglobin and Neutrophil Trends After scAlemtuzumab