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“Bendamustine monotherapy induces high response rates and STAT3 inhibition in high burden T-cell large granular lymphocytic leukemia”

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Running head: Bendamustine efficacy and STAT3 inhibition in T-LGLL

Declarations

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Disclosures

No conflicts of interest to disclose.

Contributions

L.M. designed the research, analyzed data, performed statistical analysis and wrote the manuscript; E.R., G.C., E.B., A.M. R.C., G.P., V.T., performed some of the in vitro research; L.P. provided patient samples and patient data and critically reviewed and edited the manuscript; M.F. and L.T. contributed to analyze data. GS provided funding, participated to the analysis of data and critically reviewed and edited the manuscript; A.T. performed some of

the in vitro research, contributed to analyze data and critically reviewed and edited the manuscript; R.Z. provided funding, designed the study, analyzed data, wrote the manuscript, and supervised the study.

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ABSTRACT

T-cell large granular lymphocytic leukemia (T-LGLL) is a rare chronic lymphoproliferative disorder characterized by clonal expansion of cytotoxic $CD3^+$ large granular lymphocytes (LGLs). Current first-line therapy relies on immunosuppressive agents such as methotrexate, cyclophosphamide, and cyclosporine A; however, these treatments are associated with suboptimal response rates and often require prolonged administration. Bendamustine, a bifunctional alkylating agent with purine analogue–like properties, is widely used in B-cell malignancies and has a favourable safety profile. We evaluated the efficacy and safety of bendamustine monotherapy in a retrospective cohort of 12 patients with treatment-naïve or relapsed/refractory T-LGLL and high burden disease. STAT3 mutations were detected in six patients. The main indications for treatment were severe neutropenia and severe/symptomatic anemia. After a median follow-up of 47.5 months, all patients achieved a clinical response, with a median time to response of 3 months and a complete response rate of 67%. Responses were observed irrespective of STAT3 mutational status. The estimated 3-year progression-free survival, duration of response and overall survival were 56.2%, 61.3% and 90.9%, respectively. Bendamustine was generally well tolerated: fewer than half of patients experienced grade 3–4 adverse events, mostly hematologic, and two patients discontinued treatment for toxicity. *Ex vivo* studies on patients' leukemic LGL demonstrated direct cytotoxic activity of bendamustine, associated with inhibition of STAT3 phosphorylation and induction of apoptosis. These findings support bendamustine as an effective and well-tolerated single-agent therapeutic option in high burden T-LGLL and support its further evaluation in prospective clinical studies.

INTRODUCTION

Large granular lymphocytic leukemia (LGLL) is a rare, chronic hematological malignancy caused by clonal expansion of cytotoxic T cells and accounts for approximately 2–5% of chronic lymphoproliferative disorders [1]. According to the 2022 World Health Organization (WHO) classification, T-LGLL represents the most common subtype, comprising about 85% of LGL disorders. Based on T-cell receptor (TCR) expression, T-LGLL can be further subdivided into TCRα/β ($CD8^+$ or $CD4^+$) and TCRγ/δ variants [2,3].

The clinical course of T-LGLL is highly heterogeneous. While some patients remain asymptomatic, others develop cytopenias, most commonly neutropenia with recurrent infections and anemia. T-LGLL is also strongly associated with autoimmune disorders, particularly rheumatoid arthritis, which occurs in approximately 25–32% of cases [4].

Somatic mutations affecting Signal Transducer and Activator of Transcription 3 (STAT3) and STAT5B provide important diagnostic and prognostic information. In particular, STAT3-mutated $CD8^+$ T-LGLL has been associated with a more symptomatic disease course, higher rates of neutropenia and autoimmune disease, an increased need for therapy, and inferior overall survival (OS) [5].

Treatment is indicated for symptomatic disease and primarily relies on immunosuppressive agents, including low-dose methotrexate (MTX), cyclophosphamide (CTX) and cyclosporin A (CyA) [6]. However, these therapies are associated with relevant toxicity and only moderate response rates (40–60%), and no standard treatment has been established due to the lack of large prospective studies [7].

Bendamustine is a bifunctional alkylating agent with purine analogue properties [8] that is widely used in B-cell malignancies and has a favorable safety profile. Its use in T-cell neoplasms, including LGLL, has been reported in small case series [9–11]. Importantly, preclinical studies have shown that bendamustine can inhibit STAT3 activity in cells with constitutive pathway activation [12, 13]. Given the central role of JAK/STAT signaling in LGLL pathogenesis and the presence of STAT3/STAT5B mutations in approximately 40% of patients [14,15], bendamustine represents a biological plausible strategy in this disorder.

Taken together, these clinical and biological issues provided the rationale for treating high-burden T-LGLL patients with bendamustine.

In the present study, we investigated the *in vitro* activity of bendamustine on leukemic LGLs and assessed the clinical efficacy and safety of bendamustine monotherapy in a retrospective single-center cohort of treatment-naïve and relapsed/refractory T-LGLL patients selected for high burden disease.

METHODS

Patients and treatment

Clinical and biological data from 12 patients with T-LGLL, treated with bendamustine at our Unit between 2010 and 2024, were retrospectively collected. All patients were characterized by a high burden disease, defined as $\geq 3.0 \times 10^9$/L circulating LGLs or bone marrow LGL infiltration ≥30%. STAT3 and STAT5B mutational status was evaluated by Sanger sequencing on their hot spot regions, exon 21 and exons 14-16, respectively, as previously reported [14]. Bendamustine was administered on two consecutive days every 28 days for 4 to 6 cycles, at a dose ranging from 60 to 90 mg/m², according to clinical judgment (age and clinical conditions). Treatment-related toxicity was evaluated using the Common Terminology Criteria for Adverse Events (CTCAE), version 5.0. The study was approved by the local Ethics Committee and written informed consent was obtained from all the enrolled subjects, according to the Declaration of Helsinki.

Response assessment

Treatment response was assessed using modified criteria adapted from the ECOG 5998 trial [16]. A complete response (CR) was defined as normalization of peripheral blood counts. A partial response (PR) was defined as improvement in hematologic parameters without meeting criteria for CR, according to the initial indication for treatment. Full response criteria are included in *Supplementary methods*.

In vitro and *ex vivo* cultures with Bendamustine

Fresh or cryopreserved primary T-LGLL peripheral blood mononuclear cells were cultured at 2×10^6 cells/mL in complete RPMI-1640 medium and maintained at 37°C in 5% CO_2 for 48h. Cells were treated with increasing doses of bendamustine (SelleckChem, USA) ranging from 2.5µM to 100µM. Untreated cells, exposed to the same DMSO concentration used to dissolve the drugs, served as controls to assess basal apoptosis. The same experiments were performed on Jurkat cell line.

Evaluation of apoptosis and STAT3 inhibition

Apoptosis was evaluated by flow cytometry staining primary T-LGLs with fluorochrome-conjugated anti-human CD8, CD16 or CD57, according to patients' immunophenotype, together with PE-conjugated AnnexinV antibodies (BD). Jurkat cells were stained exclusively with PE-conjugated AnnexinV. All cytometric evaluations were performed using the BD FACS Canto II. STAT3 inhibition was confirmed by western blot analysis. Cells were lysed and total protein extracts were separated by SDS-PAGE and transferred to polyvinylidene difluoride (PVDF) membranes. Membranes were incubated with antibodies against phosphorylated STAT3 (pSTAT3) and total STAT3. β-actin was used as loading control. Densitometric analyses were performed using the ImageQuant TL software.

Statistical analysis

Comparisons of biological parameters were performed using Fisher's exact test or one-way ANOVA analysis, as appropriate. Survival outcomes were estimated using the Kaplan–Meier method, with differences assessed by the log-rank test. Progression-free survival (PFS) and overall survival (OS) were calculated from the date of bendamustine initiation to disease progression, loss of response or death (for PFS), and to death from any cause (for OS), or last follow-up. Confidence intervals (CIs) were calculated using Wilson or Clopper-Pearson methods, as appropriate. Statistical analyses were performed using GraphPad Prism software version 10.4.2 (San Diego, California, USA), and a two-sided p value <0.05 was considered statistically significant.

RESULTS

Patient characteristics

Twelve consecutive patients treated with bendamustine, with a median age at treatment initiation of 66 years (range 40–83), were included. All patients had T-LGLL with a terminal effector memory phenotype.

Eleven patients had TCRα/β T-LGLL**,** and one patient had a TCRγ/δ subtype**.** STAT3 mutations were detected in 50% (n=6) of patients, with D661Y being the most frequent variant (33% of the entire cohort), followed by Y640F (n=1) and one STAT3 gene deletion (A662_N663delinsH; n=1). No STAT5B mutations were identified.

The median interval from diagnosis to bendamustine initiation was 2.5 years (range 0–16). Four patients (33%) were treatment-naïve, whereas eight (67%) had relapsed or refractory disease; half of the latter had received more than three prior lines of therapy. The median number of previous treatments among relapsed/refractory patients was 2.5 (range 1–4), including MTX, CTX, CYA, alemtuzumab, and pentostatin (Table 1).

The main treatment indication was severe neutropenia (42%; n=5), followed by transfusion-dependent anemia (25%; n=3), symptomatic anemia (17%; n=2) and moderate neutropenia with recurrent infections (8%; n=1). One patient presented with an unusual lymphoma-like manifestation, characterized by colonic infiltration and peripheral blood dissemination of leukemic LGLs without clinically significant cytopenias.

At bendamustine initiation, absolute lymphocytosis (>4.5×10⁹/L) was present in eight patients (67%). The median absolute LGL count was 8.5×10⁹/L (range 0.8–22.4), corresponding to a median of 70% of circulating lymphocytes (range 32–85). Only one patient had a concomitant autoimmune disorder (rheumatoid arthritis associated with autoimmune hemolytic anemia).

A summary of baseline patient characteristics is shown in Table 1.

Clinical response

After a median of six treatment cycles (range 3–6), all patients achieved a clinical response (12/12; 95% CI, 73.5–100%). The median time to first response was 3 months (range 3–13), and the median time to best response was 5.5 months (range 3–19). The CR rate was 67% (8/12; 95% CI, 38.9–86.1%). Half of these patients achieved CR within six months of treatment

initiation, and one patient normalized blood counts after only three months. The remaining patients (33%; n=4) achieved PR (95% CI, 13.7–61.0%). No statistically significant differences in response rates were observed according to STAT3 mutational status or between treatment-naïve and relapsed/refractory patients. Additional details regarding the clinical response of each patient are provided in Table 2 and Figure 1 (swimmer plot).

We also performed serial flow cytometric assessments of circulating LGLs, which showed normalization of the LGL count ($<0.5\times10^9$/L) in 82%**,** 83% and 38% of evaluable patients at end of treatment, after 12 months and 24 months**,** respectively. After a median follow-up of 47.5 months (range 14-147), median PFS, DoR and OS were not reached. The estimated 36-months PFS, DoR and OS were 56.2 (95% CI, 24.3 - 79.1), 61.3% (95% CI, 26.6 - 83.5) and 90.9 (95% CI, 50.8 - 98.7), respectively (Figure 2). Outcomes between STAT3 mutated and unmutated were not significantly different. Instead, patients reaching CR had a better DoR (median NR vs 8.0 months, $p<0.001$) and PFS (median of 149.0 vs 13.5 months, $p<0.001$) than those who achieved only a PR, although there was no difference in OS.

At the time of data cutoff (January 1, 2026), six patients remained in response**,** two had died from non–leukemia-related causes (the exact causes of death are unknown because they were lost to follow-up)**,** and four had lost response**,** although only two required additional therapy. Table 2 shows details regarding baseline features and clinical responses of the patients.

Safety

Overall**,** 92% of patients (n=11) experienced at least one adverse event (AE), with grade 3–4 AEs occurring in 42% (n=5)**.** Grade 3–4 treatment-related neutropenia occurred in 25% (n=3)**,** while grade 3–4 anemia was observed in one patient (8%). No grade 3–4 thrombocytopenia was reported. The most frequent non-hematologic toxicities were nausea/vomiting (25%) and diarrhea (17%). Only one patient developed a grade 3 non-hematologic AE (transient alanine aminotransferase elevation) (Table 3). Two patients discontinued therapy prematurely, one due to febrile neutropenia and one to grade 3 anemia. In the latter case, bone marrow evaluation excluded disease progression. No treatment-related deaths occurred**.**

Cytotoxicity and STAT3 inhibition analysis

The effects of bendamustine on leukemic LGLs were investigated *in vitro,* with the aim of elucidating the biological mechanisms underlying the clinical response observed in patients. Specifically, we assessed both cytotoxicity activity of bendamustine and its effects on the activation status of transcription factor STAT3.

Bendamustine was initially tested on the Jurkat cell line, already used as a model for this disease. Cells were treated with increasing concentration of the drug (ranging from 2.5μM to 100μM) and apoptosis was evaluated after 24 and 48h. Cytofluorimetric analyses showed that bendamustine significantly increases cell mortality, measured as the percentage of Annexin V–positive cells, compared with the untreated (DMSO) condition. This effect was evident at 24h starting from a concentration of 50 μM, whereas at 48h it was observed only at the highest tested dose (Figure 2A, C). Analysis of normalized data (treated versus untreated conditions) confirmed these findings, showing 2.6- and 3.2-fold increases in apoptosis at 24h with 50 μM and 100 μM bendamustine, respectively, and a 4.4-fold increase at 48h with 100 μM (Figure 2B, D).

Given the promising results obtained in Jurkat cells, the same experimental approach was applied to primary T-LGLL samples. Analysis of the normalized data revealed statistically significant effects starting at a concentration of 25 μM at both 24 and 48h. Specifically, at 24h, cell mortality increased by 1.5, 1.8, and 3.3 fold at concentrations of 25 μM, 50 μM, and 100 μM, respectively (Figure 2E-H).

Given the central role of STAT3 in T-LGLL pathogenesis, the effect of Bendamustine on STAT3 activation was evaluated by western blot analysis. As reported in Figure 3A, densitometric analysis demonstrated a significant reduction in STAT3 phosphorylation after 24h of treatment with bendamustine at concentrations of 50 μM and 100 μM. Figure 3B shows a representative western blot from a STAT3-mutated case, demonstrating a dose-dependent decrease in pSTAT3 levels upon bendamustine treatment.

DISCUSSION

In this study, we report the largest single-center series to date evaluating bendamustine monotherapy in patients with high burden treatment-naïve or relapsed/refractory T-LGLL. The estimated 3 years PFS, DoR and OS were 56.2%, 61.3% and 90.9%, respectively, with well

tolerated toxicity. Based on preclinical evidence indicating that bendamustine can inhibit STAT3 signaling [12,13], we hypothesized that this agent might exert both cytotoxic and disease-modifying effects in T-LGLL. Consistent with this hypothesis, our *ex vivo* experiments demonstrated direct cytotoxicity against leukemic LGLs together with inhibition of STAT3 phosphorylation**,** supporting a biologically plausible mechanism of action involving interference with the JAK/STAT pathway. The concordance between the inhibition of STAT3 phosphorylation observed in *ex vivo* experiments and the clinical activity seen in treated patients further supports a mechanistic link between bendamustine exposure and modulation of JAK/STAT signaling in T-LGLL.

Clinically, bendamustine showed substantial activity**,** with responses observed in all patients and complete hematologic remission achieved in two-thirds of cases. Responses were rapid**,** occurring at a median of three months, and appeared durable**,** with more than half of patients remaining progression-free at three years. Conventional immunosuppressive approaches, such as methotrexate, cyclophosphamide, or cyclosporine, typically achieve response rates of approximately 40–60% and often require prolonged treatment to maintain disease control. Compared to these, bendamustine produced rapid and deep responses in our cohort of high burden patients, suggesting that direct cytotoxic targeting of the leukemic clone may represent an effective alternative therapeutic strategy. Notably, clinical efficacy was observed irrespective of prior lines of therapy, showing activity also in the most challenging and heavily pretreated patients. As expected, patients who achieved a CR showed significantly longer DoR and PFS than those who did not, despite absence of a difference in OS.
Although not statistically significant due to sample size limitations, most relapses occurred in STAT3-wild-type patients (75%)**,** whereas all but one STAT3-mutated patient remained progression-free at last follow-up. While this observation should be interpreted with caution, it raises the possibility that STAT3 mutated disease may be particularly sensitive to bendamustine and warrants further evaluation in larger cohorts.

Given its potent lymphodepleting activity, bendamustine was preferentially administered in patients with a high circulating or medullary LGL burden and resulted in a marked and sustained reduction of leukemic clone. These findings are consistent with previous observation indicating that effective control of cytopenias in T-LGLL requires reduction of the leukemic clone**,** rather than immunomodulation alone [17]. In this context, bendamustine may represent

a particularly attractive option for patients with high leukemic burden or rapidly progressive cytopenias, in whom faster and deeper cytoreduction may be clinically advantageous.

From a safety standpoint, bendamustine was generally well tolerated**,** with manageable hematologic toxicity and limited non-hematologic adverse events. Treatment discontinuation was uncommon, and no treatment-related mortality was observed.
Therefore, we recommend a minimum administration of four cycles of bendamustine, up to six in cases of suboptimal response and good tolerance after four cycles. The dosage should be adapted according to age and performance status.
The main limitations of this study include its retrospective design and the relatively small sample size**,** which preclude definitive subgroup analyses and the generalizability of our findings. Nevertheless, given the consistency and magnitude of response observed, these data provide a strong rationale for prospective multicenter evaluation of bendamustine to define its role in both frontline and relapsed/refractory settings. Although bendamustine therapy is associated with a substantial risk of infections, the safety profile observed in our cohort should not preclude the enrollment of a large number of patients in a multicenter phase II study, which is necessary given the rarity of the disease.

Taken together, our findings identify bendamustine as a promising and well-tolerated single-agent therapy in T-LGLL**,** capable of inducing rapid and durable responses while targeting key pathogenic pathways. These results support its consideration as a valuable therapeutic option in high burden patients and provide a rationale for its inclusion in future treatment algorithms for this rare and heterogeneous disease.

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Tables and figures

Male/female	6/6
Median age at diagnosis (range); y	66 (40-83)
Laboratory data[1]	
LGL count (x10^9/L)	8.5 (0.8-22.4)
Hemoglobin (g/dL)	11.0 (8.4-15.6)
Absolute neutrophil count (x10^9/L)	0.7 (0.1-4.0)
Platelets count (x10^9/L)	185 (94-506)
BM LGL infiltrate (%)	35 (25-70)
Treatment triggers	
Severe neutropenia	5 (42%)
Neutropenia with recurrent infections	1 (8%)
Transfusion dependent anemia	3 (25%)
Symptomatic anemia (Hb<10 g/dL)	2 (17%)
Other[2]	1 (8%)
Molecular profile	
STAT3 mutation	6 (50%)
STAT5B mutation	0
TCR γδ	1 (8%)
N° of previous lines of therapy	
0	4 (33%)
1-2	4 (33%)
≥3	4 (33%)
Previous therapies	
Methotrexate	3 (38%)
Cyclosporine A	6 (75%)
Cyclophosphamide	8 (100%)
Alemtuzumab	1 (13%)
Other[3]	2 (25%)

Table 1. **Demographics and baseline characteristics**. BM: Bone Marrow; LGL: large granular lymphocyte.
[1] Data expressed as median value (range)
[2] Colonic infiltration
[3] Rituximab, pentostatin

Patient ID	Age (y)/ Sex	STAT3 status	Previous lines of therapy	Benda dosage (mg/mq)	N° of cycles	Best response	Time to BR (m)	Duration of response (m)	Relapse	Follow-up (m)	Alive at data cut-off
1	70/M	wt	1	90	6	CR	19	128	no	147	no
2	55/M	wt	3	70	6	CR	4	84	no	88	yes
3	60/M	Y640F	1	70	6	CR	3	68	no	71	yes
4	40/F	wt	3	70	6	CR	9	52	no	61	yes
5	63/M	wt	2	70	6	PR	3	8	yes	60	yes
6	72/F	del-ins	3	70	4	CR	11	41	no	52	yes
7	83/F	wt	2	60	6	PR	5	7	yes	28	yes
8	60/F	D661Y	4	90	3	PR	13	5	no	14	no
9	70/F	D661Y	0	90	6	CR	8	18	yes	43	yes
10	70/F	D661Y	0	70	6	CR	6	35	no	41	yes
11	56/M	wt	0	70	6	PR	3	12	yes	25	yes
12	71/F	D661Y	0	90	3	CR	5	15	no	20	yes

Table 2. **Baseline characteristic of the patients and clinical responses to bendamustine.** Benda: Bendamustine; BR: Best Response; CR: Complete response; m: months; PR: Partial response; wt: wild-type.

Event	n (%)	Any grade	Grade 3-4
Any adverse event		11 (92)	5 (42)
Hematological toxicities			
Neutropenia		3 (25)	3 (25)
Anemia		2 (17)	1 (8)
Thrombocytopenia		5 (42)	0 (0)
Non-hematological toxicities			
Skin disorders		1 (8)	0 (0)
Hepatic impairment		1 (8)	0 (0)
Nausea and vomiting		3 (25)	0 (0)
Constipation		1 (8)	0 (0)
Diarrhea		2 (17)	0 (0)
Febrile neutropenia		1 (8)	1 (8)
Infections		1 (8)	0 (0)
Other[1]		3 (25)	0 (0)

Table 3. **Treatment-related adverse events**

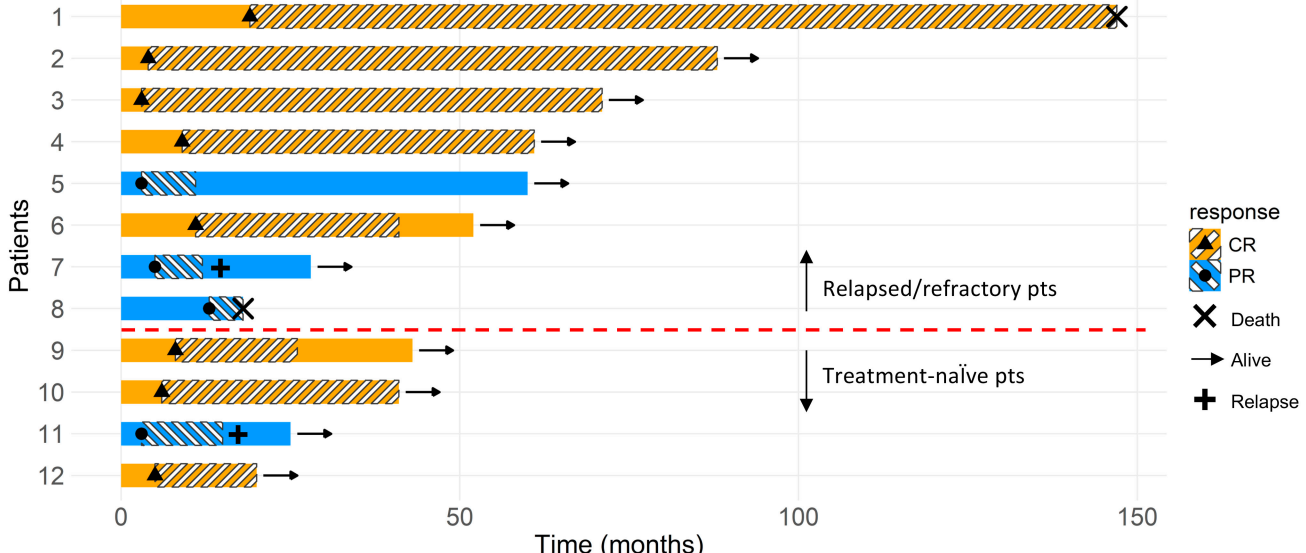
[1] Abdominal pain, back pain and left arm phlebitis

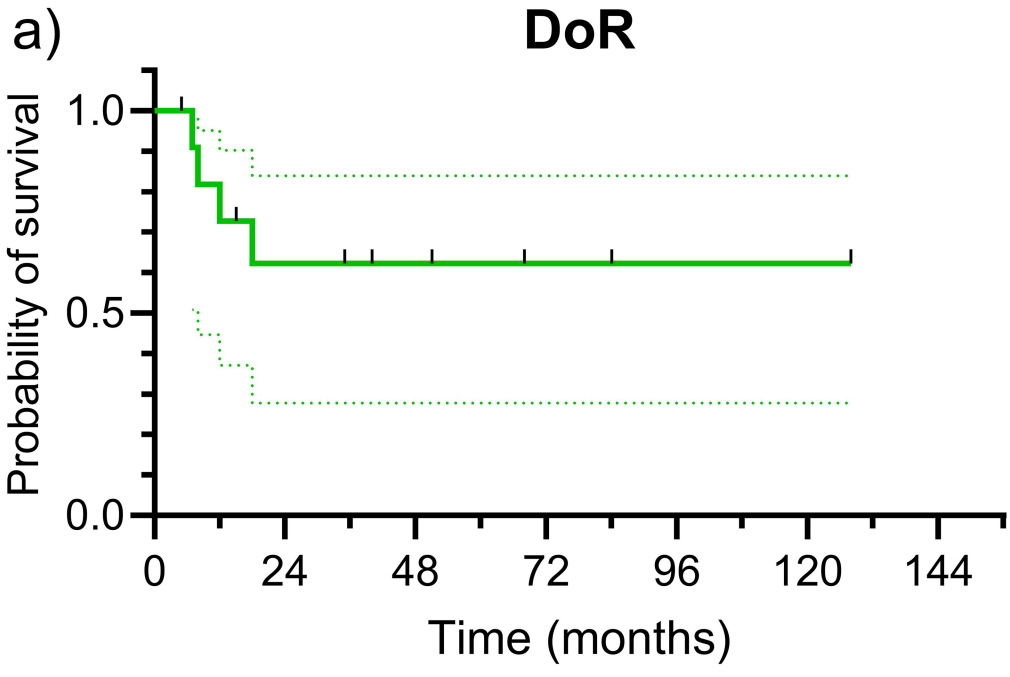
Figure 1: **Swimmer plot showing treatment response kinetics and follow-up in our cohort.** Horizontal bars represent follow-up duration. Orange bars correspond to complete response (CR) and light blue bars to partial response (PR), whereas striped areas indicate duration of response. In this graph, a "relapse" means that a new treatment has been started.

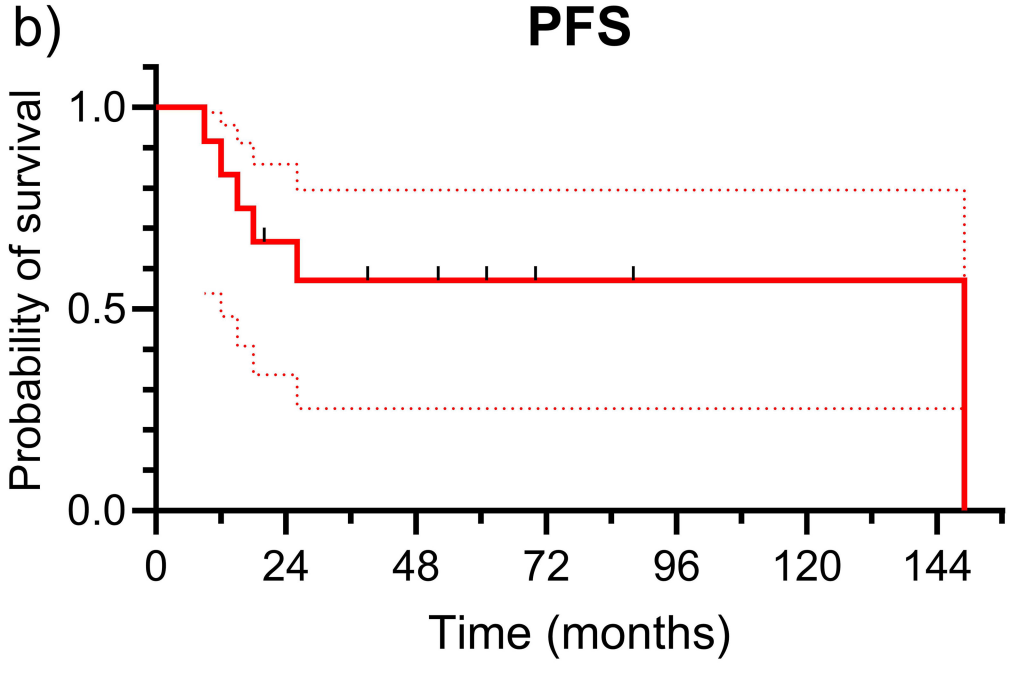
Figure 2: **Kaplan-Meyer survival graphs following treatment with bendamustine**. Dotted lines delimit confidence intervals (95%). a) Duration of Response (DoR); b) Progression-free survival (PFS); c) Overall survival (OS).

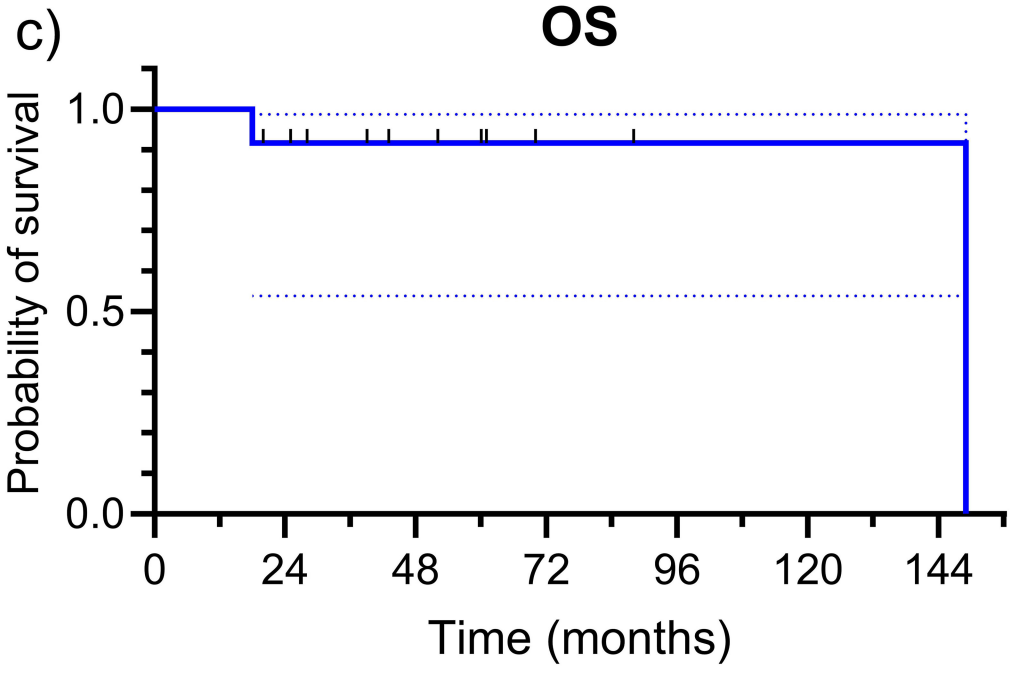
Figure 3: **Cytotoxicity analysis in the Jurkat cell line and T-LGLL primary samples at 24 and 48h.** Panels (A), (C), (E) and (G) show drug cytotoxicity expressed as absolute percentage of Annexin V+ cells (violin plots), whereas panels (B), (D), (F) and (H) show cytotoxicity expressed as fold change (T/UT), normalized by setting the untreated condition (DMSO) equal to 1 (bar plots). In panels (A-B) and (E-F) bendamustine concentrations at 24h in Jurkat cell line and T-LGLL primary samples are shown in shades of green and orange, respectively. In panels (C-D) and (G-H) data for bendamustine concentrations at 48h in Jurkat cell line and T-LGLL primary samples are shown in shades of blue and purple, respectively.
Data are presented as mean ± SD (n=4 for Jurkat cell line at 24 and 48h, n=5 and n=4 for T-LGLL samples at 24 and 48h, respectively). Statistical significance was assessed by one-way ANOVA, *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.

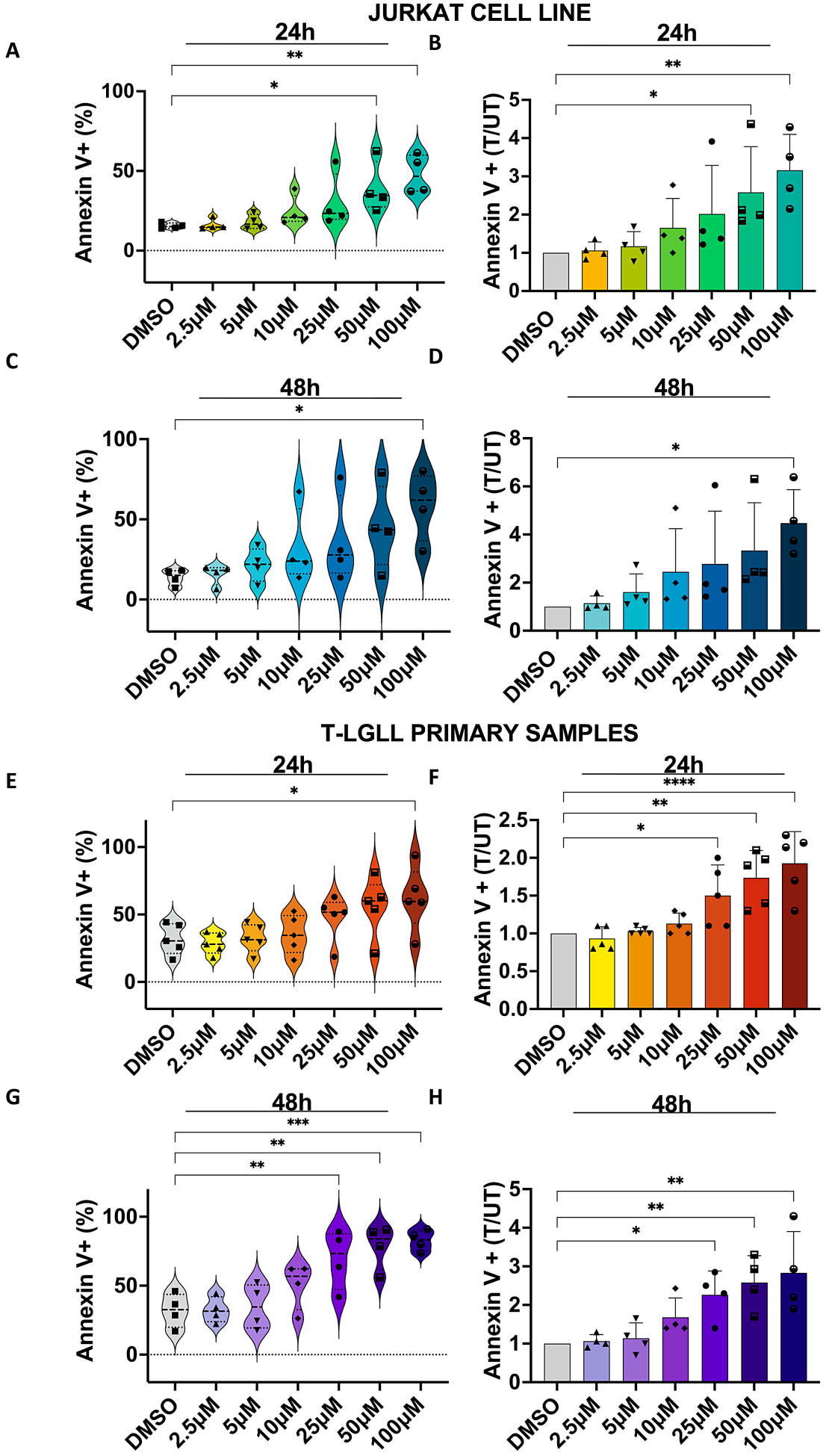
Figure 4: **Analysis of STAT3 inhibition in leukemic T-LGLs after 24h of treatment with bendamustine.** (A) Densitometric quantification of STAT3 phosphorylation at 24h after treatment with bendamustine (n=5). Data are presented as mean ± SD in bar graphs. Statistical significance was assessed by one-way ANOVA, *p<0.05. (B) Representative image from one of five independent experiments showing phosphorylated STAT3 (pSTAT3) compared with total STAT3 and β-actin at 24h following treatment with increasing concentrations of bendamustine.

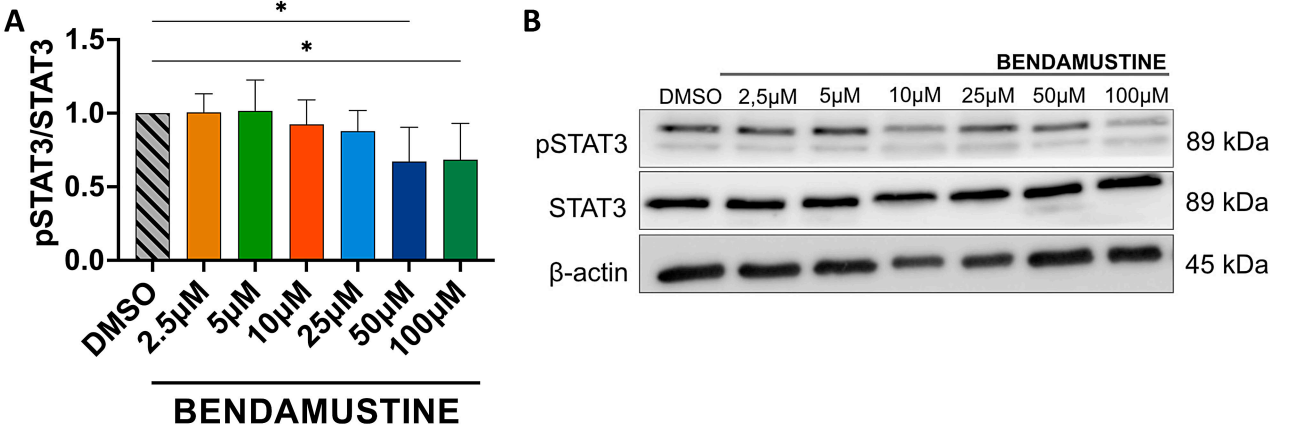

Patients
1
2
3
4
5
6
7
8
9
10
11
12
0
50
100
150
Time (months)
Relapsed/refractory pts
Treatment-naïve pts
response
CR
PR
Death
Alive
Relapse


a)
DoR
Probability of survival
1.0
0.5
0.0
0
24
48
72
96
120
144
Time (months)


b)
PFS
Probability of survival
1.0
0.5
0.0
0
24
48
72
96
120
144
Time (months)


c)
OS
Probability of survival
1.0
0.5
0.0
0
24
48
72
96
120
144
Time (months)


JURKAT CELL LINE
A
24h
Annexin V+ (%)
100
50
0
DMSO
2.5μM
5μM
10μM
25μM
50μM
100μM
B
24h
Annexin V + (T/UT)
C
48h
D
48h
T-LGLL PRIMARY SAMPLES
E
24h
F
24h
G
48h
H
48h


A
pSTAT3/STAT3
1.5
1.0
0.5
0.0
*
*
DMSO
2.5μM
5μM
10μM
25μM
50μM
100μM
BENDAMUSTINE
B
BENDAMUSTINE
DMSO
2,5μM
5μM
10μM
25μM
50μM
100μM
pSTAT3
89 kDa
STAT3
89 kDa
β-actin
45 kDa

Supplementary methods

Clinical response criteria

Treatment response was assessed using modified criteria adapted from the ECOG 5998 trial [1].

- A complete response (CR) was defined as normalization of peripheral blood counts. In addition, LGL counts as determined by repeat flow cytometry needed to be in the normal range.
- A partial response (PR) was defined as improvement in hematologic parameters without meeting criteria for CR, according to the initial indication for treatment, including at least one of the following:
    - Absolute neutrophil count (ANC) >0.5 x 10^9/L, provided this represented at least a 50% increase from baseline;
    - ANC increase of >50% over baseline values;
    - Increase in hemoglobin level of >1 g/dL sustained for at least 4 months;
    - Reduction in monthly transfusion requirements by >50% for a minimum duration of 2 months.
- Progressive disease (PD) was defined as worsening of hematologic parameters in patients who had previously achieved PR or CR.
- Stable disease (SD) was defined as failure to meet criteria for CR, PR, or PD.

T cell receptor gene rearrangement studies were not reassessed after treatment; therefore the molecular response was not included in response criteria.

References

1. Loughran, T., Zickl, L., Olson, T. et al. Immunosuppressive therapy of LGL leukemia: prospective multicenter phase II study by the Eastern Cooperative Oncology Group (E5998). Leukemia 29, 886–894 (2015). https://doi.org/10.1038/leu.2014.298