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Alemtuzumab in T-cell large granular lymphocytic leukaemia: a phase 2 study

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Abstract

Background—T-cell large granular lymphocytic leukemia (T-LGL) is a lymphoproliferative disease presenting with immune-mediated cytopenias and characterized by clonal expansion of cytotoxic $CD3^+CD8^+$ lymphocytes. Methotrexate, cyclosporine, or cyclophosphamide improve cytopenias in 50% of patients as first therapy, but the activity of an anti-CD52 monoclonal antibody, alemtuzumab, is not defined in T-LGL.

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DISCLOSURE OF CONFLICTS

The authors have no conflicts to disclose.

AUTHOR CONTRIBUTIONS

Clinical trial design: B.D., C.O.W., D.M.T., N.S.Y, J.A.B, and P.S.
Patient care: B.D., S.I., P.S., O.R, M.B., D.M.T. N.S.Y, J.A.B, and P.S.
Experiments and data analysis: B.D., S.I., X.F., N.S., M.Y., S.K., J.M., F.C., K.K, I.M., L.X., M.R., P.M., and P.S.
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Methods—Twenty-five consecutive subjects with T-LGL were enrolled from October 2006 to March 2015 at the National Institutes of Health (www.clinicaltrials.gov-NCT00345345). Alemtuzumab was administered at 10 mg/day intravenously for 10 days. The primary endpoint was haematologic response at 3 months. Analysis was intention to treat. Here we report the protocol specified interim benchmark of a phase II clinical trial using alemtuzumab in T-LGL.

Findings—In this heterogeneous, previously treated cohort, 14/25 (56%; 95% CI, 37–73%) subjects had a haematological response at 3 months. In T-LGL cases not associated with myelodysplasia or marrow transplantation, the response rate was 14/19 (74%; 95% CI, 51–86%). First dose infusion reactions were common which improved with symptomatic therapy. EBV and CMV reactivations were common and subclinical. In only 2 patients pre-emptive anti-CMV therapy was instituted. There were no cases of EBV or CMV disease. Alemtuzumab induced sustained reduction of absolute clonal population of T-cytotoxic lymphocytes, as identified by TCRBV-receptor phenotype, but the abnormal clone serendipitously persisted in responders. *STAT3* mutations in the *SH2* domain, identified in ten subjects, did not correlate with response. When compared with healthy volunteers, T-LGL subjects showed a distinct plasma cytokine and JAK-STAT signature prior to treatment, but neither correlated to response.

Interpretation—This is the largest and only prospective cohort of T-LGL subjects treated with alemtuzumab yet reported. The high activity with a single course of a lymphocytotoxic agent in a mainly relapsed and refractory suggests that haematologic response outcomes can be accomplished without the need for continued use of oral immunosuppression.

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Introduction

A syndrome of increased numbers of circulating large granular lymphocytes (LGL) associated with chronic neutropenia was recognized as a distinct clinical entity since 1977[1] and the term T-cell large granular lymphocytic leukemia (T-LGL) was coined in 1985.[2] Clonal LGL proliferations may be either $CD3^+$ (T cell LGL, or T-LGL leukemia) or $CD3^-$ (NK cell LGL, or NK-LGL leukemia).[3] LGL usually occurs in individuals over the age of 50, who may present with recurrent bacterial infections, occasional splenomegaly, and an association with rheumatoid arthritis.[3,4] Most subjects have significant neutropenia, with a "maturation arrest" in the myeloid series.[2] Some individuals have red cell aplasia with anaemia and reticulocytopenia; thrombocytopenia is uncommon and seldom severe.[2] Mortality in recent series ranges from 10–20% at 4 years.[2,5] The cause of LGL clone proliferation and the mechanism of cytopenias remain unclear. Immunosuppressive therapy can improve the cytopenias of T-LGL and responses are observed in about 50% but long-term intermittent use of cyclosporine, cyclophosphamide, or methotrexate is often required,[2,3,6] leading to toxicity and the potential for secondary leukemia or myelodysplasia, especially with long-term oral alkylator use.[7] Furthermore, the clonal population is not eradicated by these agents.[6]

The monoclonal antibody alemtuzumab targets CD52 on T cells, and is a potent and well-tolerated immunosuppressive agent at low doses with efficacy in marrow failure

syndromes.[8,9] Alemtuzumab has been reported to have activity in T-LGL in a few case reports and small case series.[10–18] Based on these early anecdotes and retrospective data we initiated in 2006 a prospective, single arm clinical trial to explore the potential of low-dose alemtuzumab to improve cytopenias in subjects with T-LGL. Here we report on the activity of alemtuzumab in T-LGL after successfully reaching a protocol specified benchmark for haematologic response.

Methods

Study design

The protocol was designed as a nonrandomized, off-label phase II study of alemtuzumab in subjects with T-LGL (Figure S1, page 1). The protocol was approved by the Institutional Review Board of the National Heart, Lung, and Blood Institute and is registered at ClinicalTrials.gov as NCT00345345.

Study eligibility

Consecutive subjects, ages 18–85, with T-LGL were enrolled from October 1st 2006 to March 1st 2015 at the National Institutes of Health Clinical Center. Eligibility criteria included a history of cytopenias with circulating LGL shown to be $CD3^+CD8^+$ $CD57^+$ T-LGL by flow cytometry, with restricted or clonal TCR rearrangement by molecular studies. At least single-lineage cytopenia was required prior to enrollment: either absolute neutrophil count (ANC) <500/μL; or symptomatic anaemia with a haemoglobin < 9 g/dL or red cell transfusion requirement of >2 units/month; or severe thrombocytopenia (< 20,000/μL) or moderate thrombocytopenia (< 50,000/μL) with active bleeding. Both treatment-naïve and treated T-LGL patients were eligible for the clinical trial. Subjects with concomitant bone marrow myelodysplasia and cytogenetic abnormalities were eligible. Exclusion criteria included reactive LGL lymphocytosis, prior history of immunosuppressive therapy with alemtuzumab, infection not adequately responding to appropriate therapy, HIV seropositivity, pregnancy and history of carcinoma not considered cured. All patients had peripheral blood flow cytometry analysis using staining for standard, CLIA certified, CD3, CD4, CD8 and CD57 antibodies performed in our clinical haematopathology laboratory at the Clinical Center. All patients prior to enrolment had to meet blood count criteria of cytopenia(s), flow cytometric and molecular presence of a T-LGL clone.

Alemtuzumab administration and supportive care

After a 1 mg intravenous test dose, 10 mg alemtuzumab (Campath®; Genzyme) was administered intravenously daily for 10 days. Monthly aerosolized pentamidine was prophylaxis for *Pneumocystis jiroveci* and valacyclovir 500 mg/day prophylaxis for herpes simplex, both continued until $CD4^+$T-cells >200/μL. Ciprofloxacin 500 mg twice daily was administered if the ANC was <200/μL. G-CSF and prophylactic antifungal therapy were not routinely administered with alemtuzumab. Molecular monitoring for EBV and CMV was performed at baseline, weekly for the first month, every two weeks in the second month, and monthly thereafter for another 6 months. EBV and CMV quantitative real-time PCR were performed as previously described.[19] A positive PCR was defined as more than 250 EBV copies/ml or more than 250 CMV copies/mL blood. Pre-emptive therapy was not routinely

administered for CMV and EBV reactivations given the self-limited nature of these reactivations in marrow failure subjects previously reported.[19] Because of reports of cardiotoxicity a 2-D echocardiogram, 24-hour Holter monitoring, and troponin levels were performed prior to and at the end of alemtuzumab treatment.[20]

Endpoints

The primary endpoint was haematologic response at three months after treatment. A complete response (CR) was defined as normalization of all affected lineages, and a partial response (PR) was defined in neutropenic subjects as 100% increase in the ANC to >500/μL, and in those with anaemia, any increase in haemoglobin of 2 g/dL or more observed in at least two serial measurements 1 week apart and sustained for one month or more without exogenous growth factors support or transfusions. Transfusion-independence, haematologic response at six months, molecular response, relapse-free and overall survival were secondary endpoints. In subjects who relapsed after alemtuzumab, a second course of the same regimen was permitted per protocol. Landmark visits occurred at 3, 6, 12 months and yearly thereafter. Hemogram, electrolytes, liver and function tests, EBV and CMV monitoring, flow cytometric and molecular analysis for T-LGL clone detection were performed at landmark visits. A bone marrow biopsy was also conducted at landmark visits with the exception of the 3-month landmark.

Statistical methods

We hypothesized that a haematologic response rate at 3 months (primary endpoint) of ≥ 50% would be achieved with alemtuzumab. We considered that a response probability of 30% or less would warrant terminating the treatment on this patient population. Thus, sample size was determined by testing the null hypothesis H0: $p \leq 30\%$ versus the alternative H1: $p \geq 50\%$ at 0.05 significance level and 0.80 of the power, with p being the overall response at 3 months. Sample size was determined using the Two-Stage Minimax Design outlined in Table 1 of Simon (1989).[21] At the first stage, 19 subjects were accrued and the null hypothesis accepted (i.e., the treatment terminated) if 6 or less of the subjects responded to the treatment at 3 months. If 7 or more subjects responded to the treatment at 3 months at the first stage, then an additional 20 subjects would be accrued. Planned analyses included descriptive statistics on the proportions of responses. Patients who died or were not evaluable for response at landmark time points were considered non-responders. The Kaplan-Meier estimates and Cox regression were used to evaluate the treatment effects on the overall survival (depicted using GraphPad Prism version 6.00, GraphPad Software, La Jolla California USA). A $p<0.05$ was considered significant.

Role of funding source

This research was supported by the Intramural Research Program of the NIH, National Heart, Lung, and Blood Institute.

Sample collection

Peripheral blood, and plasma samples were collected from subjects at baseline, 3, 6 and 12 months after alemtuzumab and yearly thereafter. Bone marrow biopsy and aspiration for

morphology and metaphase karyotyping were performed before enrolment, 6 and 12 months after immunosuppressive therapy, and then yearly. Peripheral blood mononuclear cells (PBMC) were isolated by either lymphapheresis or peripheral blood Ficoll-Hypaque density gradient centrifugation and cryopreserved in liquid nitrogen according to standard protocols. Plasma from heparinized blood was stored at −80° C. T-cell receptor gene rearrangement was performed by PCR-based assays using capillary electrophoresis.[22]

Flow cytometry and analysis

PBMC samples from 19 T-LGL subjects were available for correlative TCRBV analysis. Monoclonal antibodies and fluorescent dyes used in flow cytometry analysis are shown in Supplemental Table 1, page 8. Data acquisition was performed on a Becton Dickinson Fortessa and data analyzed using FlowJo software (Tree Star Inc. Ashland OR). At least 500 events per $CD4^+$ or $CD8^+$ cell population were acquired per TCRBV to ensure that a sufficient number of T-cells were obtained. The T-LGL clone was identified based on large clonal $CD8^+$ or $CD4^+$ TCRBV expansions when compared to a normal range of TCRBV values previously generated.[23] TCRBV clonal analysis was part of exploratory analysis.

STAT3 and STAT5 mutation analysis

For 20 patients magnetic bead sorting of $CD8^+CD57^+$ cells was done using MACS $CD8^+$ T-cell isolation kit followed by positive selection with CD57 microbeads (Miltenyi Biotec, Auburn, CA, USA), according to the manufacturer's instructions. Subsequently DNA was extracted using a Maxwell 16 blood DNA purification kit (Promega, Madison, Wisconsin) and Sanger sequencing was done using previously published primers.[24,25] STAT mutation analysis was conducted as part of exploratory analysis.

Real-time reverse transcriptase (RT)-PCR

A prepared PCR array 384 well created by Qiagen (Frederick, MD, USA) was used to check gene expression for JAK-STAT signalling pathway (Cat no: PAHS-039ZE-4). This array targets important 84 genes involved in JAK-STAT signalling pathway. Total RNA was extracted using the Qiagen RNeasy Mini kit, which is compatible with QiaCube robot. Extracted RNA from magnetic bead sorted $CD8^+CD57^+$ and $CD8^+$ $CD57^-$ cells was converted to complementary DNA (with RT^2 first strand kit) and used for PCR Array according to the manufacturer's instructions. Analysis of data was accomplished by using the ΔΔCt method (Qiagen DataAnalysis WebPortal). JAK-STAT pathway gene expression profile was conducted as part of exploratory analysis.

LGL cytokine analysis

Comprehensive cytokine analysis including 57 plasma cytokines, chemokines, and growth factors (Supplemental Table 2, Page 9) was performed using a magnetic bead based Luminex assay in samples from 14 patients (Affymetrix, CA, USA). Cytokine measurement was part of exploratory analysis.

Results

Patient characteristics

Twenty-five patients were enrolled from October 1st 2006 to March 1st 2015 and all were included in the analysis. One patient with cytopenias and a clonal population by flow cytometry could not have clonality established by TCR gene rearrangement nor TCRBV studies and was thus excluded. The average age was 57·7 years (range, 26–82). The median number of prior therapeutic interventions for T-LGL was 3 (range, 0–8) and the average time from diagnosis to alemtuzumab therapy was 34·9 months (range, 0·6 – 199). Of the subjects previously treated for T-LGL (n=23), all but one were refractory to prior immunosuppressive therapies. Details of patient characteristics are shown in Table 1. The median follow-up was 31·1 months (IQR, 6·6 – 61·1) and for surviving subjects 40·2 months (IQR, 7·1 – 65·5). One patient was lost to follow-up 4 months after alemtuzumab therapy. The bone marrow in all patients showed an interstitial lymphocytic infiltrate, as expected in T-LGL. After alemtuzumab, there was a decrease in the amount of lymphocyte interstitial infiltrate that was unquantifiable on immunohistochemical staining of bone marrow section slides. Splenomegaly was observed in only two patients. Most patients had a T-LGL clone associated with cytopenias and did not have bulky disease or other end-organ involvement.

Haematologic response and outcomes

Fourteen of twenty-five (56%; 95% CI: 37–73%) subjects responded; nine subjects had a CR and five had a PR at 3 months after treatment (Figure 1, left panel). No responses were seen in the 4 subjects with associated myelodysplasia (MDS) and in the two hematopoietic stem cell transplantation (HSCT) recipients. Thus, the response rate in nineteen subjects with typical T-LGL was 14/19 (74%; 95% CI: 51–86%; Figure 1, right panel). Of the fourteen responding patients, eight had anaemia, four had neutropenia and two had both anaemia and neutropenia at baseline. Haematologic improvement occurred quickly after treatment, usually in the first 2–3 weeks, and was durable in over half of the responders after only 1 course of alemtuzumab (Figure 2). Significant depression in non-affected lineages was not observed with this low dose alemtuzumab regimen.

Five of the responders relapsed: one achieved PR with cyclosporine; four subjects were re-treated with alemtuzumab, of whom 2 achieved CR but relapsed at 5 and 12 months later. The patient that relapsed at 5 months after retreatment achieved a durable CR to oral cyclosporine 2 months after starting the drug. Of the eleven non-responders three became responders at 6 months but, from the other eight patients, seven of them died (four of infectious complication, one from bleeding, and two of unknown causes); whereas all the alemtuzumab responders, including the relapsed subjects, were alive at the time of censor (Figure S2, page 2).

Adverse events

Alemtuzumab was well tolerated. Infusion related reactions were common and managed symptomatically. Adverse events and severe adverse events are summarized in Table 3 and a complete list of adverse events as well as severe adverse events are shown in supplemental data (Table S3 and S4, pages 10–17). Infusions were discontinued after 5 days in one patient

with post-HSCT T-LGL due to persistent hypotension; he was a non-responder at 3 and 6 months. The remaining twenty-four subjects received the full 10-day course. There were no cases of dose reductions or deaths that were treatment related. Lymphodepletion was universal (Table 3) and prolonged (Figure 3A and B and Figure S3, page 3) and subclinical EBV and CMV reactivations were common (Figure 3 C and D). At baseline, all subjects were seropositive for EBV, while CMV seropositivity was observed in 13/25 (52%) of subjects. Of the seropositive subjects, EBV reactivation occurred in 16/25 (64%; median peak copy number 500/mL). CMV reactivations occurred in 6/13 (46%; median peak copy number of 2,000/mL). There were no cases of EBV or CMV disease, and pre-emptive therapy for CMV was instituted for rising viremia in only two patients, one after HSCT receiving tacrolimus for chronic graft-versus-host disease and the other for unexplained fever. In the remainder, viral loads were only monitored and reactivations were self-limited. Hypothyroidism was present in three subjects prior to alemtuzumab and increased TSH level above the upper limit of normal, with normal T3 and T4 hormone levels, were observed in seven additional patients. There was no evidence of cardiotoxicity from alemtuzumab: troponin levels did not increase during or after the 10-day infusion, and the ejection fraction remained unchanged pre-treatment, after the 10-day infusion and at 3 months following alemtuzumab. In one patient there was a transient decrease in ejection fraction soon after completion of the alemtuzumab infusion, which returned to baseline spontaneously soon thereafter.

STAT3 and *STAT5* mutations

Sanger sequencing of DNA obtained from magnetically sorted $CD8^+/CD57^+$ cytotoxic lymphocytes was performed on twenty patients for whom samples were available before treatment. Ten subjects had *STAT3* mutations (50% of the total cases) and none had *STAT5* mutations. All *STAT3* mutations were non-synonymous single nucleotide changes as previously described: D661Y was present in seven subjects, Y640F in 2 subjects, and one had a S614R mutation. There was no correlation with clinical response to alemtuzumab, as 5 subjects with *STAT3* mutations were responders and 5 were non-responders. All four patients classified as MDS/LGL had clear morphological and cytogenetic criteria for MDS. In three cases of MDS/LGL there were sufficient CD8/CD57 positive T lymphocytes for DNA extraction for Sanger sequencing and D661Y was present in two patients.

JAK-STAT pathway and plasma cytokine profiles

Consistent with previous reports, [24,25] the JAK-STAT pathway was activated in $CD87^+CD57^+$ cytotoxic cells in T-LGL subjects as compared with healthy volunteers (Figure 4A) but there was no difference in an 84 gene panel expression between responders and non-responders to alemtuzumab. Similarly, plasma cytokine proofing identified a cluster characterizing T-LGL subjects, compared to healthy volunteers, but failed to differentiate between responders and non-responders to treatment (Figure 4B). A similar pattern of activated JAK-STAT pathway was observed in all patients (both responders and non-responders) in whom enough material was available for adequate selection of $CD8^+CD57^-$ cells (Figure S4, page 4). The previous reported correlation between HLA-DR4 and response to cyclosporine[6] was not observed in our cohort maybe due to the smaller number

of patients with this allele (only four responders and two non-responders were HLA-DR4 positive).

TCRBV clonal analysis

Despite the lymphocyte depletion in all fourteen responders the abnormal TCR gene rearrangement by PCR was still present at 3 months after alemtuzumab in the vast majority (Table 2). A $CD8^+$ TCR-Vbeta clone was identified in all patients pre-treatment. In two responders the repertoire became less skewed over time (UPN #7 and #13). In subjects with a prevalent TCR-Vbeta clone at diagnosis, the frequency of the clone remained dominant in both responders and non-responders (Figure S5, page 5). CD52 expression on the dominant TCRBV clone was identified in all patients at initial evaluation and did not correlate with response (Figure S6, page 6). The decrease in absolute numbers of the dominant clone was observed in both the CD52 positive and negative populations (Figure S7, page 7)

Discussion

Prior to this study, experience with alemtuzumab in T-LGL was limited to a few case reports and retrospective small series.[10–18] We report our 10-years experience in twenty-five subjects using low-dose alemtuzumab as previously used for subjects with autoimmune cytopenias,[26] aplastic anaemia[8] and MDS.[9] Haematologic responses were observed in over half of the patients in this refractory and heterogeneous T-LGL cohort with durable recoveries in the majority of responders after only one course of alemtuzumab.

We used a low dose alemtuzumab regimen, which was well tolerated and shown to have activity in various forms of cytopenias.[26] We have since applied this regimen in many of our protocols in aplastic anaemia and MDS, which showed activity of this agent. Different alemtuzumab regimens have been used in T-LGL case reports, small retrospective cohorts or brief descriptions within reviews with doses varying from 23 to 1,080 mg given in days to several weeks.[10–18] It not clear the optimal administration of alemtuzumab in T-LGL but it is possible that different doses, regimens with or without maintenance might be more effective. In our study alemtuzumab was given intravenously which was the preferred route in initial studies. Similar efficacy with subcutaneous compared to intravenous administration was shown in chronic lymphocytic leukemia but not in T-cell prolymphocytic leukemia.[27,28] Thus, efficacy may not be interchangeable between different routes of administration of alemtuzumab in all disease settings.

The more standard therapies in T-LGL include methotrexate, cyclosporine, and cyclophosphamide with variable response rates in the 40–60% range.[6,29] In a very large LGL cohort of 55 treatment-naïve patients a gene expression signature and mutation in STAT3 correlated with hematologic response to methotrexate of cyclophosphamide.[29] In our mostly relapsed/refractory cohort mutational and plasma cytokine analysis did not reveal predictors of response to alemtuzumab. This favourable outcome with alemtuzumab in T-LGL compares to the experience with in aplastic anaemia where one-third of refractory and about half of relapsing patients responded to the same alemtuzumab regimen.[8] In myelodysplastic syndromes, about two-thirds of patients with a higher likelihood of responding to immunosuppression improved with alemtuzumab.[9] Despite a profound and

prolonged lymphopenia, we did not observe EBV, CMV, other significant viral diseases or opportunistic infections in the current study. We did observe cases of hypothyroidism which were readily treated with hormone replacement.[8,9] This complication has been reported in other marrow failure and autoimmune disorders were alemtuzumab was employed.[8] A similar low-dose alemtuzumab was used in relapsing–remitting multiple sclerosis (MS) with favourable outcomes compared to subcutaneous IFN-β-1 in two phase 3 randomized trials.[30,31] This led to approval of the drug in this setting in Europe, Canada, and more recently in USA. In contrast to methotrexate, cyclosporine, and cyclophosphamide, alemtuzumab is a potent lymphocytotoxic agent even at low doses as administered in our study that led to universal diminution of circulating lymphocytes and consequently clones size. This represents an important distinct therapeutic effect that associates with longer remissions without the requirement of chronic intake of an oral immunosuppressant, which has its toxicities.

In the current study prophylactic acyclovir as well as *PJP* prophylaxis with inhaled pentamidine were given until $CD4^+$ count recovered over 200/μL and were sufficient to prevent these infectious complications. A similar prophylactic strategy was used in prior SAA and MDS alemtuzumab trials with similar good results.[8,9] The time to $CD4^+$ count recovery to over 200/μL in our cohort varied from 6 months to over 3 years resulting in some patients being on prophylactic therapy for years. Two subjects in present study had an identifiable (>1%) GPI-negative clone in neutrophils, diagnostic of paroxysmal nocturnal haemoglobinuria (PNH). Because $CD52^+$ is a GPI-linked anchored protein, there is a theoretical concern that alemtuzumab may increase the size of the PNH clone. However, neither subject showed an increase in the GPI-negative clone population, consistent with the reports from low-risk MDS subjects treated with alemtuzumab.[9] In all our results show that administration of low dose alemtuzumab was well tolerated in this heavily treated immunosuppressed cohort.

We attempted to identify predictors for response to alemtuzumab in our study. Curiously, sustained relief of cytopenia did not require complete eradication of the T-LGL clone and did not associate with clone size. Although alemtuzumab resulted in haematologic improvement in over 50% of T-LGL subjects, clinical improvement in marrow function occurred despite persistence of the abnormal clone, albeit at significantly lower absolute levels following profound lymphocyte depletion. Furthermore, persistence of the T-LGL clone occurred equally in responders and non-responders and was therefore not a discriminator. As has been reported, about 50% of subjects with T-LGL have an acquired activating mutation in *STAT3*.[24] We found no correlation with response to treatment and presence of the acquired *STAT3* mutations suggesting that the implication of JAK-STAT activation in T-LGL did not preclude responses to alemtuzumab.[24,32] Compared with healthy volunteers increased activation of JAK-STAT and abnormal serum cytokine profiles were observed but did not differentiate between responders and non-responders to alemtuzumab. In summary, the protein (cytokine) signalling and molecular alterations observed in T-LGL did not serve as risk stratification following therapy with alemtuzumab. Thus, the mechanism of action of alemtuzumab in T-LGL remains elusive. It is plausible that simply a diminution of a pathogenic clone below a certain threshold following the

durable lymphocytotoxic effects of alemtuzumab is sufficient to improve marrow function and cytopenias in T-LGL.

Our study is limited by being single arm, relative small sample size and heterogeneous nature of the cohort. However, the rarity of this entity precludes conduction of large randomized studies. Enrolment of additional patients with longer follow-up will allow for more precise haematological response rates and assessment of secondary endpoints.

In conclusion, we report the largest prospective clinical trial of alemtuzumab for previously treated subjects with T-LGL. This well tolerated regimen may serve as good alternative to those intolerant and/or refractory to other immunosuppressive therapies. Earlier use of alemtuzumab is likely to yield higher and/or more sustained haematologic responses but this would need to be confirmed in formal treatment protocols with less refractory cases.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Research in context

Evidence before this study

We searched PubMed and ClinicalTrials.gov with the keywords “alemtuzumab” and “large granular lymphocyte leukemia” (T-LGL). 22 articles were published between Jan 1, 1975, and June 1, 2015, that matched our search criteria. Only retrospective case reports or case series, the largest cohort had 8 patients, were published and established a role for alemtuzumab in T-LGL as hematologic responses, some long-term, were reported. Only one other trial using alemtuzumab for T-LGL was registered at ClinicalTrials.gov at it was closed for low accrual.

Added value of this study

To our knowledge, our results are the first prospective clinical data on effectiveness of alemtuzumab in treatment of refractory T-LGL. We establish a good safety profile of administration of low-dose alemtuzumab in a cohort of patients heavily pre-treated with three prior immunosuppressive regimens on average. Alemtuzumab is effective in patients with T-LGL with a hematologic response of 56% at 3 months in all treated patients and 74% at 3 months in patients without associated myelodysplastic syndromes or after allogeneic hematopoietic stem cell transplantation.

Implications of all the available evidence

Hematologic response rate to alemtuzumab in previously treated patients of over 50% is similar to other upfront therapies for T-LGL and with similar reduced side effects profile. Alemtuzumab is likely to yield higher and/or more sustained hematologic responses in less treated T-LGL patients if used earlier in the course of the disease but this would need to be confirmed in further clinical studies.

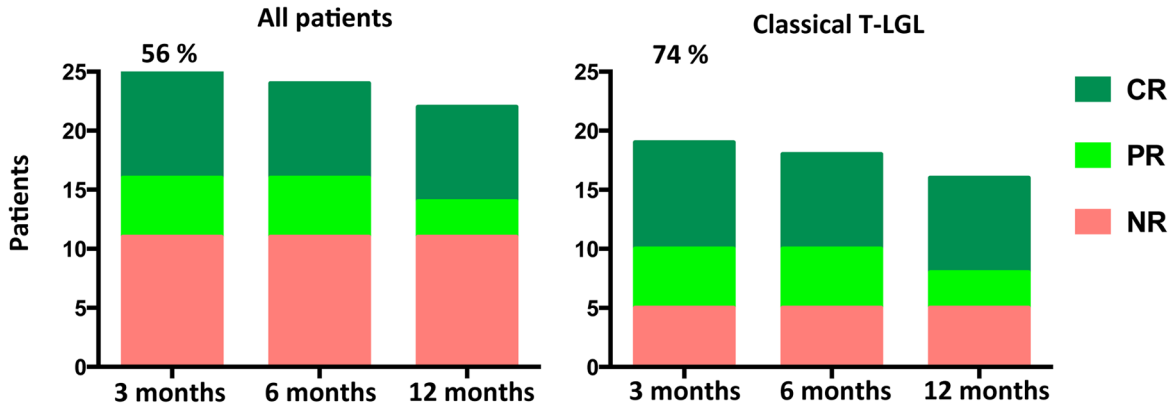


Figure 1.

Haematologic response at 3 months (primary end point) and at 6 and 12 months to treatment with alemtuzumab in all patients (n=25; left panel), and “classical” T-LGL (n=19; right panel). “Classical” T-LGL was defined as T-LGL without associated myelodysplastic syndromes (n=4) or developing after allogeneic hematopoietic stem cell transplantation (n=2). The overall response rate for all patients was 56% and for the classical T-LGL 74% as depicted above.

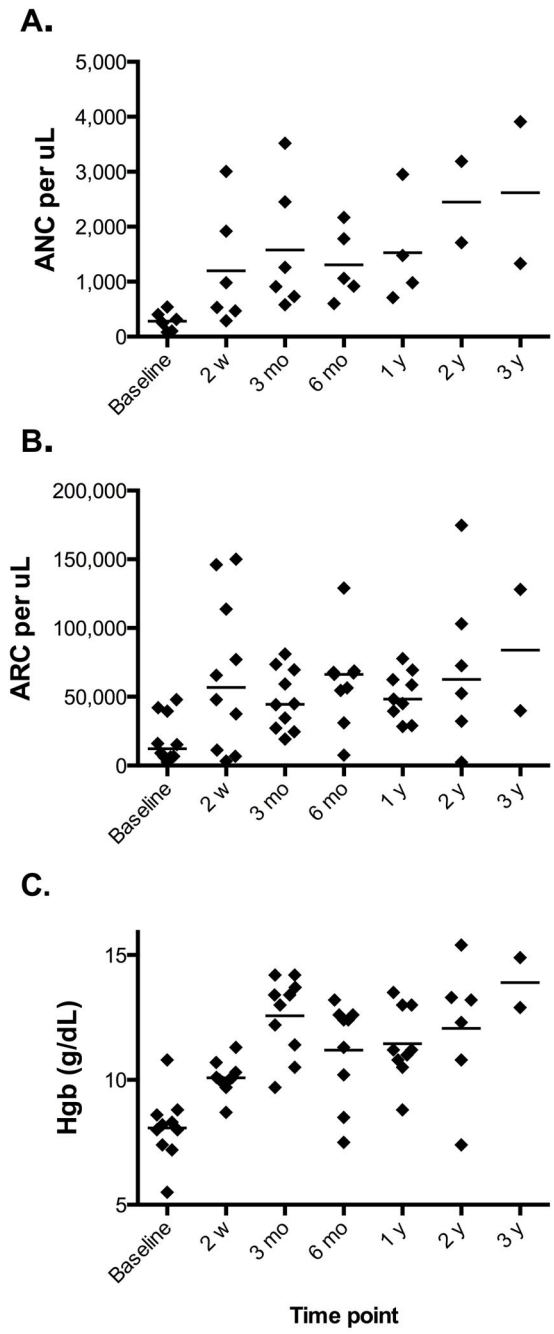
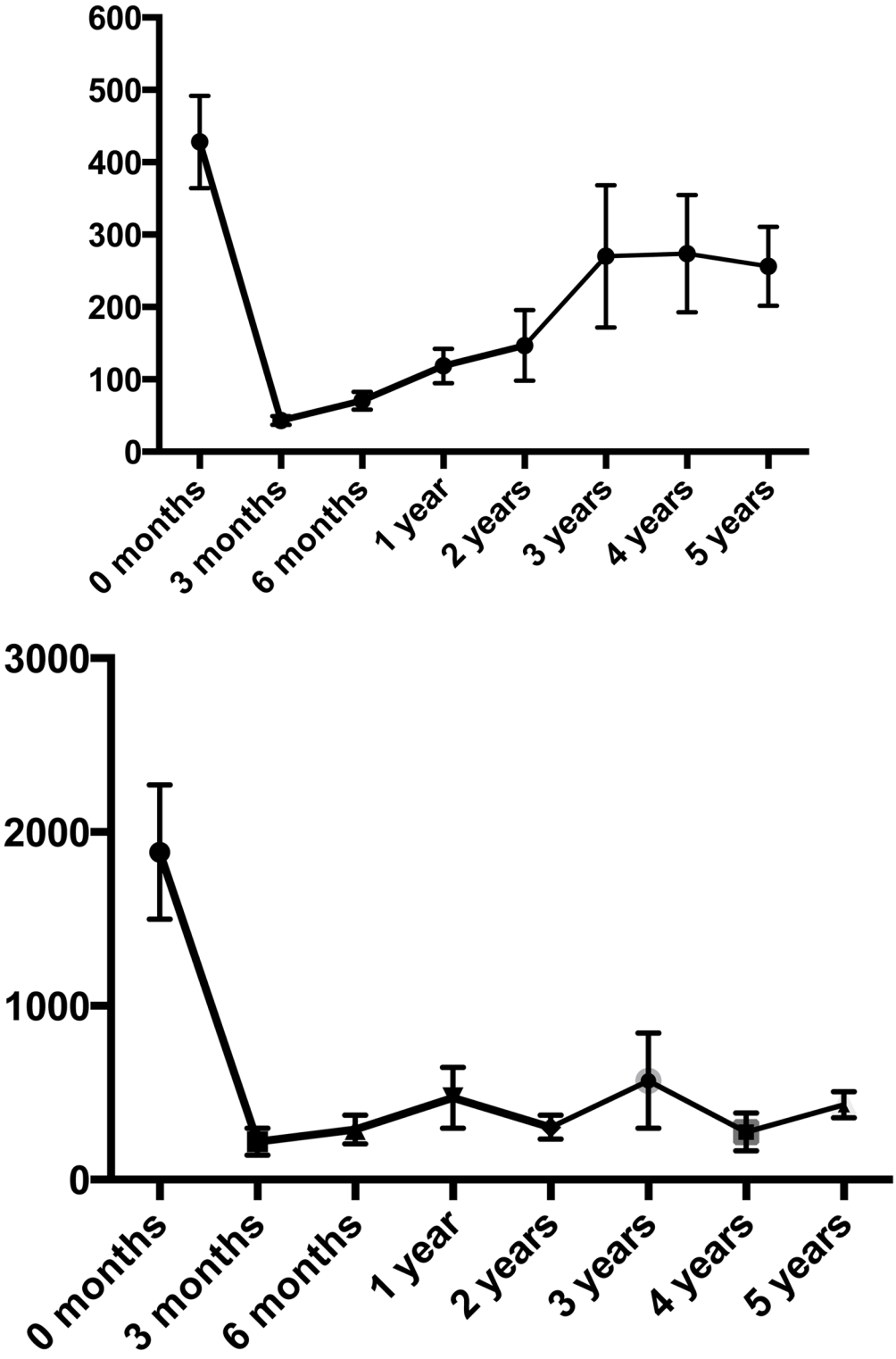


Figure 2. Blood counts in responders to alemtuzumab

A rapid and sustained improvement in absolute neutrophil count (n=6) **(A)** and in patients with anaemia (n=10) **(B, C)** was observed in over half of responding cases. In total there were 14 patients who responded at 3 months; 2 had both anaemia and neutropenia and are depicted in the corresponding panels. In relapsed patients, blood counts are depicted until the time of relapse. Scattered plot with corresponding median for each patient is depicted for each time point. ANC, absolute neutrophil count; ARC, absolute reticulocyte count; Hgb, haemoglobin.



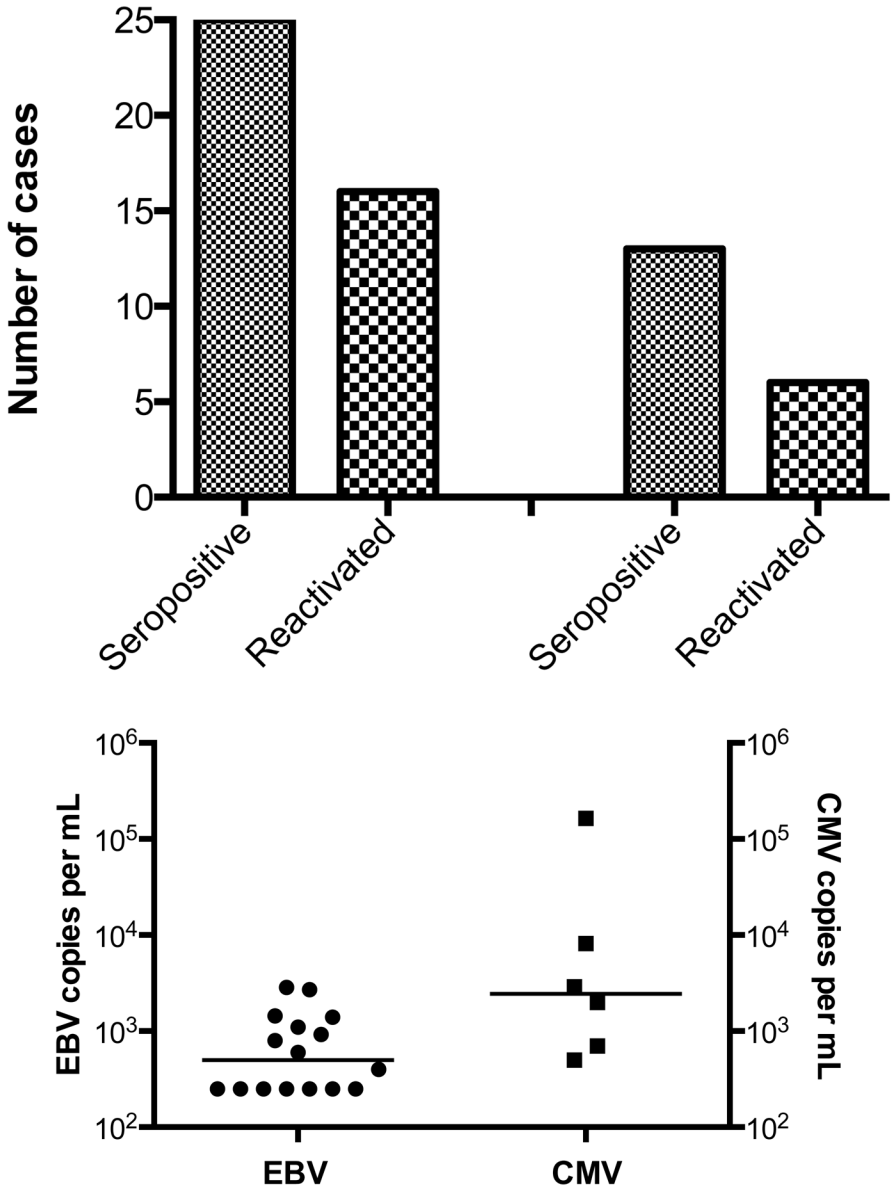
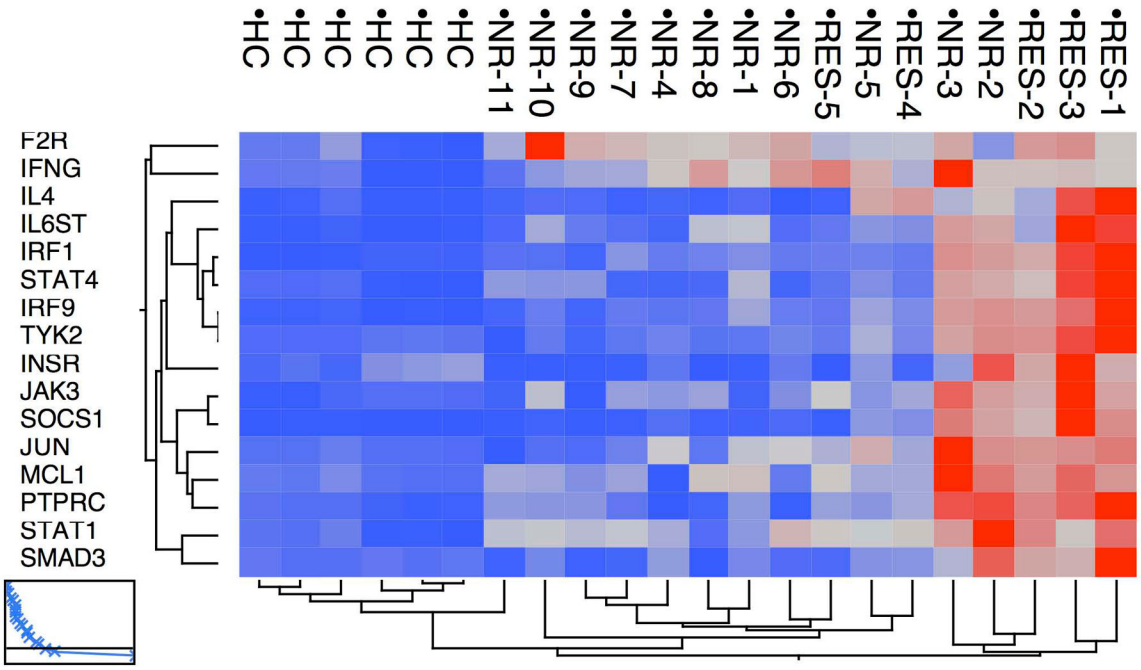


Figure 3. Lymphocyte depletion and viral reactivations

Lymphodepletion affected both helper (A) and cytotoxic (B) T-lymphocytes. Available samples before alemtuzumab and at 3, 6, 12, 24, 36 and 48 months after treatment were stained for $CD4^+$ and $CD8^+$ markers and absolute numbers were determined based on the absolute lymphocyte count from that day. Day 0 represents baseline prior to alemtuzumab therapy. Mean ± SEM is depicted for each time point. **(C) EBV and CMV reactivations following alemtuzumab**. All patients were seropositive for EBV and nearly half seropositive for CMV at baseline. About half of EBV seropositive and one-third of CMV seropositive patients reactivated (bottom right panel). These reactivations were self-limited and did not associate with disease. **(D) EBV and CMV viremia** was monitored only until copy numbers became negative. A positive PCR was defined as more than 250 EBV

copies/mL of blood or more than 250 CMV copies/mL blood. Scattered plot with peak EBV and CMV copy numbers with respective median is depicted on right lower panel.



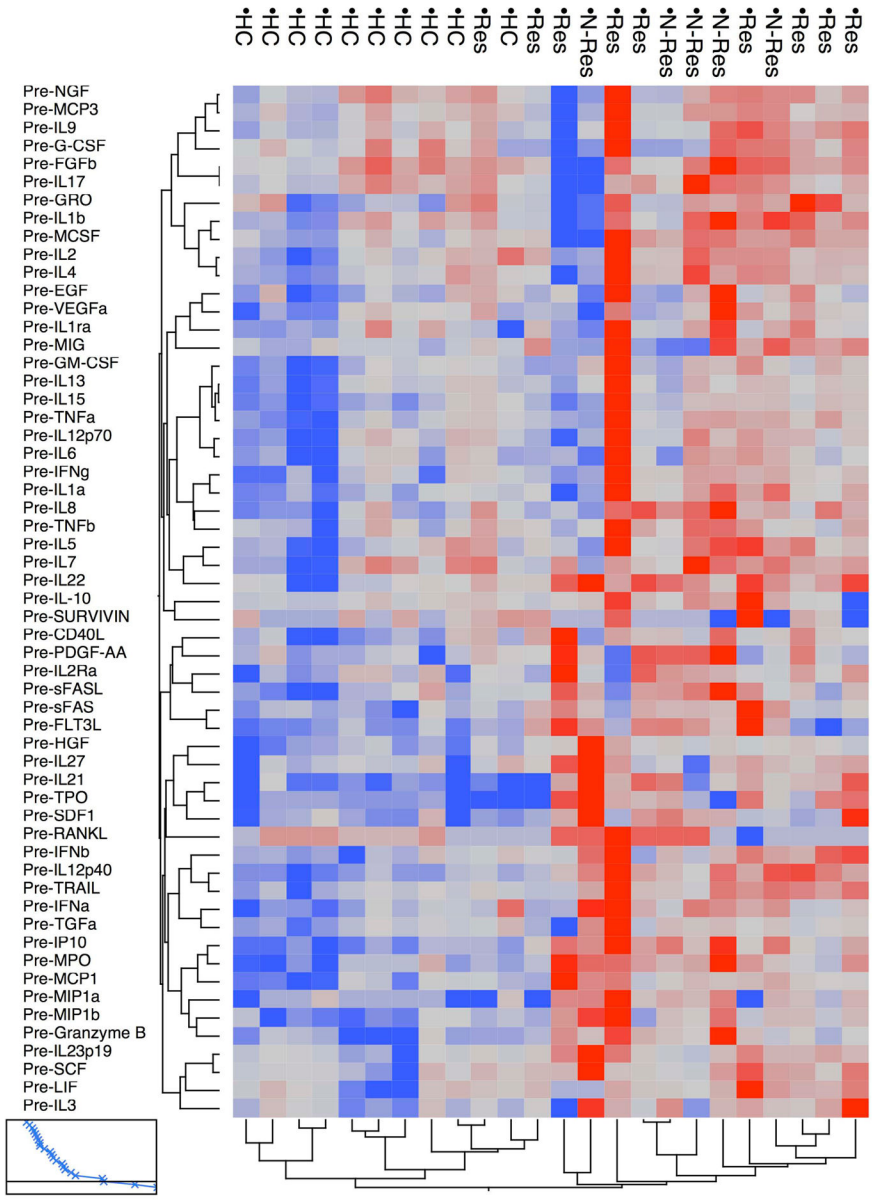


Figure 4. Activation of JAK-STAT pathway and plasma cytokine profiles are abnormal in T-LGL but do not correlate with response to alemtuzumab

A) Expression of 84 genes in the JAK-STAT pathway quantified before treatment with alemtuzumab in $CD8^{+}CD57^{+}$ lymphocytes in T-LGL subjects compared to healthy volunteers. **B)** Plasma cytokine multiplex bead assay quantification before treatment with alemtuzumab in T-LGL subjects compared to healthy volunteers. Responders to alemtuzumab are shown as Res, non-responders N-Res, and healthy controls as HC. Heat maps of gene expression and cytokines were created by two-way hierarchical cluster analysis using Ward's method. Red colour represents high levels, and blue colour low levels. N=14 for the plasma cytokine multiplex analysis.

Table 1

Subject characteristics and response to alemtuzumab treatment

UPN	Age	Gender	Prior therapies	ALC (cells/μL)	Cytopenias	MDS	Post HSCT	Response at 3 mo	Response at 6 mo	Response at 12 mo
1	51	F	Pred, CsA, splenectomy, growth factors	4731	Neutropenia	No	No	CR	CR	CR
2	67	M	MTX, MIK beta 1, MEDI 507, CsA, splenectomy, imatinib, prednisone	5857	Neutropenia	Yes	No	NR	NE, died	NE, died
3	67	M	MTX, CsA, CTX, tacrolimus	1150	Pancytopenia	Yes	No	NR	NR	NE, off
4	77	M	None	2237	Anemia	No	No	PR	Relapsed	NE, 2nd cycle
5	64	F	MTX, CsA, growth factors	5908	Anemia	No	No	PR	PR	PR
6	39	M	MTX, CsA, CTX, ATG, prednisone	2204	Anemia	No	No	NR	NE, off	NE, off
7	66	F	MTX, CsA, MEDI-507	701	Anemia	No	No	CR	PR	CR
8	51	M	Fludarabine, CsA	1408	Anemia	No	No	CR	CR	CR
9	79	F	MTX, decitabine	4879	Anemia	Yes	No	NR	NE, off	NE, off
10	61	M	MTX, CsA	4730	Neutropenia	No	No	CR	CR	CR
11	38	F	Growth factors, tacrolimus, prednisone, rituximab	4200	Anemia	No	Yes	NE, died	NE, died	NE, died
12	82	M	CsA, MTX, CTX, growth factors	860	Anemia	No	No	CR	CR	CR
13	27	F	CsA, IVIG, growth factors, rituximab	2750	Anemia	No	No	PR	PR	CR
14	64	F	MTX, CsA, CTX	870	Neutropenia	No	No	NR	PR	PR
15	48	F	MTX, Prednisone, CTX, CsA, growth factors	3850	Pancytopenia	No	No	NR	NR	NR
16	53	M	Prednisone, CsA	110	Anemia, neutropenia	No	Yes	NE, off	NE, off	NE, off
17	43	M	MTX, CsA, growth factors	2240	Anemia	No	No	NR	NR, off	NE, off
18	72	F	MTX, CsA, growth factors	2210	Anemia, neutropenia	No	No	PR	Relapsed	NE, 2nd cycle
19	29	F	MTX, Prednisone, CTX	2230	Anemia	No	No	PR	PR	PR
20	60	F	None	1590	Anemia, neutropenia	No	No	CR	CR	CR
21	61	F	MTX, Prednisone, CTX, CsA	4370	Neutropenia	No	No	CR	CR	CR
22	62	M	MTX, Prednisone, CTX, CsA	670	Anemia	Yes	No	NR	NR	NR
23	54	M	Pred, MMF, Rituximab, CsA, ATG, tacrolimus, MTX, sirolimus	1900	Anemia	No	No	NR	CR	TE
24	71	F	Pred. MTX, CsA, rituximab, CTX	1290	Anemia	No	No	CR	CR	TE

UPN	Age	Gender	Prior therapies	ALC (cells/μL)	Cytopenias	MDS	Post HSCT	Response at 3 mo	Response at 6 mo	Response at 12 mo
25	56	F	MTX, Prednisone, CTX, CsA, growth factors	2130	Neutropenia	No	No	CR	TE	TE

CsA, cyclosporine; MTX, methotrexate; MIK beta 1, humanized antibody to the interleukin-2 receptor beta chain; MEDI 507, monoclonal antibody directed against CD2; CTX, cyclophosphamide; ATG, anti-thymocyte globulin; IVIG, intravenous immunoglobulin; ALC, absolute lymphocyte count; CR, complete response; PR, partial response; TE, too early to evaluate; NE, not evaluable. The bone marrow in all patients showed an interstitial lymphocytic infiltrate, as expected in T-LGL. Splenomegaly was observed in only two patients and there were no evidence of other end-organ involvement.

Table 2

T-cell receptor gene rearrangement by PCR

UPN	Response	0 months	3 months	6 months	12 months	24 months	36 months
1	CR	Oligoclonal	Oligoclonal	Oligoclonal	Oligoclonal	Oligoclonal	Oligoclonal
2	NR	Monoclonal					
3	NR	Monoclonal	Polyclonal				
4	PR	Monoclonal	Monoclonal	Monoclonal	Oligoclonal	Monoclonal	
5	PR	Monoclonal	Monoclonal	Monoclonal	Monoclonal	Monoclonal	Monoclonal
6	NR	Monoclonal					
7	CR	Monoclonal	Monoclonal	Oligoclonal	Polyclonal		Polyclonal
8	CR	Oligoclonal	Oligoclonal	Oligoclonal	Oligoclonal	Oligoclonal	Oligoclonal
9	NR	Monoclonal	Monoclonal				
10	CR	Monoclonal	Monoclonal	Oligoclonal	Oligoclonal	Oligoclonal	
11	NR	Monoclonal					
12	CR	Monoclonal	Monoclonal	Monoclonal	Monoclonal		
13	PR	Monoclonal	Monoclonal	Oligoclonal	Polyclonal	Polyclonal	Polyclonal
14	NR	Oligoclonal	Polyclonal	Oligoclonal	Polyclonal	Oligoclonal	
15	NR	Monoclonal	Monoclonal	Monoclonal			
16	NR	Oligoclonal					
17	NR	Oligoclonal	Monoclonal	Monoclonal	Monoclonal	Polyclonal	
18	PR	Oligoclonal	Oligoclonal	Monoclonal	Monoclonal		
19	PR	Monoclonal	Monoclonal	Oligoclonal	Oligoclonal		
20	CR	Monoclonal	Monoclonal	Monoclonal	Monoclonal		
21	CR	Monoclonal	Monoclonal	Monoclonal			
22	NR	Oligoclonal	Monoclonal	Monoclonal			
23	NR	Monoclonal	Monoclonal				
24	CR	Monoclonal	Monoclonal				
25	CR	Monoclonal	Monoclonal				

Different repertoire patterns (monoclonal, oligoclonal, and polyclonal) are depicted in different shades of gray. In 2 responders (UPN 7 and 13) the repertoire became more gaussian over time. Some responders maintained an oligoclonal repertoire despite sustained haematologic improvement. Time 0 months is baseline prior to alemtuzumab administration. CR, complete response; PR, partial response; NR, no response.

Table 3

Adverse events

		Grade 1–2	Grade 3	Grade 4
Haematological	Lymphopenia	3 (12%)	10 (40%)	12 (48%)
	Thrombocytopenia	0	0	2 (8%)
	Leukopenia	0	4 (16%)	4 (16%)
	Bleeding	4 (16%)	0	0
General	Infusion reaction*	24 (96%)	1 (4%)	0
	Consitutional+	8 (32%)	1 (4%)	0
	Pain	15 (60%)	4 (16%)	0
	Mood changes	0	1 (4%)	0
Cardiovascular	Elevated BP	4 (16%)	0	0
	Decreased EF	0	1 (4%)	0
Pulmonary	Hypoxia	0	1 (4%)	0
	Lung nodules	0	1 (4%)	0
Gastrointestinal	Diarrhea	1 (4%)	1 (4%)	0
	Nausea and vomiting	6 (24%)	1 (4%)	0
	Elevated liver enzymes	5(20%)	4 (16%)	0
Infectious	Neutropenic infections	3 (12%)	5 (20%)	0
	Non-neutropenic infections	7(28%)	1 (4%)	0
Dermatological	Skin rashes	9 (36%)	0	0
	Hair loss	1 (4%)	0	0
Endocrinological	Elevated TSH	7 (28%)	0	0

Data are n (% of 25 subjects). No patients had grade 5 adverse events.

* Fever, chills, hypotension, hypertension associated with alemtuzumab infusion and without any other identified cause

+ Fatigue, night sweats, weight loss (UPN#13 had chronic diarrhea, nausea, vomiting and weight loss - all grade 3 - as part of her autoimmune enteritis associated with her T-LGL).

BP=blood pressure. EF=ejection fraction. TSH=thyroid stimulating hormone.