Original Study

Alemtuzumab and CHOP Chemotherapy for the Treatment of Aggressive Histology Peripheral T Cell Lymphomas: A Multi-Center Phase I Study

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Abstract

We conducted a phase I study of alemtuzumab combined with CHOP (cyclophosphamide, adriamycin, vincristine and prednisone) chemotherapy in peripheral T cell lymphomas. The objectives were to establish the safest dose with the highest antibody levels and evaluate the effects on the immune system. Twenty patients were enrolled across 4 dose levels. Maximally tolerated dose was not reached with alemtuzumab 60 mg subcutaneously every 3 weeks and antibody levels were highest at this dose level. The results of this study inform other investigators about possibilities for optimal dosing in T cell lymphomas when used in combination with chemotherapy and highlight the high levels of baseline and post-treatment immune suppression observed in this patient group.

Background: Alemtuzumab has single-agent activity in relapsed peripheral T cell lymphoma (PTL), but the optimal dose and/or schedule in combination with chemotherapy for first-line use is unknown. The primary objectives were to establish the maximally tolerated dose and pharmacokinetics (PK) of alemtuzumab combined in this way. **Patients and Methods:** Adult patients with untreated CD52-positive ($CD52^+$) PTL were enrolled in a phase I trial. Alemtuzumab was given subcutaneously in escalating doses and/or schedules in combination with CHOP (cyclophosphamide, adriamycin, vincristine and prednisone) using a 3+3 design. Trough PK of alemtuzumab were measured on day 1 of each 21-day cycle and B and T cell subsets were serially measured. **Results:** Twenty patients were enrolled across 4 dose levels. Dose-limiting toxicities necessitated expansion at 10 mg weekly (fatal tuberculosis reactivation) and 60 mg every 3 weeks (grade 4 thrombocytopenia) dose levels. Maximally tolerated dose was not reached. Ten patients developed asymptomatic cytomegalovirus reactivations at a median of 39 days (range, 4-99 days). Two patients developed fungal pneumonias. The overall and complete response rates were 68% and 37%, respectively. Highest day 1 alemtuzumab trough levels were achieved at 60 mg (1973 ng/mL), but with significant inter- and intradose variability. Lymphopenia at baseline was common and T cell recovery was significantly delayed. **Conclusion:** With monitoring and prophylaxis, alemtuzumab 60 mg combined with CHOP showed activity in $CD52^+$ PTL and achieved the highest drug levels.

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Introduction

The mature T-cell and natural killer (NK)-cell neoplasms are uncommon heterogeneous lymphoid cancers that comprise 10% to 15% of all aggressive lymphomas.[1] Historically, clinical outcomes of T cell lymphoma patients who receive CHOP (cyclophosphamide, adriamycin, vincristine and prednisone)-like regimens have been inferior to those of patients with B cell lymphoma with this disparity magnified by the advent of anti-CD20 chemoimmunotherapy. The reported 5-year overall survival (OS) rates for mature T cell lymphomas are 32% to 37% in international registries[2,3] with minimal improvement despite schedule intensification and the combination of etoposide.[4] No published randomized trials exist for peripheral T cell lymphoma (PTL) because of its rarity and diverse subtypes and the role for upfront consolidative high dose therapy with autologous stem cell transplant is inconclusive.[5-8] Because of the poor outcomes with initial therapy for T-cell lymphomas, novel therapies and approaches need to be tested.

Alemtuzumab is a humanized murine monoclonal antibody directed against the antigen CD52, a 21- to 28-kD cell-surface glycoprotein found on 95% of normal B and T lymphocytes, monocytes, and eosinophils, but not on human hematopoietic progenitor cells.[9,10] Clinical activity of alemtuzumab monotherapy has been observed in relapsed chronic lymphocytic leukemia (CLL),[11-13] T-cell prolymphocytic leukemia,[14-16] mycoses fungoides/Sézary syndrome,[17] and relapsed refractory peripheral T cell lymphoma (PTL).[18] With no previous pharmacokinetics (PK) studies to inform dose, schedule, or safety, we performed a phase I dose escalation study of alemtuzumab in combination with CHOP in patients with previously untreated PTL. The primary objectives were to establish the maximum tolerated dose of combination therapy and the trough PK of alemtuzumab in different subcutaneous doses and schedules. The secondary objectives were to examine efficacy and the effects on T cell reconstitution, immunoglobulins, and cytomegalovirus (CMV) reactivation.

Patients and Methods

Study Design

This was a multicenter phase I study coordinated through the Ontario Clinical Oncology Group (OCOG). The study was approved by all center research ethics boards and participating patients provided written informed consent. See Appendix A in the online version at http://dx.doi.org/10.1016/j.clml.2015.11.008 for further details.

Patients

Patients $\geq$ 18 years of age with untreated, histologically proven, CD52-positive ($CD52^+$) T cell lymphomas were eligible. Patients had measureable stage II to IV disease, a life expectancy of at least 16 weeks, an Eastern Cooperative Oncology Group performance status <3 and adequate hematologic, renal, and liver function. The following lymphoma subtypes were eligible: PTL not otherwise specified (NOS), angioimmunoblastic T cell lymphoma (AITL), anaplastic lymphoma kinase-1 negative ($ALK1^-$) anaplastic large cell lymphoma (ALCL), hepatosplenic, enteropathy-associated, and subcutaneous panniculitic T cell lymphomas.[19] Patients with HIV, central nervous system involvement at baseline, Sézary syndrome, or human T-cell lymphotropic virus-1 associated or natural killer (NK)/T cell histologies were excluded. All patients had confirmation of expression of CD52 according to immunohistochemistry (IHC) performed at a central laboratory[20,21] (see Appendix A in the online version at http://dx.doi.org/10.1016/j.clml.2015.11.008). To increase patient accrual, the protocol was amended after the completion of dose level 2 to permit up to 1 cycle of CHOP chemotherapy for those with rapidly progressive disease who required treatment while awaiting confirmation of CD52 positivity.

Methods

CHOP chemotherapy was given at standard doses (see Appendix A in the online version at http://dx.doi.org/10.1016/j.clml.2015.11.008) for 6 to 8 cycles repeated every 21 days for up to 8 cycles. Treatment with subcutaneous alemtuzumab occurred at increasing dose levels using a standard 3+3 design: (1) 10 mg on day 1; (2) 10 mg on days 1, 8, and 15; (3) 30 mg on day 1; (4) 60 mg on day 1; and (5) 30 mg on days 1, 8, and 15. Three patients were sequentially enrolled at a given dose level. Dose-limiting toxicities (DLTs) were defined as: Grade $\geq$3 neutropenia during granulocyte colony stimulating factor (GCSF) treatment by day 1 of the next cycle, platelet count of $<10 \times 10^9$/L at any time or $<50 \times 10^9$/L by day 1 of the next cycle, death during the study, febrile neutropenia exceeding 10 days' duration, any Grade $\geq$ 3 infection attributed to study drugs, any nonhematologic toxicity of Grade $\geq$ 3 (excluding nausea, vomiting, and alopecia) likely related to study medication, and any interruption of study medicine because of toxicity that exceeded 14 days. If no DLTs were observed by the end of cycle 3, enrollment at the next dose level proceeded. If 1 of 3 patients experienced a DLT before the end of cycle 3, an additional 3 patients were enrolled at that dose level.

Cytomegalovirus reactivation in the absence of overt infection was not considered a DLT. The maximally tolerated dose of this drug combination was defined as the dose level below the one in which escalation was stopped because of DLTs.

Therapy was discontinued in case of progressive lymphoma, physician discretion, DLT, or consent withdrawal. For the purpose of safety assessment the treatment period included the time from the first dose of alemtuzumab until 8 weeks after the final cycle of CHOP chemotherapy (see Appendix A in the online version at http://dx.doi.org/10.1016/j.clml.2015.11.008).

Anti-Infective Prophylaxis

Prophylaxis for herpes simplex virus, varicella zoster virus, and pneumocystis jerovecii (PJP) included famciclovir and sulfamethoxazole-trimethoprim (see Appendix A in the online version at http://dx.doi.org/10.1016/j.clml.2015.11.008).

Evaluations During Study

Clinical. Baseline staging included a physical examination, complete blood count (CBC), electrolyte levels, hepatic and renal function tests, lactate dehydrogenase level, serologic testing for CMV immunoglobulin G antibody, computed tomography (CT) scanning, and bone marrow trephine biopsy. A lumbar puncture was done if clinically indicated. Patients were assessed every 3 weeks with repeat CBC, and renal and hepatic blood tests. After chemotherapy was completed, patients were assessed every 3 months for 2 years and then every 6 months until 5 years.

Cytomegalovirus Monitoring. Cytomegalovirus monitoring was performed every 2 weeks, initially using enzyme-linked immunosorbent assay (ELISA; pp65 antigen) and then using polymerase chain reaction (PCR) analysis after August 2008 (see Appendix A in the online version at http://dx.doi.org/10.1016/j.clml.2015.11.008). Any confirmed asymptomatic CMV antigenemia was treated pre-emptively with oral valganciclovir 450 mg orally twice per day for 2 weeks until biweekly CMV monitoring was negative for 2 consecutive readings. Patients who developed clinical signs or symptoms of overt CMV infection (retinitis, pneumonitis, enteritis) with confirmed CMV in antigen testing, were considered to have experienced a DLT and removed from the study.

Immunologic Analyses. At baseline, all patients underwent serum quantitative immunoglobulin testing, which was repeated every 6 months for 2 years during the study. T- and B-cell subset analysis was done at baseline and at 3, 6, and 12 months after study enrollment.

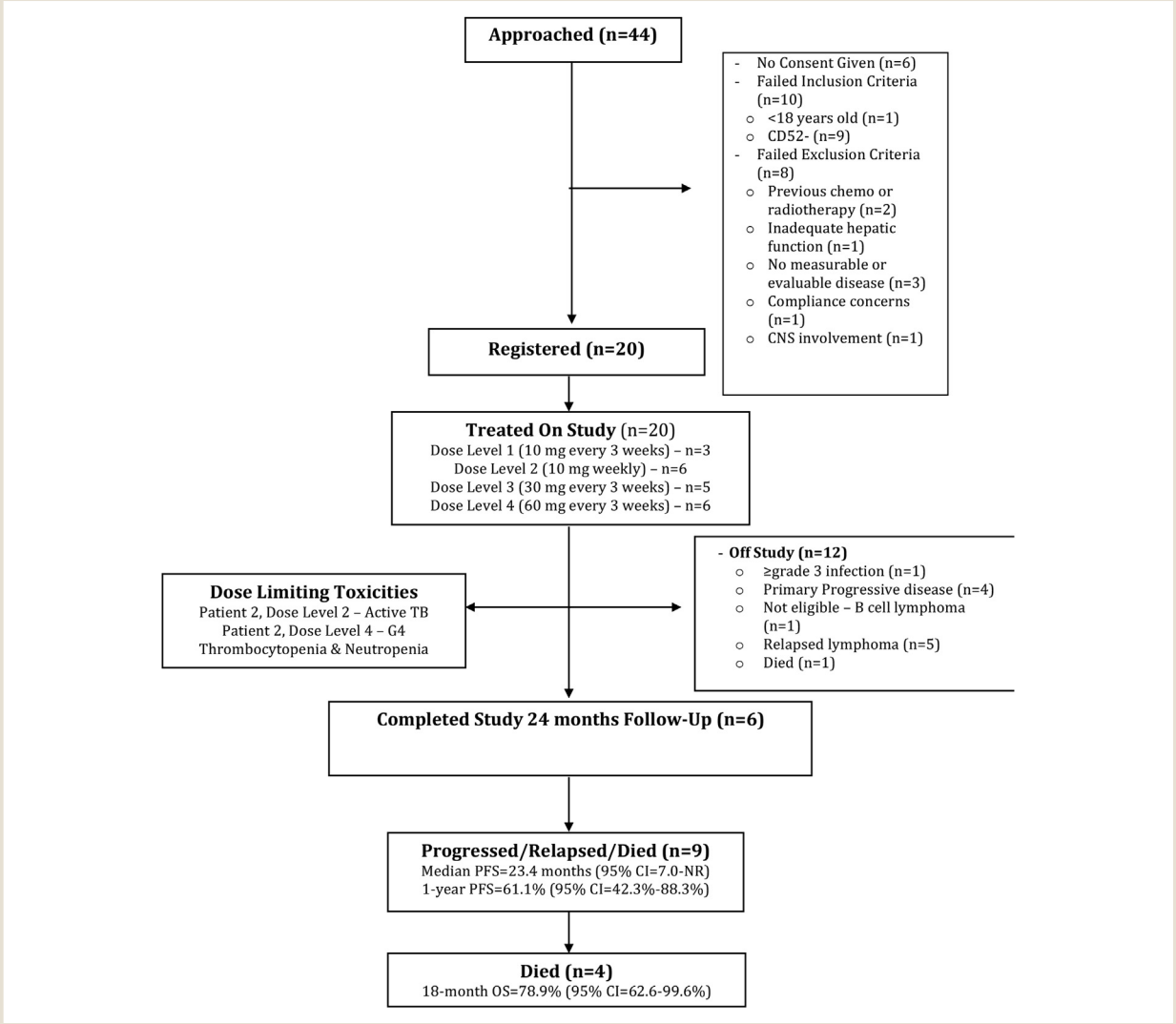
Efficacy Definitions

After the third and the final alemtuzumab-CHOP cycles, patients were restaged according to CT scans and responses defined according to 1999 International Working Group guidelines of Cheson et al.[22] In the absence of overt progressive disease, restaging CT scans continued every 6 months for 2 years. OS and progression-free survival (PFS) were defined from time of enrollment.

Pharmacokinetics

Predose (trough) levels were drawn on day 1 of every cycle. Where possible, kinetics of elimination were determined after termination of alemtuzumab therapy from plasma samples drawn

Figure 1 Study Flow Diagram



Abbreviations: chemo = chemotherapy; CNS = central nervous system; G4 = grade 4; OS = overall survival; PFS = progression-free survival; TB = tuberculosis.

twice weekly for 2 weeks (see Appendix A in the online version at http://dx.doi.org/10.1016/j.clml.2015.11.008).

Toxicity. Adverse events (AEs) were reviewed using the Common Terminology Criteria for Adverse Events Version 3.0 (CTCAE v3.0; see Appendix A in the online version at http://dx.doi.org/10.1016/j.clml.2015.11.008).

Statistics

Sample Size. Using the modified Fibonacci phase I design,[23] cohorts of 3 patients at each dose level were enrolled. The minimum sample size would be 15 patients and the maximum 30. Although the intention was to expand the maximum tolerated dose (MTD) for phase II testing, the study was closed after phase I completion of dose level 4 because of slow accrual.

Analysis

Data were analyzed descriptively using SAS version 9.2 (SAS Institute, Cary, NC) and R version 2.13.1 (www.r-project.org). OS and PFS curves were calculated using the Kaplan–Meier method.[24] All evaluations were 2-sided and a P value of $\leq .05$ was considered statistically significant (see Appendix A in the online version at http://dx.doi.org/10.1016/j.clml.2015.11.008).

Results

Between November 2006 and December 2012, 44 patients were screened and 20 patients were enrolled, at a median time from diagnosis of 1.8 months (range, 0.2-14 months). Follow-up data until April 11, 2014 are presented (Figure 1). The median age was 55 (range, 23-77) years, 85% had advanced stage disease and 65% had extranodal involvement (Table 1). Of 29 patients who consented to screening of their biopsy specimens for CD52 expression, 9 (31%) were negative (Figure 1). One patient was deemed ineligible for response or survival after cycle 2 when central pathology review disclosed the presence of AITL and diffuse large B cell lymphoma.

Four patients (20%) received a single cycle of CHOP chemotherapy before study enrollment. The median number of cycles of CHOP-alemtuzumab was 6 (range, 2-8) with only 6 patients who completed all 8 cycles. A total of 122 cycles were given with a median cycle length of 21 days (range, 19-48 days). The median cumulative dose of alemtuzumab received was 185 mg (range, 60-420 mg), with medians of 60, 205, 180, and 270 mg at dose levels 1 through 4, respectively (Table 2).

Toxicity

Twenty patients were evaluable for toxicity (according to dose level, shown in Table 3). There were 14 patients (70%) with Grade 3 to 4 neutropenia and 4 (20%) with Grade 3 to 4 thrombocytopenia overall, but 50% with Grade 3 to 4 thrombocytopenia at the highest alemtuzumab dose level. Most nonhematological toxicities were Grade 2 and infectious. There were 6 episodes of febrile neutropenia in 4 patients. Eleven patients (55%) received GCSF and 1 patient experienced Grade 3 bacterial pneumonia.

Ten patients (50%) developed asymptomatic CMV reactivation (n = 9) or new infection (n = 1) at a median time of 39 days (range, 4-99 days); 1 patient had a second CMV reactivation and 2 had 3 reactivations. Patients whose CMV reactivated more than twice were given pre-emptive valganciclovir until completion of therapy.

Table 1 Baseline Characteristics (n = 20)

Characteristic	Value
Median Age at Time of Diagnosis (Range)	55.3 (22.7-76.8)
Male Sex	15 (75.0)
ECOG Performance Status	
0	7 (35.0)
1	11 (55.0)
2	1 (5.0)
3	1 (5.0)
Median LDH, U/L (Range) (n = 18)	227 (125-1111)
Treatment Dose	
10 mg Q3 weekly (10 mg)	3 (15.0)
10 mg weekly (10 mg × 3)	6 (30.0)
30 mg Q3 weekly (30 mg)	5 (25.0)
60 mg Q3 weekly (60 mg)	6 (30.0)
Histology	
Peripheral T-cell lymphoma, NOS	6 (30.0)
Angioimmunoblastic (AILD)	7 (35.0)
ALK1$^-$ anaplastic	3 (15.0)
Hepatosplenic	1 (5.0)
Enteropathy-associated	1 (5.0)
Panniculitic	2 (10.0)
Ann Arbor Stage	
2	3 (15.0)
3	6 (30.0)
4	11 (55.0)
Extranodal Sites	
n (%) with ≥1	13 (65.0)
Median number of sites (Range)	1 (0-3)
Sites of Extranodal Disease	
Liver	4 (20.0)
Lung	4 (20.0)
Bone Marrow	11 (55.0)
Skin	1 (5.0)
Other (jejunum, mesentery/ peritoneum)	2 (10.0)
Received Previous CHOP for 1 Cycle	4 (20.0)
Presence of B Symptoms	8 (40.0)
IPI (n = 18)	
Low (0-1)	7 (38.9)
Low-Intermediate (2)	4 (22.2)
High-Intermediate (3)	4 (22.2)
High (4-5)	3 (16.7)

Data are presented as n (%), except where otherwise noted.
Abbreviations: AILD = angioimmunoblastic lymphoma; ALK = anaplastic lymphoma kinase; CHOP = cyclophosphamide, adriamycin, vincristine and prednisone; ECOG = Eastern Cooperative Oncology Group; IPI = International Prognostic Index; LDH = lactate dehydrogenase; NOS = not otherwise specified; Q3 = every 3 weeks.

Table 2 Outcomes

Outcome	n	Value
Number of Cycles, CHOP	20	6 (2-8)
Number of Cycles, Alemtuzumab	20	
2		2 (10)
3		2 (10)
4		2 (10)
5		1 (5)
6		5 (25)
7		2 (10)
8		6 (30)
Cumulative Dose, Alemtuzumab	20	185 (60-420)
Dose Level 1	3	60 (60-80)
Dose Level 2	6	205 (60-240)
Dose Level 3	5	180 (60-240)
Dose Level 4	6	270 (180-420)
Duration of Response, Months	13	19.1 (3.3-44.2)
Duration of Follow-Up, All Patients, Months	20	28.4 (1.3-77.4)
Duration of Follow-Up, Patients Alive at Last Follow-Up, Months	15	35.8 (16.0-77.4)
Best Response, All Patients, CR:CRu:PR:SD:PD:NE/Dead	20	**4:3:6:1:4:2**
Dose Level 1	3	1:0:2:0:0:0
Dose Level 2	6	1:1:1:0:2:1
Dose Level 3	5	1:1:0:1:1:1
Dose Level 4	6	1:1:3:0:1:0
Overall Survival	19	
Events, n (%)		4
Median, months		Not reached
2-Year (95% CI)		78.9% (62.6%-99.6%)
Progression-Free Survival	19	
Events, n (%)		10 (52.6%)
Median, months		23.4
2-Year (95% CI)		47.5% (28.6%-75.6%)

Values are presented as n (%) or median (range) except where otherwise noted.
Abbreviations: CHOP = cyclophosphamide, adriamycin, vincristine and prednisone; Cru = complete remission unconfirmed; NE = not evaluable.

The median time of valganciclovir treatment was 44 days (range, 14-151 days). No patient developed overt CMV disease. One patient in dose level 4 with asymptomatic CMV reactivation developed increasing CMV titers during valganciclovir treatment because of the acquisition of viral UL97 and UL54 resistance mutations and required treatment with intravenous (I.V.) foscarnet. This patient received only 4 cycles of CHOP-alemtuzumab and 2 additional cycles of CHOP alone.

Two cases of Grade 3 fungal pneumonia were documented, 1 in the setting of febrile neutropenia (dose level 2, cycle 8, associated with cryptococcal cellulitis) and another 3 weeks after cycle 7 (dose level 4, bronchoscopy biopsy was positive for invasive fungi, culture was negative). Both were successfully treated.

Dose-Limiting Toxicity

There were 2 DLTs necessitating expansion of dose levels 2 and 4. In dose level 2, a 62-year-old Asian man with subpanniculitic lymphoma developed fulminant tuberculosis hepatitis and pneumonitis after 2 cycles of CHOP-alemtuzumab and succumbed from liver failure after an inability to tolerate isoniazid/rifampin. The second DLT at dose level 4 occurred in a patient with baseline Grade 2 thrombocytopenia (platelet count, 52×10^9/L), who experienced persistent Grade 4 thrombocytopenia after 3 cycles of alemtuzumab/CHOP chemotherapy. He completed 3 additional cycles of dose-reduced CHOP alone.

Response

Of the 19 response-evaluable patients, 7 (37%) achieved complete response or complete remission unconfirmed, 6 (32%) partial response, 1 (5%) stable disease, and 1 (5%) died (according to dose level, see Table 2). The median duration of response was 19 months (range, 3-44 months). Four patients had primary progressive disease at a median time of 4.1 months (range, 2.7-5.7 months). With a median follow up of 28 months (range, 1.3-77.4), 9 patients (50%) had relapsed or had disease progression, and 4 (21%) had died. Actuarial 2-year PFS, and OS were 47.5% (95% confidence interval [CI], 28.6%-79.0%), and 78.9% (95% CI, 62.6%-99.6%; Figure 2).

Immune Parameters

B- and T-cell subsets over time are shown in Figure 3. Eight of 17 patients (47%) with absolute lymphocyte count (ALC) measured before chemotherapy had an ALC of $< 1.0 \times 10^9$/L. Notably, 50%, 71%, 93%, and 50% of patients had lower than normal $CD19^+$, $CD3^+$, $CD4^+$, and $CD8^+$ cell counts, respectively at baseline. T cell depletion was profound until the 6-month mark, when recovery in all T-cell subsets began, although only 45% of patients recovered or surpassed baseline $CD3^+$ levels by 10 to 12 months. Recovery to baseline $CD4^+$ T cells by 12 months was seen in only 20% of patients, and recovery of $CD8^+$ T cells was seen in 73% of patients. In contrast, B cell recovery began earlier and surpassed baseline in 89% of patients. NK cell levels (reduced in 35% at baseline) declined during the study but recovered to baseline or higher in 80%. When immunoglobulins were measured serially at least three times, increases were seen in 11 patients (see Supplemental Figure 1 in the online version at http://dx.doi.org/10.1016/j.clml.2015.11.008). Most patients had declines in their serum immunoglobulins of 40% to 80%, with less than half who recovered to their baseline levels at 1 year. Nevertheless, most immunoglobulin measurements remained within the normal reference ranges.

Pharmacokinetics

Trough levels of alemtuzumab measured on day 1 of every cycle for patients who remained in 3 or more cycles were evaluable in 17

Table 3 Adverse Events According to CTCAE

Adverse Event Grade	2	3	4	5
Dose Level 1 = 10 mg (n = 3)				
Febrile neutropenia	–	1	–	–
Varicella zoster	1	–	–	–
Nausea/vomiting	1	–	–	–
Neuropathy	1	–	–	–
Mucositis	1	–	–	–
Pain	1	–	–	–
Leukopenia		–	1	–
Lymphopenia		1	2	–
Neutropenia		2	1	–
Dose Level 2 = 10 mg × 3 (n = 6)				
Febrile neutropenia	1	–	–	–
CHF; CAS	1	–	–	–
Constipation	–	1	–	–
Cryptoccus right lower limb	–	1	–	–
BK cystitis	–	1	–	–
Cellulitis	1	–	–	–
Dyspnea	–	2	–	–
Increased ALP/AST levels	–	1	–	–
Hyperglycemia	–	1	–	–
Infection and fever (Hickman)	–	1	–	–
Cryptococcal pneumonia	–	1	–	–
Bacterial pneumonia	–	1	–	–
Hepatic failure (TB)	–	–	–	1
Varicella zoster	1	–	–	–
Mucositis	1	–	–	–
Neuropathy	1	–	–	–
Nausea/vomiting	1	–	–	–
Depression	2	–	–	–
Fatigue	3	–	–	–
Anemia	1	1	–	–
Leukopenia	–	1	1	–
Lymphopenia	–	–	1	–
Neutropenia	1	1	1	–
Dose Level 3 = 30 mg (n = 5)				
Hyperglycemia	–	1	–	–
Peripheral neuropathy	–	1	–	–
Fatigue	2	–	–	–
Iritis	1	–	–	–
Neuropathy	1	–	–	–
Pain	2	–	–	–
Vomiting	1	–	–	–
Dyspnea	1	–	–	–
Anemia	1	1	–	–
Leukopenia	–	1	2	–
Lymphopenia	–	1	4	–
Neutropenia	1	1	2	–
Thrombocytopenia	–	–	1	–

Table 3 Continued

Adverse Event Grade	2	3	4	5
Dose Level 4 = 60 mg (n = 6)				
Hyponatremia	–	1	–	–
Febrile neutropenia	–	2	–	–
Cystitis	1	–	–	–
Pain	4	–	–	–
Pneumonia	–	1	–	–
Bacterial infection	2	–	–	–
Nausea/vomiting	1	–	–	–
Constipation	2	–	–	–
Dyspnea	–	1	–	–
Anemia	–	1	2	–
Leukopenia	1	1	4	–
Lymphopenia	–	1	5	–
Neutropenia	–	1	5	–
Thrombocytopenia	1	1	2	–

Abbreviations: ALP = alkaline phosphatase; AST = aspartate aminotransferase; BK = BK virus; CAS = coronary artery syndrome; CHF = congestive heart failure; CTCAE = Common Terminology Criteria for Adverse Events; TB = tuberculosis.

patients and ranged from 41.7 ng/mL to 1973 ng/mL with the median highest trough levels of 307 ng/mL (Figure 4). Only 3 patients achieved trough levels that exceeded 1000 ng/mL, and 2 were treated at the highest dose level. Because we primarily measured trough levels of alemtuzumab we did not have the data to observe a 2-compartment model previously reported.[24,25] We did observe a large variability in concentrations between patients at each dose level of at least fourfold, as had been observed by others in CLL.[24,25] Although trough levels appeared to increase with increasing doses in most instances, there was no discernable relationship between maximum trough concentrations and response (197.6 ng/mL for relapse and/or disease progression vs. 388.1 ng/mL for durable response; $P = .7239$; see Supplemental Figure 2 in the online version at http://dx.doi.org/10.1016/j.clml.2015.11.008). Estimated terminal half-life (measurable in 8 patients) was a median of 12 days (range, 3.5-58 days).

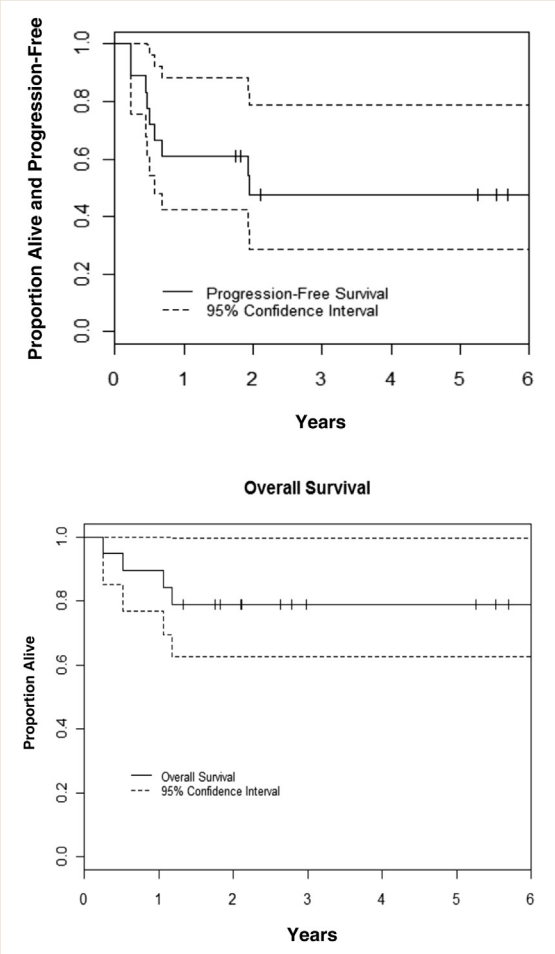
Discussion

In this phase I study, the safety, PK, efficacy, and immunological effects of combining alemtuzumab in various doses and schedules with CHOP chemotherapy for patients with aggressive $CD52^+$ T cell lymphomas were evaluated. The maximum dose level tested was 60 mg subcutaneously (S.C.) on day 1 every 21 days, and this dose level was tolerable and resulted in the highest trough antibody levels. PK were nonlinear, with high interdose and patient variability. This combination was immunosuppressive and we observed a high rate of CMV reactivation and documented pneumonia (2 fungal, 1 bacterial). Almost half of the patients (47%) enrolled in this study were lymphopenic at baseline and T-cell reconstitution was delayed and incomplete at 1 year.

With evidence that alemtuzumab has single agent activity in relapsed PTL (overall response rate, 36%),[18] other groups have combined alemtuzumab with different forms and schedules of cytotoxic chemotherapy for the management of relapsed/refractory[18,26-29] or newly diagnosed[29-35] disease. In these studies, the alemtuzumab dose and/or schedule was arbitrarily selected and with the exception of 2 studies,[30,34] CD52 expression was not determined before treatment. There is only 1 previous unpublished phase I study of I.V. alemtuzumab in doses that ranged from 30 to 90 mg combined with dose-adjusted EPOCH (etoposide, prednisone, vincristine, cyclophosphamide, and adriamycin). In this National Cancer Institute (NCI) study of 14 patients, 30 mg I.V. every cycle was the dose recommended for phase II testing because of excessive bone marrow suppression observed at the higher doses.[30] We also observed the highest rates of Grades 3 to 4 thrombocytopenia and neutropenia at dose level 4 (1 Grade 4 thrombocytopenia was a DLT), but in contrast to results of the NCI study, the MTD was not reached at 60 mg. Possible explanations for these differences could include the different routes of alemtuzumab administration (I.V. vs. S.C.) and toxicities of the selected chemotherapy regimens.[36] In the recently enrolled alemtuzumab and CHOP in T-cell lymphoma ($ACT1^-$) and ACT-2 studies (randomized controlled trials that compared CHOP-14 with CHOP-14 with alemtuzumuab), an early dose reduction from 360 mg of alemtuzumab (30 mg for days 1-2 of CHOP-14) to 120 mg (30 mg on day 1 of cycles 1-4 of CHOP-14 only) was prompted because of the emergence of 2 systemic fungal infections. This led to the reduction in the number of serious AEs (SAEs) per patient to levels comparable with those seen within the control arm.[37] Although we also observed 2 fungal infections, none were fatal nor counted as DLTs to prompt dose reduction or expansion. Furthermore, the administration of higher-dose alemtuzumab in concert with a more cytotoxic chemotherapy schedule (CHOP-14 not 21) might have compounded toxicity.

Comparisons across the largely phase II studies is difficult because of the heterogeneous patient populations, doses, schedules, and routes of alemtuzumab administration, and chemotherapy regimens (see Supplemental Table 1 in the online version at http://dx.doi.org/10.1016/j.clml.2015.11.008). In these studies, rates of CMV reactivation ranged from 9% to 54% with CMV infection rates of 5% to

Figure 2 Kaplan–Meier Progression-Free (Top) and Overall Survival (Bottom) Curves From Time of Enrollment (in Years)



15%[33,34] and treatment-related mortality from 0% to 36%.[18,34] Opportunistic infections caused by pathogens associated with severe T-cell dysfunction including tuberculosis,[18,31] John Cunningham virus,[34] PJP,[34] CMV,[29,31,33] and aspergillosis[18,32,34] have all been reported and some studies did not complete enrollment because of excess toxicity.[29,30,33] It is interesting that Grady et al found that 27% of patients with PTL and cutaneous T cell lymphoma had CMV viremia before systemic therapy and viral loads correlated with degrees of $CD8^+$ lymphopenia.[38] This suggests that patients with PTL might be uniquely vulnerable to CMV infections. In our study, CMV reactivation was a significant complication with 3 patients who received a median of 42 days of valganciclovir because of repeated activation. However, we did not observe CMV infections nor related deaths, possibly because of our frequent screening and use of preemptive valganciclovir. We did not implement primary prophylaxis with valganciclovir,[39,40] because of concerns regarding enhanced myelosuppression. The future prophylactic use of the highly effective new nonmyelosuppressive antiviral letermovir could be evaluated and if successful at eliminating CMV reactivation, would greatly simplify the clinical management of such patients.[41]

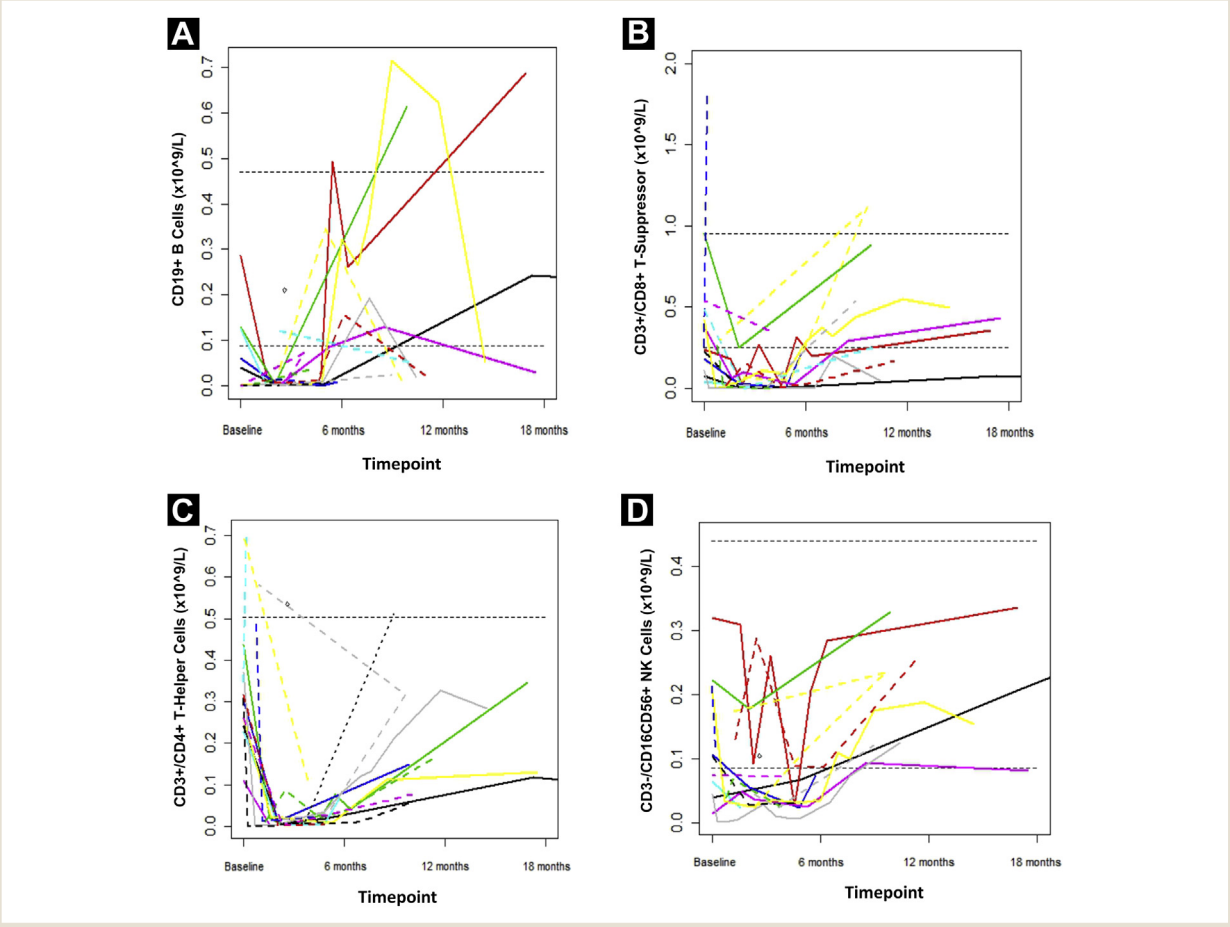
Although the degree of T- and B-cell immunosuppression induced by CHOP-alemtuzumab was expected, the extent of baseline lymphopenia that preceded chemotherapy (47%) was greater than reported in diffuse large B cell lymphoma, where it ranges from 19% to 39%.[42-45] This is important to note because host immunocompetence (as reflected by the ALC) might affect the depths and durations of response to chemotherapy alone[43] or with rituximab in B cell lymphomas.[42,44] One cannot know whether alemtuzumab was responsible for the delay in T-cell recovery after chemotherapy because it has not been specifically studied in T-cell lymphomas. Furthermore, cytotoxic chemotherapy alone might lead to prolonged periods of T-cell immunosuppression in adults.[46,47] Although we did not observe opportunistic infections beyond 3 months, our findings suggest that more protracted courses of prophylactic antimicrobials and vigilance for virus, fungus, and PJP might be warranted. In addition, consolidative cellular immunotherapy or immunomodulation might be ineffectual in the first year with such degrees of T cell depletion.

To our knowledge, this is the first study to measure alemtuzumab PK when used S.C. in combination with chemotherapy in PTL. In vitro, the concentrations of alemtuzumab required for complement and antibody dependent cellular cytotoxicity and apoptosis in CLL range from 0.1 to 10 μg/mL to 0.01 μg/mL, respectively.[48,49] As observed in CLL, we hypothesized that higher trough concentrations (achievable with higher alemtuzumab doses) would associate with superior clinical outcomes.[24,25,36,50,51] Despite achievement of the highest trough concentrations at 60 mg every 21 days, we observed significant interpatient variability within and between dose levels. For example, at 60 mg dosing, highest trough levels ranged from 225 ng/mL to 1973 ng/mL in patients who received ≥4 doses of alemtuzumab. Peak trough levels plateaued early in some patients and in others, no plateaus were seen. The concentrations for dose level 2 were higher than dose level 3 despite the identical cumulative dose and suggested a threefold change in clearance and is in agreement with previously observed nonlinear PK.[24] The median terminal half-life was 12 days but ranged from 3.5 to 58.5 days and was determined in a limited number of patients. This wide range might be because of the time-dependent PK of alemtuzumab in which clearance decreases as tumour burden decreases.[24]

Only 3 patients achieved the targeted 1 μg/mL reported to be associated with higher response and minimal residual disease attainment in CLL.[36] These differences might be explainable by variations in tumor burden, intensity of CD52 expression, degrees of immunosuppression and soluble CD52. Although we observed no discernable relationship between peak trough levels and clinical response/durability, the small sample sizes and different histologies preclude any decisive conclusions about drug concentration and response.

This phase I study was challenged by slow accrual for 2 major reasons: drug and patient eligibility on the basis of CD52 expression. Over the duration of this study, the pharmaceutical ownership of alemtuzumab changed 3 times, which halted study accrual for many months as drug supply contracts were renegotiated. At the time the study was designed, we assumed, on the basis of available literature specific to T cells, that CD52 would be expressed on nearly all mature T-cell lymphomas. Other studies have since emerged and reported lower incidence of CD52 expression; < 40%

Figure 3 B- and T-Cell Subsets Over Time. Horizontal Dotted Lines Represent Upper and Lower Limits of Normal Reference Range. (A) CD3/CD8$^+$ Suppressor T Cells; (B) CD19$^+$ B Cells; (C) CD3$^+$/CD4$^+$ T Helper Cells; (D) CD3-CD16$^+$CD56$^+$ Natural Killer Cells (NK Cells). In (C) Only the Lower Limit of Normal is Shown



to 60% of PTL measured using IHC of paraffin-embedded sections.[52,53] Furthermore, CD52 expression appears to vary according to T cell subtype, the methods used to detect CD52, and the intensity and/or sites of staining (membranous vs. cytoplasmic) judged to be positive. Using IHC, the reported rates of CD52 positivity range from 100% for adult T-cell leukemia/lymphoma, 40% for NK/T cell and angioimmunoblastic lymphoma, 35% to 40% for PTL-NOS, and 0% to 22% for ALCL.[20,54] The relevance of CD52 expression intensity using this therapy cannot be answered by our study but should be explored.

Potential additive benefits of alemtuzumab with CHOP can only be assessed in a prospective randomized trial. Fortunately, there are 2 phase III studies in the first-line setting that are testing alemtuzumab 30 mg combined in the first 4 cycles of CHOP-14 chemotherapy, and have completed enrollment: ACT-1 (NCT00646854) and ACT-2 (NCT00725231) of the German and the Nordic lymphoma cooperative study groups, respectively. Despite selection of an unvalidated dose and schedule of alemtuzumab, it is hoped that these studies will more definitively determine if there is a survival benefit to this approach.

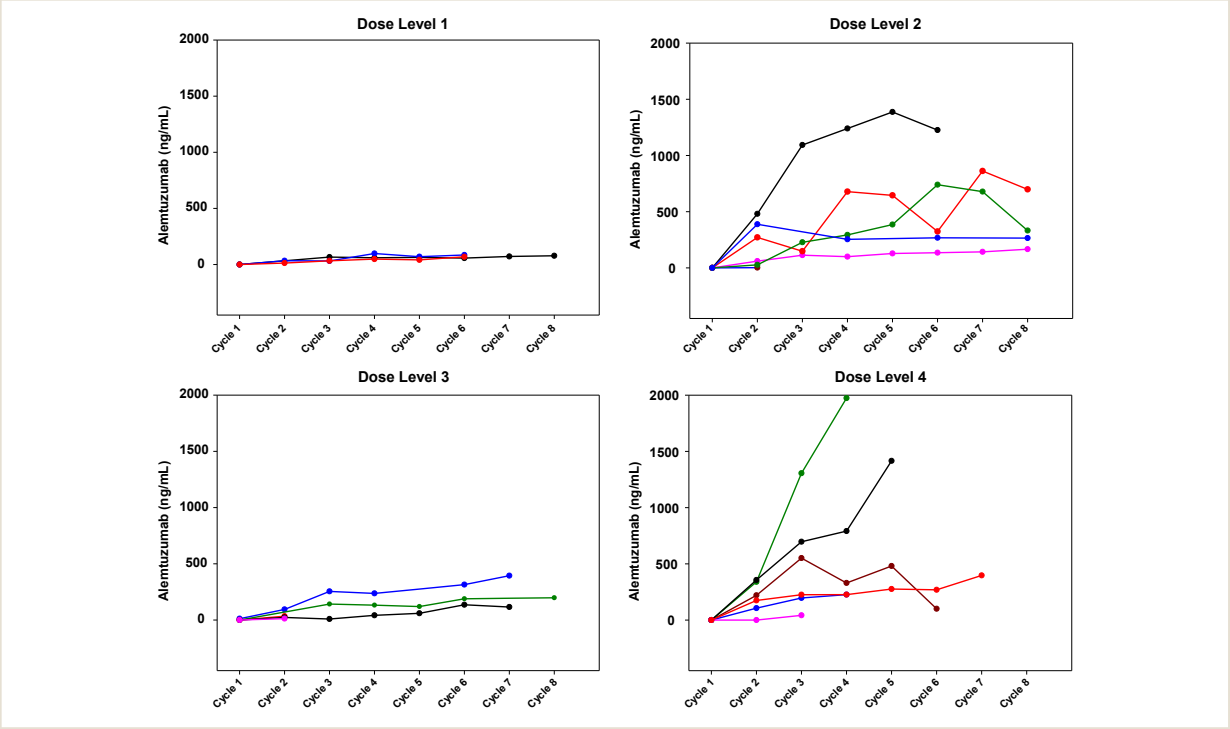
Conclusion

The maximally tolerated dose of alemtuzumab with CHOP chemotherapy is still unknown. With close monitoring for CMV and the anticipation of enhanced myelosuppression, alemtuzumab 60 mg S.C. every 21 days can be safely administered and achieved the highest trough levels of all dose levels tested. Lymphopenia at baseline is common and T-cell lymphopenia is protracted after this chemoimmunotherapy but its clinical significance is currently unknown. If the recently completed randomized trials establish a signal of improved survival outcomes compared with CHOP alone, additional PK studies of 60 mg every 21 days or 20 mg weekly, that evaluate the relationship between alemtuzumab concentrations, CD52 expression, and clinical outcomes might still be warranted to help optimize patient outcomes.

Clinical Practice Points

- Alemtuzumab has been combined with chemotherapy for T-cell lymphomas in several phase II trials but the safest dose, trough PK, and immunological effects have not been studied.

Figure 4 Trough Pharmacokinetics of Alemtuzumab According to Cycle and Dose Level. Y Scale Concentrations Vary According to Graph. Dose Level 1: Alemtuzumab 10 mg Every 3 Weeks. Dose Level 2: Alemtuzumab 10 mg Weekly. Dose Level 3: Alemtuzumab 30 mg Every 3 Weeks. Dose Level 4: Alemtuzumab 60 mg Every 3 Weeks



- In a prospective multicenter phase I study, the maximally tolerated dose of alemtuzumab was not reached; alemtuzumab 60 mg can be safely combined with CHOP and showed activity in $CD52^+$ PTL.
- B- and T-cell lymphopenia at baseline is observed in 50% and 70%, respectively, and T-cell lymphopenia is sustained after chemoimmunotherapy in most patients.
- The highest day 1 alemtuzumab trough levels were achieved at 60 mg (1973 ng/mL), but with significant inter- and intradose variability.
- If alemtuzumab is shown to improve OS in ongoing randomized trials of aggressive T-cell lymphomas, our findings might direct additional research regarding optimal dosing and schedule, and highlight the importance of sustained antimicrobial therapy after treatment completion.

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Disclosure

The authors have stated that they have no conflicts of interest.

Supplemental Data

Supplemental figures, table, and appendix accompanying this article can be found in the online version at http://dx.doi.org/10.1016/j.clml.2015.11.008.

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Appendix A

Patients and Methods

Study Design Monitoring and Steering Committee

A medical monitor at OCOG reviewed all accumulating AEs and SAEs. The trial steering committee reviewed all AEs and DLTs after each stage of accrual and before dose level expansion or dose escalation. The trial data were maintained at the OCOG Coordinating and Methods Centre in Hamilton. The study statistician (G. P.) was responsible for the analyses. The principal investigator and coauthors had input into the analyses and preparation of the report.

Measurement of CD52 Using IHC

Primary rat anti-human CD52 antibody (clone YTH34.5; Serotec, Oxford, United Kingdom; 1:100 dilution) was applied on formalin-fixed paraffin-embedded tissue. Rabbit anti-rat secondary antibody (Zymed) was applied at a dilution of 1:750. Before antibody exposure, the sections underwent enzyme-induced epitope retrieval using protease 2 (Ventana) for 4 minutes. The CD52 stain was considered positive when the neoplastic cells exhibited strong cytoplasmic or membranous staining. Staining in $< 30\%$ of the cells was reported as focally positive. Nuclear staining was considered nonspecific and not counted.

Dosing for CHOP Chemotherapy

We used dosages of cyclophosphamide 750 mg/m^2 I.V. on day 1, doxorubicin 50 mg/m^2 I.V. on day 1, vincristine 1.4 mg/m^2 I.V. (dose capped at 2 mg) on day 1, and prednisone 100 mg orally on days 1 to 5.

Methods of CMV Monitoring

Before August 2008, CMV antigenemia testing was performed using monoclonal anti-human anti-cytomegalovirus antibodies directed against pp65 (Biotest Clonal CMV; Biotest) followed by monoclonal anti-mouse-fluorescein isothiocynate conjugate with Evans Blue (Baxter). The presence of ≥ 1 positive cell per 10^5 polymorphonuclear cells was reported as positive. In August 2008, samples were tested using RealStar CMV PCR kits (Astra Diagnostics, Hamburg, Germany). The lower limit of detection of the PCR was 100 copies per mL.

Pharmacokinetics

Serum antibody levels were assayed using a sensitive ELISA technique (range, 6.3-400 ng/mL) with a precision of 11.1% and an overall accuracy of $\pm 5.3\%$ (Merck Millipore, Discovery & Development Solutions, Abingdon, Oxon, United Kingdom).

The elimination half-life was calculated assuming a first order decline in concentrations and standard pharmacokinetic methods (see Supplemental Table 1 in the online version at http://dx.doi.org/10.1016/j.clml.2015.11.008).[23] Mean trough, and end of interval concentration time data for patients after dose level 1 was best fit to a first order, 1-compartment model. An assumed fixed time-to-peak of 3 hours (ka = 1.25 hours $-$ 1) and an unchanging bioavailability (F) was used. This model is insensitive to reasonable variations in Tmax. The end of interval trough concentrations were best-fit to standard multiple dose pharmacokinetic equations through a sum of squares minimization procedure to determine a volume and half-life.

Anti-Infective Prophylaxis

Granulocyte colony stimulating factor was recommended but not mandated, unless the patient had a previous episode of febrile neutropenia.

Toxicity

Patients who had received at least 1 dose of alemtuzumab were eligible for toxicity assessment. Any AEs that occurred but could not be categorized according to the CTCAE v3.0 were graded by a physician and recorded using a scale of (1) mild, (2) moderate, (3) severe, or (4) life-threatening.

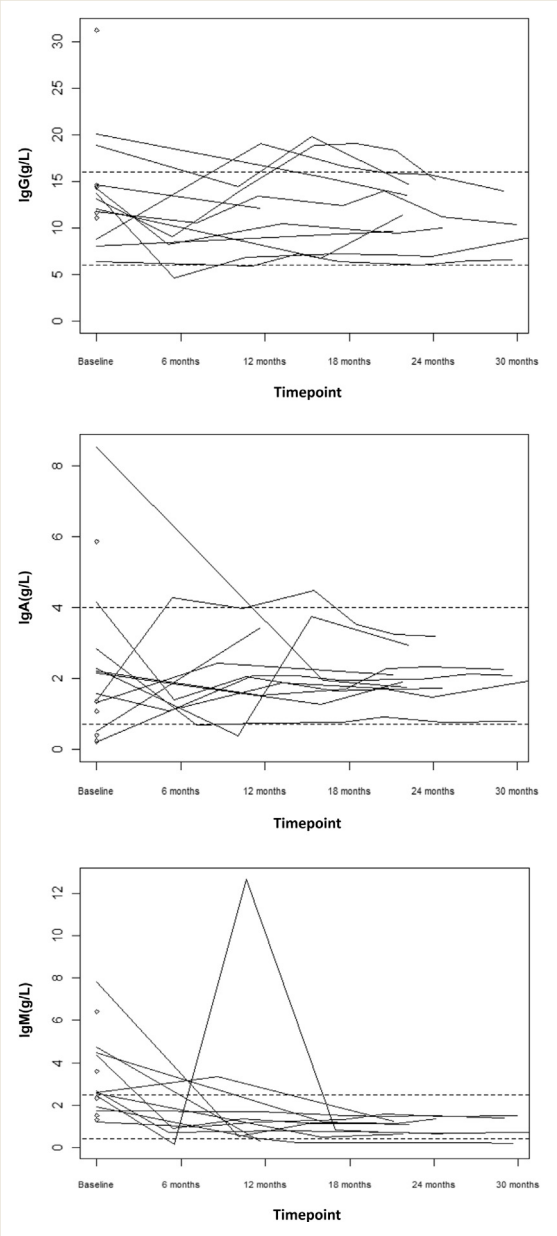
Adverse Event Reporting

After this period, any SAE or Grade 3 to 4 drug-related AEs that occurred subsequent to the AE reporting period deemed to be possibly or probably related to the study drug were also documented.

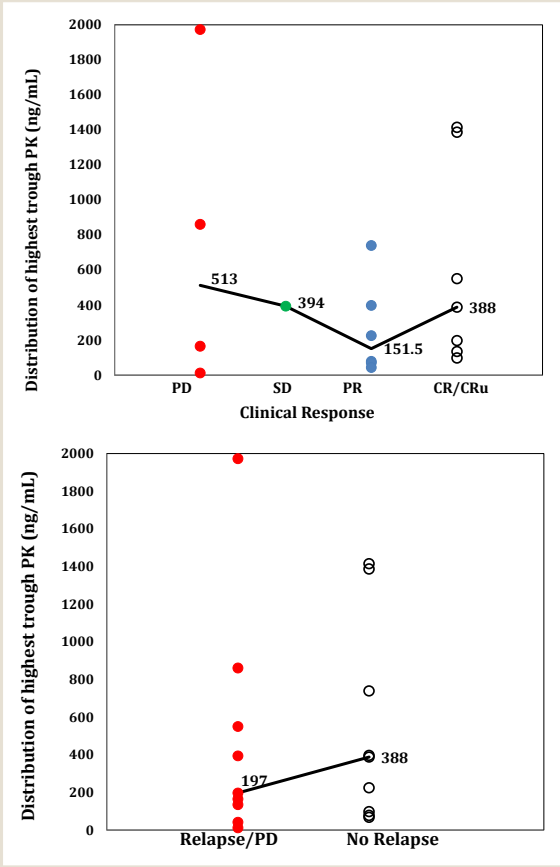
Statistics

Summary statistics, such as the median, range, and frequency were used to describe the patient characteristics, overall response rates, safety profile, and follow-up. The frequency and severity of all Grade 2 to 5 AEs were summarized according to dose level. Pharmacokinetic data were conveyed using graphs that depicted trough levels according to dose level and response and the Wilcoxon rank sum test was used to explore potential associations between peak trough levels and response and/or relapse and/or progression.

Supplemental Figure 1 Immunoglobulins IgG, IgA, and IgM Over Time. Dotted Lines Represent the Upper and Lower Limits of Normal



Supplemental Figure 2 Peak Alemtuzumab Trough Levels According to Relapse (Top Panel) and Best Response (Bottom Panel). Labeled Numbers Represent Median Values



Abbreviation: Cru = complete remission unconfirmed.

Supplemental Table 1 Summary of Alemtuzumab With CHOP Chemotherapy Studies in T Cell Lymphoma

Reference	n	Age (Range), Years	Phase Study/ Chemotherapy	Route/ Dose/ Schedule/ Total Dose/ Cycle	Untreated/ Relapsedor Refractory	CD52+	ORR/CR, %	Follow-Up, Months	EFS/DFS, %	OS	CMV Reactivation %	Infection	TRM, %
Enblad et al[18]	14	60 (53-78)	2/None	I.V./30 mg/tiw × 12 wk NA	—/14	No	36/21	—	—	—	43	Miliary TB (n = 1), zoster (n = 1), aspergillosis (n = 2)	36
Janik et al[30,a]	14	35 (17-77)	1/DA-EPOCH 21	I.V./30/60/90 mg D 1 30/60/90 mg	14/—	Yes	—/42	—	—	—	36	Bacterial/ fungal/ viral (n = 11)	—
Intragumtornchai et al[31]	13	44 (21-56)	2/CHOP 21/ESHAP 28	S.C./30 mg/D I-3 cycles 1-5 90 mg	13/—	No	90/80 (n = 10 evaluated)	8	75	48%	54	TB (n = 2), CMV (n = 1), FN (n = 7)	—
Kim et al[33,b]	20	50 (20-65)	2/CHOP-21[c]	I.V./30 mg/D 1 cycles 1-6 30 mg	20/—	No	80/65	7	43 1 y	44% 1 y	25	CMV (n = 3), pseudomonas pneumonia (n = 1), FN (n = 11)	10
Gallamini et al[34]	25	52 (28-69)	2/CHOP 28	S.C./30 mg/D I cycles 1-8[d] 30 mg	25/—	Yes 11/15 +	75/71	16	54 1 y, 48 2 y	70% 1 y, 53% 2 y	9	JC virus/ PML (n = 1), aspergillosis (n = 2), PJP (n = I), staph aureus (n = 1)	0
Kim et al[28]	24	49 (23-60)	2/DHAP 21	I.V./70 mg/40 mg 10 mg D 1, 30 mg D 1, 2 cycles 1-3,[e] 70 mg (n = 16), 40 mg (n = 8)	0/24	No	50/21	32	—	6 mo	33	CMV (n = 2), HBV liver failure (n = 1)	8

Supplemental Table 1 Continued

Reference	n	Age (Range), Years	Phase Study/ Chemotherapy	Route/ Dose/ Schedule/ Total Dose/ Cycle	Untreated/ Relapsedor Refractory	CD52+	ORR/CR, %	Follow-Up, Months	EFS/DFS, %	OS	CMV Reactivation %	Infection	TRM, %
Weidmann et al[29]	38	56 (21-77)	2/FCD 21	I.V., S.C. (I0)/30 mg/10 mg D 1, 30 mg D 2-3 cycles 1-6 70 mg	27/11	No	61/39	17	12 mo,[f] 2.5 mo[g]	26 mo,[f] 6 mo[g]	26	FN (n = 15), CMV (n = 2)	18
Kluin-Nelemans[35]	20	50 (20-65)	2/CHOP 14	S.C./30 mg D 1,5, 10 cycles 1-8 90 mg	20/0	No	90/65	29	10 mo, 45 1 y, 27 2 y	27 mo 77% 1 y, 55% 2 y	35	FN (n = 8), CMV (n = 1), EBV and NHL (n = 3)	20
Binder et al[32]	41 29[h]	55 (19-70)	2/CHOEP 14 age ≤60 CHOP 14 age >60	I.V./S.C. as consolidation D 1-3: 3, 10, 30 mg I.V. 30 mg S.C. weekly × 3/133 mg over 4 weeks consolidation CR/Cru or PR patients only	29/0	No	61/58 (after chemo)	46	42 3 y	75% 3 y	14	Aspergillus (n = 1), Candida (n = 1), CMV (n = 2), zoster (n = 1)	0

Abbreviations: ASCT = autologous stem cell transplant; chemo = chemotherapy; CHOEP = cyclophosphamide, adriamycin, vincristine, etoposide and prednisone; CHOP = cyclophosphamide, adriamycin, vincristine and prednisone; CMV = cytomegalovirus; Cru = complete remission unconfirmed; D = day; DA-EPOCH = dose-adjusted infusional etoposide, prednisone, vincristine, cyclophosphamide, and adriamycin; DFS = disease-free survival; DHAP = Dexamethasone, high dose ara-C and cisplatin; EBV = Ebstein-Barr virus; EFS = event-free survival; ESHAP = etoposide, high-dose cytarabine and cisplatin; FCD = fludarabine, cyclophosphamide and doxorubicin; FN = febrile neutropenia; HBV = hepatitis B virus; JC = John Cunningham; NHL = non-Hodgkinapos;s lymphoma; OS = overall survival; PJP = pneumocystis jiroveci pneumonia; PML = progressive multifocal leukoencephalopathy; S.C. = subcutaneous; TB = tuberculosis; tiw = three times weekly; TRM = treatment-related mortality.

[a]Abstract only: bone marrow suppression prevented further treatment at 60 mg dose cycles 3, and 5 (n = 2) and 90 mg at cycles 4, and 5 (n = 2).

[b]The study closed earlier than the planned with 43 patients because of infectious complications.

[c]ASCT in 4 patients followed.

[d]For the first 4 patients, alemtuzumab given only for 4 cycles.

[e]First 16 patients received 30 mg on D 2 as well, total dose 70 mg; 8 responders went on to ASCT.

[f]New diagnosis.

[g]Relapsed refractory.

[h]Received alemtuzumab as consolidation.