A phase I study of escalated dose subcutaneous alemtuzumab given weekly with rituximab in relapsed chronic lymphocytic leukemia/small lymphocytic lymphoma

Jennifer R. Brown, Bradley Messmer, Lillian Werner, Matthew S. Davids, Evgeny Mikler, Jeffrey G. Supko, David C. Fisher, Ann S. LaCasce, Philippe Armand, Eric Jacobsen, Virginia Dalton, Bethany Tesar, Stacey M. Fernandes, Sean McDonough, Jerome Ritz, Laura Rassenti, Thomas J. Kipps, Donna Neuberg, and Arnold S. Freedman

Department of Medical Oncology, Department of Biostatistics and Computational Biology, Dana-Farber Cancer Institute, Boston, MA, USA; Moores Cancer Center, UC San Diego, LaJolla, CA, USA; Department of Medicine, Harvard Medical School, Boston, MA, USA

ABSTRACT

This study assessed the safety and preliminary efficacy of escalated dose subcutaneous alemtuzumab in combination with rituximab in chronic lymphocytic leukemia. Twenty-eight patients with relapsed refractory chronic lymphocytic leukemia were treated on four dosing cohorts of weekly rituximab at 375 mg/m^2 and alemtuzumab doses that started at 30 mg three times per week and escalated to weekly dosing over four weeks, culminating with 90 mg weekly. One dose limiting toxicity of a rituximab infusion reaction was seen in cohort 2, but the regimen was otherwise well tolerated without evidence of differential toxicity by cohort. The overall response rate by National Cancer Institute-Working Group criteria was 61%, and the rate of complete bone marrow response was 43%, most of whom were negative for minimal residual disease. The addition of CT scan evaluation per International Workshop on Chronic Lymphocytic Leukemia 2008 criteria reduced the overall response rate to 14%. Median overall survival was 35 months, with 12 patients able to proceed to stem cell transplantation. Pharmacokinetic studies showed that chronic lymphocytic leukemia involving more than 80% of the bone marrow at study start was associated with lower trough concentrations of alemtuzumab and rituximab, and that higher trough serum concentrations of alemtuzumab were associated with complete bone marrow clearance. We conclude that escalated subcutaneous doses of alemtuzumab given weekly are well tolerated and result in excellent bone marrow clearance of chronic lymphocytic leukemia, helping patients to proceed to stem cell transplantation. *This study is registered at ClinicalTrials.gov (Identifier:00330252).*

Introduction

Alemtuzumab is an anti-CD52 antibody with significant activity in genetically high-risk treatment-resistant CLL. When given intravenously three times per week to CLL patients refractory to fludarabine and previously exposed to alkylators, alemtuzumab had a 33% overall response rate, with a time to progression of 4.7 months and median overall survival of 16 months.[1] However, intravenous administration was associated with significant infusional toxicity leading to the introduction of subcutaneous administration, still three times per week, which was substantially better tolerated.[2] The German CLL Study Group subsequently redid the original alemtuzumab registration trial using subcutaneous administration, and found efficacy equivalent to intravenous administration, and again independent of high-risk genetic features including 11q and 17p deletions.[3] Furthermore, they observed that alemtuzumab allowed patients to proceed to allogeneic SCT who would not otherwise have been able to, and these patients had what appeared to be a significantly better overall survival compared to patients receiving other therapies after alemtuzumab.[3]

However, single agent alemtuzumab has some disadvantages. Particularly in nodal disease, its activity is relatively poor. This problem has led to efforts to combine alemtuzumab with chemotherapy.[4-8] In general, the increase in efficacy with these regimens has not been sufficient to outweigh the increase in toxicity and displace the pre-existing chemotherapy regimen.[4,6] Given the known toxicities of both alemtuzumab and purine analog chemotherapy, a more rational combination is to pair alemtuzumab with other antibodies or with steroids that are potentially less toxic and act in a TP53-independent fashion similar to alemtuzumab. Combinations with rituximab have shown promising efficacy in high-risk disease,[9] as have combinations with high-dose glucocorticoids in patients with 17p deletion,[10] both of which had manageable toxicity. Another recent approach has been to test whether the addition of GM-CSF might enhance response without increasing toxicity, and no benefit, but rather a possible detrimental effect, was observed.[11]

Although these regimens show promise, they are cumbersome to administer, involving frequent dosing. A parallel line of investigation has, therefore, developed to focus on simplifying the dosing and administration and/or reducing the toxicity. Multiple groups have been interested in giving alemtuzumab weekly, sometimes in the context of lower doses to improve toxicity.[12-14] These studies have generally found preserved response rates but have not reported on efficacy in cytoreduction for SCT.

 doi:10.3324/haematol.2013.086207
The online version of this article has a Supplementary Appendix.
Manuscript received on February 14, 2013. Manuscript accepted on April 23, 2013.
Correspondence: jbrown2@partners.org

In this study, we sought to develop a well tolerated and more convenient alemtuzumab regimen that would nonetheless reduce disease burden sufficiently to allow patients to proceed to allogeneic SCT. We, therefore, assessed the tolerability and efficacy of administering alemtuzumab subcutaneously weekly rather than three times per week, at full dose, i.e. up to 90 mg per dose. In addition, since pre-clinical and clinical studies suggest that rituximab in combination with alemtuzumab may enhance efficacy,[15,16] we added weekly rituximab to alemtuzumab in the hope of enhancing activity in lymph nodes. Our study shows that alemtuzumab given subcutaneously at 90 mg weekly in combination with rituximab is well tolerated, associated with a 61% response rate, and helped 12 of 23 transplant eligible patients to proceed to allogeneic SCT.

Design and Methods

Eligibility

This prospective study enrolled patients with relapsed or refractory CLL/SLL who required therapy by NCI-WG 1996 criteria.[17] Prior therapy with alemtuzumab or rituximab was permitted. Active infection was an exclusion criterion. There were no restrictions for cytopenias. The study was approved by the Dana-Farber Harvard Cancer Center Institutional Review Board, and all patients signed informed consent prior to initiation of therapy. This study is registered at *ClinicalTrials.gov (Identifier:00330252)*.

Treatment program

The planned therapy included an initial dose escalation of alemtuzumab over the first four weeks, as shown in the treatment schema in *Online Supplementary Table S1,* culminating in a single weekly 90 mg dose on dose level 4. Up to 45 mg was given at a single subcutaneous injection site; higher doses were split between two injection sites. Rituximab was given weekly intravenously at 375 mg/m^2 in all cohorts, with the option of splitting the dose between Days 1 and 3 in the first week for patients with elevated absolute lymphocyte counts. Subjects completed an initial 8-week cycle followed by response evaluation; they then had the option of completing a second 8-week cycle at their maximum prior dose. Ten patients completed eight weeks and 13 patients completed 16 weeks.

All patients received infectious prophylaxis with trimethoprim-sulfamethoxazole and acyclovir (or equivalent). During the first cycle, all patients received allopurinol and intravenous hydration. CMV viral load monitoring was performed weekly and acyclovir was switched to valgancyclovir at the time of a first positive result. Pegfilgrastim was administered when the ANC dropped below 800/μL but prophylactic antibiotics were not routinely given.

Table 1. Patients' characteristics.

N.	28
Median age (range)	62 (47-76)
Median time from diagnosis to study treatment	94 mos (14-236)
Median number of prior therapies	4 (1-11)
Patients refractory to fludarabine	15 (54%)
Stage	
0-2	5 (18%)
3	6 (21%)
4	17 (61%)
Median beta-2-microglobulin	3.9 (1.2-7.2)
Median absolute lymphocyte count (ALC)	20.5K (0.06-80.6)
Median platelet count	89K (23-353)
Bulky lymphadenopathy > 5 cm	15 (54%)
Bone marrow cellularity >80% CLL	17 (61%)
IGHV unmutated	13/16 (81%)
ZAP-70 positive	14/19 (74%)
Cytogenetics	
Del 17p	9 (32%)
Del 11q	11 (39%)
Complex (7 with 17p, 2 with 11q)	9 (32%)
Trisomy 12	2 (7%)
Del 13q	4 (14%)
Normal	2 (7%)

Definition of dose limiting toxicity

The study used a standard '3+3' dose escalation design, with occurrence of dose limiting toxicity (DLT) assessed in the first seven weeks given the progressive dose escalation. DLT was defined as greater than or equal to grade 3 non-hematologic toxicity, except grade 4 infection or grade 2 irreversible neurological, cardiac or renal events. Hematologic toxicity was only considered dose-limiting if therapy had to be held more than twice for absolute neutrophil count (ANC) below 0.2x10^9/L or platelets below 20x10^9/L without recovery within two weeks. Hematologic toxicity was assessed according to NCI-WG 1996 criteria,[17] while non-hematologic toxicity was assessed according to Common Terminology Criteria for Adverse Events version 3.0 (CTCAE 3.0).

Study evaluations

Screening evaluations included laboratory testing for hepatitis B and C and HIV, as well as bone marrow biopsy with fluorescence *in situ* hybridization (FISH) cytogenetics and computed tomography (CT) scans of the chest / abdomen / pelvis. CT scans and bone marrow biopsy were repeated after eight and 16 weeks in patients who completed 16 weeks. The primary end-point of this trial as designed was overall response rate (ORR) based on NCI-WG 1996 criteria, i.e. without the CT scan data.[17] However, a planned secondary end-point was to assess the impact of CT scanning on overall response rate.

Details of the evaluation of CLL prognostic factors, peripheral blood B, T and NK cell subsets, pharmacokinetic assays and statistical analysis are available in the *Online Supplementary Appendix*.

Results

Patients' characteristics

Twenty-eight patients were enrolled on this study between July 2006 and January 2010; patients' characteristics are detailed in Table 1. Median age was 62 years (range 47-76) and 75% were male. Eighty-two percent had stage 3-4 disease at time of study entry and the median number of prior therapies was 4 (range 1-11). Seventy-one percent of patients had high-risk deletions of 17p or 11q, including 32% with deletion 17p, and 81% had unmutated *IGHV*, suggesting that this was an extremely high-risk patient population.

Dose escalation and toxicity

The therapy was generally well tolerated with only one observed DLT: a grade 3 rituximab infusion reaction on

dose level 2 prior to any escalation of alemtuzumab dose (*Online Supplementary Table S1*). Dose level 2 was, therefore, expanded to include 6 evaluable patients (total 8 enrolled) and no further DLTs were observed on the study. Ten additional patients were, therefore, accrued to dose level 4 as an expansion cohort. Injection site reactions were minimal even with escalated doses, primarily grade 1, with two grade 2 events (Table 2). Other toxicities were as expected with alemtuzumab in a relapsed refractory CLL population, and included 50% grade 3-4 neutropenia and 57% grade 3-4 thrombocytopenia. Seventeen of 28 patients (61%) received pegfilgrastim support during study therapy. CMV reactivation was seen in 7 patients (25%), and no clinical CMV disease occurred. Notable individual grade 3 toxicities included a persistent methicillin resistant *Staphylococcus Aureus* (MRSA) bacteremia, an Epstein-Barr virus (EBV)-related lymphoma and metastatic colon cancer identified shortly after the completion of study therapy. No deaths were attributable to study therapy. Early study withdrawals occurred due to pre-existing and persistent thrombocytopenia requiring study therapy to be held (n=2), persistent fever attributed to alemtuzumab (n=1), progressive multifocal leukoencephalopathy (PML) which in retrospect was present prior to study entry (n=1), and DLT (grade 3 rituximab reaction n=1).

Response to therapy

The primary end point of this trial, ORR by NCI-WG 1996 criteria, was 61% at week 8 (17 of 28; 90%CI: 43-76%), with CR rate 11% (3 of 28; 90%CI: 3-25%). Two of 13 patients who completed a second 8-week cycle improved their response (one PR from SD, and one CR from PR). Given that CT scanning in clinical trials was not yet recommended as a routine part of CLL staging when this trial was designed, a planned study endpoint was to evaluate the impact of CT scans on ORR evaluation, and, indeed, we found that incorporating CT scan evaluation into the week 8 disease restaging, similar to the IWCLL 2008 criteria,[18] decreased the ORR to 14% (4 of 28; 90%CI: 5-30%), largely due to the prevalence of significant abdominal lymphadenopathy in this relapsed refractory patient population. The ORR in the 17p deletion patients without CT was 8 of 9 (89%; 90%CI: 57-99%), and with CT was 3 of 9 (33%; 90%CI: 10-66%).

Bone marrow response was excellent, however, showing complete clearance of disease by eight weeks in 8 patients and by 16 weeks in an additional 4 patients, for a total of 12 patients who achieved bone marrow complete remission (43%). Eight of 10 of these who were evaluable for MRD status were negative for bone marrow MRD by 4-color flow cytometry. Eighty percent of those evaluable (at least 67% of bone marrow CR patients) were, therefore, MRD negative in bone marrow. Again, the patients with 17p deletion had similar responses, with 5 of 9 demonstrating a bone marrow CR (56%; 90%CI: 25-83%).

Survival

The median PFS for this refractory high-risk population was 26 months (Figure 1A). The time to treatment failure (TTF) was a significantly shorter 8 months (Figure 1B), however, as several patients went to SCT in remission and others who had stable disease or partial response rapidly received alternative therapy directed at residual nodal disease, typically high-dose methylprednisolone with rituximab,[19-21] with the goal of getting them to SCT. To date, 13 patients have died, 7 of disease, 4 of other malignancies, one of PML and one of SCT complications. The median overall survival is 35 months (Figure 1C), with 12 patients having undergone subsequent allogeneic SCT.

Among the 9 17p deletion patients, the median progression-free survival (PFS) was 35 months, median TTF was 18 months and median OS 38 months. These results again suggest that these patients fared as well or better than other subgroups with an alemtuzumab-based regimen.

Lymphocyte subsets

Analysis of lymphocyte subsets in peripheral blood demonstrated rapid and parallel declines in CLL cells as well as B ($CD20^+$), T ($CD3^+$) and NK ($CD56^+CD3^-$) cells in the first three weeks after initiation of therapy, with minimal effect on granulocytes and monocytes (Figure 2A, *Online Supplementary Table S2*). Therapy was completed by week 8 or 16, but all circulating lymphocytes remained very low for at least six months post treatment. T cells began to recover by 40 weeks, but the counts were still below $0.4x10^9$/L (Figure 2B), and these were mostly CD4 memory cells ($CD45RO^+$; Figure 2C). CD4 counts were above $0.4x10^9$/L by one year (Figure 2C). CD8 T cells remained very low at 40 weeks but also recovered by one year (Figure 2D). NK cells showed recovery approximately parallel to CD4 T cells (Figure 2E).

Table 2. Toxicities.

Toxicities	Cohort				
	1	2	3	4	Total (%)
Injection site reactions					
Grade 1	1	4	2	4	11 (39%)
Grade 2		1		1	2 (7%)
Neutropenia					14 (50%)
Grade 3	1	1		4	6
Grade 4	2	1	1	4	8
Thrombocytopenia					16 (57%)
Grade 3	1	3	1	4	9
Grade 4	1	2	1	3	7
Toxicities (≥ Grade 3)					
Hyperglycemia	1			2	3 (11%)
Fatigue	1			1	2 (7%)
Allergic reaction				2	2 (7%)
Hyperkalemia				1	1 (3.6%)
Rash				1	1 (3.6%)
Hyponatremia				1	1 (3.6%)
Pulmonary embolism				1	1 (3.6%)
EBV lymphoma				1	1 (3.6%)
Metastatic colon cancer				1	1 (3.6%)
Immune hemolytic anemia				1	1 (3.6%)
Cytokine release syndrome		1			1 (3.6%)
Retinal detachment		1			1 (3.6%)
Headache				1	1 (3.6%)
Constipation				1	1 (3.6%)
Elevated ALT			1		1 (3.6%)
Infection					4 (14%)
MRSA bacteremia				1	1 (3.6%)
Diverticular abscess				1	1 (3.6%)
Pulmonary cryptococcus			1		1 (3.6%)
Other				1	1 (3.6%)

Pharmacokinetics

Maximum trough concentrations of rituximab and alemtuzumab in serum were obtained from 23 and 24 patients, respectively. As illustrated in Figure 3, the range of maximum trough serum concentration of both drugs achieved in the different dose cohorts of patients was similar. The overall median values of the maximum trough serum concentrations were 237.0 μg/mL (n=23; range <2.5-434.0 μg/mL) for rituximab and 4.10 (n=24; range <0.50-14.08 μg/mL) for alemtuzumab. The maximum trough serum concentrations of the two drugs were not correlated (r=0.038). There were no significant differences between the maximum trough serum concentrations of either drug in patients with an objective response compared to those who did not respond. The mean maximum trough concentration of alemtuzumab was significantly greater in patients with a bone marrow CR as compared to those who did not clear their bone marrow (5.64±3.24 μg/mL (n=12) *vs.* 3.03 ± 2.13 μg/mL (n=11); *P*=0.029). In contrast, there was no significant difference (*P*=0.54) in the mean maximum trough concentration of rituximab in patients with a bone marrow CR (222.8±135.6 μg/mL, n=11) as compared to those without bone marrow CR (243.4±115.3 μg/mL, n=11). Maximum trough serum concentrations of the two drugs were not associated with either bulky lymphadenopathy over 5 cm on physical examination or CT scan, or with absolute lymphocyte count grouped as less than $5x10^9$/L, 5-$20x10^9$/L, 20-$100x10^9$/L, or more than $100x10^9$/L. A trend to an association of lower alemtuzumab and rituximab trough serum concentrations was seen with packed bone marrow in which more than 80% of the intertrabecular space was CLL (alemtuzumab 1.8 *vs.* 4.5 μg/mL and rituximab 134 *vs.* 282 μg/mL; *Online Supplementary Table S3*).

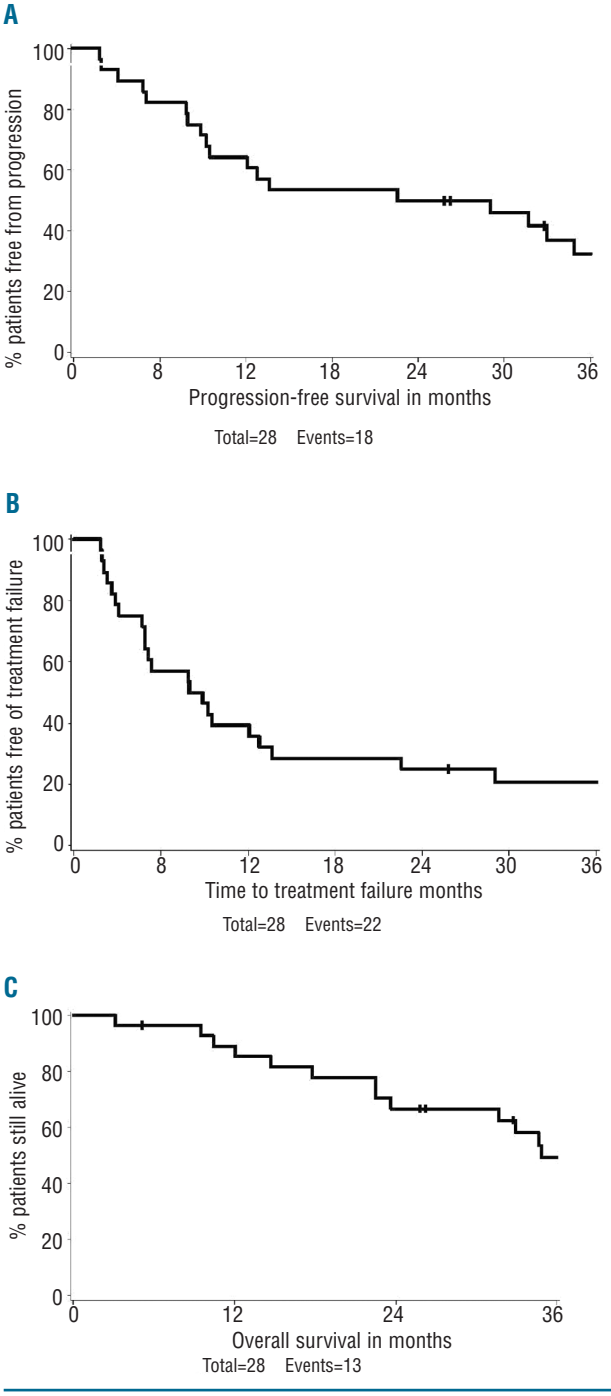

Figure 1. (A) **Progression-free survival. Median 26 months.** (B) **Time to treatment failure. Median 8 months.** (C) **Overall survival. Median 35 months. All curves include the 28 enrolled patients.**

Discussion

We report on the safety and efficacy of a novel subcutaneous weekly dosing regimen of alemtuzumab in combination with rituximab, designed to increase ease and convenience for patients without compromising clinical benefit. After an initial escalation, the entire weekly alemtuzumab dose of 90 mg is administered in a single day. This dosing was well tolerated with only minor injection site reactions, and the expected incidence of cytopenias and infection, which were generally easily managed. Depletion of circulating CLL cells was extremely rapid, as was depletion of T and NK cells, but lymphocyte recovery was significant within 6-8 months of completing therapy.

In this very heavily pre-treated and high-risk patient population, 82% of whom had advanced stage disease and 71% of whom had deletions of 11q or 17p, the response rate of the regimen by NCI-96 standard criteria was 61%. The bone marrow CR rate was 43% and the majority of these patients were negative for CLL in bone marrow by 4-color flow cytometry. These results underscore that the primary efficacy of alemtuzumab is in blood and bone marrow clearance, which it can do quite effectively even in very high-risk patients. Reduction of bulky particularly abdominal lymphadenopathy is relatively poor, however, as has been described previously by many groups,[1,22] and probably explains why the addition of CT scans to the response evaluation in these patients substantially reduced the response rate. Our study results do not suggest that rituximab added substantially to alemtuzumab in terms of nodal responses. Nonetheless, these patients still did well in terms of their PFS and OS, possibly because of the excellent bone marrow clearance.

Prior studies have demonstrated that the response of B-cell malignancies to treatment with either rituximab or alemtuzumab is significantly associated with higher maximum trough serum concentrations of both drugs.[23-26] No association between response and the maximum trough serum concentration of rituximab was observed even

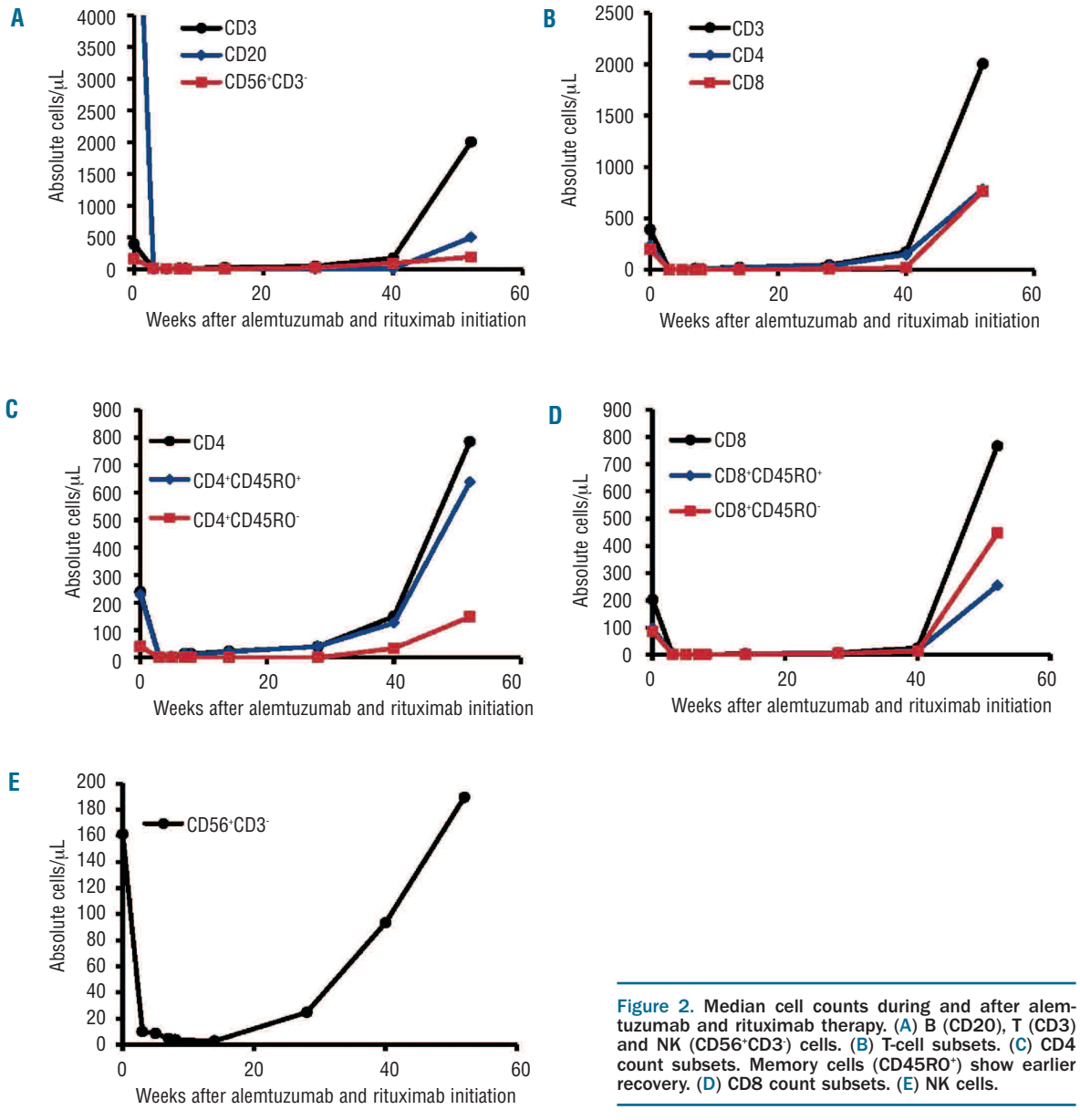

Figure 2. **Median cell counts during and after alemtuzumab and rituximab therapy. (A) B (CD20), T (CD3) and NK (CD56$^+$CD3$^-$) cells. (B) T-cell subsets. (C) CD4 count subsets. Memory cells (CD45RO$^+$) show earlier recovery. (D) CD8 count subsets. (E) NK cells.**

though the overall median concentration observed in this study, 237.0 μg/mL, was slightly greater than reported in prior studies employing the same dose given once a week.[23,25] This observation may be because rituximab is not as effective in CLL as in follicular lymphoma. Maximum trough alemtuzumab concentrations of at least 8 μg/mL in serum have been previously associated with CR in patients with relapsed CLL.[24] Consistent with previous studies, the maximum trough serum concentration of alemtuzumab achieved in our patients was highly variable, ranging from less than the lower limit of quantitation of the assay to 14.1 μg/mL. The findings of this study suggest that differences in bone marrow disease burden may be an important contributor to the interpatient variability in the systemic exposure to the drug. Specifically, the extent of bone marrow disease burden was correlated with lower maximum trough serum levels whereas higher trough levels were associated with disease clearance from bone marrow. However, the chances of achieving bone marrow clearance were not substantially reduced for patients with a packed bone marrow (33% *vs.* 43% for the overall population), suggesting that sensitivity to the drug is also an important determinant of response, independent of disease burden.

One of the goals of this study was to allow patients to achieve adequate cytoreduction to undergo allogeneic stem cell transplantation (SCT). By the time SCT is typically considered in CLL, the disease is often refractory, with limited remaining options to achieve cytoreduction. Here we show that the use of alemtuzumab facilitated SCT for 12 out of 23 patients who were otherwise good candidates, and previous work has suggested that undergoing SCT is associated with better survival.[3] The alemtuzumab typically cleared bone marrow disease and resolved cytopenias, while high-dose glucocorticoids were sometimes required after patients came off study to further shrink lymphadenopathy. Based on this observation from this trial, we are conducting a trial for 17p CLL

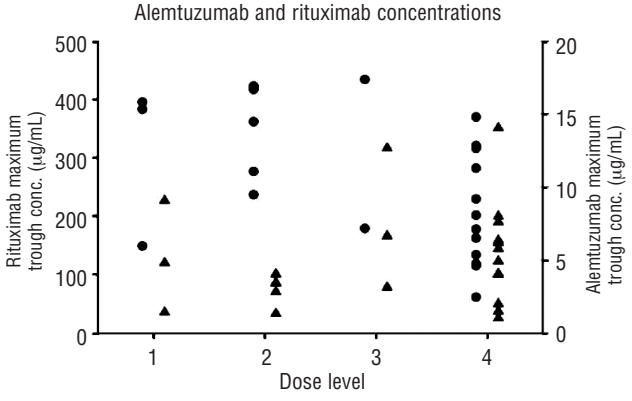

Figure 3. Maximum trough serum concentrations of rituximab (solid circles) and alemtuzumab (solid triangles) in patients evaluated at each dose cohort. No association is seen between drug levels and dose cohort.

patients in which nodal disease is first cytoreduced with high-dose methylprednisolone and ofatumumab, and bone marrow is then cleared with alemtuzumab and ofatumumab prior to SCT. Although in a couple studies alemtuzumab immediately prior to SCT has been associated with worse outcomes,[27] we have not observed that in our data,[28] and this association may be in part due to the adverse CLL biology of patients who require alemtuzumab for cytoreduction.

Recently the role of alemtuzumab therapy in CLL has been questioned since it has been withdrawn from the commercial market in order for its manufacturer to market it for multiple sclerosis. Nonetheless, the drug is still easily available on a compassionate use basis. Although use of alemtuzumab certainly requires active management of the infection risk, at present no other therapy is so effective at clearing bone marrow disease in relapsed refractory or genetically high-risk CLL. It remains to be seen if any of the novel targeted kinase inhibitors, BCL-2 family inhibitors or antibodies like GA101 will have similar activity. For now, alemtuzumab still fills a critical niche for a subset of relapsed refractory CLL patients, as illustrated by our results in this study.

Acknowledgments

We are indebted to the nurses and clinical research staff of the Dana-Farber Cancer Institute for their excellent care of these patients, and to the patients for their participation in this study.

Funding

JRB was supported by NIH grant K23 CA115682-01 and is a Scholar of the American Society of Hematology as well as a Scholar in Clinical Research of the Leukemia and Lymphoma Society.

Authorship and Disclosures

Information on authorship, contributions, and financial & other disclosures was provided by the authors and is available with the online version of this article at www.haematologica.org.

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