Citation: Blood Cancer Journal (2011) 1, e22; doi:10.1038/bcj.2011.20

www.nature.com/bcj

LETTER TO THE EDITOR

Virus reactivations and serology patterns following first-line therapy with alemtuzumab or fludarabine-based combination therapy in patients with chronic lymphocytic leukemia

Blood Cancer Journal (2011) **1,** e22; doi:10.1038/bcj.2011.20; published online 10 June 2011

Alemtuzumab (Campath, MabCampath) is a monoclonal antibody targeting the CD52 antigen. Recent guidelines recommend that this agent should be considered as first-line treatment in selected patients, in particular, those with 17p deletion.[1]

Importantly, alemtuzumab treatment requires special infection-related considerations, as the antibody causes severe and prolonged lymphocytopenia.[2] Anti-infective prophylaxis treatment and weekly monitoring for cytomegalovirus (CMV) are recommended.[1] However, viruses other than CMV may be reactivated following alemtuzumab therapy and cause complications.[3–6] This prompted us to analyze clinical and subclinical virus reactivations as well as serological changes in patients who had received alemtuzumab as first-line monotherapy[7] and to compare the results with patients treated with fludarabine-based combination therapy.

Eighteen chronic lymphocytic leukemia (CLL) patients (A1-18) who participated in a phase 2 study on subcutaneous alemtuzumab (30 mg three times per week for up to 18 weeks) as first-line therapy[7] were compared with 27 patients (C1-27, control group) treated with FC(R) (three received combination therapy with rituximab, FCR). Dosing of FC(R) was as follows: fludarabine given orally 40 mg/m^2 or intravenously (IV) 25 mg/m^2, days 1–3; cyclophosphamide 250 mg/m^2 orally or IV, days 1–3; rituximab 375 mg/m^2 IV, day 1 (first cycle) and 500 mg/m^2, day 1 (subsequent cycles). FC(R) was given at 28-day intervals. Patient characteristics at baseline are summarized in Table 1. All but two patients had CLL; one (C11) had small lymphocytic lymphoma and one (C21) had B-cell prolymphocytic leukemia.

Quantitative PCR was used to detect and measure the presence of CMV, Epstein-Barr virus (EBV) and human herpesvirus 6 (HHV-6) genomes at baseline, months 1, 2 and 3 during therapy, end of treatment, 6 and 8–12 months after end of therapy. Values $<$200 DNA copies/ml were considered negative. Qualitative PCR was used for parvovirus B19 detection.

Serology analyses were done at baseline; end of treatment and 6–12 months after end of treatment. A significant change of specific IgG serum content was defined as follows: difference in absorbance $>$0.4 for CMV, varicella zoster virus and EBV p107 (enzyme-linked immunosorbent assay); threefold change of the U value for measles (Enzygnost, Dade Behring Marburg GmbH, Marburg, Germany); and a fourfold change of the titer for EBV VCA (immunofluorescence).

Simultaneously, phenotyping of lymphocyte subpopulations was performed. The frequencies of major subpopulations, that is, CD4+/CD3+, CD8+/CD3+, CD3+/CD56+, CD3−/CD56+, CD19+/CD5−, were estimated by flow cytometry.

Response evaluation at end of therapy was performed using the NCI-IWCLL response criteria.[8] Assessment of adverse events was conducted according to the Common Terminology Criteria for Adverse events v.3.0 (CTCAE, 12 December 2003).

All alemtuzumab-treated patients received anti-infective prophylaxis consisting of valacyclovir, cotrimoxazole and fluconazole during therapy and for 8 weeks after completion of treatment (standard type and length of prophylaxis at time of trial).[7] One patient (A14) was not treated with cotrimoxazole because of hypersensitivity to this agent. In the control group 10 patients received acyclovir/valacyclovir and cotrimoxazole, one had valacyclovir only, 12 received cotrimoxazole only and four had no prophylaxis.

Fisher exact test (two-tailed) or χ^2-test (d.f. = 1) was utilized for comparison of the incidence of virus reactivations and changes of IgG levels. For comparison of different cell counts non-parametric independent Mann–Whitney signed-rank test was used.

A total of 440 PCR analyses were performed in the alemtuzumab-treated group, among which 11 (2.5%) were positive. All of these occurred during the time between baseline and 2 months of therapy (Table 2). In the control group, none of the 455 PCR analyzed samples were positive. The difference between the two groups was statistically significant ($P<0.001$). Three of the 11 positive PCR analyses (all EBV) in the alemtuzumab group occurred at baseline; two of these were also positive in the subsequent analysis. Thus, the incidence of treatment-related virus reactivations was 6/440 (1.4%) in the alemtuzumab-treated group and 0/455 in the control group ($P<0.05$).

All episodes of PCR positivity, of which all but three CMV reactivations were asymptomatic, resolved spontaneously. There was only one patient (A18) with a late reactivation during unmaintained follow-up. This patient had recurrence of symptomatic EBV reactivation (grade 3) 20 months after completion of alemtuzumab therapy. All patients with virus reactivation had responded to their anti-CLL treatment.

Sixteen of the alemtuzumab-treated patients and 17 of the control patients were evaluable with regard to differences in the IgG levels. Between baseline and 6–12 months post-therapy there were seven (8.9%) significant decreases and five (6.3%) significant increases detected among the alemtuzumab-treated patients, the corresponding figures for the controls were three (3.5%) and one (1.2%), respectively, (not significant) (Tables 3a and b).

The results of long-term analyses of immune subpopulations in the alemtuzumab group have been published.[2] We compared these results with the control group (data not shown). The median number of cells within each lymphocyte subpopulation at baseline was not statistically different. At end of treatment, the values for all subsets were significantly lower in the alemtuzumab group. At 8–12 months after alemtuzumab therapy, the number of cells had recovered and there was no statistically significant difference between the median values for the lymphocyte subpopulations. Interestingly, the alemtuzumab-treated patients with virus reactivation had, at end of treatment,

Table 1 Patient characteristics at baseline

Characteristic	Alemtuzumab (n = 18)		Fludarabine combination (n = 27)	
	No. of patients	%	No. of patients	%
Age, years				
Median	68		64	
Range	56–74		57–83	
Sex				
Male	10	56	18	67
Female	8	44	9	33
Rai stage				
0	0	0	0	0
I–II	5	28	14	52
III–IV	13	72	13	48
Time since initial diagnosis, months				
Median	28		29	
Range	1–264		1–131	
WHO performance status				
0–1	18	100	26	96
2–3			1	4
No. of prior anti-tumor regimens				
0	18	100	17	63
1			9	33
2			1	4
Type of prior anti-tumor therapy[a]				
Chlorambucil ± steroids			8	
Fludarabine+cyclophosphamide			2	
Alemtuzumab			1	
No. of patients with IgG below reference interval (<6.7 g/l)	10	56	10	37

Abbreviations: IgG, immunoglobulin G; WHO, World Health Organization.
[a]Median time from last prior treatment, months (range): 16 (4–100).

significantly higher median values of CD4+/CD3+, CD8+/CD3+ and CD3+/CD56+ cells than those without.

Seventeen of the 18 patients (94%) in the alemtuzumab group met the criteria for partial or complete remission.[7] The corresponding figures for the fludarabine combination-treated group were 24 of 26 evaluable patients (92%).

This study had some limitations. The reduced number of patients affected the statistical power, and it was a non-randomized comparison, even though we used consecutive control patients that were analyzed in a prospective fashion.

Table 3a Alemtuzumab-treated patients with one or more significant change of antivirus IgG level, values at 6–12 months post-therapy compared with baseline

Patient	Alemtuzumab (n = 16)[a]				
	CMV IgG	VZV IgG	Measles IgG	EBV p107 IgG	EBV VCA IgG
A4	0	0	0	0	+
A5	0	0	0	0	+
A8 (=C9)	–[b]	–	–	–	0
A12	0	0	–	0	0
A14	0	0	0	0	–
A15	0	0	+	–	0
A16	+	0	0	0	0
A18	0	+[c]	0	0	0
Total	1 decrease 1 increase	1 decrease 1 increase	2 decrease 1 increase	2 decrease	1 decrease 2 increase

Abbreviations: CMV, cytomegalovirus; EBV, Epstein-Barr virus; IgG, immunoglobulin G; VZV, varicella zoster virus; 0, no significant change; –, significant decrease; +, significant increase.
[a]Two of the totally 18 patients were excluded; one because of IV γ-globulin treatment (A1) and the other because of shorter follow-up than 6 months (A16). *N* = 15 for EBV p107G because of sample shortage in one patient.
[b]Symptomatic reactivation, grade I, 2 months after start of treatment.
[c]Symptomatic reactivation, grade I, 10 months after end of treatment.
All, but patient A16, responded to alemtuzumab treatment.

Table 2 Alemtuzumab-treated patients with positive virus PCR; virus, copy numbers (no of genome equivalents/ml) and symptoms

Patient (n = 18)	Time point of PCR analyses						
	Baseline (n = 18)	1 month (n = 15)	2 months (n = 13)	3 months (n = 12)	End of treatment (n = 18)	6 months post-therapy (n = 17)	12 months post-therapy (n = 17)
A4	N	N	CMV 11 600	N	N	N	N
A5	N	N	CMV 81 400 cough	N	N	N	N
A6	EBV 2000	EBV 1800[a]	ND	N	N	N	N
A7	EBV 2600	EBV 2000	ND	ND	N	N	N
A7	N	HHV-6 1300	ND	ND	N	N	N
A8	N	N	CMV 8600 fever	N	N	N	N
A11	N	CMV 12 900	N	N	N	N	N
A12	N	N	CMV 7900 fever	N	N	N	N
A18	EBV 2600	N	N	N	N	N	N
Total 8 (44%)	Total 3 (17%)	Total 4 (27%)	Total 4 (31%)	Total 0	Total 0	Total 0	Total 0

Abbreviations: CMV, cytomegalovirus; EBV, Epstein-Barr virus; HHV-6, human herpesvirus 6; N, negative; ND, not determined.
[a]2 weeks after start of therapy.

Table 3b Fludarabine combination-treated patients with one or more significant change of antivirus IgG level, values at 6–12 months post-therapy compared with baseline

Patient	*Fludarabine combination* (n = 17)[a]				
	CMV IgG	*VZV IgG*	*Measles IgG*	*EBV p107 IgG*	*EBV VCA IgG*
C3[b]	0	0	0	0	+
C11	0	0	0	0	−
C17	0	0	0	0	−
C18	−	0	0	0	0
Total	1 decrease	0	0	0	2 decrease 1 increase

Abbreviations: CMV, cytomegalovirus; EBV, Epstein-Barr virus; IgG, immunoglobulin G; VZV, varicella zoster virus; 0, no significant change; −, significant decrease; +, significant increase.
[a]Nine of the totally 27 patients were not analyzed because of short follow-up (<6 months). One patient (C13) was excluded because of alemtzumab-treatment before the +12 month sample.
[b]C3 = second-line patient (FC), all the others first-line patients.
All patients responded to fludarabine combination treatment.

Our data demonstrates that, except for CMV, there was no major increase in incidence of virus reactivation following first-line subcutaneous alemtuzumab compared with the FC(R)-treated controls. The number of significant antivirus IgG decreases or increases did not differ significantly between the two treatment groups; however, the titer decreases noted in individual patients raises the question of whether such patients might need special infection-preventive measures to avoid reinfection.

Conflict of interest

Claes Karlsson, Jeanette Lundin and Anders Österborg have received research support and honoraria for lectures from Genzyme Corporation.

Acknowledgements

This study was supported by the Swedish Cancer Society, the Cancer Society in Stockholm, the Cancer and Allergy Foundation, the Karolinska Institutet Foundations, Roche AB, Sweden, Bayer-Schering Pharma, Germany and Genzyme Corporation, USA. We thank Leila Relander for her excellent secretarial help and Leslie Fenton for linguistic check of the manuscript.

C Karlsson[1,2], H Dahl[3], J Lundin[1,2], E Rossmann[1,2], M Brytting[3], H Mellstedt[1], A Linde[4] and A Österborg[1,2]
[1]Department of Oncology-Pathology, Karolinska Institutet, Stockholm, Sweden;
[2]Department of Hematology, Karolinska University Hospital, Stockholm, Sweden;
[3]Department of Diagnostics and Vaccinology, Swedish Institute for Communicable Disease Control, Stockholm, Sweden and
[4]Department of Analysis and Prevention, Swedish Institute for Communicable Disease Control, Stockholm, Sweden
E-mail: claes.karlsson@karolinska.se

References

1 Osterborg A, Foa R, Bezares RF, Dearden C, Dyer MJ, Geisler C *et al.* Management guidelines for the use of alemtuzumab in chronic lymphocytic leukemia. *Leukemia* 2009; **23**: 1980–1988.
2 Lundin J, Porwit-MacDonald A, Rossmann ED, Karlsson C, Edman P, Rezvany MR *et al.* Cellular immune reconstitution after subcutaneous alemtuzumab (anti-CD52 monoclonal antibody, CAMPATH-1H) treatment as first-line therapy for B-cell chronic lymphocytic leukaemia. *Leukemia* 2004; **18**: 484–490.
3 O'Brien SM, Kantarjian HM, Thomas DA, Cortes J, Giles FJ, Wierda WG *et al.* Alemtuzumab as treatment for residual disease after chemotherapy in patients with chronic lymphocytic leukemia. *Cancer* 2003; **98**: 2657–2663.
4 Wendtner CM, Ritgen M, Schweighofer CD, Fingerle-Rowson G, Campe H, Jager G *et al.* Consolidation with alemtuzumab in patients with chronic lymphocytic leukemia (CLL) in first remission—experience on safety and efficacy within a randomized multicenter phase III trial of the German CLL Study Group (GCLLSG). *Leukemia* 2004; **18**: 1093–1101.
5 Herbert KE, Prince HM, Westerman DA. Pure red-cell aplasia due to parvovirus B19 infection in a patient treated with alemtuzumab. *Blood* 2003; **101**: 1654.
6 Crowley B, Woodcock B. Red cell aplasia due to parvovirus b19 in a patient treated with alemtuzumab. *Br J Haematol* 2002; **119**: 279–280.
7 Lundin J, Kimby E, Bjorkholm M, Broliden PA, Celsing F, Hjalmar V *et al.* Phase II trial of subcutaneous anti-CD52 monoclonal antibody alemtuzumab (campath-1H) as first-line treatment for patients with B-cell chronic lymphocytic leukemia (B-CLL). *Blood* 2002; **100**: 768–773.
8 Cheson BD, Bennett JM, Grever M, Kay N, Keating MJ, O'Brien S *et al.* National Cancer Institute-sponsored Working Group guidelines for chronic lymphocytic leukemia: revised guidelines for diagnosis and treatment. *Blood* 1996; **87**: 4990–4997.