Alemtuzumab as Remission Induction Therapy in Behçet Disease: A 20-year Experience

Aladdin J. Mohammad, Rona M. Smith, Yok W. Chow, Afzal N. Chaudhry, and David R.W. Jayne

ABSTRACT. ***Objective.*** To study the efficacy and safety of anti-CD52 antibody (alemtuzumab) in the treatment of refractory and relapsing Behçet disease (BD).

Methods. Thirty-two patients (22 women) with BD received 60 courses of alemtuzumab between 1994 and 2013. Three-dose regimens were used: 134 mg in 21 courses (Group 1), 95 mg in 18 courses (Group 2), and 60 mg in 21 courses (Group 3). Immunosuppressive drugs were stopped at the time of alemtuzumab, and prednisolone was reduced according to clinical response. Treatment response was assessed by clinical status, inflammatory activity, prednisolone dose, and the need for subsequent immunosuppressive drugs and disease relapse.

Results. After the first alemtuzumab course, 27 of 32 patients (84%) achieved partial or complete remission (CR). Fifty of 60 courses (83%) resulted in remission (66% CR) without differences in remission rates between dosing regimens. Profound lymphocyte depletion occurred after all courses. Relapse-free survival rates were 83.6% at 6 months and 52.8% at 12 months, and were higher among Group 1 patients (Group 1: 100% and 77.8%, Group 2: 81.3% and 37.5%, and Group 3: 65.0% and 37.1%, $p < 0.001$). Mild to moderate infusion reactions occurred after 16 courses (27%). Eight patients (25%) developed symptomatic thyroid disease.

Conclusion. Alemtuzumab led to remission in the majority of patients with difficult-to-treat BD. Relapse was common and may be associated with lower dosing. Adverse events included infusion reactions and new autoimmunity. Achieving complete lymphocyte depletion did not affect the remission rate or duration. (J Rheumatol First Release August 15 2015; doi:10.3899/jrheum.141344)

Key Indexing Terms:
BEHÇET DISEASE REMISSION INDUCTION ALEMTUZUMAB
LYMPHOCYTE DEPLETION THERAPY THYROID DISEASE

Behçet disease (BD) is a chronic relapsing inflammatory disease of unknown etiology characterized by recurrent oral and genital ulcerations with other systemic manifestations, the most serious of which are neurological, gastrointestinal, and vascular, and most are believed to be vasculitic in nature. BD lacks pathognomonic symptoms and laboratory or histological findings, and diagnosis relies on clinical criteria, such as those of the International Study Group (ISG) for BD[1]. The pathogenesis of BD is likely to be multifactorial, with infectious agents and aberrations in B and T cell functions being implicated on a background of genetic predisposing factors, such as the presence of the HLA-B51 allele[2,3].

There is evidence that T cells are involved in the pathogenesis of BD. It has been postulated that heat shock proteins or bacterial antigens are involved in stimulating oligoclonal T cell expansion[4,5]. Adenosine deaminase, an enzyme involved in lymphocyte proliferation, maturation, and differentiation, is activated in BD, particularly in disease exacerbations[6]. Histopathology specimens demonstrate predominantly T cell infiltrates in skin and oral lesions[7,8]. Studies have shown both a Th1-predominant response with corresponding increases in Th1-produced cytokines, namely interleukin (IL) 2, IL-6, IL-8, IL-12, IL-18, tumor necrosis factor-α (TNF-α), and interferon-γ, and a Th2 response with the production of IL-4, IL-10, and IL-13. It is likely that there is a combination of Th1 and Th2 activity[9,10,11]. Analysis of T cell subpopulations has shown relative increases in the CD8:CD4 ratio with reductions in the percentage of regulatory T cells, particularly in patients with active disease[12].

From the Department of Renal Medicine, Addenbrooke's Hospital, Cambridge, UK; Department of Clinical Sciences, Rheumatology, Lund University, Lund, Sweden; Department of Medicine, Hospital Pantai Ayer Keroh, Melaka, Malaysia.

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A.J. Mohammad, MD, PhD, Department of Renal Medicine, Addenbrooke's Hospital, and Department of Clinical Sciences, Rheumatology, Lund University; R.M. Smith, MA, MB BChir, MRCP, Department of Renal Medicine, Addenbrooke's Hospital; Y.W. Chow, MD(USM), MRCP(UK), MRCPE, FRCP(Edinburgh), AM(MAL), Department of Medicine, Hospital Pantai Ayer Keroh Melaka; A.N. Chaudhry, MBBS, PhD, FRCP, FHEA, FACad MEd, Department of Renal Medicine, Addenbrooke's Hospital; D.R. Jayne, MD, FRCP, Department of Renal Medicine, Addenbrooke's Hospital.

Address correspondence to Dr. A.J. Mohammad, Box 57, Renal Medicine, Addenbrooke's Hospital, Hills Road, Cambridge, CB20QQ, UK. E-mail: aladdin.mohammad@med.lu.se

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Therefore, T cell-directed agents would seem to be an appropriate therapeutic strategy in BD. Cyclosporine has shown efficacy in uveitis of BD[13]. Lymphocyte depletion using antithymocyte globulin has also induced remission in BD, but its use is limited by the development of an antiglobulin response[14]. It was thought that humanized antibodies would avoid significant antiglobulin responses and allow repeated dosing that is important in a chronic relapsing condition such as BD. Alemtuzumab is an anti-CD52 humanized monoclonal antibody. CD52 is expressed on lymphocytes and macrophages, and alemtuzumab causes profound T cell depletion that is particularly prolonged for the CD4+ subset and leads to a relative expansion of regulatory T cells[15,16]. Alemtuzumab is capable of inducing sustained remission in other autoimmune conditions, such as granulomatosis with polyangiitis, multiple sclerosis, and inflammatory eye disease[17,18,19,20]. We present a single center's 20-year experience of using alemtuzumab in BD, including longterm followup data on a report of a previous cohort in 2003[21], as well as data on patients subsequently treated with alemtuzumab.

MATERIALS AND METHODS

Thirty-three patients with refractory or relapsing BD have been treated at Addenbrooke's Hospital, Cambridge, UK, with alemtuzumab between 1994 and 2013. One patient (female, 49 yrs old) developed a severe reaction during the test dose of 4 mg alemtuzumab. No further alemtuzumab was given and the patient was not included in further analyses. All patients were over 18 years of age and met the ISG diagnostic criteria for BD[1]. Contraindications to alemtuzumab included (1) coexistence of another multisystem autoimmune disease; (2) hepatitis B or C, human immunodeficiency virus infection, or other active infection requiring antibiotic therapy; (3) previous history of malignancy; and (4) current pregnancy. Data on 18 patients included in our report were reported in 2003[21]. In accordance with the UK National Health Service (NHS) Research Ethics Committee guidelines, ethical approval was not required for this work because it consisted of retrospective data and all treatment decisions were made prior to our evaluation.

Treatment. Alemtuzumab was administered intravenously. Premedication with 250 mg intravenous (IV) methylprednisolone and 10 mg IV chlorpheniramine was given before each infusion. Acyclovir, nystatin, and cotrimoxazole were recommended as infection prophylaxis for up to 3 months after each infusion. Any other immunosuppressive agent that the patient was taking was withdrawn at the time of alemtuzumab administration. Prednisolone was continued after alemtuzumab and tapered according to the clinical status. Three different dose schedules and alemtuzumab preparations were used: Group 1 (18 patients, total 21 courses) received a total dose of 134 mg (4 mg, 10 mg, 40 mg, 40 mg, and 40 mg on 5 consecutive days) between 1994 and 2000; Group 2 (7 patients, 18 courses) received 95 mg (5 mg, 30 mg, 30 mg, and 30 mg over 4 consecutive days) between 2002 and 2009; and Group 3 (7 patients, 21 courses) received 60 mg (30 mg/day for 2 consecutive days) from 2010, the most recent cohort of patients. During the study time, alemtuzumab doses were reduced from 134 mg to 95 mg and currently 60 mg per course. The reasons for dose reduction have been, first, that lower doses achieve lymphocyte depletion efficiently; second, a desire to reduce the infective toxicity that is related to the duration of lymphopenia, in turn related to the alemtuzumab dose; and third, patient convenience and cost.

Data collection. Data collection included baseline demographics and clinical data at time of diagnosis of BD. In addition, laboratory variables (including total lymphocyte count, CD4+, CD8+, and CD19+ cell levels, and thyroid function tests) were collected prior to each infusion of alemtuzumab and sequentially at 3, 6, 9, and 12 months after each infusion course. Complete depletion of lymphocytes was defined as lymphocytes $< 0.01 \times 10^9$/l. Date of lymphocyte recovery was defined as the first date that the level returned to normal limits ($1.00–2.80 \times 10^9$/l). Complete remission (CR) was defined as complete resolution of all clinical symptoms and signs attributable to BD. Partial remission (PR) was defined as substantial resolution of clinical symptoms and signs attributed to BD. Relapse was defined as the recurrence of symptoms and signs of BD that led to reinstitution of any immunosuppressive therapy or an increased prednisolone requirement. Refractory disease was defined as the persistence of disease manifestations not responding to conventional treatments including high-dose steroids or addition of immunosuppressive agent including biologic agents. Infusion reactions were defined as any allergic skin reactions, hypotension, difficulty in breathing, or unexplained circulatory deterioration that developed during or within a few hours postinfusion. Thyroid disease was defined as abnormal thyroid function tests accompanied by signs and symptoms of thyroid disease that developed after the onset of treatment with alemtuzumab. Severe disease was defined as the presence of at least 1 of any of the following manifestations: eye disease (panuveitis, bilateral uveitis, retinal vasculitis, and severe visual impairment), nervous system disease (parenchymal or nonparenchymal brain disease or severe peripheral nervous system disease), vascular disease (major vessel occlusion or thromboembolic disease), and gastrointestinal disease (gastrointestinal bleeding or perforation).

Statistical analyses. Differences in categorical variables between groups were studied using the Fisher's exact test and chi-square test. Continuous variables were presented as medians and interquartile ranges (IQR) unless otherwise stated. Differences in continuous variables between groups were first tested by the nonparametric Kruskal-Wallis test. If significant differences were present, then further analysis by the nonparametric Mann-Whitney U test was done to study differences between groups. A p value of < 0.05 was considered significant. Patients' survival and relapse-free survival were estimated by using the Kaplan-Meier method. Statistical analyses were performed using the Statistical Package for the Social Sciences; SPSS 20.0 for Windows (IBM SPSS 20.0.0).

RESULTS

Patients. Thirty-two patients (22 women) with refractory or relapsing BD received 60 courses of alemtuzumab during 20 years (1994–2013). Seventeen received 1 course, 7 received 2 courses, 5 received 3 courses, 1 received 4 courses, and 2 received 5 courses.

The clinical and demographic characteristics of patients and treatment prior to alemtuzumab are shown in Table 1. Eighteen patients had at least 1 severe disease manifestation (Supplementary Table 1 available online at jrheum.org). The remaining 14 patients received alemtuzumab because of failure of other treatments or unacceptable adverse events from other medications.

Remission. After the first alemtuzumab course, 27 of 32 patients (84%) achieved PR or CR: 16/18 (89%) in Group 1, 5/7 (71%) in Group 2, and 6/7 (86%) in Group 3 ($p = 0.801$). Remission was achieved after 50 of 60 alemtuzumab courses (83%): 19/21 (90%) in Group 1, 16/18 (89%) in Group 2, and 15/21 (71%) in Group 3 ($p = 0.257$). CR was achieved in 33/50 courses (66%) and PR in 17/50 courses (34%). The distribution of CR to PR (%CR:%PR) in the treatment groups was 68:32 for Group 1, 75:25 for Group 2, and 53:47 for Group 3. Ten courses [Group 1: 2 courses (9%), Group 2: 2 courses (11%), and Group 3: 6 courses (28%)] did not result

Table 1. Clinical and demographic characteristics in 32 patients with BD treated with ALM (alemtuzumab). Values are median (IQR) and n (%) unless otherwise specified.

Variables/features	Results
Female/male, n	22/10
Age at diagnosis, yrs	32 (25–38)
Time from diagnosis to first ALM, mos	29 (8–84)
Time from first ALM course to last followup or death, mos	153 (39.5–180)
Time from most recent, last, ALM course to death, 4 patients, mos	43.5 (17.2–87.7)
Time from first ALM course to thyroid disease, 8 patients, mos	17 (12–62)
Time from remission to relapse, 50 courses, mos	11 (7–28)
Organ involvement at presentation or any time during disease course	
Recurrent oral ulcerations	31 (97)
Recurrent genital ulcerations	30 (94)
Eye lesions	21 (66)
Skin disease	25 (78)
Joint involvement	25 (78)
CNS disease	15 (47)
Gastrointestinal disease	12 (38)
Vascular, including DVT	7 (22)
Peripheral nervous system disease	3 (9)
Pulmonary disease	3 (9)
Renal involvement	1 (3)
Patients with severe disease*, n (%)	18 (56)
Nervous system disease	7 (22)
Eye disease	11 (34)
Vascular disease, including VTE and peripheral vascular disease	4 (13)
Gastrointestinal disease	2 (6)
Patients treated with > 5 immunosuppressives prior to ALM	17 (53)
Patients treated with at least 1 anti-TNF-α prior to ALM	12 (37.5)
Treatment prior to ALM, n	
Glucocorticoids	26
Glucocorticosteroids, monotherapy	3
Plasma exchange	1
Other immunosuppressive drugs	
Azathioprine	14
Cyclosporine	12
Anti-TNF-α	12
MMF, thalidomide, hydroxychloroquine, each	10
Tacrolimus	9
Colchicine	8
MTX and IVIG, each	6
CYC	5
Rituximab	3

* Severe disease manifestation in at least 1 organ system. BD: Behçet's disease; IQR: interquartile range; CNS: central nervous system; DVT: deep vein thrombosis; VTE: venous thromboembolic disease; TNF-α: tumor necrosis factor-α; MMF: mycophenolate mofetil; MTX: methotrexate; IVIG: intravenous immunoglobulin; CYC: cyclophosphamide.

in remission. There were no differences in age at diagnosis, BD duration, or clinical features when compared with those achieving remission (data not shown). Complete lymphocyte depletion was achieved in only 2/10 (20%) of courses that failed to induce remission, compared with 25/50 (50%) for courses that resulted in remission (p = 0.162). When considering the 50 courses that resulted in remission, complete lymphocyte depletion was achieved in 4/17 (24%) of courses that resulted in PR compared with 21/33 (64%) of courses that led to CR (p = 0.007). Among courses that resulted in CR, median prednisolone dose at entry was 20 mg per day (IQR 10–30), and decreased to 4 mg (IQR 0–10) per day after 12 months. The corresponding figures for single courses, which resulted in remission, were 15 mg per day (IQR 7–20) decreased to 7 mg per day (IQR 0–12.5). Sixteen out of 18 patients (89%) with severe disease achieved remission (11 CR, 5 PR). All patients with severe nervous system disease and severe eye disease achieved remission (data not shown). Of the 11 patients previously treated with anti-TNF-α, 8 (73%) achieved remission. No difference was evident in rates of remission among patients treated with < 5 immunosuppressive drugs prior to alemtuzumab (87%) compared with those who were treated with > 5 immunosuppressive drugs (82%). However, 12/13 patients treated with < 5 drugs who

achieved remission had CR compared to 6/14 among those treated with > 5 drugs (p = 0.006). The 3 patients previously treated with rituximab (RTX) achieved remission in 6 months and 2 were in remission 12 months after alemtuzumab.

Relapse rates and relapse-free survival. Relapse occurred after 34/50 courses (68%) in 16/27 patients (59%) during followup (median 153.5 mos, IQR 39.5–180). Among 43 repeated courses, 6 courses (14%) had secondary failure (moved from any remission to no response to subsequent course). Relapses were not related to normalization of lymphocytes or CD4+ or CD8+ T cell levels at last followup. For all the alemtuzumab courses administered, the estimated relapse-free survival rate was 84% at 6 months and 53% at 12 months (Supplementary Figure 1 available online at jrheum.org). When analysis was restricted to the first alemtuzumab treatment (n = 32 patients), the relapse-free survival rate for all patients was 88.5% at 6 months and 69% at 12 months (Supplementary Figure 2 available online at jrheum.org). After stratifying courses into the 3 different dose groups, the relapse-free survival rates for Group 1 were 100% and 77.8%; for Group 2, 81% and 38%; and for Group 3, 65% and 37.1% at 6 months and 12 months, respectively (p < 0.001; Figure 1). When considering relapse-free survival rates after the first alemtuzumab course only, the corresponding figures for Groups 1 and 2 were 100% and 80%, and for Group 3, 50% and 33% (p = 0.038; Supplementary Figure 3 available online at jrheum.org). There were no differences in relapse-free survival between those with and without complete lymphocyte depletion (data not shown). Of note, 8 of the 50 courses that achieved remission had remission duration of > 60 months (mean 144.6 mos, range 66–219), and 6 of them had remission duration of more than 100 months (mean 168 mos, range 106–219). Eleven patients (9 in Group 1) did not have any relapse during the followup since alemtuzumab treatment.

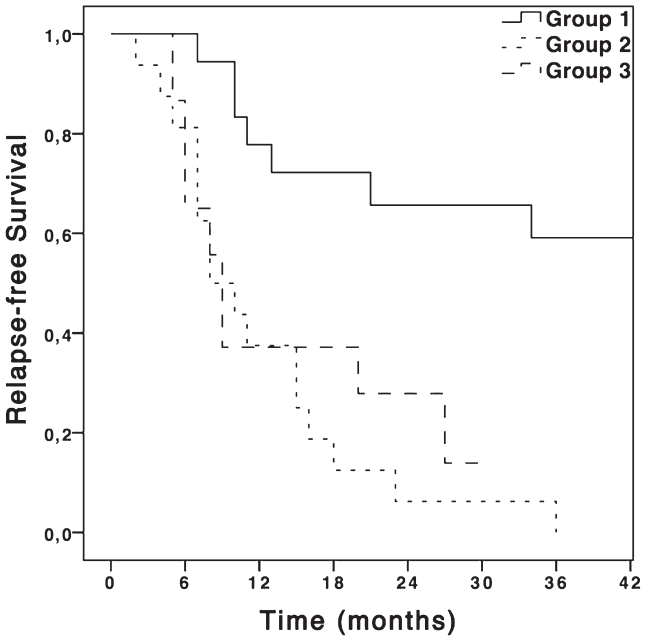

Figure 1. Kaplan–Meier curves showing relapse-free survival after all courses of alemtuzumab (50 remissions) per treatment groups (p < 0.001).

Laboratory investigations. Changes in the levels of lymphocytes and their subsets are shown in Table 2 and Figure 2. Total lymphocytes, CD4+, and CD8+ T cells remained low during 12 months of followup after alemtuzumab (Figure 2). Laboratory data on lymphocyte subsets from last followup were available for 27 patients. Median time from most recent alemtuzumab course to last laboratory assessment of lymphocyte subsets was 18 months (IQR 8–78), and was shorter for Group 3 (8.5 mos, IQR 2–22.2) than Group 1 (78 mos, IQR 17.5–122.5) and Group 2 (62.5 mos, IQR 11.7–89; p = 0.003). At last followup, the median total lymphocyte count was 1.20 (IQR 0.63–1.72), and the corresponding figures for the CD4+ and CD8+ cells were 0.38 (IQR 0.16–0.62) and 0.33 (IQR 0.18–0.48), respectively.

Lymphocyte depletion. Lymphocyte depletion occurred after all treatment courses. Data on the total lymphocyte count within 1 month from treatment were available for 52 of 60 courses (87%); after that, profound lymphocyte depletion (more than 72% decline from baseline lymphocyte count) occurred in all cases. However, complete lymphocyte depletion occurred after just 27/60 courses (45%): 15/21 (71%) in Group 1 compared with 11/18 (61%) and 1/21 (5%) in Groups 2 and 3, respectively (p < 0.001). There were no differences in remission rates between courses with and without complete lymphocyte depletion (93% vs 76%, p = 0.162). However, among patients who achieved complete lymphocyte depletion and entered remission, 84% had CR and 16% had PR compared with 48% and 52%, respectively, among those without complete depletion (p = 0.007). There were no differences in remission duration, relapse rate, occurrence of thyroid disease, or infusion reactions between patients with and without complete lymphocyte depletion (data not shown). After courses that resulted in complete lymphocyte depletion, recovery of lymphocytes to normal level occurred in 20 patients (12 in Group 1 and 8 in Group 2) at a median time of 9 months (IQR 5–23): 5.5 months (IQR 4.25–34.5) in Group 1 and 10.5 months (IQR 8–13.7) in Group 2 (p = 0.723). No differences in level of total lymphocyte, CD4+, and CD8+ cell counts during the 12 months following alemtuzumab were observed when comparing patients who experienced relapse with those who did not (data not shown).

Infections. Five patients experienced respiratory infections (including pneumonia) that were successfully treated with antibiotics (Group 1: 4 patients, Group 2: 1 patient). One developed *Clostridium difficile* colitis (Group 1) that was antibiotic-related, and another developed neutropenia (with no infectious complication) that responded to granulocyte colony-stimulating factor (Group 3). Opportunistic infections were not seen.

Table 2. Prednisolone doses and levels of lymphocytes, CD4+, CD8+, and CD19+ cells in 32 patients with BD treated with 60 courses of alemtuzumab. Treatment Group 1 took 134 mg, treatment Group 2, 95 mg, and treatment Group 3, 60 mg. Measures of CD4+, CD8+, and lymphocytes are given in $\times 10^9$/l. The reference normal value ($\times 10^9$/l) of the total lymphocytes is 1.00–2.80, for CD4+, 0.30–1.40, and for CD8+, 0.20–0-90. Values are median (IQR) unless otherwise specified.

Variables	All Courses, n = 60	Group 1, n = 21	Group 2, n = 18	Group 3, n = 21	p
Prednisolone, mg, 0M	12.5 (10.0–20.0)	15.0 (8.0–25.0)	11.0 (10.0–18.5)	12.5 (7.5–22.5)	0.925
Prednisolone, mg, 3M	7.5 (0–10.0)	7.5 (1.5–13.1)	7.5 (0–10.0)	8.0 (0–10.0)	0.931
Prednisolone, mg, 6M	6.0 (0–10.0)	5.0 (0–15.0)	10.0 (0–13.0)	7.5 (0–10.0)	0.904
Prednisolone, mg, 9M	7.7 (2.8–10.0)	6.7 (1.8–11.6)	10.0 (2.0–13.0)	8.0 (3.7–10.0)	0.920
Prednisolone, mg, 12M	10.0 (2.0–13.0)	7.0 (1.0–10.0)	13.0 (2.5–41.5)	10.0 (4.0–15.0)	0.530
CD4+ 0M	0.54 (0.23–1.26)	1.09 (0.43–1.36)	0.39 (0.18–1.31)	0.54 (0.18–1.16)	0.487
CD4+ 3M	0.07 (0.04–0.11)	0.07 (0.03–0.15)	0.08 (0.06–0.25)	0.07 (0.03–0.09)	0.254
CD4+ 6 M	0.12 (0.09–0.23)	0.16 (0.11–0.30)	0.11 (0.07–0.15)	0.11 (0.08–0.16)	0.346
CD4+ 9M	0.17 (0.09–0.29)	0.18 (0.09–0.32)	0.18 (0.09–0.24)	0.16 (0.03–0.32)	0.923
CD4+ 12M	0.18 (0.13–0.43)	0.18 (0.13–0.45)	0.19 (0.15–0.43)	0.18 (0.06–0.42)	0.809
CD8+ 0M	0.48 (0.17–0.81)	0.72 (0.39–0.82)	0.51 (0.20–0.81)	0.29 (0.13–0.82)	0.419
CD8+ 3M	0.14 (0.06–0.22)	0.13 (0.07–0.22)	0.22 (0.16–0.31)	0.06 (0.02–0.09)	0.004*
CD8+ 6M	0.16 (0.08–0.28)	0.25 (0.15–0.40)	0.19 (0.12–0.30)	0.07 (0.04–0.16)	0.019**
CD8+ 9M	0.15 (0.09–0.34)	0.25 (0.13–0.34)	0.13 (0.09–0.40)	0.13 (0.02–0.19)	0.326
CD8+ 12M	0.20 (0.12–0.36)	0.26 (0.17–0.56)	0.17 (0.11–0.46)	0.13 (0.04–0.24)	0.180
L 0M	1.5 (1.0–2.4)	2.1 (1.5–2.8)	1.35 (1.07–1.82)	1.28 (0.52–2.3)	0.043***
L 3M	0.50 (0.27–0.75)	0.45 (0.28–0.80)	0.50 (0.40–1.01)	0.43 (0.17–0.58)	0.223
L 6M	0.72 (0.50–1.10)	1.1 (0.55–1.3)	0.69 (0.50–1.00)	0.65 (0.46–0.81)	0.241
L 9M	0.83 (0.50–1.30)	0.70 (0.50–1.3)	0.83 (0.40–1.4)	0.96 (0.38–1.21)	0.926
L 12M	1.0 (0.65–1.48)	0.91 (0.65–1.75)	1.00 (0.70–1.4)	1.13 (0.38–1.33)	0.740
CD19+ 0M	0.28 (0.05–0.45)	0.17 (0.06–NA)	0.15 (0.04–0.30)	0.37 (0.06–0.58)	0.232
CD19+ 3M	0.19 (0.04–0.31)	0.03 (0.0–NA)	0.11 (0.05–0.49)	0.23 (0.19–0.31)	0.266
CD19+ 6M	0.23 (0.04–0.50)	0.40 (0.0–NA)	0.07 (0.02–0.39)	0.28 (0.11–0.59)	0.395
CD19+ 9M	0.15 (0.02–0.44)	NA	0.07 (0.02–0.50)	0.38 (0.0–0.41)	0.964
CD19+ 12M	0.27 (0.03–0.51)	NA	0.27 (0.05–0.35)	0.41 (0.0–0.83)	0.638

Differences among groups were first studied by the nonparametric Kruskal-Wallis test. If significant differences were present, then further analysis by the nonparametric Mann-Whitney U test was performed; the results were as follows: * significant differences between Group 1 and Group 3, p = 0.027, Group 2 and Group 3, p = 0.002; ** significant differences between Group 1 and Group 3, p = 0.016, Group 2 and Group 3, p = 0.018; *** significant differences between Group 1 and Group 2, p = 0.045, Group 1 and Group 3, p = 0.026. BD: Behçet disease; IQR: interquartile range; p: differences between groups (all); 0M, 3M, 6M, and 12 M: at time of treatment, 3 months, 6 months, 9 months, and 12 months posttreatment, respectively; L: total lymphocyte count; NA: not available.

Malignancies. One patient had developed esophageal carcinoma, successfully treated with radiotherapy in 2007, 9 years after alemtuzumab (Group 1), and 1 was diagnosed with glioblastoma multiforme 5 years after his single alemtuzumab course (Group 2).

New autoimmunity. Eight patients (25%) developed symptomatic thyroid disease after treatment: 4 received a single course and 4 were treated with repeated courses. Four had thyrotoxicosis, 3 had hypothyroidism, and 1 developed autoimmune thyroiditis. One of these patients had known thyroid disease that deteriorated 9 months after alemtuzumab. All patients were women and all were from either Group 1 (5 patients) or Group 2 (3 patients). The median time from first alemtuzumab infusion and the onset of thyroid disease was 17 months (IQR 12.2–26.2). One patient (3%) developed autoimmune hemolytic anemia that required glucocorticoids and RTX; of note, this patient received a large cumulative dose and developed this after a fifth course of alemtuzumab.

Infusion reactions. There were 16 infusion reactions (2 in Group 1, 5 in Group 2, and 9 in Group 3; p = 0.058) after 60 courses (27%) in 8 patients. Fourteen reactions were mild and 2 were moderates. All reactions abated at the end of the infusion.

Patient survival. Four patients (1 woman), 2 in Group 1 and 2 in Group 2, died during followup. The ages of patients who died were 33, 39, 43, and 47 years at diagnosis and 69, 45, 61, and 51 at date of death, respectively. Deaths occurred after a median time of 43.5 months from the most recent alemtuzumab therapy (IQR 17.2–87.7). For all patients, the estimated survival rate was 97% at 5 years and 93% at 10 years. The causes of death were glioblastoma, cardiac events while in remission (Group 1: 3 courses), infection (Group 1: 96 mos after single course), and active pulmonary vasculitis (Group 2: 2 courses within 6 mos).

DISCUSSION

The management of BD, a rare condition with wide-ranging clinical manifestations, is a challenge, particularly in those patients with relapsing or refractory disease. However, response rates to alemtuzumab were impressive in this cohort. Overall, 84% of patients achieved remission. This is notable considering the refractory nature of disease in these

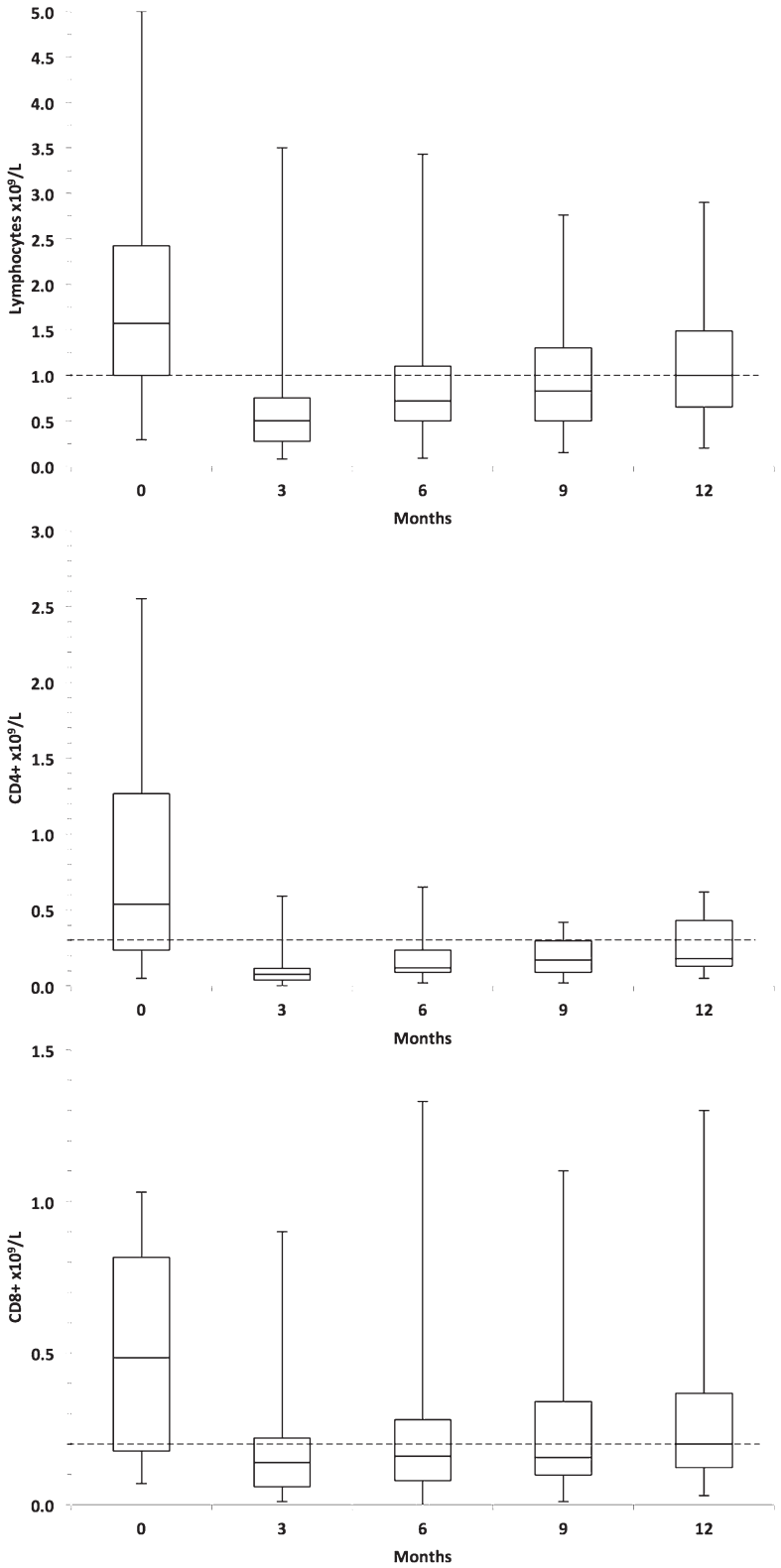

Figure 2. Box plot of total lymphocytes, CD4+, and CD8+ positive cell counts at baseline, 3, 6, 9, and 12 months after treatment with alemtuzumab in patients with relapsing/refractory Behçet disease treated with a total of 60 courses alemtuzumab. Horizontal dotted lines indicate the lower normal value for cells.

patients. The advent of anti-TNF therapy means that many of the more recently treated patients have also failed this newer treatment modality, further emphasizing the refractory nature of their disease because reported response rates to infliximab, particularly in Behçet eye disease, have been excellent[22,23]. However, remissions are not always sustained and there is an unmet need for further therapeutic agents to induce lasting remission[24]. Alemtuzumab decreased the use of prednisolone in all patients who experienced remission despite stopping all other immunosuppressive drugs. This was most evident in those with CR.

As in other conditions, there is no clear dose-dependent effect in terms of clinical or biochemical response following alemtuzumab[25]. Response was sustained in the majority, with 69% of patients maintaining remission at 12 months following their first course of alemtuzumab. Relapses were more frequent in the lowest dose group, although further alemtuzumab was effective, and we have now adopted the strategy of routine retreatment at 6 months, regardless of disease activity. However, attention must also be drawn to the longevity of some of these remissions, with a few patients continuing to be free of disease and not taking any immunosuppression over 10 years after treatment.

In our patients, alemtuzumab therapy was well tolerated. However, infusion reactions were common, occurring after 27% of courses of treatment, but did not extend beyond the infusion period, and were managed symptomatically while the infusion proceeded. Despite the prolonged and profound lymphopenia following alemtuzumab therapy, opportunistic infections were not seen, although routine antifungal and antiviral prophylaxis was administered.

Thyroid dysfunction was seen in 25% of patients. This phenomenon of new-onset thyroid autoimmunity is also seen in similar proportions of patients treated with alemtuzumab for other conditions, such as multiple sclerosis and anti-neutrophil cytoplasmic antibody-associated vasculitis (AAV)[26,27,28]. The time period elapsed to the development of thyroid dysfunction following alemtuzumab in our cohort was comparable with other studies[29]. Although no cases of thyroid disease were seen in the lowest dose group, this may well reflect shorter followup, rather than a dose-dependent effect. In multiple sclerosis, the development of new autoimmunity after alemtuzumab has been associated with higher levels of IL-21, which is proposed to lead to increased T cell apoptosis and cell cycling, thus increasing the opportunities of T cells to encounter self-antigen and hence develop autoimmunity. This potential mechanism is yet to be explored in other conditions. However, it is now routine practice at our unit to measure thyroid-stimulating hormone levels pretreatment and 3 months thereafter, and patients are aware before beginning alemtuzumab therapy that there is the possibility of new-onset autoimmunity.

The retrospective design of our study has limitations. The evolution of clinical practice, in particular the advent of anti-TNF therapy during the last 20 years and the inevitable loss of data or underreporting of adverse events over such a long followup period, makes comparisons between dosing groups difficult. In addition, the demography of patients in this cohort differs from other studies, where severe disease is more common in men, and cardiac and major arterial disease often occur more frequently. This may reflect the selective referral policy to our unit, or the wide geographical variation seen in BD. Therefore, it is possible that therapeutic responses to alemtuzumab will vary in other BD cohorts.

However, aside from a single case report, our center is the only institution to publish its experience of the treatment of patients with BD with alemtuzumab[30]. The number of patients included in this cohort, and the longterm followup data available on many, are key strengths. Alemtuzumab is recommended as a third line step-up treatment for patients with moderate and severe BD, according to the NHS National Specification Patient Pathway[31]. Therefore, in the era of evidence-based medicine, the publication of a cohort such as this is important.

We have demonstrated that alemtuzumab is effective at inducing sustained remission in BD and is well tolerated in patients with severe disease who have often been exposed to large amounts of prior immunosuppression. We have previously showed that relapse risk is reduced following 2 courses of alemtuzumab, and a fixed-interval retreatment strategy as with RTX in AAV may become a future approach[28,32]. However, the risk of cumulative exposure against the benefits of longterm disease control must be carefully evaluated prior to the adoption of such a course of action. It must be remembered that the clinicians treating these patients have had considerable experience using this agent, both in BD and other vasculitides. Thorough assessment prior to the administration of alemtuzumab, to ensure that infectious risk, in particular, is minimized, and vigilance for infection and new-onset autoimmunity following administration is imperative from a safety perspective. However, based on our evidence, alemtuzumab offers an alternative therapeutic strategy for BD in patients with refractory or relapsing disease.

ONLINE SUPPLEMENT

Supplementary data for this article are available online at jrheum.org.

REFERENCES

1. Criteria for diagnosis of Behçet's disease. International Study Group for Behçet's Disease. Lancet 1990;335:1078-80.
2. Direskeneli H. Behçet's disease: infectious aetiology, new autoantigens, and HLA-B51. Ann Rheum Dis 2001;60:996-1002.
3. de Menthon M, Lavalley MP, Maldini C, Guillevin L, Mahr A. HLA-B51/B5 and the risk of Behçet's disease: a systematic review and meta-analysis of case-control genetic association studies. Arthritis Rheum 2009;61:1287-96.
4. Suzuki Y, Hoshi K, Matsuda T, Mizushima Y. Increased peripheral blood gamma delta+ T cells and natural killer cells in Behçet's disease. J Rheumatol 1992;19:588-92.

5. Skin hypersensitivity to streptococcal antigens and the induction of systemic symptoms by the antigens in Behçet's disease—a multicenter study. The Behçet's Disease Research Committee of Japan. J Rheumatol 1989;16:506-11.
6. Calis M, Ates F, Yazici C, Kose K, Kirnap M, Demir M, et al. Adenosine deaminase enzyme levels, their relation with disease activity, and the effect of colchicine on adenosine deaminase levels in patients with Behçet's disease. Rheumatol Int 2005;25:452-6.
7. Gül A, Esin S, Dilsen N, Koniçe M, Wigzell H, Biberfeld P. Immunohistology of skin pathergy reaction in Behçet's disease. Br J Dermatol 1995;132:901-7.
8. Poulter LW, Lehner T. Immunohistology of oral lesions from patients with recurrent oral ulcers and Behçet's syndrome. Clin Exp Immunol 1989;78:189-95.
9. Hamzaoui K, Hamzaoui A, Guemira F, Bessioud M, Hamza M, Ayed K. Cytokine profile in Behçet's disease patients. Relationship with disease activity. Scand J Rheumatol 2002;31:205-10.
10. Raziuddin S, al-Dalaan A, Bahabri S, Siraj AK, al-Sedairy S. Divergent cytokine production profile in Behçet's disease. Altered Th1/Th2 cell cytokine pattern. J Rheumatol 1998;25:329-33.
11. Dalghous AM, Freysdottir J, Fortune F. Expression of cytokines, chemokines, and chemokine receptors in oral ulcers of patients with Behcet's disease (BD) and recurrent aphthous stomatitis is Th1-associated, although Th2-association is also observed in patients with BD. Scand J Rheumatol 2006;35:472-5.
12. Gündüz E, Teke HU, Bilge NS, Cansu DU, Bal C, Korkmaz C, et al. Regulatory T cells in Behçet's disease: is there a correlation with disease activity? Does regulatory T cell type matter? Rheumatol Int 2013;33:3049-54.
13. Masuda K, Nakajima A, Urayama A, Nakae K, Kogure M, Inaba G. Double-masked trial of cyclosporin versus colchicine and long-term open study of cyclosporin in Behçet's disease. Lancet 1989; 1:1093-6.
14. Bonnet M. [Immunosuppressive therapy of Behcet's syndrome. Long term follow-up evaluation (author's transl)]. [Article in French] J Fr Ophtalmol 1981;4:455-64.
15. Zhang X, Tao Y, Chopra M, Ahn M, Marcus KL, Choudhary N, et al. Differential reconstitution of T cell subsets following immunodepleting treatment with alemtuzumab (anti-CD52 monoclonal antibody) in patients with relapsing-remitting multiple sclerosis. J Immunol 2013;191:5867-74.
16. Havari E, Turner MJ, Campos-Rivera J, Shankara S, Nguyen TH, Roberts B, et al. Impact of alemtuzumab treatment on the survival and function of human regulatory T cells in vitro. Immunology 2014;141:123-31.
17. Lockwood CM, Thiru S, Isaacs JD, Hale G, Waldmann H. Long-term remission of intractable systemic vasculitis with monoclonal antibody therapy. Lancet 1993;341:1620-2.
18. Lockwood CM. Refractory Wegener's granulomatosis: a model for shorter immunotherapy of autoimmune diseases. J R Coll Physicians Lond 1998;32:473-8.
19. Paolillo A, Coles AJ, Molyneux PD, Gawne-Cain M, MacManus D, Barker GJ, et al. Quantitative MRI in patients with secondary progressive MS treated with monoclonal antibody Campath 1H. Neurology 1999;53:751-7.
20. Dick AD, Meyer P, James T, Forrester JV, Hale G, Waldmann H, et al. Campath-1H therapy in refractory ocular inflammatory disease. Br J Ophthalmol 2000;84:107-9.
21. Lockwood CM, Hale G, Waldman H, Jayne DR. Remission induction in Behçet's disease following lymphocyte depletion by the anti-CD52 antibody CAMPATH 1-H. Rheumatology 2003; 42:1539-44.
22. Sfikakis PP, Markomichelakis N, Alpsoy E, Assaad-Khalil S, Bodaghi B, Gul A, et al. Anti-TNF therapy in the management of Behçet's disease—review and basis for recommendations. Rheumatology 2007;46:736-41.
23. Lindstedt EW, Baarsma GS, Kuijpers RW, van Hagen PM. Anti-TNF-alpha therapy for sight threatening uveitis. Br J Ophthalmol 2005;89:533-6.
24. Furuta S, Chow YW, Chaudhry AN, Jayne D. Switching of anti-TNF-α agents in Behçet's disease. Clin Exp Rheumatol 2012;30 Suppl 72:S62-8.
25. CAMMS223 Trial Investigators, Coles AJ, Compston DA, Selmaj KW, Lake SL, Moran S, et al. Alemtuzumab vs. interferon beta-1a in early multiple sclerosis. N Engl J Med 2008;359:1786-801.
26. Coles AJ. Alemtuzumab treatment of multiple sclerosis. Semin Neurol 2013;33:66-73.
27. Daniels GH, Vladic A, Brinar V, Zavalishin I, Valente W, Oyuela P, et al. Alemtuzumab-related thyroid dysfunction in a phase 2 trial of patients with relapsing-remitting multiple sclerosis. J Clin Endocrinol Metab 2014;99:80-9.
28. Walsh M, Chaudhry A, Jayne D. Long-term follow-up of relapsing/refractory anti-neutrophil cytoplasm antibody associated vasculitis treated with the lymphocyte depleting antibody alemtuzumab (CAMPATH-1H). Ann Rheum Dis 2008;67:1322-7.
29. Azzopardi L, Thompson SA, Harding KE, Cossburn M, Robertson N, Compston A, et al. Predicting autoimmunity after alemtuzumab treatment of multiple sclerosis. J Neurol Neurosurg Psychiatry 2014;85:795-8.
30. Perez-Pampin E, Campos-Franco J, Blanco J, Mera A. Remission induction in a case of refractory Behçet disease with alemtuzumab. J Clin Rheumatol 2013;19:101-3.
31. National Health Service. Service specification and standards – Beçhet's syndrome service, 2012. [Internet. Accessed June 25, 2015.] Available from: www.webarchive.org.uk/wayback/archive/20130325152307/http://www.specialisedservices.nhs.uk/document/10502
32. Smith RM, Jones RB, Guerry MJ, Laurino S, Catapano F, Chaudhry A, et al. Rituximab for remission maintenance in relapsing antineutrophil cytoplasmic antibody-associated vasculitis. Arthritis Rheum 2012;64:3760-9.