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Complete List of Authors:	Hill-Cawthorne, Grant; King Abdullah University of Science and Technology, Pathogen Genomics Laboratory Button, Tom; University of Cambridge, Department of Neurology Tuohy, Orla; University of Cambridge, Department of Neurology Jones, Joanne; University of Cambridge, Department of Neurology May, Karen; University of Cambridge, Department of Neurology Somerfield, Jennifer; University of Cambridge, Department of Neurology Green, AJE; University of Edinburgh, The National CJD Surveillance Unit Giovannoni, G; University College London, Institute of Neurology; Blizard Institute of Cell and Molecular Science, Neuroscience and Trauma Compston, Alastair; University of Cambridge, Department of Neurology Fahey, Michael; University of Cambridge, Centre for Applied Medical Statistics, Department of Public Health and Primary Care; Commonwealth Scientific and Industrial Research Organisation (CSIRO), Division of Mathematics, Informatics and Statistics Coles, A J; University of Cambridge, Department of Neurology
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TITLE PAGE

Long-term lymphocyte reconstitution after alemtuzumab treatment of multiple sclerosis

Grant A. Hill-Cawthorne,[a,b] Tom Button,[a] Orla Tuohy,[a] Joanne L. Jones,[a] Karen May,[a] Jennifer Somerfield,[a] Alison Green,[c] Gavin Giovannoni,[d,e] D. Alastair S. Compston,[a] Michael T. Fahey,[f,g] and Alasdair J. Coles[a]

[a]Department of Neurology, University of Cambridge, UK; [b]Pathogen Genomics Laboratory, King Abdullah University of Science and Technology (KAUST), Thuwal, Saudi Arabia; [c]The National CJD Surveillance Unit, University of Edinburgh, Scotland, UK; [d]Institute of Neurology, University College London, UK; [e]Centre for Neuroscience and Trauma, Blizard Institute, Barts and The London School of Medicine and Dentistry, Queen Mary University of London, UK; [f]Centre for Applied Medical Statistics. Department of Public Health and Primary Care, University of Cambridge, UK; and [g]Division of Mathematics, Informatics and Statistics, Commonwealth Scientific and Industrial Research Organisation (CSIRO), Clayton South, Victoria, Australia

Corresponding author

Alasdair J. Coles, PhD, FRCP

Department of Neurology

Box 165, Addenbrooke's Hospital

Cambridge CB2 2QQ, UK

Telephone: +44-(0)1223-217091

Fax: +44-(0)1223-336941

E-mail: ajc1020@medschl.cam.ac.uk

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Abstract

Background: Alemtuzumab is a lymphocyte-depleting monoclonal antibody that has demonstrated superior efficacy over interferon beta-1a for relapsing-remitting multiple sclerosis, and is currently under investigation in phase 3 trials. One unresolved issue is the duration and significance of the lymphopenia induced. We examined the long-term effects on lymphocyte reconstitution of a single course and the consequences that this has on disability, morbidity, mortality, and autoimmunity.

Methods: We report the lymphocyte reconstitution (n=36; 384 person-years) and crude safety data (n=37; 447 person-years) for the first patients with progressive MS to receive alemtuzumab (1991-1997). Reconstitution time was expressed as a geometric mean or, when a non-negligible number of individuals failed to recover, as a median using survival analysis.

Results: Geometric mean recovery time (GMRT) of total lymphocyte counts to the lower limit of the normal range ("LLN";$\geq 1.0 \times 10^9$ cells/L) was 12.7 months (95%CI: 8.8, 18.2 months). For B cells, GMRT to LLN ($\geq 0.1 \times 10^9$/L) was 7.1 months (95%CI: 5.3, 9.5); median recovery times for CD8+ (LLN$\geq 0.2 \times 10^9$ cells/L) and CD4+ lymphocytes (LLN$\geq 0.4 \times 10^9$ cells/L) were 20 months and 35 months. However, CD8+ and CD4+ counts recovered to baseline levels in only 30% and 21% of patients respectively. No infective safety concerns arose during 447 person-years of follow-up.

Conclusions: Lymphocyte counts recovered to the lower limit of normal after a single course of alemtuzumab in around 8 months (B cells) and 3 years (T cell subsets), but usually did not recover to baseline values. However, this long-lasting lymphopenia in patients with a previously normal immune system is not associated with an increased risk of serious opportunistic infection.

INTRODUCTION

Campath-1H is a humanised monoclonal antibody that binds CD52 and depletes lymphocytes, monocytes and NK cells.[1] Marketed as alemtuzumab, now Lemtrada, it was approved for the treatment of chronic lymphocytic leukaemia in 2001.[2] Since 1991, we have investigated its use as a treatment for multiple sclerosis: a phase 2 trial has been published[3] and phase 3 trials are ongoing.[4, 5] However, despite the potential for its widespread use in young systemically healthy adults with multiple sclerosis, the extent and clinical significance of the lymphopenia that alemtuzumab induces is not well known. Experience from alemtuzumab treatment of other conditions is not representative, as in lymphocytic malignancies there is abnormal lymphocyte proliferation, and in treatment-resistant systemic autoimmune disease, patients are older, unwell[6] and have been exposed to multiple immunotherapies.[7]

The aim of this study was to describe the long-term safety effects of a single course of alemtuzumab in treatment-naïve people with multiple sclerosis. We report data from the first 37 patients, each with progressive multiple sclerosis, treated between 1991 and 1997. Whilst alemtuzumab successfully reduced the relapse, we have previously reported that patients continued to experience progressive disability.[8] Previously, assuming linear kinetics of reconstitution after alemtuzumab, data from this cohort led to estimates of median recovery time to baseline levels for CD4+ and CD8+ T-cells of 61 and 30 months respectively, with B cells reaching baseline levels, and “overshooting” more rapidly.[9] Linear reconstitution is a reasonable model for the first 12-18 months, but we now show that after 18 months the rate decelerates to a point that linear kinetics becomes an inappropriate model. We now re-address the extent of lymphocyte recovery in this cohort after a longer interval using analysis techniques that do not assume linear reconstitution.

We also report the long-term safety profile of this small cohort. We previously reported low rates of infections in the first few years after alemtuzumab, strikingly lower than patients with HIV infection with similar CD4+ counts;[10] one potential explanation being that, after alemtuzumab, lymph nodes retain a substantial number of healthy lymphocytes that escape deletion.[11-13] We report the effect of alemtuzumab on cerebrospinal fluid (CSF) oligoclonal bands in the context of progressive multiple sclerosis. Finally, we explore the relationship between lymphocyte reconstitution and the development of secondary autoimmune diseases: up to 30% of alemtuzumab-treated multiple sclerosis patients develop autoimmune thyroid disease,[13] and other autoimmune diseases such as Goodpasture's disease are also seen.[9]

METHODS

This is a review of safety and lymphocyte reconstitution of the first 37 patients to receive alemtuzumab as a treatment of multiple sclerosis, in Cambridge, UK.

Patients and treatment

The first cohort of patients, treated between 1991 and 1993, consisted of seven patients, six with secondary progressive and one with primary progressive disease.[14] Six patients were treated with 12mg of alemtuzumab daily for 10 days and one patient received 60mg in total. Early[14] and later[8, 9] data on efficacy have already been reported. Five of the seven patients were re-treated between two and four years after the first dose. The second cohort, treated between 1995 and 1997, consisted of 29 patients, all with secondary progressive multiple sclerosis.[8] All patients received 100mg of alemtuzumab over 5 days. 14 of the 29 patients were also treated with a novel humanised IgG4 anti-CD4 antibody (200mg over the subsequent 5 days), which was designed, successfully, to be non-depleting. For the purposes of analysis both cohorts are considered together. No antimicrobial prophylaxis was administered to either cohort and no other immunosuppressant medications were taken during follow-up. One additional patient with progressive multiple sclerosis, treated in 1997, is also included in the analysis of reconstitution. This study was approved by the Local Research Ethics Committees and all patients gave their written informed consent. G.A.H.C., T.B. and M.T.F. analysed the data and all authors had access to the primary clinical data.

Assessments

All patients were offered continued follow-up at our site, three-monthly for the first three years after alemtuzumab, then biannually for two years, and annually thereafter. At each of these visits, blood was taken for total lymphocyte count, subsets (CD4+, CD8+, CD19+), autoantibody screen, liver function and renal function. Blood tests were variably available

between 1990 and 2009 depending upon the patient's treatment date and availability for follow-up. Disability was assessed annually using Kurtzke's Expanded Disability Status Score, EDSS.[15] Eleven patients, who lived at a distance, declined these assessments. All living patients were reviewed within the last 2 years or received a telephone interview. As a minimum, data on autoimmune disease, major illnesses or death were collected, as well as a crude estimate of disability. Autoimmunity was defined as the clinical development of secondary autoimmune disease or persistently abnormal thyroid function tests in the presence of autoantibodies indicating thyroid disease, as in our previous studies.[16]

Assessment of Recovery

Total lymphocyte counts and subset analyses were carried out whenever patients attended the clinic. Analysis was restricted to measurements taken at least one day after completing the first cycle of alemtuzumab and not confounded by any subsequent treatment. That is, all data recorded after subsequent treatments were excluded in this analysis. The number of repeated measurements per patient ranged from 5 to 46 (median=18, IQR = 12 to 23). Four components of immune reconstitution were examined: total lymphocyte counts, CD19+, CD4+ and CD8+ lymphocyte subsets.

Total and T lymphocyte cell counts were analysed to two endpoints. The first endpoint is time taken for recovery to a pre-defined "lower limit of normal" level (LLN). These LLNs were derived from our laboratory ranges and were $1.0x10^9$, $0.4x10^9$ and $0.2x10^9$ cells/L for total, CD4+ and CD8+ lymphocytes respectively. Throughout this manuscript we shall refer to this endpoint as reconstitution to "normal". The second endpoint is time to recovery to the patient's relevant lymphocyte count before alemtuzumab treatment. This second endpoint will be referred to as reconstitution to "baseline level".

B lymphocytes were only analysed to the first endpoint, namely reconstitution to "normal" due to a lack of baseline measurements. For B lymphocytes LLN was $0.1x10^9$ cells/L.

Fifteen patients from the second cohort consented to lumbar puncture before and after alemtuzumab (Table 1). Median disease duration was 4 years (range 1-13 years). Unconcentrated CSF and paired serum samples were assessed using isoelectric focusing in agarose gel with immunofixation. The blots were read independently by two experienced observers blinded with respect to the identity of each patient and the time-point of the sample.

Table 1. Details of the fifteen patients with secondary progressive multiple sclerosis that consented to lumbar punctures before and after alemtuzumab treatment.

Number of patients	15
Number of females (%)	10 (67)
Median age at time of treatment, years (range)	40 (23-49)
Median disease duration, years (range)	4 (1-13)
Median EDSS pre-treatment (range)	6 (4-7)
Median time of pre-treatment LP in days (range)	1 (0-37)
Median time of post-treatment LP in months (range)	11 (3-28)

CSF cerebrospinal fluid

Statistical analysis

The median and interquartile range (IQR) were used to describe data. To make statistical inference on recovery time for reconstitution the geometric mean or the median was used. When the number of individuals who failed to recover was negligible, recovery time was log transformed to improve symmetry and the geometric mean computed. When there was a non-negligible number of individuals failing to recover, their recovery times were treated as

censored and a median was estimated using survival analysis. The relationship with age at baseline and autoimmune status was examined using a scatterplot for the former and a box and whisker plot for the latter. To examine the effect of autoimmunity, a t-test of zero mean difference in log recovery time was calculated. This result was back-transformed to the original time scale and reported as the ratio of the geometric means for autoimmunity-absent divided by autoimmunity-present along with its 95% confidence interval (CI) and p-value. Median recovery times to baseline level (as well as to normal level for CD4+ and CD8+) were estimated using survival analysis to allow inclusion of patients who did not recover to their baseline level in the analysis. These results were presented in cumulative incidence (Kaplan-Meier) plots of recovery to baseline level against time since first treatment, and summarised by percentiles of the recovery time distribution.

RESULTS

Patients

Thirty-seven patients (17 males and 20 females) were followed altogether. All of the patients had primary or secondary progressive multiple sclerosis, with a median age of 39 years (23-56), at the time of first alemtuzumab treatment and median duration since disease onset of 9 years (range 1-23). Median disability at treatment was EDSS 6 (4-8). 28/37 patients received a single cycle of alemtuzumab; 9/37 patients received an additional cycle, a median of 3.5 years after the first exposure. Baseline lymphocyte subset levels were not available in 3/37 patients; follow-up lymphocyte counts were available for a median of 12 years (0.5-16) and clinical information (including by telephone contact) for a median of 14 years (2-18). No patients were lost to follow-up. The total duration of follow-up was 384 person-years for lymphocyte counts and 447 person-years for clinical data. 12/37 developed secondary autoimmune disease.

Lymphocyte reconstitution

Figure 1 illustrates the total, CD19+, CD4+, and CD8+ lymphocyte counts from our cohort for a maximum 16 years after a single cycle of alemtuzumab. Total, CD4+ and CD8+ lymphocyte counts all increase at a high rate of reconstitution for the first 12-18 months before the rate decreases. The median baseline lymphocyte counts (with interquartile range (IQR)) and upper and lower normal limits [ULN, LLN, all $x10^9$ cells/L] were: total lymphocyte count 1.8 (IQR 1.4-2.3) [LLN 1.0, ULN 3.5]; CD4+ 0.8 (0.71–1.01) [0.4, 1.5]; for CD8+ 0.42 (0.35–0.45) [0.2, 0.9]; and CD19+ 0.19 (0.17–0.25) [0.1, 0.5].

Total lymphocyte counts reconstituted to a "normal level" (defined as $\geq 1.0x10^9$ cells/L) in 34/36 patients over a median of 12 years; the two patients not recovering to normal are

excluded from this analysis. The distribution of time to recovery was strongly skewed to the right, with most patients' total lymphocyte counts recovering within two years. Median recovery time was 12.6 months (IQR 6.1-29.8 months). The log-transformed distribution of time taken to recovery was approximately symmetric (Figure 2A) with the geometric mean closely approximating the median. The geometric mean of recovery to the normal level was 12.7 months (95%CI: 8.8-18.2 months). The total lymphocyte count recovery time to normal showed little association with baseline age (r = 0.11), and a moderate (negative) association with baseline total lymphocyte count (r = -0.29). Total lymphocyte recovery tended to be 1.8 times slower (95%CI: 0.92-3.6, p=0.08) for those patients who did not develop autoimmunity (15.4 months) compared to those who did (8.5 months, Figure 2B).

Recovery of total lymphocyte counts to individual baseline levels was achieved in only 14/36 patients (39%). To avoid excluding these individuals, median recovery time was estimated using survival analysis, yielding a figure of 151 months (Figure 2C; 95%CI: 91-212). The 25th percentile of recovery time is 38 months and more reliably estimated than the median as indicated by the confidence bands around the curve at this point.

34/36 patients recovered B cell lymphocyte counts to a normal level ($\geq 0.1 \times 10^9$/L), mostly within two years (Figure 3A). Median recovery time was 8.4 months. After log-transformation the distribution showed greater symmetry than on the original scale, but with a visual indication of bi-modality (Figure 3B). The geometric mean was 7.1 months (95%CI: 5.3-9.5). There was no association between autoimmune status and CD19+ recovery to "normal" levels.

In 31/36 patients (86%), CD8+ lymphocyte counts recovered to a normal level ($\geq 0.2 \times 10^9$/L); 27 patients in the first 4 years and four in the next 8 years of follow-up (Figure 4A). Median

recovery time was 20 months (95%CI: 5-36). The 25th percentile of recovery time was 6 months. No relationship was found between mean CD8+ recovery time to normal and autoimmune status. In only 10/33 patients did CD8+ lymphocyte counts recover to baseline levels (Figure 4B); median recovery time was 155 months, while the 25th percentile of recovery time was 66 months.

Reconstitution of CD4+ lymphocytes was slower; only 28/36 patients recovered to a normal CD4+ level ($\geq 0.4 \times 10^9$/L) over a median of 12 years (Figure 4C). In the cumulative incidence plot, the 25th percentile of recovery was more reliably estimated than the median; being 27 months and 35 months respectively. Geometric mean recovery time to normal varied little by autoimmunity and the differences were not significant. CD4+ counts recovered to baseline in only 7/33 patients (3 patients having missing baseline measurements). The median cannot be estimated but the 25th percentile of recovery time was 112 months (Figure 4D).

Disability

Disability in all but one of the 37 patients continued to worsen progressively despite alemtuzumab treatment, as previously reported.[8, 9] At the last recorded follow-up, a median of 14 years post-treatment, the median disability estimated in 35/37 patients, was EDSS 7.5 (range 4.5–9). Relapses were uncommon, but were not systematically captured.

Morbidity and mortality

Information on infections was not systematically collected from the patients and many minor infections, common in the normal population, will neither have been reported by patients nor have generated a hospital record. From the information available there were eleven major infections among the 37 patients, with two occurring in the same patient. Most of these were pneumonia at advanced stages of disability with all five cases contributing to death (one of

which was noted to be secondary to aspiration). Three cases of urinary sepsis were classified as severe because they were notified as the cause of death. Deaths rates in the two original cohorts of patients were 29% for the first cohort and 33% for the second cohort. Three further episodes of infection were caused by necrotising gingivitis (previously reported),[9] a cervical epidural abscess and septicaemia secondary to a breast abscess. Five patients reported segmental varicella-zoster virus reactivations, the majority occurring within two to three years of treatment, with one reactivation eight years after treatment. Again, major infection rates were similar in the first and second cohorts (29% and 30% respectively). For patients undergoing surgical procedures, including two cholecystectomies, a hernia repair and a hemiarthroplasty, there was no excess post-surgical morbidity.

14 patients developed Graves's disease after treatment with alemtuzumab (37.8%). Autoimmune disease in these two cohorts appeared to be associated with female sex; 12 females out of 21 (57%) versus 2 males out of 15 (13%). However, this has not been a consistent finding in the larger clinical trial cohorts.[3, 9]

Two malignant tumours were recorded in the 37 patients at follow-up: a case of prostatic adenocarcinoma in a patient who was 55 years old at the time of treatment; and one example of skin basal cell carcinoma. One benign tumour was recorded: an incidental meningioma found during MRI scanning. As of August 9, 2010 there had been 12 deaths among the 37 patients, giving a mortality rate of 2.68 per 100 person-years. Leaving aside the two patients who committed suicide, the median disability of the patients who died was EDSS 8 (range 6-9), measured on average three years before death. Subsequent clinic visits to measure EDSS became impossible as the patients became increasingly dependent and institutionalised. Causes of death in this group were infections associated with advanced disability (Table 2).

Table 2. Details of the twelve patients that died from the cohort of 37 treated with alemtuzumab for progressive multiple sclerosis.

Patient No.	Age at first alemtuzumab treatment, years	Time from first dose to death, years	Last EDSS recorded	Time from last EDSS to death, years	Cause of death
2	33	9	8	5.5	Pneumonia/MS
3	33	6	7.5	0.5	Urinary sepsis
4	48	10	7	4.0	Urinary sepsis
9	30	11	7.5	4.5	Unknown
17	31	10	8	0.5	Pneumonia
22	55	11	7.5	1.5	Prostate carcinoma
24	36	11	9	4.0	Pneumonia
25	34	13	9	7.0	Pneumonia
29	40	13	9	1.5	Pneumonia
35	23	6	8	1.0	Suicide
36	40	12	8.5	1.5	Urinary sepsis
37	42	3	6	1.0	Suicide

Oligoclonal bands in cerebrospinal fluid

Paired CSF samples were taken before (range 0-37 days) and after (range 3-28 months) the first (12 patients) or second (3 patients) cycle of alemtuzumab treatment. In all 15 cases, analysis of the CSF demonstrated the persistence of oligoclonal bands (OCB) following treatment with alemtuzumab.

DISCUSSION

We report the longest follow-up to date of patients with multiple sclerosis after a single course of alemtuzumab treatment: 384 person-years of lymphocyte counts and 447 person-years of clinical data from 37 patients treated between 1991 and 1997. We show that lymphocyte counts recovered to the lower limit of the normal range within 8 months (B cells) and 3 years (T cell subsets), but rarely return to baseline values. No long-term safety signal emerges from this small cohort, other than confirmation of the increased risk of autoimmunity.

As previously reported,[3, 9] we observed faster reconstitution of B cells after alemtuzumab than T cells; we have previously shown that the B cell subtypes return at varying rates.[17] There was a suggestion that those patients with a high baseline total lymphocyte count recover to a normal, albeit lower, lymphocyte count more rapidly than others. Interestingly, T cell numbers returned to the normal range in nearly all patients (78% of patients for CD4+, 86% for CD8+), but rarely to baseline levels (21% of patients for CD4+ and 30% for CD8+). We speculate that in adults with little thymic function, reconstitution of the T cell pool after alemtuzumab may be "reset" to a lower threshold. Furthermore, there may be sustained alterations within the lymphocyte subsets – such as those shown at 12 months.[18] This suggests that simple lymphocyte counts may not be a reliable assessment of immunocompetence.

Our previous study of lymphocyte reconstitution in this cohort, assuming linear kinetics, suggested more rapid reconstitution of T cells.[9] However, it is now clear that reconstitution is initially linear and rapid, followed either by slowing of the rate of increase and/or a subsequent fall in lymphocyte count. A similar pattern of immune reconstitution is seen after

alemtuzumab treatment of other autoimmune diseases or for organ transplantation,[13, 19-21] and after alemtuzumab in an hCD52 transgenic mouse, albeit on a much contracted timescale.[12] Long-term follow-up was possible in patients treated with alemtuzumab for refractory rheumatoid arthritis (RA) between 1991 and 1994.[22, 23] This cohort was relatively older (median age 54 years, range 25.5-70) and had received a median of four disease-modifying anti-rheumatic drugs (DMARDs) (range 1-8) before treatment. At a median of 11.8 years (range 10.5-13.3 years) post-treatment CD4+, CD8+ and CD19+ counts were 0.5, 0.26 and $0.11x10^9$ cells/L respectively.[23] Most had CD4+ and CD8+ T cell counts within the normal range, but, in contrast to this study, B cell counts were subnormal in 50%. No excess mortality or infections were seen in patients treated with alemtuzumab compared to a hospital-based RA cohort. Likewise, this lymphocyte reconstitution profile after alemtuzumab is similar to that seen following lymphopenia in other contexts, suggesting that it is driven by common homeostatic mechanisms, for instance after haematopoietic stem cell transplantation.[6, 13, 24, 25] The lag in CD4+ cell recovery correlates with age of the recipient and probably reflects impaired thymic function. Indeed, thymus enlargement is evident radiographically in younger patients following HSCT[26] and is seen in individuals treated with HSCT for both non-Hodgkin's lymphoma and multiple sclerosis.[27] Interestingly, CD4+ reconstitution after HSCT for rheumatoid arthritis is considerably delayed, an observation attributed to poor memory T-cell expansion associated with low levels of circulating IL-7.[28] However, in people with multiple sclerosis, serum IL-7 levels rise significantly after alemtuzumab.[18]

As expected from previous reports,[3, 13] one third of multiple sclerosis patients develop autoimmunity after alemtuzumab, particularly those prone to excessive IL-21 secretion.[16] Here we demonstrate a non-significant trend that those patients who develop autoimmunity reconstituted their total lymphocytes quicker (x1.8) than those without autoimmunity. There

are too few data on lymphocyte subsets to judge whether their reconstitution differentiates between those with and without autoimmunity.

We have also shown that alemtuzumab does not alter the persistence of oligoclonal bands in the CSF of patients with progressive multiple sclerosis following treatment with alemtuzumab. Treatment with other effective immunotherapies, such as rituximab [29] and autologous haematopoetic stem cell transplantation, also do not eradicate intrathecal antibody production.[30-36] These therapies, which so radically alter the peripheral immune compartment, are clearly unable either to access or influence the plasma cells producing antibodies detected in CSF.

No particular safety signal emerged from this study. No patient was lost to follow-up and our data on death and major safety events are complete. Segmental VZV reactivation, a feature of alemtuzumab treatment in the CAMMS223 trial,[3] was seen in 5/37 patients. However, minor adverse events were not systematically collected and the cohort described here is small. The phase 3 trials of alemtuzumab will add further information on long-term safety after alemtuzumab in the context of multiple sclerosis.

The fact that 12/37 of this progressive multiple sclerosis cohort died is consistent with our experience of managing people with untreated multiple sclerosis at this level of disability. Two of these 12 committed suicide, which is more common in multiple sclerosis.[37] The mean disability of the remaining ten patients was EDSS 8.1 on average three years before their death. The cause of death in these patients, overwhelmingly due to sepsis from a urinary or chest source, is typical of that in untreated patients with advanced disability from multiple sclerosis.[38] Mortality increases with disability in multiple sclerosis: in a Canadian cohort, people with an EDSS of ≥ 7.5 had an increased fatality rate of four times that of controls,[39]

whereas it was increased eightfold in a French cohort with similar levels of disability.[40] There is no indication that alemtuzumab treatment or its complications were directly implicated in the deaths of any patient in our study.

We conclude, from this small cohort, that one cycle of alemtuzumab has long lasting effects on the immune system, possibly by resetting the target for reconstitution of T lymphocytes to below baseline values: CD4+ and CD8+ T counts enter the normal range by 3 years. Throughout, our patients appear immunocompetent, and the main complication of alemtuzumab treatment remains autoimmunity. A caveat is that current trial protocols for the treatment of relapsing-remitting multiple sclerosis require two cycles of alemtuzumab, 12 months apart, with possible re-treatment with evidence for the return of disease activity. The long-term effects of such multiple alemtuzumab treatments have yet to be studied.

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COMPETING INTERESTS

Alasdair Coles, Alastair Compston and Joanne Jones have received personal travel costs, occasional honoraria and departmental support from Genzyme Corporation. Gavin Giovannoni has received honoraria and consulting fees from Genzyme Corporation and Sanofi-Aventis. The other authors have no relationships to declare.

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FIGURE LEGENDS

Figure 1. Raw data plots of total lymphocyte counts and CD4+, CD8+ and CD19+ subgroups (all x10^9 cells/L) for years after alemtuzumab. Dotted lines indicate upper and lower limits of normal range. The solid grey line indicates the median (and the grey shaded area the upper and lower quartile) of the baseline values.

Figure 2. Recovery of total lymphocyte counts (LC) following alemtuzumab treatment. 34 of the 36 patients recovered their total LC to a normal level defined as ≥1.0x10^9 cells/L. (**A**) Distribution of time taken to recovery to a normal level after log transformation. (**B**) Box-whisker plot of log total LC recovery time to a normal level by autoimmune status. 0=No autoimmune disease, 1=Autoimmune disease (see methods for definition, p=0.08). One outlier (greater than 1.5x IQR) can be seen in the Autoimmune status=1 group. (**C**) Cumulative incidence curve plotting the recovery distribution where an event is the occurrence of total LC recovery to the patient's baseline level (solid line). Vertical lines on the curve indicate censored observations i.e. the patients are not followed up further due to death, retreatment or lack of lymphocyte data. Dashed lines are 95% confidence intervals.

Figure 3. Recovery of CD19+ B cell counts following alemtuzumab treatment. 34 of the 36 patients recovered their B cells to a normal level defined as ≥0.1x10^9 cells/L. (**A**) Distribution of time taken for B cell recovery to a normal level. (**B**) Log transformation of time taken for B cell recovery to a normal level.

Figure 4. Recovery of CD8+ and CD4+ T cell counts following alemtuzumab treatment. (**A**) Cumulative incidence curve plotting the recovery distribution where an event is the occurrence of CD8+ lymphocyte recovery to a normal level defined as ≥0.2x10^9 cells/L (solid

line). Vertical lines on the curve indicate censored observations i.e. the patients were not followed up further due to death, retreatment or lack of lymphocyte data. Dashed lines are 95% confidence intervals. (**B**) Similar cumulative incidence curve where an event is defined as CD8+ lymphocyte recovery to the patient's baseline level. (**C**) Cumulative incidence curve where an event is the recovery of CD4+ T cells to a normal level defined as $\geq 0.4 \times 10^9$ cells/L. (**D**) Similar curve for recovery of CD4+ T cells to patient's baseline level.

Figure 1

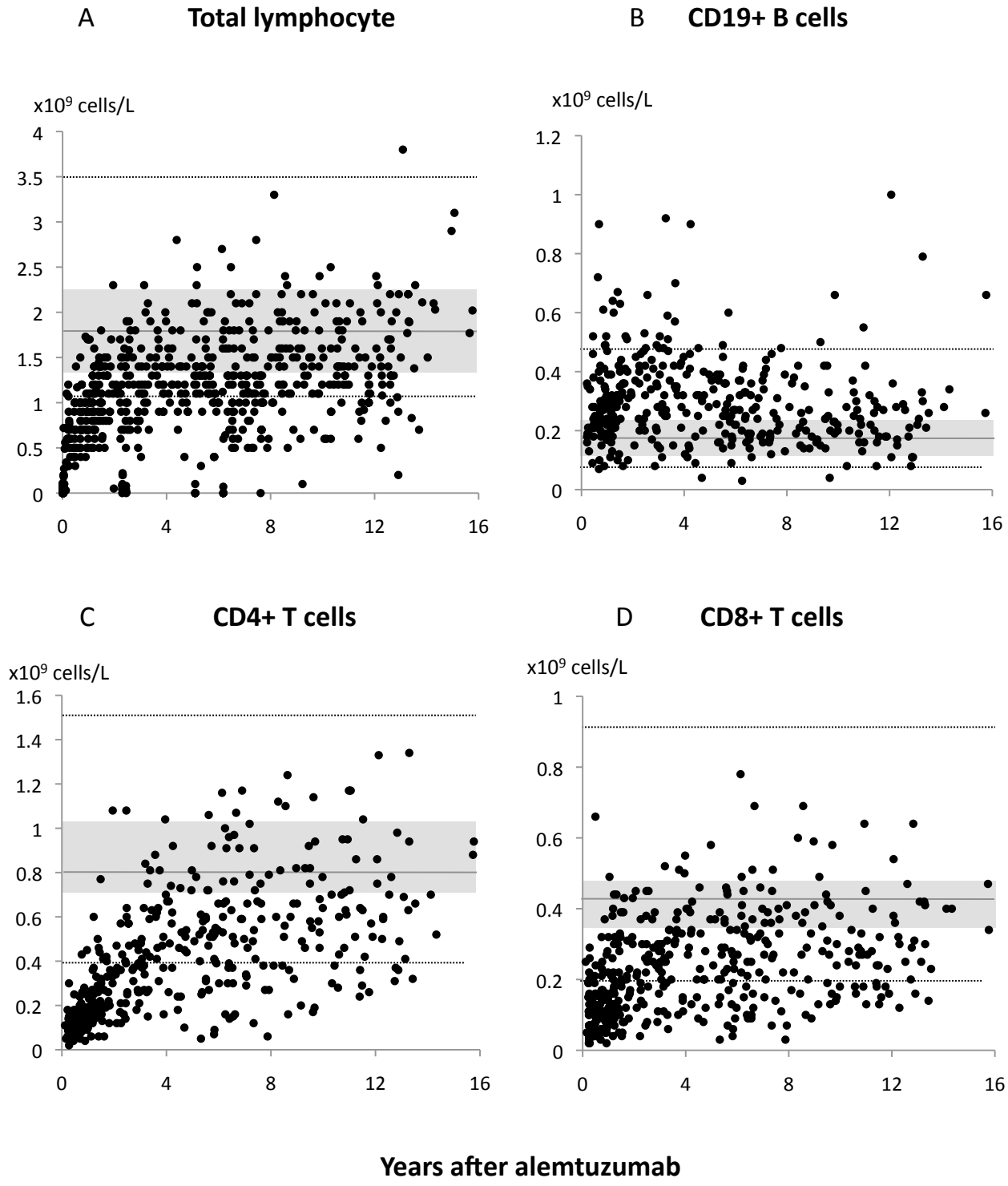

A Total lymphocyte
x10⁹ cells/L
B CD19+ B cells
x10⁹ cells/L
C CD4+ T cells
x10⁹ cells/L
D CD8+ T cells
x10⁹ cells/L
Years after alemtuzumab

Figure 2

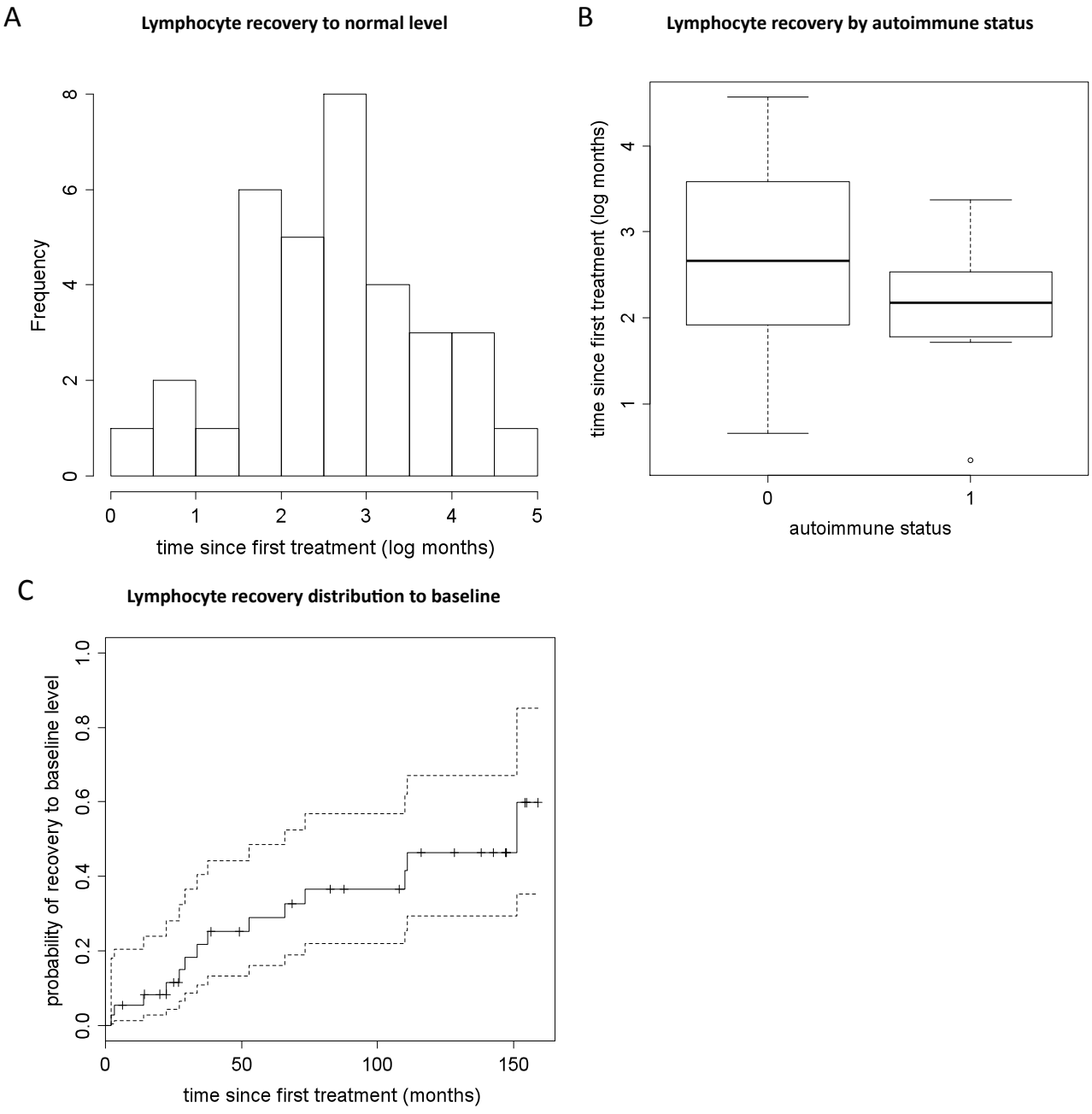
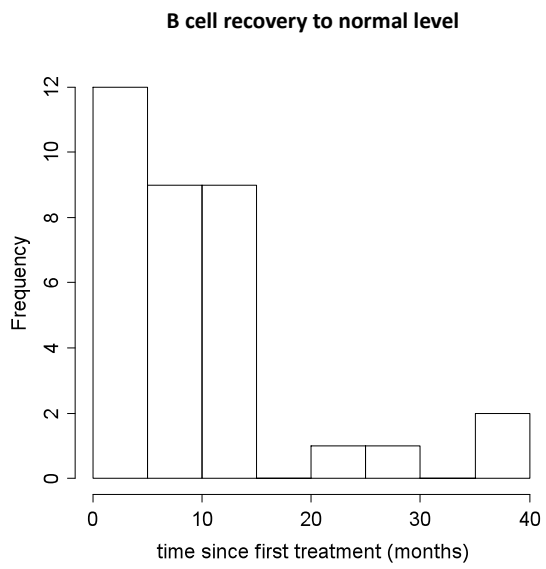

A
Lymphocyte recovery to normal level
Frequency
0
2
4
6
8
0
1
2
3
4
5
time since first treatment (log months)
B
Lymphocyte recovery by autoimmune status
time since first treatment (log months)
1
2
3
4
0
1
autoimmune status
C
Lymphocyte recovery distribution to baseline
probability of recovery to baseline level
0.0
0.2
0.4
0.6
0.8
1.0
0
50
100
150
time since first treatment (months)

Figure 3

A


B cell recovery to normal level
Frequency
0
2
4
6
8
10
12
0
10
20
30
40
time since first treatment (months)

B

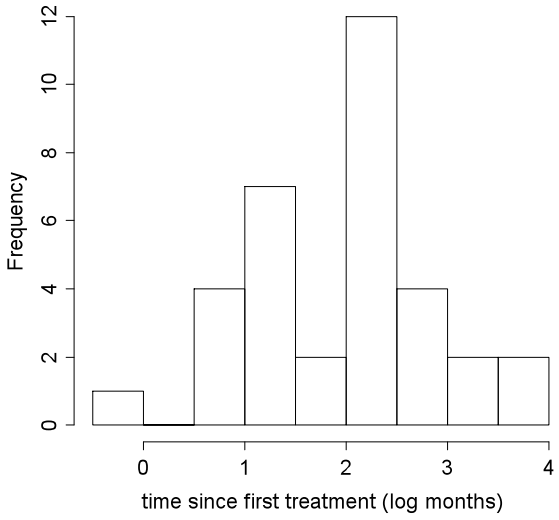
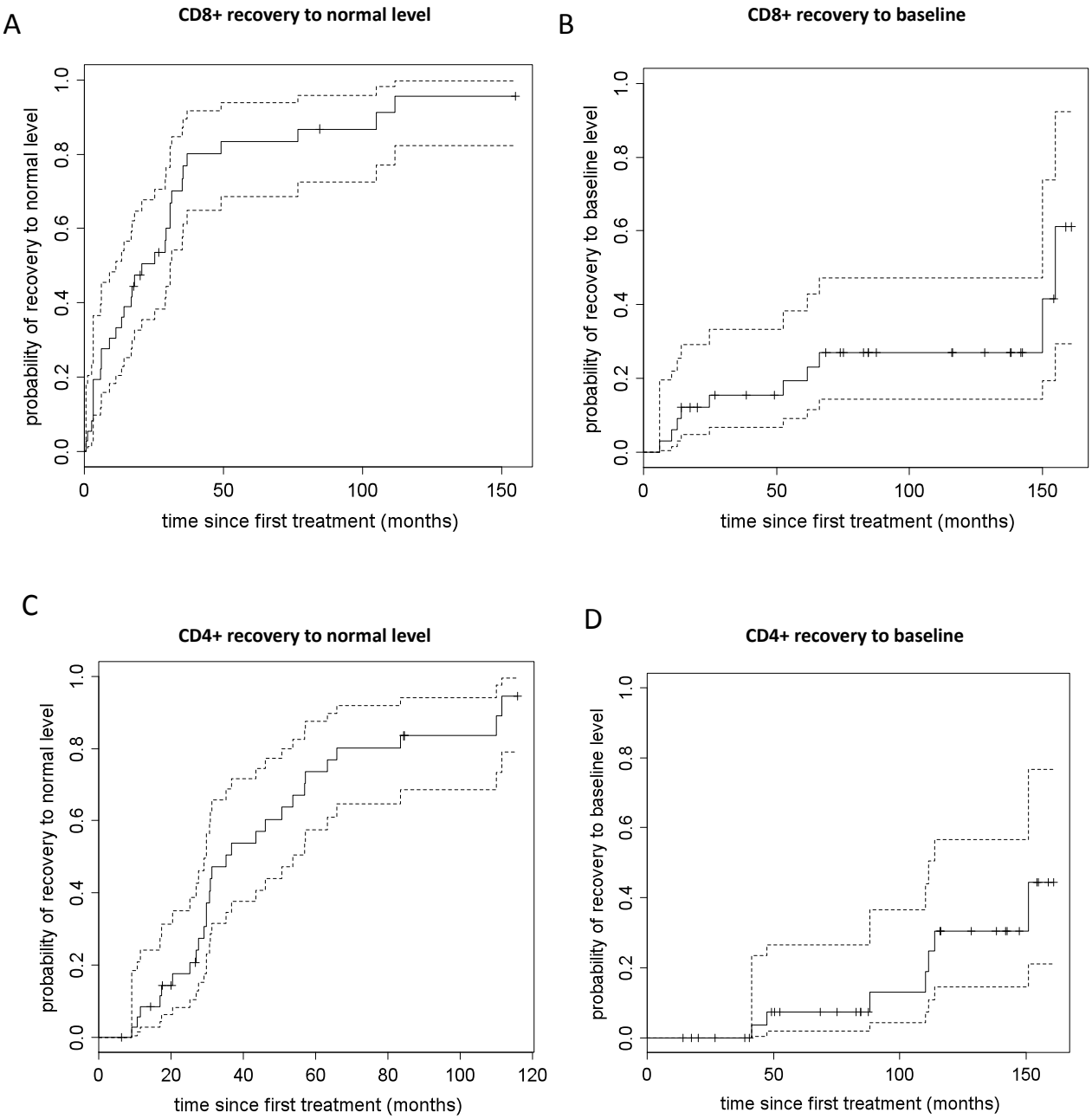

Log transformation of B cell recovery to normal level
Frequency
0
2
4
6
8
10
12
0
1
2
3
4
time since first treatment (log months)

Figure 4


A
CD8+ recovery to normal level
probability of recovery to normal level
time since first treatment (months)
B
CD8+ recovery to baseline
probability of recovery to baseline level
time since first treatment (months)
C
CD4+ recovery to normal level
probability of recovery to normal level
time since first treatment (months)
D
CD4+ recovery to baseline
probability of recovery to baseline level
time since first treatment (months)