


Alemtuzumab depletion failure can occur in multiple sclerosis

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Introduction

Multiple sclerosis (MS) is an immune-mediated, demyelinating disease of the central nervous system.¹ The inflammatory element responds to lymphocyte, notably B cell, depleting agents.² Alemtuzumab is one of the most-effective treatments for relapsing MS,^{3,4} but it causes delayed B-cell autoimmunities in a significant number of people with MS.⁵ We have postulated that this is a consequence of B-cell hyper-repopulation in the relative absence of T-cell regulation.⁶ The loss of immune tolerance may also allow anti-alemtuzumab responses to form,⁶ which have

Summary

Alemtuzumab is a lymphocyte-depleting antibody and one of the most effective treatments for relapsing multiple sclerosis. However, it also causes loss of immune-tolerance leading to secondary autoimmunity and marked anti-drug antibody responses. Although these anti-drug responses have been reported to be of no significance, we hypothesized that they will affect the depleting capacity and treatment response in some individuals. This was found following analysis of the regulatory submission of the pivotal phase III trials, which was obtained from the European Medicines Agency. At the population level there was lack of influence of 'ever-positive' alemtuzumab-specific antibody responses on lymphocyte depletion, clinical efficacy and adverse effects during the 2-year trial. This was not surprising as no one before the first infusion, and only 0.6% of people before the second-infusion, had pre-infusion, neutralizing antibodies (NABs). However, at the individual level, NABs led to poor lymphocyte depletion. Importantly, it was evident that 31% of people had NABs and 75% had binding antibodies at the end of treatment-cycle 2, which suggests that problems may occur in people requiring additional alemtuzumab cycles. In addition, we also identified individuals, following 'post-marketing' alemtuzumab use, whose lymphocyte level was never effectively depleted after the first infusion cycle. Hence, although alemtuzumab depletes lymphocytes in most individuals, some people fail to deplete/deplete poorly, probably due to biological-response variation and NABs, and this may lead to treatment failure. Monitoring depletion following infusion and assessment of the neutralizing response before re-infusion may help inform the decision to retreat or switch therapy to limit treatment failure.

Keywords: antibodies; CD52; immunotherapy; multiple sclerosis; tolerance.

been reported to be of no consequence to safety or efficacy.^{3,4,7}

Alemtuzumab (CAMPATH-1H) was the first humanized antibody, which was generated to reduce the immunogenicity of the parent CD52-specific rat monoclonal antibody.⁸ A low anti-globulin response was inferred to occur from the phase II study report in people with MS, with only 1 in 208 people (0.5%) having binding antibodies (BAb), above a predefined limit, within 12 months.⁹ Even within the phase III trial reports in MS, only 29% of people were reported to have BAb at 12 months.^{3,4} However, analysis of data

from rheumatoid arthritis (63% BAbs after first infusion),¹⁰ other MS studies,¹¹ and importantly the regulatory submission to the European Medicines Agency (EMA) containing the phase III trial results, showed that a substantial number of people with MS (579/811 (71.5%) produced drug-specific antibody responses within 12 months.⁶ It was evident that alemtuzumab induced a high-frequency [667/789 (84.9%) within 24 months] and high-titre (up to 6 553 600) BAB response that was boosted by repeated cycles of treatment.⁶ Surprisingly, it was also found that 623/667 (93.4% within 24 months) of those people with binding antibodies also produced neutralizing antibodies (NABs).⁶ The occurrence of NABs was not disclosed in the pivotal trial reports^{3,4} and their importance was also dismissed in a meeting report.⁷ In addition, no effect on depletion was reported in people with MS requiring multiple cycles of alemtuzumab in phase II and phase III extension studies.^{12,13} Although it is possible that NABs are not significant at the population level, it was hypothesized that NABs would be important for some individuals, particularly those requiring additional alemtuzumab infusions,^{5,13} as NABs may become persistent in some people.⁶

Materials and methods

Clinical trial reports

Redacted copies of the regulatory submission of the Comparison of Alemtuzumab and Rebif Efficacy in Multiple Sclerosis, study one (CARE-MS I. NCT00530348³) and two (CARE-MS II. NCT0054805⁴), were supplied by the EMA.⁶ The trials were performed in people with MS who were treatment-naïve (CARE-MS I) or who had previously been treated (CARE-MS II) with interferon- β or glatiramer acetate. The data presented here only concern the 12 mg/day alemtuzumab dose, used in clinical practice. This information was derived from the tabulated documents supplied since Q2 2016. Tabulated data concerning BAbs and NABs during MS-CARE II have not yet been supplied. The primary raw data were not supplied by the EMA and requests to access data on antibody responses, via the clinicalstudydatarequest.com website, of which Sanofi is a sponsor, have not yet been supported.

Audit

An audit of 126 people with MS receiving alemtuzumab as part of their clinical care at The Royal London Hospital (Barts Health NHS Trust) was performed to determine their lymphocyte counts following five daily 12-mg alemtuzumab infusions (first cycle) or three 12-mg infusions (second cycle). Cell numbers were monitored as part of

standard care. Analysis of these data did not require ethical review. Informed consent was obtained to report individual case reports.

Results

At the population level, alemtuzumab-specific antibodies do not influence the efficacy of alemtuzumab during the first two treatment cycles

Analysis of the tabulated, unpublished CARE-MS I³ data provided by the EMA was consistent with published statements^{3,4,7} that alemtuzumab-specific antibodies did not impact on lymphocyte depletion (Table 1a), clinical efficacy (Table 1b) or safety (Table 1c; see Supplementary material, Table S1). This was perhaps not surprising because, at the time of infusion, 0% of the participants had NABs before the first cycle of antibodies and only 5/789 (0.6% from CARE-MS I and II) had NABs before the second cycle of antibody. Hence, at the population level, the presence of drug-specific antibodies appeared to be of no concern to the regulators within the EMA.¹⁴

However, interpretation of an online scatter diagram (Fig. 1) from an EMA assessment report,¹⁴ which related lymphocyte number to time from infusion and alemtuzumab-specific antibody status, suggested two previously unreported issues. These are particularly evident when focusing on the first month after treatment, when lymphocyte depletion would not be masked by the rapid B-cell repopulation that occurs following alemtuzumab infusion.⁶ It appears that there are: (i) alemtuzumab antibody-negative individuals who do not deplete, or poorly deplete, their lymphocytes, and (ii) individuals who produce alemtuzumab-specific antibodies (Fig. 1), who do not deplete or poorly deplete lymphocytes.¹⁴

Alemtuzumab-specific antibodies can influence the efficacy of alemtuzumab at the individual level

The data incorporated in the online EMA report (Fig. 1), are consistent with the supplied data from CARE-MS I studies, which indicated that the depletion of lymphocytes was more marked in antibody-negative individuals (Baseline $2.01 \pm 0.64 \times 10^9/l$, $n = 91$; 0.22 ± 0.32 cells $\times 10^9/l$, $n = 45$; 1 month after cycle 2) compared with ever-antibody-positive individuals (BAB only: Baseline $2.08 \pm 0.77 \times 10^9/l$ $n = 68$; $0.42 \pm 0.24 \times 10^9/l$ $n = 14$. BAbs & NABs: Baseline $2.09 \pm 0.61 \times 10^9/l$, $n = 210$; $0.37 \pm 0.22 \times 10^9/l$, $n = 283$) (Table 2). However, without access to the individualized data, it is not possible to determine whether: (i) this was statistically significant, and (ii) the reduced-depletion was due to NABs or an inherent characteristic of people generating anti-drug antibodies, given differences were evident 1 month after the first cycle (Table 2). Nevertheless,

Table 1. Influence of ever-positive alemtuzumab-specific binding and neutralizing antibodies on clinical activity, lymphocyte depletion and adverse events

Time	Always Ab negative		Ever BAb positive/NAB negative		Ever BAb positive/NAB positive	
	<i>n</i>	Outcome	<i>n</i>	Outcome	<i>n</i>	Outcome
(a) Influence of ever-positive anti-neutralizing antibodies and lymphocyte depletion						
<i>Mean ± SD CD4 T cells × 10⁹/l</i>						
Baseline	91	0.96 ± 0.35	68	0.97 ± 0.40	210	0.98 ± 0.35
1 month	85	0.03 ± 0.11	65	0.03 ± 0.02	211	0.05 ± 0.04
3 month	90	0.09 ± 0.11	65	0.09 ± 0.04	213	0.11 ± 0.06
6 month	90	0.15 ± 0.12	68	0.16 ± 0.06	214	0.17 ± 0.09
9 month	92	0.22 ± 0.10	67	0.22 ± 0.10	214	0.24 ± 0.11
12 month	91	0.27 ± 0.19	65	0.29 ± 0.12	215	0.28 ± 0.12
13 month	45	0.06 ± 0.12	18	0.06 ± 0.04	300	0.06 ± 0.04
15 month	50	0.11 ± 0.22	16	0.10 ± 0.04	291	0.11 ± 0.08
18 month	51	0.17 ± 0.14	18	0.16 ± 0.08	296	0.18 ± 0.09
21 month	49	0.23 ± 0.15	18	0.23 ± 0.12	294	0.26 ± 0.12
24 month	47	0.30 ± 0.22	18	0.28 ± 0.13	288	0.32 ± 0.17
<i>Mean ± SD CD8 T cells × 10⁹/l</i>						
Baseline	91	0.48 ± 0.19	68	0.53 ± 0.27	210	0.50 ± 0.22
1 month	85	0.05 ± 0.08	65	0.07 ± 0.09	211	0.08 ± 0.10
3 month	90	0.12 ± 0.14	65	0.11 ± 0.08	213	0.13 ± 0.11
6 month	90	0.16 ± 0.14	68	0.17 ± 0.13	214	0.16 ± 0.13
9 month	92	0.23 ± 0.19	67	0.21 ± 0.14	214	0.22 ± 0.16
12 month	91	0.26 ± 0.19	65	0.26 ± 0.18	215	0.24 ± 0.16
13 month	45	0.07 ± 0.11	14	0.08 ± 0.07	300	0.06 ± 0.08
15 month	50	0.11 ± 0.13	16	0.12 ± 0.09	291	0.11 ± 0.08
18 month	51	0.16 ± 0.14	17	0.17 ± 0.13	296	0.16 ± 0.09
21 month	49	0.19 ± 0.14	18	0.19 ± 0.10	294	0.20 ± 0.12
24 month	47	0.23 ± 0.18	18	0.22 ± 0.12	288	0.24 ± 0.14
<i>Mean ± SD CD19 B cells × 10⁹/l</i>						
Baseline	91	0.25 ± 0.14	68	0.27 ± 0.14	210	0.27 ± 0.12
1 month	85	0.02 ± 0.02	65	0.03 ± 0.03	211	0.02 ± 0.01
3 month	90	0.20 ± 0.13	65	0.21 ± 0.12	213	0.21 ± 0.12
6 month	90	0.26 ± 0.14	68	0.28 ± 0.19	214	0.28 ± 0.17
9 month	92	0.30 ± 0.17	67	0.30 ± 0.16	214	0.32 ± 0.18
12 month	91	0.33 ± 0.28	65	0.33 ± 0.18	215	0.35 ± 0.18
13 month	45	0.03 ± 0.03	14	0.06 ± 0.10	283	0.03 ± 0.05
15 month	50	0.15 ± 0.11	16	0.19 ± 0.10	291	0.18 ± 0.11
18 month	51	0.26 ± 0.18	17	0.25 ± 0.14	296	0.27 ± 0.16
21 month	49	0.29 ± 0.20	18	0.28 ± 0.11	294	0.31 ± 0.17
24 month	47	0.36 ± 0.22	18	0.31 ± 0.12	288	0.35 ± 0.18
(b) Influence of ever-positive anti-neutralizing antibodies and clinical events						
<i>Number (annualized rates, 95% CI) of relapses</i>						
Overall	49	13 (0.22, 0.13–0.37)	22	2 (0.18, 0.03–0.97)	305	67 (0.15, 0.12–0.19)
Cycle 1	92	15 (0.19, 0.12–0.31)	68	10 (0.26, 0.12–0.52)	216	31 (0.16, 0.11–0.22)
Cycle 2	51	7 (0.16, 0.08–0.34)	18	0	300	32 (0.13, 0.09–0.18)
<i>Mean ± SD overall T2-hyperintense volume</i>						
Baseline	48	7.47 ± 7.72	21	7.91 ± 7.28	302	7.40 ± 9.33
24 month	48	6.61 ± 7.38	20	7.74 ± 7.31	298	6.56 ± 8.57
(c) Influence of ever-positive anti-neutralizing antibodies and treatment-related adverse events						
<i>Number (percentage) of people with MS with adverse event</i>						
Overall	49	45 (91.8)	22	19 (86.4)	305	274 (89.8)
Cycle 1	92	77 (83.7)	68	59 (86.8)	216	187 (86.6)
Cycle 2	51	32 (62.7)	18	9 (50)	300	202 (67.3)
<i>Number (percentage) of administration site reactions</i>						
Overall	49	26 (53.1)	22	13 (59.1)	305	164 (53.8)

Table 1 (Continued)

Time	Always Ab negative		Ever BAb positive/NAb negative		Ever BAb positive/NAb positive	
	n	Outcome	n	Outcome	n	Outcome
Cycle 1	92	36 (39.1)	68	27(39.7)	216	91 (42.1)
Cycle 2	51	12 (23.5)	18	5 (27.8)	300	92 (30.7)

The presence of binding (BAb) and Binding and neutralizing antibodies (NAb) was assessed as being present or absent during each cycle of treatment of alemtuzumab. The results were extracted from tabulated data within the EMA dataset. They represent the mean and standard deviation, the number and annualized relapse rate and 95% confidence intervals. The mean and standard deviation of T2 lesions and the number of adverse events and (percentage).

inspecting responses before and after alemtuzumab reveals that pre-cycle alemtuzumab NAb did affect lymphocyte depletion at the individual level (Table 3). Examination of immunophenotyping, 1 month after the second infusion cycle, demonstrated that one individual with a high-titre response had failed to adequately deplete (Table 3). This raises concerns that neutralization responses would become more problematic during and following a third treatment cycle, as 623/789 (79.0%; titre 30–102, 400) people had made a BAb response and 239/764 (31.3%; titre 20–640) had a persistent NAb response at 24 months from treatment onset.¹⁴ Indeed a blunted lymphocyte depletion response was seen following a third treatment

cycle in an individual, in the MS-CARE extension programme, who received multiple treatment cycles due to disease activity (see Supplementary material, Fig. S1). A clinical relapse triggered a fourth cycle about 20 months later. Although depletion was comparable to the first and second treatment cycles, inflammatory MS activity occurred, triggering a fifth infusion, which induced limited depletion. Following requests about information relating to antibody neutralization. The manufacturer confirmed the presence of BAbs and the presence of NAb (maximum titre = 200), which were boosted with each cycle. This alerted us to the fact that NAb occurred, in part prompting our investigations. Although this individual was clearly depleting, it was possible that NAb were contributing to treatment failure. Indeed an online search of meeting presentations revealed that 2/6 (33.3%) people who received at least three infusions of alemtuzumab before being switched to fingolimod, showed a relative lack of depletion upon the third treatment cycle,¹⁵ suggesting that some people may generate a neutralizing response that prevents depletion.

This prompted us to audit the lymphocyte levels of people with MS treated with alemtuzumab in our centre in the post-marketing setting (see Supplementary material, Fig. S2). All 57 people with who received two cycles of treatment had lymphocyte depletion following infusion cycle 1 and 2. The lymphocytes were depleted from $2.21 \pm 1.06 \times 10^9$ cells/l to $0.27 \pm 0.21 \times 10^9$ cells/l ($P < 0.001$ compared with baseline) at month 1 and $0.36 \pm 0.20 \times 10^9$ cells/l ($P = 0.001$ compared with baseline) at month 13, 1 month after the second cycle. However, paired analysis indicated that there was significantly ($P = 0.004$) more depletion after the first infusion compared with the second infusion cycle, perhaps consistent with more antibody being infused on the first cycle (60 mg) compared with subsequent (36 mg) cycles. It was found that 7/57 people did not deplete below 0.7×10^9 cells/l after the second infusion cycle and one person depleted by only 36.4% compared with month 0, despite a depletion of over 80% following the first infusion. However, the status of alemtuzumab-specific antibodies was unknown. Outside the CARE MS extension

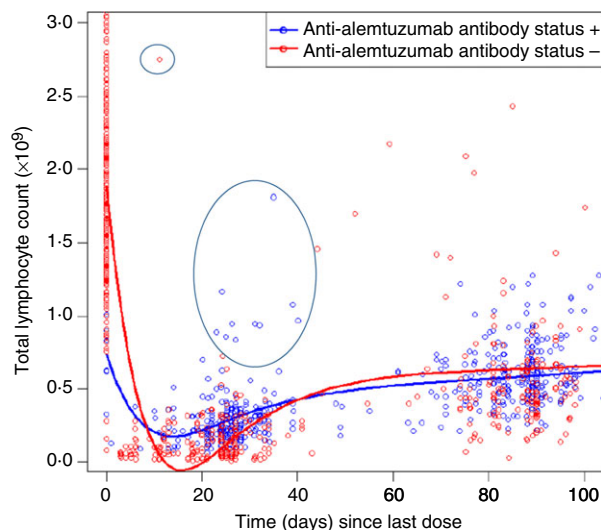


Figure 1. Lymphocyte depletion and anti-alemtuzumab antibody status. Scatterplot of peripheral blood lymphocyte levels over time and anti-alemtuzumab antibody status in people with in the MS CARE studies. The diagram is reproduced from documents in the public domain,¹⁴ following correspondence with both the European Medicines Agency and Sanofi Genzyme. The latter allowed the reproduction. Data within the first month will show the depletion kinetics. At later time-points, the data may be confounded by rapid re-populators. The circles highlight an antibody-negative individual who was a poor depleter and antibody-positive individuals who are poor depleters.

Table 2. Influence of ever-positive alemtuzumab-specific binding and neutralizing antibodies on lymphocyte depletion events

Time	Always negative		Ab Ever Bab+/Nab negative		Ever Bab/NAb positive	
	N	Mean ± SD	N	Mean ± SD	N	Mean ± SD
Baseline	91	2.01 ± 0.64	68	2.08 ± 0.77	210	2.09 ± 0.61
1 month	85	0.15 ± 0.52	65	0.22 ± 0.18	211	0.33 ± 0.20
3 month	90	0.63 ± 0.33	65	0.63 ± 0.33	213	0.68 ± 0.33
6 month	90	0.83 ± 0.33	68	0.85 ± 0.37	214	0.90 ± 0.36
9 month	92	1.04 ± 0.40	67	1.01 ± 0.35	214	1.07 ± 0.40
12 month	91	1.15 ± 0.52	65	1.15 ± 0.41	215	1.16 ± 0.42
13 month	45	0.22 ± 0.32	14	0.42 ± 0.24	283	0.37 ± 0.22
15 month	50	0.57 ± 0.41	16	0.65 ± 0.21	291	0.65 ± 0.26
18 month	51	0.83 ± 0.34	17	0.85 ± 0.38	296	0.89 ± 0.32
21 month	49	0.99 ± 0.39	18	0.99 ± 0.34	300	1.08 ± 0.38
24 month	47	1.15 ± 0.51	18	1.11 ± 0.36	288	1.21 ± 0.46

The presence of binding (BAB) and Binding and neutralizing antibodies (NAb) was assessed as being present or absent during each cycle of treatment of alemtuzumab. The results represent the mean and standard deviation of cells $\times 10^9/l$ at various times post infusion at baseline and at 12 months or the number of adverse events and (percentage).

trial participants, people with MS generally do not receive a third treatment cycle, though there is some inconsistency in provision of the drug across the National Health Service within the UK. People with disease breakthrough were often switched to other disease-modifying

treatments. The data suggest that a few individuals fail to deplete, consistent with the generation of a NAB response.

Some individuals do not develop leucocyte depletion following alemtuzumab-treatment

Although NABs may cause lack of response to alemtuzumab, the EMA report¹⁴ data indicated that there may also be alemtuzumab antibody-negative individuals who fail to deplete, or deplete poorly (Fig. 1). We were able to identify such cases in the EMA data set supplied. At baseline there were $2.07 \pm 0.65 \times 10^9$ cells/l ($n = 369$; range $0.93\text{--}5.83 \times 10^9$ cells/l) lymphocytes. There was depletion within 1 month after alemtuzumab ($0.27 \pm 0.22 \times 10^9$ cells/l; $n = 361$) but the upper values of the range ($0.02 \times 10^9\text{--}2.00 \times 10^9$ cells/l) indicated that some people either did not deplete or depleted poorly.

In the audit at The Royal London Hospital, 126 people with MS who received at least one dose of alemtuzumab (see Supplementary material, Fig. S2) had $2.05 \pm 0.89 \times 10^9$ lymphocytes/l (range $0.4 \times 10^9\text{--}6.2 \times 10^9$ cells/l) at baseline and depleted to $0.27 \pm 0.19 \times 10^9$ lymphocytes/l (range $0.0 \times 10^9\text{--}1.0 \times 10^9$ cells/l) 1 month later. Only 3/126 people did not deplete below $0.7 \times 10^9/l$. Two people depleted by $< 50\%$ after first administration, although one of these was lymphopenic at baseline. Of note, the individual who was a poor-depletor (11.1%) had been treated with fingolimod, a risk factor for lack of alemtuzumab activity,¹⁶ before switching to alemtuzumab.

Table 3. Pre-cycle neutralizing antibodies can influence depletion at the individual level

Time	No Ab		BAB Positive		NAB Positive		Titre NAB positive individual results
	N	Mean ± SD	N	Mean ± SD	N	Mean ± SD	
Lymphocytes							
Baseline	362	2.07 ± 0.64	3	2.29 ± 0.80	0	n/a ± n/a	
1 month	352	0.27 ± 0.22	3	0.18 ± 0.22	0	n/a ± n/a	
13 month	233	0.33 ± 0.25	92	0.37 ± 0.20	3	0.83 ± 0.88	LT: 0.11, 0.56. HT: 1.81
CD4 T cells							
Baseline	362	0.97 ± 0.36	3	1.18 ± 0.62	0	n/a ± n/a	
1 month	352	0.04 ± 0.03	3	0.03 ± 0.01	0	n/a ± n/a	
13 month	233	0.05 ± 0.06	97	0.06 ± 0.04	3	0.19 ± 0.28	LT: 0.02, 0.04. HT: 0.51
CD8 T cells							
Baseline	362	0.50 ± 0.22	3	0.50 ± 0.21	0	n/a ± n/a	
1 month	352	0.07 ± 0.10	3	0.02 ± 0.01	0	n/a ± n/a	
13 month	233	0.06 ± 0.09	97	0.07 ± 0.07	3	0.10 ± 0.11	LT: 0.02, 0.05. HT: 0.23
CD19 T cells							
Baseline	362	0.27 ± 0.13	3	0.26 ± 0.14	0	n/a ± n/a	
1 month	352	0.02 ± 0.02	3	0.03 ± 0.01	0	n/a ± n/a	
13 month	233	0.03 ± 0.04	97	0.03 ± 0.02	3	0.27 ± 0.44	LT: 0.02, 0.02. HT: 0.78

The presence of binding antibodies (BABs) and neutralizing antibodies (NABs) was assessed before each cycle of treatment of alemtuzumab. The results represent the mean and standard deviation of cells $\times 10^9/l$ and the individual responses of the three people who had pre-cycle NABs of either low titre (LT; first quartile) or high titre (HT; fourth quartile).

However other individuals were identified who depleted very poorly upon the first infusion, but had not used fingolimod previously. Their most recent therapy had been dimethyl fumarate. In one individual, their baseline white blood cell count was 7.6×10^9 cells/l and this only decreased to 7.0×10^9 /l 1 month after alemtuzumab infusion and their lymphocyte count went from 2.0×10^9 cells/l to 2.58×10^9 cells/l 1 month after alemtuzumab. In another individual, poor depletion was also evident (Fig. 2). Hence, there are people with MS who do not deplete, or deplete poorly, following alemtuzumab treatment. Further clinical investigations into the causes of this can be made once ethical approval for such research is acquired.

Discussion

Although alemtuzumab was the first humanized antibody designed to reduce the immunogenicity of therapeutic rodent antibodies,⁸ it is clear that its biology prevents this from being adequately achieved and it is, perhaps, rather poor at limiting immunogenicity.⁶ It is well understood that NAb responses lead to failure of therapeutic drugs, including therapeutic antibodies,¹⁷ as indicated here following alemtuzumab treatment. CD52-specific antibodies deplete CD4 regulatory T cells and CD8 suppressor cells and appear to block immunological tolerance induction,^{6,18} at a time of immature B-cell hyper-population, seen in the blood.⁶ This may create the environment for secondary B-cell autoimmunities that occur with high frequency as a consequence of alemtuzumab treatment and may also create an environment for NAb production.⁶ Although alemtuzumab depletes peripheral blood leucocytes, it appears to use antibody-dependent cellular cytotoxicity as a central depleting mechanism and may purge

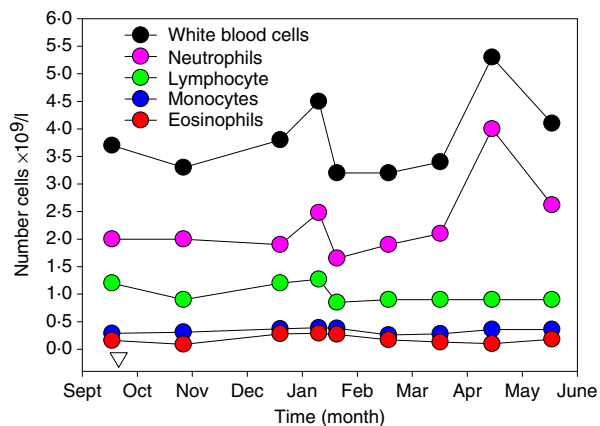


Figure 2. Failure to respond to alemtuzumab from first infusion. The absolute numbers of white blood cells, lymphocytes, monocytes and granulocytes were assessed in an individual receiving a single cycle of alemtuzumab treatment (inverse triangle).

the lymphoid tissues, notably the bone marrow, less effectively, as seen in CD52-depleting antibody in human CD52-transgenic and wild-type mice.^{18–20} Therefore, anti-globulin responses may be generated during the 5-day infusion period when high-titre blood concentrations are present and account for the high frequency of BAbs and NABs produced within a month of first infusion.⁶

The influence of BAbs and NABs is not a major issue within the first 2 years of the trial analysis examined by the regulators.¹⁴ However, the concern should be that alemtuzumab neutralization may be more of a problem following subsequent cycles. At the population level, depletion occurred with each treatment in the MS-CARE extension study,¹³ consistent with earlier studies ($n = 108^{12}$), without mention of non-depleters. However, as reported here, there is no doubt that NABs have led to some people being repeatedly treated with agents with limited efficacy¹⁵ and they do become problematic for some individuals.^{21,22}

The failure to adequately report this issue has probably exposed people to the unnecessary risks of the procedures and risks of disease reactivation due to lack of efficacy. Indeed our own experience prompted us to investigate and expose¹⁶ the occurrence of alemtuzumab neutralization. This study indicates that at the individual level NABs may indeed be very important, and shows that it is critical that lymphocyte-depleting potential is monitored. This could easily be scheduled due to the requirement for monthly safety blood tests and the titres of antibodies should be monitored before re-infusion, in particular in relation to the third, fourth and fifth treatment cycles. In addition, BAbs may also contribute to loss of function of alemtuzumab. They may serve to augment cross-linking of CD52 that can expand CD52-deficient lymphocytes.²³ These CD52-deficient cells occur following alemtuzumab infusion and can persist for months within the B-cell compartment and for years within the T-cell compartment.²⁴ Further work will be needed to determine pre-treatment levels that contribute to physical antibody neutralization and lack of treatment response.

In addition to the generation of NABs, it is also evident that some people do not deplete/delete well on first infusion. It is interesting that people destined to make alemtuzumab-specific antibodies appeared to deplete lymphocytes less well than those who did not make antibodies. This occurred even after the first infusion and probably before NABs would influence the outcome, suggesting a genetic predisposing influence. Analysis of over 60 000 exomes has not indicated loss of function variants of CD52.²⁵ There are, however, two common single nucleotide polymorphisms (SNP; rs1071849 and rs17645) in the 3' untranslated region that are in linkage disequilibrium (allele frequency in Europeans = 0.7541²⁵), which are suggested to impact the efficacy of the glycosylphosphatidylinositol anchor in CD52 to influence responses to

alemtuzumab, in some circumstances.^{26,27} Furthermore, Latino people have a common SNP (rs77928789; allele frequency = 0.1931) in the 5' untranslated region.²⁵ Whether these alleles influence expression of CD52 is unclear. In addition, there are Fc receptor (FCGR3A, SNP rs396991 and FCGR2A, SNP rs1801274) variants that influence affinity for human IgG1 and affect efficacy of antibody-dependent cellular cytotoxicity,^{28–30} which is reported to be an important depletion mechanism of alemtuzumab.¹⁹ Although these Fc receptor variants did not affect alemtuzumab (CAMPATH-1H)-induced depletion at doses used to treat cancer,³¹ this dose is significantly higher than the alemtuzumab (Lemtrada) formulation used in MS. Therefore, these genetic variants may be relevant in MS, as the Fc receptor depletion influences are known to be antibody dose-dependent, as seen with rituximab-induced depletion.^{29,32} It will be important to determine whether these alleles are important in depletion caused by alemtuzumab in MS, as it may influence switching to other IgG1 treatments or non-antibody alternatives.

Alternatively it has also been suggested that prior use of fingolimod, to sequester lymphocytes into lymphoid tissue and bone marrow, may block the activity of alemtuzumab and allow more rapid repopulation.¹⁶ Therefore, it is of interest that one of the poor depleters reported here was treated with fingolimod prior to alemtuzumab. However, the people in the MS-CARE studies were naive with respect to fingolimod and therefore poor depletion in some people with MS has alternative explanations and requires further investigation.

Although it is evident that alemtuzumab is a useful treatment, and despite being a potent T-cell and B-cell depletion agent, alemtuzumab is not immune to the problems associated with NAb. Our study suggests that it is important to actively monitor lymphocyte depletion potential to determine whether individual people with MS are, perhaps genetically, resistant to alemtuzumab depletion, or perhaps whether there is resistance to IgG1 depletion. It will also be important to monitor whether high-titre NAb are present before re-infusion of additional treatment cycles. This may affect decisions to retreat or switch treatment and may influence which treatments people are switched to.

It is likely that the frequency of non-depletors/poor depletors on first and subsequent treatment cycles and the pre-treatment titre of anti-alemtuzumab antibodies that are associated with loss of depletion function is known or readily accessible by the manufacturer. This is because people with MS were immunophenotyped during the original MS-CARE studies,⁶ and many people with MS ($n = 742/811$) entered the CARE-MS extension studies and were immune-phenotyped.¹³ Many people with MS ($n = 412$) received no re-treatment or other disease-modifying treatments, but some people received a third

($n = 156$), fourth ($n = 48$) or fifth ($n = 8$) treatment cycle.¹³ Alternatively, examination and compilation of post-marketing data will allow some of these issues to be investigated. However, this is yet another example of why it is important that full and unrestricted access to all anonymized clinical trial data should be granted within a reasonable time frame, both to incentivize companies to publish their data, and to ensure that efficacy and, importantly, safety issues can be investigated independently. This information should be deposited, in an accessible format, prior to and as part of initial, and subsequent, regulatory approval processes.

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Disclosures

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References

- 1 Compston A, Coles A. Multiple sclerosis. *Lancet* 2008; **372**:1502–17.
- 2 Baker D, Marta M, Pryce G, Giovannoni G, Schmierer K. Memory B cells are major targets for effective immunotherapy in relapsing multiple sclerosis. *EBiomed* 2017; **16**:41–50.
- 3 Cohen JA, Coles AJ, Arnold DL, Confavreux C, Fox EJ, Hartung HP *et al.* Compston DA; CARE-MS I investigators. Alemtuzumab versus interferon β 1a as first-line

- treatment for patients with relapsing–remitting multiple sclerosis: a randomised controlled phase 3 trial. *Lancet* 2012; **380**:1819–28.
- 4 Coles AJ, Twyman CL, Arnold DL, Cohen JA, Confavreux C, Fox EJ *et al.* Compston DA; CARE-MS II investigators. Alemtuzumab for patients with relapsing multiple sclerosis after disease-modifying therapy: a randomised controlled phase 3 trial. *Lancet* 2012; **380**:1829–39.
 - 5 Tuohy O, Costelloe L, Hill-Cawthorne G, Bjornson I, Harding K, Robertson N *et al.* Alemtuzumab treatment of multiple sclerosis: long-term safety and efficacy. *J Neurol Neurosurg Psychiatry* 2015; **86**:208–15.
 - 6 Baker D, Herrod SS, Alvarez-Gonzalez C, Giovannoni G, Schmierer K. Interpreting lymphocyte reconstitution data from the pivotal phase III trials. *JAMA Neurol* 2017; **74**:961–9.
 - 7 Ziemssen T, Arnold DL, Cohen JA, Coles AJ, Fox EJ, Hartung H-P *et al.* Immunogenicity of alemtuzumab does not impact safety and efficacy in relapsing remitting multiple sclerosis patients in the CARE-MS I study. *Mult Scler J* 2013; **19**(Suppl. 1):212–3.
 - 8 Rebello PR, Hale G, Friend PJ, Cobbold SP, Waldmann H. Anti-globulin responses to rat and humanized CAMPATH-1 monoclonal antibody used to treat transplant rejection. *Transplantation* 1999; **68**:1417–20.
 - 9 Coles AJ, Compston DA, Selmaj KW, Lake SL, Moran S, Margolin DH *et al.* Alemtuzumab vs. interferon β 1a in early multiple sclerosis. *N Engl J Med* 2008; **359**:1786–801.
 - 10 Weinblatt ME, Maddison PJ, Bulpitt KJ, Hazleman BL, Urowitz MB, Sturrock RD *et al.* CAMPATH-1H, a humanized monoclonal antibody, in refractory rheumatoid arthritis: an intravenous dose-escalation study. *Arthritis Rheum* 1995; **38**:1589–94.
 - 11 Sommerfield J, Hill-Cawthorne GA, Lin A, Zandi MS, McCarthy C, Jones JL *et al.* A novel strategy to reduce the immunogenicity of biological therapies. *J Immunol* 2010; **185**:763–8.
 - 12 Kousin-Ezewu O, Azzopardi L, Parker RA, Tuohy O, Compston A, Coles A *et al.* Accelerated lymphocyte recovery after alemtuzumab does not predict multiple sclerosis activity. *Neurology* 2014; **82**:2158–64.
 - 13 Boyko AN, Havrdovaz E, King J, Limmroth V, Selmaj KW, Sharrack B *et al.* Lymphocyte depletion and repopulation is consistent across alemtuzumab treatment courses in patients with relapsing–remitting multiple sclerosis: 6-year analysis of patients from the CARE-MS studies. *Mult Scler J* 2016; **22**:312–3.
 - 14 European Medicines Agency. Committee for Medicinal Products for human use Assessment report Lemtrada Procedure No EMEA/H/C/003718/000. EMA/563018/2013 27 June 2013. URL http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_Public_assessment_report/human/003718/WC500150522.pdf [accessed on 16 September 2017].
 - 15 Selmaj K, Bass AD, Edwards KR, Sorensen PS, Margolin DH, Kasten L *et al.* Lymphocyte pharmacodynamics and safety of fingolimod use in patients previously treated with alemtuzumab. *Eur J Neurol* 2015; **1**(Suppl. 1):759.
 - 16 Willis M, Pearson O, Illes Z, Sejbaek T, Nielsen C, Duddy M *et al.* An observational study of alemtuzumab following fingolimod for multiple sclerosis. *Neurol Neuroimmunol Neuroinflamm* 2017; **4**:e320.
 - 17 Cohen BA, Oger J, Gagnon A, Giovannoni G. The implications of immunogenicity for protein-based multiple sclerosis therapies. *J Neurol Sci* 2008; **275**:7–17.
 - 18 von Kutzleben S, Pryce G, Giovannoni G, Baker D. Depletion of CD52-positive cells inhibits the development of central nervous system autoimmune disease, but deletes an immune-tolerance promoting CD8⁺ T-cell population. Implications for secondary autoimmunity of alemtuzumab in multiple sclerosis. *Immunology* 2017; **150**:444–55.
 - 19 Hu Y, Turner MJ, Shields J, Gale MS, Hutto E, Roberts BL *et al.* Investigation of the mechanism of action of alemtuzumab in a human CD52 transgenic mouse model. *Immunology* 2009; **128**:260–70.
 - 20 Turner MJ, Lamorte MJ, Chretien N, Havari E, Roberts BL, Kaplan JM *et al.* Immune status following alemtuzumab treatment in human CD52 transgenic mice. *J Neuroimmunol* 2013; **261**:29–36.
 - 21 Coles AJ. Alemtuzumab therapy for multiple sclerosis. *Neurotherapeutics* 2013; **10**:29–33.
 - 22 Eggers C, Thomas K, Hofer T, Egger M, Ziemssen T. Abrogation of the lymphocyte depleting action of alemtuzumab by neutralizing antibodies – a case report. *Mult Scler J* 2017; **23**(Suppl. 3):655.
 - 23 Rowan W, Tite J, Topley P, Brett SJ. Cross-linking of the CAMPATH-1 antigen (CD52) mediates growth inhibition in human B- and T-lymphoma cell lines, and subsequent emergence of CD52-deficient cells. *Immunology* 1998; **95**:427–36.
 - 24 Brett SJ, Baxter G, Cooper H, Rowan W, Regan T, Tite J *et al.* Emergence of CD52-, glycosylphosphatidylinositol-anchor-deficient lymphocytes in rheumatoid arthritis patients following Campath-1H treatment. *Int Immunol* 1996; **8**:325–34.
 - 25 Lek M, Karczewski kj, Minikel EV, Samocha KE, Banks E, Fennell T *et al.* Analysis of protein-coding genetic variation in 60,706 humans. *Nature* 2016; **536**:285–91.
 - 26 Hale C, Bartholomew M, Taylor V, Stables J, Topley P, Tite J. Recognition of CD52 allelic gene products by CAMPATH-1H antibodies. *Immunology* 1996; **88**:183–90.
 - 27 Oko A, Wyrwicz LS, Glyda M, Idasiak-Piechocka I, Bińczak-Kuleta A, Kaczmarczyk M *et al.* CD52 gene polymorphism and its potential effect on the response to alemtuzumab in renal transplant recipients. *Ann Acad Med Stetin* 2009; **55**:22–6.
 - 28 Robledo G, Márquez A, Dávila-Fajardo CL, Ortego-Centeno N, Rubio JL, Garrido Ede R *et al.* Association of the FCGR3A-158F/V gene polymorphism with the response to rituximab treatment in Spanish systemic autoimmune disease patients. *DNA Cell Biol* 2012; **31**:1671–7.
 - 29 Mellor JD, Brown MP, Irving HR, Zalberg JR, Dobrovic A. A critical review of the role of Fc γ receptor polymorphisms in the response to monoclonal antibodies in cancer. *J Hematol Oncol* 2013; **6**:1.
 - 30 Liu D, Tian Y, Sun D, Sun H, Jin Y, Dong M. The FCGR3A polymorphism predicts the response to rituximab-based therapy in patients with non-Hodgkin lymphoma: a meta-analysis. *Ann Hematol* 2016; **95**:1483–90.
 - 31 Lin TS, Flinn IW, Modali R, Lehman TA, Webb J, Waymer S *et al.* FCGR3A and FCGR2A polymorphisms may not correlate with response to alemtuzumab in chronic lymphocytic leukemia. *Blood* 2005; **105**:289–91.
 - 32 Rezvani AR, Maloney DG. Rituximab resistance. *Best Pract Res Clin Haem* 2011; **24**:203–16.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Influence of ever-positive alemtuzumab-specific binding and neutralizing antibodies on alemtuzumab-related adverse events.

Figure S1. Blunted lymphocyte depleting response in a person with multiple sclerosis who received multiple alemtuzumab infusion cycles.

Figure S2. Lymphocyte count profile on people with multiple sclerosis treated with alemtuzumab in the post-marketing setting.