Alemtuzumab for multiple sclerosis: Long term Follow-up in a multi-centre cohort

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Abstract
Background: Alemtuzumab has recently been approved for treatment of relapsing MS, but concerns remain about its use since long-term studies of adverse events remain limited. Furthermore, a clear understanding of its application and durability of effect in clinical practice has yet to evolve.
Objectives: To investigate long-term efficacy and safety outcomes in a multicentre cohort of patients treated with alemtuzumab.
Methods: Patients treated from 2000 and followed-up at three regional centres were identified. Baseline and prospective data were obtained and validated by clinical record review.
Results: One hundred patients were identified with a mean follow-up of 6.1 years (range 1–13). Forty patients were retreated with at least one further treatment cycle. Annualized relapse rates fell from 2.1 to 0.2 ($p<0.0001$) post-treatment and were sustained for up to eight years of follow-up. Mean change in EDSS score was +0.14. Forty-seven patients developed secondary autoimmunity.
Conclusion: Observed reduction in relapse rates reflected those reported in clinical trials, but we were unable to corroborate previous observations of disability reversal. 40% of patients required additional treatment cycles. Autoimmune adverse events were common, occurring at a higher rate than previously reported, but were largely predictable, and could be managed effectively within a rigorous monitoring regime.

Introduction
The range of effective treatments for relapsing multiple sclerosis (MS) is rapidly expanding, leading to an ever greater choice for both patients and clinicians. Although the new disease-modifying therapies have undergone rigorous clinical trials before reaching the clinic, post-marketing surveillance and reporting will be essential in order to fully understand safety and efficacy, and in some cases have been key in modifying use in clinical practice. Alemtuzumab is a humanized monoclonal antibody which targets CD52, a 12 amino acid glycosylated glycosylphosphatidylinositol-linked protein expressed on the cell surface of lymphocytes, monocytes, macrophages, eosinophils and NK cells.[1,2] The anti-CD52 effect of alemtuzumab results in rapid and profound depletion of circulating lymphocytes following intravenous infusion, as a result of antibody-dependent cell-mediated cytotoxicity.[3] However, CD52 is not expressed on haematopoietic precursors, so allowing beneficial immune reconstitution.[4,5] Although exhibiting a degree of individual variability, the pattern of immune reconstitution is not thought to reliably predict disease activity.[6–8]

Early open label studies demonstrated a marked reduction of relapse rates and slowing of disability accumulation when given early in the course of disease.[9–14] One subsequent phase II trial[15] and two phase III trials,[16,17] both against an active comparator, have confirmed this effect, and open

label follow-up data for the phase II trial also demonstrates durability of effect over five years of follow-up.18 Despite the clear beneficial effects of alemtuzumab on MS disease activity, there have been concerns regarding its side-effect profile, initially cited by the US Food and Drug Administration as a reason not to approve its use in the United States, although later revoked. In particular, secondary autoimmune disease (AID) is said to affect approximately 30% of patients, with the thyroid gland the most common target.19 Other serious, but less common, forms of AID include idiopathic thrombocytopaenic purpura (ITP), haemolytic anaemia, autoimmune neutropaenia and glomerulonephritis (Goodpasture's syndrome).15–17,19 In addition, predictable adverse infusion related reactions, including headaches, rigors, pyrexia and rash, affect the majority of patients.20 Because of these issues, long-term follow-up data from both controlled trials and open label studies will continue to be of value in informing patient selection, retreatment strategies and long-term surveillance protocols.

Alemtuzumab has been used in selected centres in the UK since 2000 as a practical, alternative treatment for patients with early, aggressive disease with poor prognostic indicators, at a time when access to more effective treatments was limited, and therefore offers a unique opportunity to access long term follow- up data collected in routine clinical practice. Patients presenting with high relapse rates,21 together with poor prognostic features,22–24 were considered candidates for treatment. We present data of a 'realworld' experience of the use of alemtuzumab in MS across three UK MS centres, focusing on relapse rates, disability data, re-treatment rates and adverse events.

Methods
Patients and data collection
Patients referred to and assessed in the neurology department at the University Hospital of Wales, Cardiff, were identified as candidates for treatment with alemtuzumab if they had a relapsing disease course and evidence of aggressive disease, characterized by a high relapse rate, active disease on cranial MR imaging, rapidly accumulating disability, early motor, cerebellar or cognitive dysfunction or combinations of these factors, and were considered to have poor prognosis. A smaller number of patients were treated locally at regional specialist neuroscience centres in Swansea and Bristol following regional network case-based discussions. Patients receiving alemtuzumab as part of externally sponsored clinical trials were excluded from analysis.

Prior to treatment all patients had a normal blood count, thyroid function tests, routine blood indices and white cell immunophenotyping. At the time of treatment no patient had evidence of active infection, and treatment during relapse was avoided whenever possible. Consent for treatment was obtained and explanation of potential risks and benefits provided.

Treatment regimen and adverse event monitoring
Prior to 2006, patients received an initiation dose of 24–30 mg alemtuzumab intravenously per day for five days, with 1 g intravenous methylpredniso-lone given as pre-treatment on the first three days only in order to ameliorate the expected infusion reaction side-effects related to cytokine release.20 After 2006, the daily dose of alemtuzumab was reduced to 12 mg. Routine top-up treatment was administered after 12 months, consisting of three daily doses of alemtuzumab with concurrent steroid pre-treatment. Additional courses were given as indicated after intervals of not less than 12 months, as a result of one or more of the following factors: (1) disabling clinical relapse; (2) evolving disability with or without objective change in EDSS;(3) the development of new or enhancing lesions on MRI performed 12 months or more after a prior treatment cycle.

A monitoring programme for adverse autoimmune events included monthly full blood count and urea and electrolytes, in addition to thyroid function tests with anti-thyroperoxidase antibodies at least six-monthly intervals. Urinalysis was performed when indicated or during concomitant illness. Additional tests for relevant AID-related antibodies were per-formed when appropriate.

Data analysis
Patients were identified from a regional clinical data-base,25 and a systematic review of notes was per-formed to validate the dataset. Data was collected on demographics, EDSS scores, relapses, adverse events and prior medication use in order for further analysis to be performed. Final data capture was performed on 23 April 2015. Retreatment rates, annualized relapse rates (ARR) pre- and post-treatment, disability out-comes, adverse events, including rates of AID, and outcomes of

pregnancies were investigated. Six-month sustained accumulation (SAD) and reduction of disability (SRD) was calculated according to established definitions.26

Table 1. Demographics and baseline characteristics of 100 patients treated with alemtuzumab.

Demographics	All patients	Responders	Non-responders
Number of patients	100	96	4
Female	67	63	4
Relapsing disease	97	93	4
Mean age at disease onset (SD)	28.4 years (8.9)	28.5 (8.9)	26.5 (10.5)
Mean baseline EDSS (SD)	4.0 (1.9)	3.8 (1.9)	4.5 (1.1)
Mean time from disease onset to first treatment (SD)	4.4 years (3.7)	4.4 (3.7)	4.4 (2.1)
Mean time from diagnosis to first treatment (SD)	2.0 years (2.0)	2.0 (2.1)	1.3 (1.7)
Mean follow-up post first treatment	6.1 years	6.0	8.2
Median follow-up post first treatment (range)	6.2 years (0.2–12.9)	6.0 (0.2–12.9)	8.5 (6.2–9.7)
Prior DMT use	27	26	1

SD: Standard deviation.

Results
Demographics
One hundred patients treated with alemtuzumab since 2000 were identified (female 67, male 33) with a total follow-up of 607 patient years. Ninety-seven patients had relapsing onset disease at the time of first treatment. Three patients were subsequently re-classified as secondary progressive disease with frequent relapses with the benefit of hindsight. Demographic characteristics of the cohort are summarized in Table 1.

The majority of patients (79%) have been followed-up for between two and ten years, with a small pro-portion being followed-up for less than two (9%) and more than ten (12%) years, respectively. 27% patients had been on at least one prior disease-modifying therapy (DMT). These included avonex (4), azathioprine (2), betaferon (5), copaxone (5), extavia (1), methotrexate (1), mycophenolate mofetil (2), natalizumab (3) and rebif (18). Of the three patients treated with natalizumab, two patients developed thyroid autoimmunity; another patient was subsequently diagnosed with haemolytic anaemia and ITP. Three patients commenced alternative DMTs a mean of 3.4 years following first alemtuzumab infusion. Two patients from this cohort have died; one following an ischaemic stroke 6.9 years after initial treatment and the other patient eight years after the first treatment infusion from aspiration pneumonia.

Retreatment rates
The majority of patients (53%) underwent or were planned to complete the standard two cycles of treatment. 28% patients received three treatments, 11% four treatments and one patient five treatments. Seven patients received one treatment cycle only, with the commonest reasons being concerns related to monitoring adherence (n=2), development of precancerous comorbidity (n=1) or severe infusion reactions (n=3). One of the first patients to be treated also only received one cycle when experience of using alemtuzumab was more limited. Indications for 53 re-treatment cycles in 40 patients are outlined in Table 2. Figure 1 demonstrates the temporal relationship between retreatment events and duration of follow-up. Between two and five years, 27% of patients had been retreated, increasing to 51% and 58% at five to ten years and greater than ten years follow-up, respectively.

Relapses
One hundred patients experienced a total of 766 relapses, of which 170 (22%) followed the initial treatment cycle. The mean pre-treatment annualized relapse rate (ARR) was 2.1 (median 1.8). Following the first treatment cycle the ARR reduced to 0.2 (median 0.1) (p<0.0001) (Figure 2). A small number of patients were unresponsive to treatment and continued to experience frequent clinical relapses: four patients had ⩾10 post treatment relapses and had a post treatment ARR of >1. The reduction in ARR was sustained over follow-up of up to eight years (Figure 3).

Disability

Mean baseline EDSS was 4.0. Mean change in EDSS from treatment baseline was +0.14. For two patients who died of non-MS related causes, the last EDSS recorded in life was selected as their final EDSS assessment. A negative change in EDSS was seen for each of the first three years of follow up, and then again towards the later years of follow up, although the numbers were small in these latter groups (Figure 4). 27% had a SAD, although none had developed this status within two years of follow-up. 25% of patients achieved sustained reduction in disability (SRD). Twelve patients (12%) were considered to have developed secondary progressive disease a mean of 3.8 years after initial treatment.

Table 2. Reasons for retreatment.

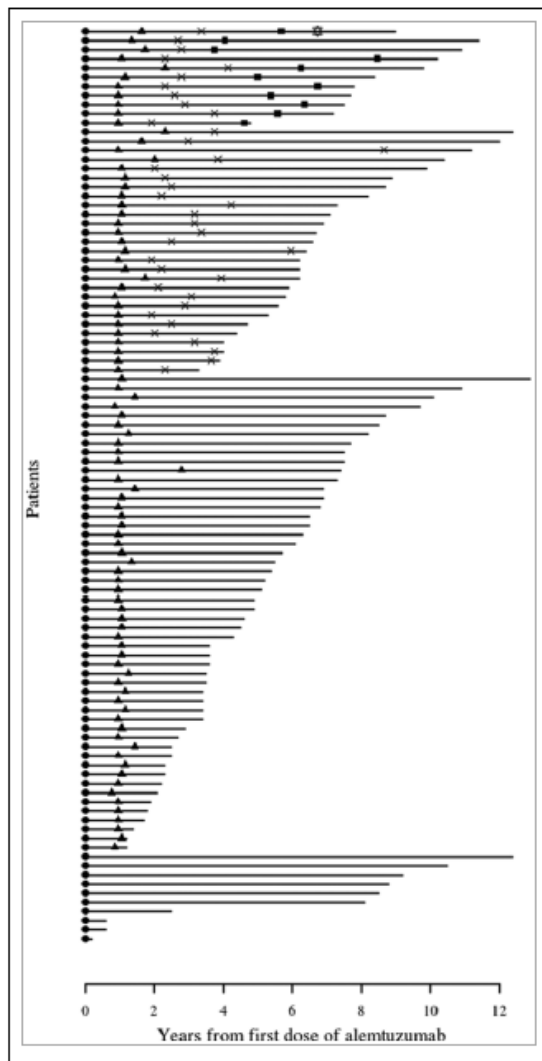| Reason for retreatment	Number of treatment cycles	%
Clinical relapse only	13	25
New radiological lesions (with or without enhancement) only	14	26
Clinical relapse and new lesions (with or without enhancement)	19	36
Worsening disability and new lesions	2	4
Worsening disability without change in EDSS	5	9

Figure 1. Temporal variation in retreatment rates. Horizontal lines represent duration of follow-up. Treatments indicated by different symbols.

Adverse events
Infusion reactions. 87% of patients experienced early infusion related adverse events, which occurred despite concomitant steroid use, but tended to be mild, and responded to conservative treatment.

Acquired autoimmune disease. Fifty-one AID diagnoses were made in a total of 47 patients. As noted in previous studies, the thyroid gland was the most com-mon site of autoimmunity, with 35% patients affected. Three patients developed idiopathic thrombocytopaenic purpura (ITP) and 13 other separate autoimmune disorders were diagnosed; one case each of haemolytic anaemia, pancytopaenia, autoimmune hepatitis, type II diabetes mellitus and anti-phospholipid syndrome, and two cases each of alopecia, neutropaenia, autoimmune alveolitis and vitiligo. Mean time to development of AID was 995 days (median 898, range 30–3180 days, Figure 5) following first treatment and a mean of 578 days (median 394, range 0–3180 days, supplementary figure 1) after the most recent treatment. The risk of developing secondary autoimmunity was greatest in the first five years of follow-up (Figure 5) and reduced after this time. No autoimmune kidney disease was observed in this cohort. In addition to the reported three cases of ITP, a transient infusion related thrombocytopaenia was observed in two patients, but resolved without intervention.

A total of 34 different novel auto-antibodies (excluding thyroid receptor and anti-TPO antibodies) were detected in 30 different patients during the period of follow-up; 13 ANA, nine ANCA, three anti-smooth muscle antibodies, two anti-centromere antibodies, two parietal cell antibodies, one rheumatoid factor, one anti-dsDNA, one anti-cardiolipin, one Beta 2 Glycoprotein 1 antibody and one anti-GBM antibody. Importantly, the patient who developed anti-GBM antibodies had normal renal function throughout the course of treatment. The majority of these detected auto-antibodies were transient, with only four anti-bodies persisting after subsequent testing. These included one anti-centromere antibody, two ANCA and one anti-cardiolipin antibody.
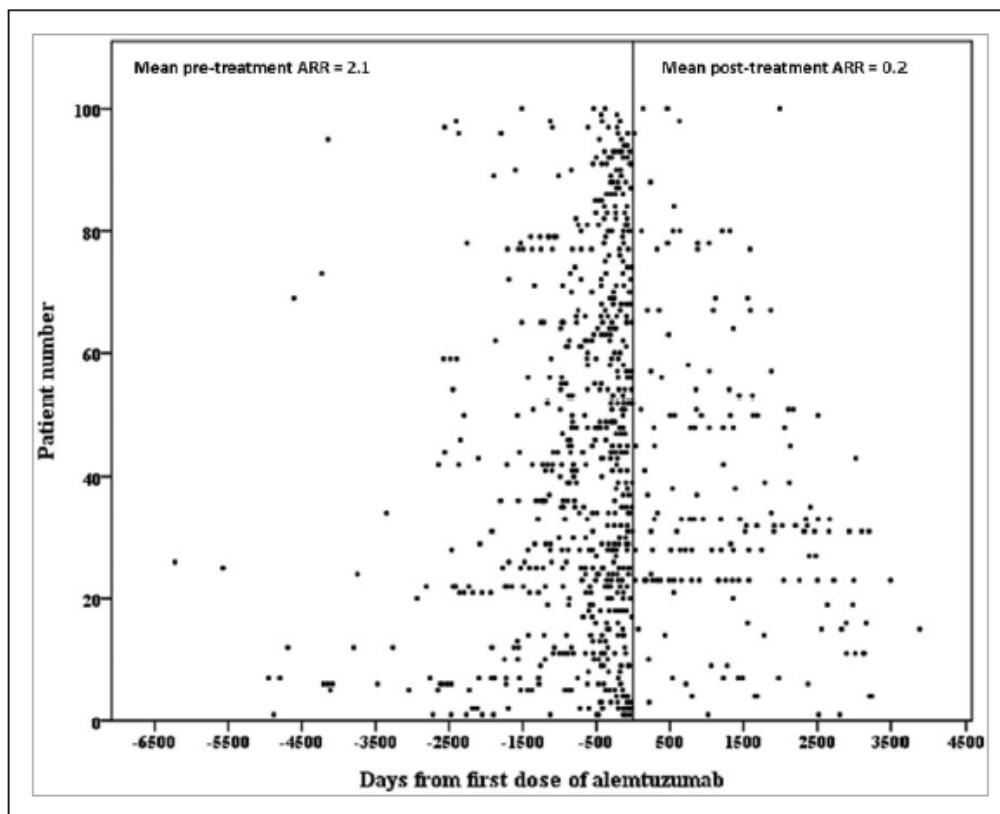

Figure 2. Pre- and post-treatment relapses for patients treated with alemtuzumab.

Infections. Forty-two documented infections occurred in 23 patients. All infections were mild or moderate in severity and responded to standard treatment. Urinary tract infections were most common (12%). Eight (8%) patients developed herpes zoster and six (6%) respiratory tract infections. Other diagnoses were less common, and included influenza (3%) pityriasis (2%), sinusitis (2%), tonsillitis (2%), genital herpes simplex (1%), conjunctivitis (1%), mastitis (1%), mumps (1%), acute cholangitis (1%) and cellulitis (1%). One patient (1%) developed cryptosporidium infection during a hospital in-patient stay for a surgical operation.
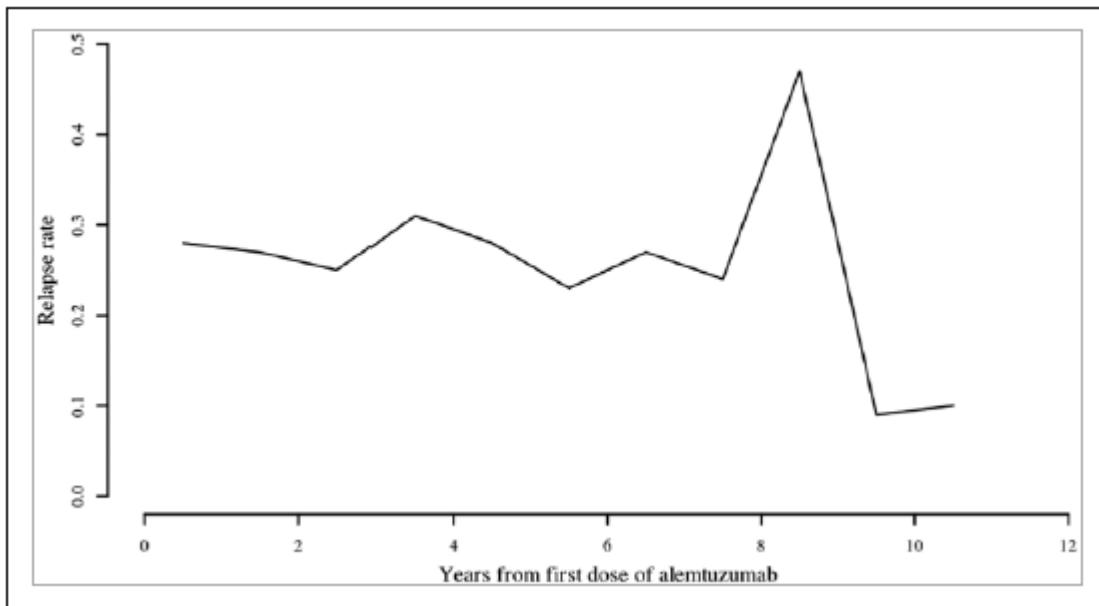

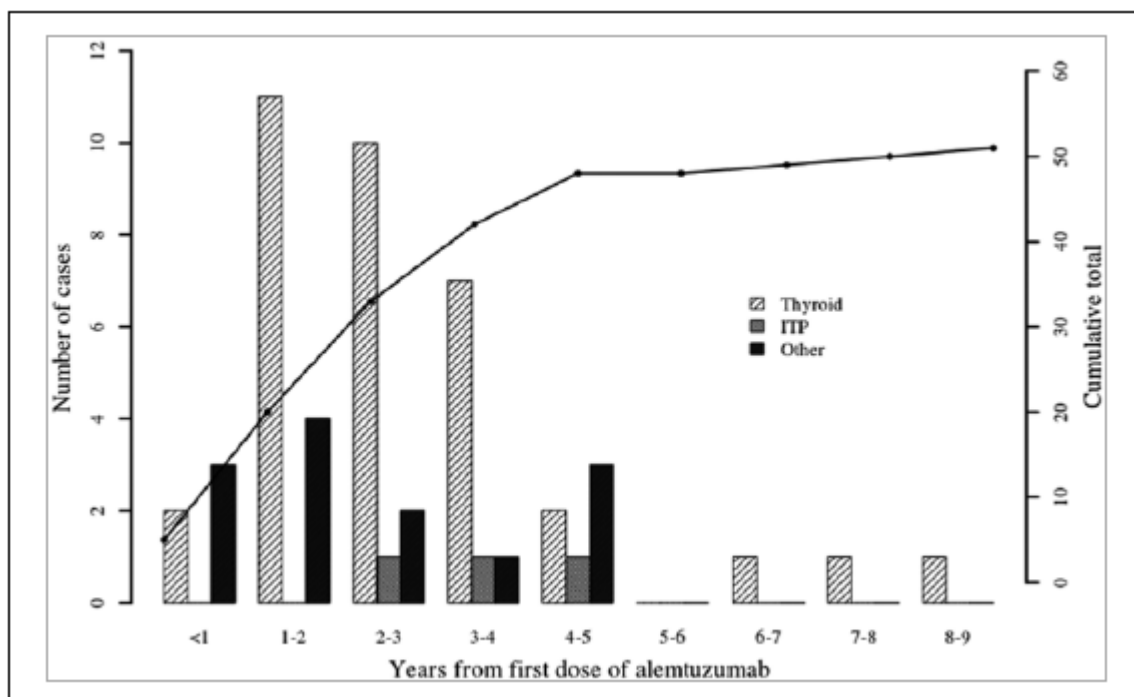**Figure 3.** Annualized relapse rate by year of follow-up.

Figure 5. Risk of developing autoimmune disease by duration of follow-up. Lined columns: thyroid autoimmune disease; grey columns: idiopathic thrombocytopaenic purpura; black columns: other autoimmune conditions.

Pre-malignant/malignant conditions. Ten patients developed pre-malignant or malignant conditions during the period of follow-up. Five patients (5%) developed cervical dysplasia, three patients (3%) were identified with a low level IgG paraprotein or monoclonal gammopathy of uncertain significance (one of which was also diagnosed with meningioma) and two patients were diagnosed with basal cell carcinoma.

Pregnancy. Thirteen pregnancies were recorded in twelve women (18%). Two pregnancies resulted in miscarriage and one was terminated. The child of one patient who developed thyroid AID following treatment experienced transient neonatal hyperthyroidism.

Discussion

Alemtuzumab has had encouraging results in both clinical trials and open-label studies, but long-term follow up data remains sparse. Tuohy et al.26 have recently published long term results of a cohort of patients treated in open-label studies in Cambridge, UK, but additional data from other centres is also required tounderstand practical application and longer-term adverse events in routine clinical practice.

In the phase II (CAMMS223) and phase III trials (CARE-MSI and CARE-MSII), alemtuzumab was shown to reduce the ARR by 74%, 55% and 49.4%, respectively. The findings in this study confirm that up to a mean 6.1 year follow-up the percentage reduction in ARR is maintained, and in this cohort was 90%. In particular, the treatment seems to be durable in relation to relapses up to eight years following treatment. After this time the ARR reduces, but the number of patients in this group is small. Four patients clearly did not respond to treatment, with ⩾10 post-treatment relapses and a combined post-treatment ARR of >1, and represent an interesting sub-group which may warrant more detailed analysis of disease biology. Further characteristics of this subgroup are detailed in Table 1. However these data are commensurate with the long-term efficacy outcomes of the Cambridge cohort, where 52% received the standard two cycles of treatment, 36% received three cycles, 8% four cycles and one patient five cycles.26 In our dataset the trend for requiring re-treatment increased over time, implying that the majority of patients are likely to require further treatment cycles. So far 40% of patients have required retreatment. An important practical consideration was that significant cognitive deficits were identified as a barrier to informed consent and adherence to long term monitoring protocols, and we have now altered our local selection criteria to offer alternative treatments for these patients. Three (3%) patients were also intolerant of treatment as a result of severe infusion reactions or pancytopaenia following infusion.

Previous studies have suggested an expectation of an improvement in EDSS from baseline following treatment with alemtuzumab when compared with an active comparator (interferon beta 1-a). In the CAMMS223 phase II study, the mean change in EDSS from baseline was −0.39 (p<0.001),15 CARE-MSI 0.14 (p=0.97),16 CARE-MSII −0.17 (p<0.0001)17 and CAMMS223 five-year follow-up −0.3 (p=0.0002).27 Although the improvement in EDSS in the CAMMS223 cohort achieved statistical significance over five years of follow-up, this significance was not observed when months 36–60 only were analysed. This would imply that the proposed effect on disability was short-lived and not sustained. Conversely, our cohort experienced an overall worsening in EDSS score of +0.14 from treatment baseline. The previous phase II and two phase III trials have shown rates of SAD in the alemtuzumab treatment groups of 9%, 8% and 13% at three, three, and two years, respectively. Similarly, 11% of patients had SAD in the CAMMS223 five-year follow-up study. We identified a much higher proportion of patients who had SAD over six years follow-up, so that 27% of patients

were deemed to have progressed in our cohort. These results, however, were comparable to the levels of SAD found in the open-label Cambridge long-term follow-up study, with 32% of patients found to have SAD over a median seven-year follow-up.26 Only 27% of patients had a SRD in our cohort, compared to 43.5% in the Cambridge cohort.26 Differences in disability outcomes may, in part, be explained by the fact that in our cohort there were a larger number of EDSS assessors, as would be expected in a 'real-world' clinical setting, but this may have created some increased variability. Although the mean change in EDSS score was +0.14, we would still consider this an encouraging outcome, given the particularly aggressive disease profile of this cohort of patients.

The most significant adverse event of alemtuzumab treatment is secondary AID. Secondary thyroid auto-immunity has previously been shown to be unaffected by the cumulative dose, dosage interval or dosage frequency, suggesting that total risk is acquired at the time of first dose.28 47% of patients in this study developed AID, with 35% developing thyroid autoim-munity.10,12,15–17,28 Rates of ITP (3%) were also com-parable to published data. Other AIDs were seen at lower frequencies, but without a control group for comparison, the relationship of these to alemtuzumab treatment is difficult to confirm. This risk of developing secondary AID appears to be maximum in the first five years following initial treatment, with only three cases (all thyroid AID) seen after this time. This would seem to suggest that autoimmune surveillance should be continued for a minimum of five years after the first treatment cycle. This is commensurate with the current monitoring guidelines of four years after the last dose of alemtuzumab. Although 34 novel anti-bodies were detected during post-treatment monitoring only four of these persisted, and have not so far been associated with relevant disease.

Despite alemtuzumab causing profound and prolonged lymphopaenia, serious infections are rare. This is thought to be due to the relative preservation of the innate immune system, haemopoetic stem cells and the nature of subsequent immune reconstitution.4 In addition, as a result of the interval between treatments, lymphocyte repopulation occurs. Most infections following alemtuzumab treatment are mild to moderate and respond to conventional therapies. We observed similar findings in this study, with urinary, respiratory and herpes zoster infections being most common. One case of cryptosporidium infection was associated with an in-patient stay for a surgical procedure. We are unaware of any serious infections occur-ring in our cohort.

Within the clinical trials, the rate of pre-malignant or malignant conditions was 0.5%–2.8%, although the studies were not powered in such a way as to detect small changes as compared with interferon beta-1a. Outside of trials one case of malignant melanoma has been reported,29 and a further patient developed Castleman's disease (a prelymphomatous condition) and is now in remission following R-CHOP chemo-therapy.30 We observed pre-malignant/malignant conditions in 10% of patients. In particular, we are aware of five female patients who developed cervical dysplasia (5%). The occurrence of cervical dysplasia may be affected by immunosupression,31 and these data perhaps suggest that stringent pre- and post-treatment cervical screening should be performed, and this is now included as routine in our protocols. In addition to these findings, an IgG paraprotein or MGUS was also detected on screening in three patients. The significance of this is difficult to ascertain at present, but is a novel finding in MS patients receiving alemtuzumab, although persistent paraproteinaemias have previously been reported following alemtuzumab therapy in the context of stem cell transplantation.32

The pregnancy rate of 18% in this cohort, despite advice on appropriate contraception, may reflect the positive effect on quality of life following alemtuzumab that many patients reported, leaving them more confident to start families. No unexpected adverse pregnancy outcomes have so far been observed in this cohort.

In conclusion, this follow-up study in a highly selected group of MS patients with poor prognostic indicators, treated with alemtuzumab in routine clinical practice, confirms a durable effect on relapse rates, but no improvement in disability. AID has affected nearly half of the cohort to date, but this figure is likely to rise with longer follow-up. Unexpected findings included three cases of a low level IgG paraprotein and five cases of cervical dysplasia. Although the lack of a comparative control group does not allow definitive conclusions to be drawn, it will be important to monitor these in larger post-marketing surveillance studies. However, alemtuzumab appears to be an effective treatment for relapsing MS in routine clinical practice, and its side-effects, for the most part, are predictable and treatable.

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