Manuscript Review Cover Sheet		
Review Details		
Review Due Date		
Manuscript Objectives	Post hoc analysis to examine efficacy and safety through 9 years among alemtuzumab-treated patients from CARE-MS I and II with HAD at baseline	
Audience	Neurologists	
Data Summary	• Baseline characteristics • ARR • Percentage of relapse-free patients • EDSS score (improved, stable, or worsened) vs core study baseline • EDSS score over time • Freedom from 6-month CDW • 6-month CDI • Freedom from MRI disease activity • Annual and cumulative NEDA • Brain volume loss • Safety (primary HAD definition only) 4 HAD definitions Studies comprising the analysis: • CARE-MS I, CARE-MS II, and the CAMMS03409 and TOPAZ extensions	
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Tables	6 (incl. 3 e-Tables)	NA
Figures	5	NA
Questions/Comments for Reviewers / Outstanding Issues		

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Efficacy and Safety of Alemtuzumab Through 9 Years of Follow-up in Patients with Highly Active Disease: Post Hoc Analysis of CARE-MS I and II Patients in the TOPAZ Extension Study

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Thank you.

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Running heading: Alemtuzumab efficacy and safety over 9 years in CARE-MS patients with highly active disease

Abstract *429/450 words*

Background: Alemtuzumab efficacy versus subcutaneous interferon beta-1a (SC IFNB-1a) was demonstrated over 2 years in patients with RRMS, with continued efficacy over 7 additional years. Alemtuzumab is included as a recommended treatment for patients with highly active disease (HAD) by the American Academy of Neurology Practice Guidelines, and the label indication in Europe was recently restricted to the treatment of HAD patients. There is currently no consensus definition for HAD, and alemtuzumab efficacy across various HAD definitions has not been explored previously.
Objectives: To evaluate post hoc alemtuzumab efficacy and safety in CARE-MS patient subgroups defined by four definitions of HAD, compared with SC IFNB-1a over 2 years, and with extended follow-up of the alemtuzumab arm through Year 9.

Methods: Patients in the CARE-MS studies received either alemtuzumab (baseline: 5 days; 12 months later: 3 days) or SC IFNB-1a (3-times weekly). Alemtuzumab-treated patients who enrolled in the extensions could receive additional courses ≥ 12 months apart. Four definitions of HAD were applied to assess alemtuzumab efficacy: the pre-specified primary definition (two or more relapses in the year prior to baseline and at least one gadolinium [Gd]-enhancing lesion at baseline) and three alternative definitions that focused on either relapse, MRI, or prior treatment response criteria. Efficacy outcomes were annualized relapse rate, change in Expanded Disability Status Score, 6-month confirmed disability worsening, 6-month confirmed disability improvement, MRI disease activity, and brain volume change. Adverse events were summarized for HAD patients meeting the primary definition.
Results: In the pooled CARE-MS population, 208 alemtuzumab-treated patients met the primary HAD definition. Annualized relapse rate was 0.27 in Years 0–2 and 0.16 in Years 3–9. Over 9 years, 62 % of patients were free of 6-month confirmed disability worsening, 50 % had 6-month confirmed disability improvement, and median cumulative change in brain volume was – 2.15 %. During Year 9, 62 % had no evidence of disease activity, and 69 % were free of MRI disease activity. Similar efficacy outcomes were observed using an alternative relapse-driven HAD definition. For patients meeting alternative HAD definitions focused on either higher MRI lesion counts or disease activity while on prior therapy, reduced efficacy for some endpoints were seen. Safety was consistent with the overall CARE-MS population through Year 9.
Conclusions: Over 9 years, alemtuzumab efficacy was maintained in CARE-MS HAD patients based on four HAD definitions. These results support intervention with alemtuzumab in patients with early indicators of HAD, including frequent relapse without high MRI activity. No safety signals were observed over 9 years that were unique to the HAD populations.
ClinicalTrials.gov Identifiers: NCT00530348; NCT00548405; NCT00930553, NCT02255656.

Key Points

- Using 4 definitions of highly active disease with varying criteria, alemtuzumab improved outcomes in patients with HAD, and efficacy was maintained over 9 years in highly active relapsing-remitting multiple sclerosis
- Efficacy in the HAD population was generally consistent with and similar to the overall CARE-MS population

- No new safety signals were seen in alemtuzumab-treated patients with highly active disease over 9 years

1. INTRODUCTION

Highly active RRMS disease (HAD) is associated with a more aggressive disease course, leading to an accelerated rate of disease progression [1]. Frequent relapses and high MRI lesion counts early on are key factors that may predict more rapid disability onset and progression [2, 3]. Therefore, HAD patients have a greater need for early relapse prevention and improved MRI outcomes. Need for aggressive, early treatment in HAD patients is more widely accepted, with some moderate- to high-efficacy disease-modifying therapies (DMTs) already indicated for HAD [4-7]. Recent updates to treatment guidelines for the American Academy of Neurology and the European label include alemtuzumab for HAD patients.[8, 9]

Despite therapy indications and recommendations for HAD, neither a consensus definition nor a comprehensive treatment algorithm have been established.[10] Generally, HAD has been defined as rapid accumulation of disability, frequent relapses, and high MRI activity. Criteria vary, with some defining HAD using Expanded Disability Status Scale (EDSS) score and response to treatment along with relapse and MRI activity.[10-12] Efforts to refine the definition of HAD have been complicated by the potential to be either too restrictive (ie, inadvertently eliminating patients) or too lax (ie, consequently including patients without need for aggressive treatment).[10] To be comprehensive in our analysis and better understand the effect of alemtuzumab on HAD according to definitions varying in restrictiveness, we employed four definitions (one primary and three alternatives) that emphasize different disease parameters to assess the efficacy and safety of alemtuzumab over 9 years in HAD patients.

In the phase 3 Comparison of Alemtuzumab and Rebif® Efficacy in Multiple Sclerosis (CARE-MS) studies (NCT00530348; NCT00548405), alemtuzumab improved clinical and MRI outcomes versus subcutaneous interferon beta-1a (SC IFNB-1a) in patients with active RRMS [13, 14]. Efficacy was maintained over an additional 7 years in 2 consecutive extension studies (CARE-MS extension [NCT00930553] and the ongoing TOPAZ study [a long-Term follow-up study for multiple sclerOsis Patients who have completed the AlemtuZumab extension study; NCT02255656]) [15-18]. Adverse events (AEs) associated with alemtuzumab treatment in clinical trials and postmarketing experience include infusion-associated reactions (IARs), increased frequency of infection and the potential for opportunistic infections, secondary autoimmunity (thyroid disorders, immune thrombocytopenia [ITP], nephropathies, autoimmune cytopenias, autoimmune hepatitis, and other less common autoimmune events), acute acalculous cholecystitis, and cardiovascular and pulmonary events possibly related to infusion [8, 13-15, 17, 19-23].

Here, we evaluate post hoc alemtuzumab efficacy and safety in CARE-MS patients with HAD as defined by each of four definitions, compare with the efficacy and safety of SC IFNB-1a over 2 years in these HAD populations, and follow-up those in the alemtuzumab arm through Year 9.

2. METHODS

2.1 Design of CARE-MS Core Studies and Extension Studies

The CARE-MS core studies and their extensions have been described previously [13, 14]. Briefly, the 2-year, core phase 3 CARE-MS studies compared alemtuzumab with SC IFNB-1a in patients with active RRMS who were either treatment-naive (CARE-MS I) or had an inadequate response to prior therapy (CARE-MS II) [13, 14]. In the core studies, patients received either alemtuzumab 12 mg/day IV (baseline: 5 consecutive days;

12 months later: 3 consecutive days), or SC IFNB-1a 44 μg (3 times per week) [13, 14]. Alemtuzumab-treated patients who completed the core studies and entered the 4-year CARE-MS extension could receive additional, as-needed, alemtuzumab (12 mg/day; 3 consecutive days ≥ 12 months apart) for relapse or MRI activity, or receive other licensed DMTs per investigator's discretion [15, 17]. SC IFNB-1a–treated patients could also enter the extension and receive alemtuzumab; however, outcomes for these patients beyond Year 2 are not presented because SC IFNB-1a treatment was discontinued thereafter. Follow-up of patients beyond 6 years is continuing in the TOPAZ extension, wherein patients can receive additional alemtuzumab courses (12 mg/day; 3 consecutive days ≥ 12 months apart) or other DMT at any time, both per investigator's discretion (no criteria) [16, 18].

2.2 Clinical Efficacy Assessments

Relapses were defined as new neurologic symptoms attributable to MS lasting ≥ 48 hours with an objective change in neurologic examination; annualized relapse rate (ARR) and proportions of patients free of relapse were reported. EDSS score was assessed quarterly and at the time of suspected relapse by raters who were blinded throughout the follow-up period to core study treatment assignment and treatment history. Changes in EDSS score were defined as improved (≥ 1.0-point decrease from core study baseline), stable (≤ 0.5-point change in either direction from core study baseline), and worsened (≥ 1.0-point increase from core study baseline). Confirmed disability worsening (CDW) was defined as a ≥ 1.0-point EDSS score increase (or ≥ 1.5 points if baseline EDSS = 0) confirmed over 6 months. Confirmed disability improvement (CDI) was defined as a ≥ 1.0-point EDSS score decrease from baseline confirmed over 6 months (assessed only in patients with baseline EDSS score ≥ 2.0). MRI was assessed annually by imaging specialists blinded to core study treatment assignment and treatment history. Freedom from MRI disease activity was defined as no new gadolinium (Gd)-enhancing T1 lesions on current MRI and no new/enlarging T2 hyperintense lesions since last MRI. No evidence of disease activity (NEDA) was defined as the absence of relapse, 6-month CDW, and MRI disease activity. Brain volume loss (BVL) was assessed by brain parenchymal fraction (BPF); blinded scans were read at the Cleveland Clinic (Cleveland, OH, USA).

2.3 Safety Monitoring

Safety monitoring occurred for ≥ 48 months following the last alemtuzumab administration, according to the recommended risk-minimization protocol, which included hematology (complete blood counts with differential; at least monthly), renal examinations (serum creatinine and urinalysis with microscopy; monthly), and thyroid function (at least quarterly). All AEs, serious AEs, and medical events of interest were recorded. IARs were defined as any AE with onset during or ≤ 24 hours after the end of infusion.

2.4 HAD Definitions and Analyses

Four different definitions of HAD were applied to the core CARE-MS I and II populations (Fig. 1a), resulting in four groups of HAD patients (i.e. primary definition and alternative definitions 1–3). Some patients met more than one HAD definition and were, therefore, included in more than one group.

The primary HAD definition was pre-specified in the CARE-MS I and II trial protocols, and was defined as patients having two or more relapses in the year prior to baseline and at least one Gd-enhancing

lesion at baseline (Fig. 1a), and is one of the most used in the literature.[5, 7, 24, 25] Outcomes from the CARE-MS I and II studies individually, as well as the pooled CARE-MS studies, were assessed for those fitting this primary HAD definition.

Three alternative HAD definitions (Fig 1a) were also applied to the pooled population from the CARE-MS studies, and sensitivity analyses of efficacy outcomes were assessed for the subgroups fitting each alternative definition. The first alternative HAD definition focused on relapse; patients met the criteria for the first definition if they had two or more relapses in the year prior to core study baseline, independent of baseline Gd-enhancing lesion count. The second alternative HAD definition focused on MRI lesions; patients met the criteria for the second definition if they had at least one relapse in the year prior and three or more Gd-enhancing lesions at core study baseline. The third alternative HAD definition focused on prior therapy failure; patients met the criteria for the third definition if they had at least one relapse and at least one Gd-enhancing lesion in the year prior to baseline while on therapy with another DMT. Because all CARE-MS I patients were treatment-naive at core study baseline, none met the third alternative definition.

2.5 Statistical Analyses

The statistical analyses for alemtuzumab were based on all available interim data through Year 9 (TOPAZ Year 3); data cut-off date was 20 October 2018. ARR was estimated using a negative binomial model with robust variance estimation. Mean EDSS scores from core study baseline through Year 9 were evaluated. Ranked analysis of covariance (ANCOVA) with adjustment for geographic region and baseline EDSS score was used to compare changes in EDSS scores from baseline through Year 2 in alemtuzumab- versus SC IFNB-1a–treated patients. Proportions of patients free of 6-month CDW or achieving 6-month CDI were assessed using Kaplan-Meier estimates. Proportions of patients free of MRI disease activity, new Gd-enhancing T1 lesions, new/enlarging T2 hyperintense lesions, and new T1 hypointense lesions were analyzed using logistic regression with covariate adjustment for baseline values; 95 % confidence intervals (CIs) were obtained by normal approximation to the binomial distribution. Percentage change in BPF from core study baseline was evaluated at each time point; distribution-free estimates were obtained for the CI of the median. All analyses were carried out using SAS statistical software (version 9.4, The SAS Institute, Cary, NC).

2.6 Standard Protocol Approvals, Registrations, and Patient Consents

CARE-MS I, CARE-MS II, CAMMS03409, and TOPAZ are registered with ClinicalTrials.gov (NCT00530348; NCT00548405; NCT00930553; NCT02255656). Patients provided written informed consent, and all procedures were approved by local institutional ethics review boards of participating sites.

3. RESULTS

3.1 Patients and Baseline Characteristics: Primary HAD Definition

At core study baseline, 28 % of CARE-MS I and 24 % of CARE-MS II alemtuzumab-treated patients met the primary HAD definition; of these, 70 %–76 % remained on study through Year 9 (Fig. 1b). These retention rates were similar to those in the overall CARE-MS populations (66 %–75 %) and in the CARE-MS patients without HAD (65 %–75 %). Total follow-up time over 9 years in CARE-MS alemtuzumab-treated

HAD patients is presented in Supplementary Table 1. Over Years 3–9, 56 % of CARE-MS I and 43 % of CARE-MS II alemtuzumab-treated HAD patients received neither additional alemtuzumab nor another DMT, comparable to 55 % and 41 % of patients in the overall CARE-MS I and II populations, respectively.

At baseline, HAD patients were slightly younger, with shorter disease duration, more prior relapses, higher T2 hyperintense lesion volumes and more Gd-enhancing lesions when compared with the overall CARE-MS population; baseline T2 hyperintense lesion counts were not available. More CARE-MS II patients with HAD received two or more previous DMTs compared with the overall CARE-MS II population (Table 1). Baseline characteristics for the overall CARE-MS I and II populations were published previously.[13, 14]

3.2 Efficacy: Primary HAD Definition

In HAD patients from CARE-MS I and II, ARR over Years 0–2 was significantly reduced with alemtuzumab versus SC IFNB-1a ($p \leq 0.01$) and remained low over Years 3–9, with ≥ 53 % remaining free of relapses (Fig. 2a, Fig. 3a). Mean changes in EDSS scores did not significantly differ in CARE-MS I patients with HAD treated with SC IFNB-1a (-0.19) versus alemtuzumab (–0.17), but were significantly improved in CARE-MS II HAD patients treated with alemtuzumab (–0.21) versus SC IFNB-1a (0.16; $p = 0.0351$). Over 9 years, mean EDSS score remained unchanged in CARE-MS I patients with HAD patients and increased 0.35 points in CARE-MS II patients with HAD. Sixty-five percent or more of patients with HAD from either CARE-MS I or II achieved stable or improved EDSS scores at Year 9 versus core study baseline (Fig. 2b, Fig. 3b). In CARE-MS I and II patients with HAD, 91 % of those treated with alemtuzumab were free of 6-month CDW over Years 0–2 versus 85 %–90 % of those treated with SC IFNB-1a, whereas 34 %–36 % vs 19 %–32 % achieved CDI over Years 0–2, respectively. Through Year 9, ≥ 55 % of CARE-MS patients with HAD treated with alemtuzumab were free of 6-month CDW, and ≥ 49 % achieved 6-month CDI (Fig. 2c–d, Fig. 3c–d).

In Year 2, significantly more alemtuzumab-treated patients with HAD were free of MRI disease activity than those treated with SC IFNB-1a ($p \leq 0.0007$). Over 60 % of patients with HAD treated with alemtuzumab were free of MRI disease activity in each of Years 3–9 (Fig. 4a, Fig. 5a). More alemtuzumab-treated versus SC IFNB-1a–treated patients with HAD achieved annual NEDA during Year 2 in both CARE-MS I and II, and ≥ 53 % of alemtuzumab-treated HAD patients attained NEDA in each of Years 3–9 (Fig. 4b, Fig. 5b). Over Years 3–9, 21 % of CARE-MS I and 14 % of CARE-MS II patients with HAD treated with alemtuzumab had sustained NEDA. Cumulative median BVL from core study baseline was 2.65 % and 1.68 % for alemtuzumab-treated CARE-MS I and II patients with HAD over 9 years, respectively (Fig. 4c, Fig. 5c).

3.3 Sensitivity Analyses of Efficacy: Alternative Definitions of HAD

To better understand how criteria for HAD of varying restrictiveness affect clinical benefit, sensitivity analyses were carried out using three alternative definitions of HAD applied to the CARE-MS pooled population. Efficacy outcomes for those in the overall pooled CARE-MS I and II population are presented in Supplementary Table 2.

At baseline in the pooled CARE-MS studies, 54 % of alemtuzumab-treated patients satisfied the first alternative definition of HAD (i.e. two or more relapses in year prior to baseline, independent of Gd-enhancing lesion count at baseline). Efficacy data were similar between these patients and those meeting the primary HAD definition, except the former had less cumulative BVL over Years 0–9 (Table 2).

Of the pooled CARE-MS alemtuzumab patients, 21 % met the second alternative definition of HAD (i.e. at least one relapse in the year prior and three or more Gd-enhancing lesions at baseline) at core study baseline. Efficacy outcomes, specifically ARR, proportions with improved/stable EDSS score, freedom from 6-month CDW, and attainment of 6-month CDI, for those who met the second alternative HAD definition were similar to those who met the primary HAD definition. Compared with those who met the primary definition of HAD, patients who met the second alternative definition of HAD experienced a greater EDSS score increase through Year 9, were less likely to be relapse-free or to attain sustained NEDA over Years 3–9, were less likely to be MRI disease activity-free or to attain annual NEDA during Year 9, and experienced slightly greater cumulative BVL through Year 9 (Table 2).

Twenty-two percent of pooled CARE-MS patients met the criteria for the third alternative definition of HAD at study baseline (i.e. at least one relapse and at least one Gd-enhancing lesion in the year prior to baseline while on therapy with another DMT). Compared with those who met the primary definition of HAD, those who met the third alternative definition of HAD had similar ARR, attainment of 6-month CDI, freedom from MRI disease activity in Year 9, and attainment of NEDA in Year 9. Differences in mean EDSS score through Year 9, proportions with stable or improved EDSS scores, proportions of relapse-free over Years 3–9, proportions free of 6-month CDW, proportions achieving sustained NEDA, and cumulative BVL through Year 9 were observed between those who met the third alternative and the primary definitions of HAD. Of these differentiating endpoints, only BVL was favored in those who met the third alternative definition versus those who met the primary HAD definition (Table 2).

3.4 Safety: Primary HAD Definition

Incidences of AEs were similar in alemtuzumab-treated patients who met the primary definition of HAD versus the overall alemtuzumab-treated patient population over Years 0−9 (Table 3; Supplementary Table 3). In alemtuzumab-treated patients who met the primary HAD definition, annual incidences for overall AEs, IARs, and infections (time trend not shown) declined over time; incidences of serious AEs were ≤ 10.2 % and ≤ 12.6 % in any year in CARE-MS I and II patients, respectively. Autoimmune AEs were predominantly thyroid events, with annual incidences peaking in Year 3 (CARE-MS I, 13.7 %; CARE-MS II, 19.8 %) and subsequently declining; no thyroid events were reported in Year 9. Three cases of ITP occurred in patients meeting the primary HAD definition in CARE-MS I, and none occurred in CARE-MS II [13, 26]. One case of nephropathy in a patient meeting the primary HAD definition was reported in each of CARE-MS I and II [22]. Malignancies occurred in three CARE-MS I HAD patients and in no CARE-MS II HAD patients [13, 17, 27]. One death occurred in Year 8 in a CARE-MS I HAD patient due to an unknown cause approximately 14 months after the last alemtuzumab dose; the patient had a history of acute systolic congestive heart failure [27]. Two deaths occurred in CARE-MS II HAD patients: a motor vehicle accident in Year 2 [14] and a death due to

atrioventricular block in Year 8 [28], approximately 26 months after the last alemtuzumab dose, which was assessed as not related to alemtuzumab.

4. DISCUSSION

Frequent relapses and increased lesion counts early in the RRMS disease course are harbingers of rapid disease progression [2, 3], making reduction of early disease activity in HAD patients a clinically important unmet need. Alemtuzumab is recommended by the American Academy of Neurology DMT guidelines for treatment of HAD patients [9]. Furthermore, a putative treatment model for HAD patients advocates as a first step either biologic immunomodulation with alemtuzumab or chemotherapeutic immunosuppression with cladribine, cyclophosphamide, and mitoxantrone [1]. Recent label changes in Europe, which now indicate alemtuzumab for HAD, also highlight the relevance of this treatment as an option for patients with HAD.[8]

Without an available consensus definition of HAD, further evaluation of alemtuzumab based on multiple definitions varying in focus and restrictiveness is needed for this high-risk population. Proposed definitions for highly active MS, previously referred to as "aggressive" MS, have evolved, and have included various combinations of clinical disease markers such as frequent relapses with incomplete recovery, accumulation of early physical/cognitive impairment, and MRI markers of disease activity (e.g., high lesion frequency despite MS therapy, early brain atrophy) [10]. Our primary definition, which has also been referred to as “rapidly evolving severe MS,” was used previously in post hoc analyses of highly active patients from large clinical trials of fingolimod and natalizumab, and is one of several definitions specified by the European Medicines Agency for highly active MS phenotypes [5, 7, 24, 25].

Differences in study populations, study design, and follow-up time prevent direct comparison of alemtuzumab treatment effects with other DMTs. Yet, unlike studies with other DMTs, which examined efficacy and safety in HAD patients over 2 years [24, 25, 29], this analysis assessed outcomes with alemtuzumab in HAD patients over 9 years and demonstrated sustained efficacy in two clinical trials of treatment-naive and inadequate response to prior therapy RRMS populations. Efficacy findings were generally consistent between CARE-MS I and II HAD patients, supporting alemtuzumab as an effective option regardless of prior DMT exposure. In the CARE-MS I and II HAD populations, relapse rates were significantly reduced over 2 years with alemtuzumab compared with SC IFNB-1a, and were accompanied by reductions in MRI lesion activity and BVL. Disability outcomes differed between patients with HAD in each of the CARE-MS studies. In CARE-MS I (i.e. treatment-naive), disability outcomes in alemtuzumab-treated HAD patients were similar to SC IFNB-1a-treated patients; whereas, in CARE-MS II (i.e. previously exposed to DMTs), disability outcomes in alemtuzumab-treated HAD patients were superior to those of SC IFNB-1a-treated HAD patients. These outcomes align with previous findings from CARE-MS I, wherein the study was underpowered due to unexpectedly few SC IFNB-1a-treated patients experiencing CDW.[13] No safety signals were unique to the CARE-MS HAD populations over 9 years.

Efficacy results for any of the HAD subgroups analyzed here were generally consistent with those of the overall CARE-MS populations (Supplementary table 2). Alemtuzumab efficacy was maintained through Year 9 in HAD patients, with retention rates and rates of additional alemtuzumab administration similar to the overall CARE-MS population. However, BVL was greater in the primary HAD population than in the overall

CARE-MS populations (median BPF change for overall alemtuzumab populations over Years 0–9: −2.04 % in CARE-MS I [18] and −1.22 % in CARE-MS II [16]), potentially due to higher baseline Gd-enhancing lesion burden in the HAD patients. Published evidence has shown the correlation between increased BVL and Gd-enhancing lesion burden in MS patients [30]. In the case of CARE-MS I, the results also indicated stronger disability improvement in the HAD patients treated with alemtuzumab when compared with the overall patients treated with alemtuzumab, as evidenced by higher attainment of 6-month CDI (50 % vs. 41 %) and a higher proportion of patients with clinically meaningful EDSS score improvement (30 % vs. 20 %) [18]. A recent post hoc analysis of CARE-MS patients through Year 6 identified a total of 20 (3 %) alemtuzumab-treated patients from the overall population who converted to secondary progressive MS (SPMS) after initiating treatment [31]. Of these converting patients, nine met the primary HAD definition at baseline, translating to 4 % of alemtuzumab-treated HAD patients converting to SPMS over 6 years. This low level of SPMS conversion from RRMS among HAD patients further supports the efficacy of alemtuzumab in this high-risk population. These observations in CARE-MS I patients promote the strategy of minimizing damage with aggressive treatment during the critical window of opportunity in the early stages of HAD.

The goals of RRMS treatment continue to evolve with increased availability of high-efficacy agents; the attainment of NEDA has become a realistic possibility in some HAD patients [30, 32]. In this analysis, alemtuzumab treatment resulted in sustained NEDA over Years 3–9 in 21 % of CARE-MS I patients and 14 % of CARE-MS II patients with HAD. Studies of DMTs have reported rates of NEDA over similar timeframes for the general MS population, but not specifically in HAD populations, limiting comparisons with this post hoc analysis [32-35].

To account for clinical and MRI contributions toward HAD, additional analyses were carried out on the pooled populations using three alternative definitions. Results observed with the first alternative definition, which emphasized on relapse activity in the absence of Gd-enhancing lesions at baseline, were similar to those observed with the primary definition for most endpoints, with exception of BVL. In patients meeting the first alternative definition, BVL was similar to that in the overall CARE-MS alemtuzumab populations. This likely reflects the substantial proportion of patients free of Gd-enhancing lesions at baseline (52 %) who satisfied the first alternative definition. Because this first alternative definition of HAD accounts only for increased relapse activity, it reveals the treatment benefit in patients with indicators of HAD at early stages based on relapse activity alone. Since these patients may be missed for early intervention due to lack of MRI disease activity, these findings support earlier intervention based on higher relapse rate in order to mitigate disability progression and help minimize brain atrophy. Efficacy of alemtuzumab on patients with either multiple MRI lesions at baseline (i.e., the second alternative HAD definition) or treatment failure in association with clinical and MRI activity (i.e., the third alternative HAD definition) was reduced, with more patients requiring additional alemtuzumab compared with the overall CARE-MS population. Since these two alternative definitions account for contributions of MRI lesions, the data support the argument that earlier intervention with high-efficacy DMT prior to lesion accumulation may render more favorable outcomes.

A limitation of this post hoc analysis was selection bias due to non-stratification of disease activity at randomization. However, in the CARE-MS studies, similar percentages of patients across treatment arms had highly active RRMS, based on the four HAD definitions used here, and retention rates over 9 years were also

similar to the overall population. Another limitation was the underpowering of assessments for detection of between-treatment differences for subgroup analyses, which complicated comparisons.

5. Conclusions

These findings demonstrate alemtuzumab is associated with greater improvements in clinical and radiological measures of disease activity compared with SC IFNB-1a over 2 years in patients with highly active RRMS, and may help to control HAD for at least 7 additional years. Efficacy of alemtuzumab in HAD patients was consistent with that seen in the overall CARE-MS population, and there were no safety signals unique to the HAD population in this study.

6. References

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7. Author contributions

TZ, ADB, RB, GC, SE, JH, SFH, CL, VL, DP, CP, SS, LS, AT, BMJU, BVW, and BAS contributed to study design, data collection, drafting and critical review of the manuscript, and approval of the final submission. ZC contributed to drafting and critical review of the manuscript, and approval of the final submission. ND led the statistical support, contributed to the drafting and critical review of the manuscript, and approved the final submission.

8. Compliance and Ethical Standards

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8.4. Ethical approval and informed consent

Patients provided written informed consent, and all procedures were approved by local institutional ethics review boards of participating sites.

8.5. Data Availability

Qualified researchers may request access to patient level data and related study documents including the clinical study report, study protocol with any amendments, blank case report form, statistical analysis plan, and dataset specifications. Patient level data will be anonymized and study documents will be redacted to protect the privacy of trial participants. Further details on Sanofi's data sharing criteria, eligible studies, and process for requesting access can be found at: https://www.clinicalstudydatarequest.com.

TABLES

Table 1 Baseline characteristics for the HAD alemtuzumab-treated patients in CARE-MS I and II

	Alemtuzumab-Treated HAD Patients	
Baseline Characteristic	**CARE-MS I (*N* = 105)**	**CARE-MS II (*N* = 103)**
Age, years	32.1 (8.0)	32.7 (7.7)
Female, n (%)	69 (66)	69 (67)
EDSS score	2.0 (0.8)	2.6 (1.2)
Years since initial relapse	1.7 (1.4)	4.0 (2.6)
Number of relapses in prior 1 year	2.3 (0.6)	2.4 (0.8)
Number of relapses in prior 2 years	2.9 (1.0)	3.4 (1.4)
Gd-enhancing lesion count	5.6 (7.1)	5.2 (6.2)
Patients with Gd-enhancing lesions, n (%)	105 (100)	103 (100)
T2-hyperintense lesion volume, cm^3	9.9 (10.1)	12.1 (12.2)
Brain parenchymal fraction	0.82 (0.02)	0.82 (0.02)
Number of previous DMTs received, n (%)		
0	105 (100)	0
1	-	69 (67)
2	-	26 (25)
3	-	5 (5)
≥ 4	-	3 (3)
Previous DMTs received, n (%)		
IFNB-1a	-	59 (57)
IFNB-1b	-	32 (31)
Glatiramer acetate	-	42 (41)
Natalizumab	-	7 (7)
Immunoglobulin	-	3 (3)
Azathioprine	-	4 (4)

Values are mean (SD) unless otherwise noted
Primary HAD definition: ≥ 2 relapses in the year prior to baseline and ≥ 1 Gd-enhancing lesion at baseline
CARE-MS Comparison of Alemtuzumab and Rebif Efficacy in Multiple Sclerosis, *DMT* disease-modifying therapy, *EDSS* Expanded Disability Status Scale, *Gd* gadolinium, *HAD* highly active disease, *IFNB* interferon beta; *SD*, standard deviation

Table 2 Efficacy through 9 years in alemtuzumab-treated patients with HAD according to different definitions (CARE-MS I and II pooled)

	Alemtuzumab-Treated Patients			
Parameter	**Primary HAD Definition (*N* = 208)[a]**	**Alternative HAD Definition 1 (*N* = 438)[b]**	**Alternative HAD Definition 2 (*N* = 174)[c]**	**Alternative HAD Definition 3 (*N* = 180)[d]**
Proportion of the overall pooled CARE-MS alemtuzumab-treated population satisfying each HAD definition, %	26	54	21	22
Proportion of patients who received neither additional alemtuzumab nor another DMT in the extensions, %	50	50	41	40
Proportion of patients who received additional alemtuzumab, %	48[g]	48[h]	55[i]	55[j]
3 total courses, %	26[g]	25[h]	26[i]	29[j]
4 total courses, %	14[g]	15[h]	18[i]	16[j]
5 total courses, %	5[g]	4[h]	8[i]	7[j]
6 total courses, %	2[g]	2[h]	1[i]	2[j]
7 total courses, %	0.5[g]	0.5[h]	2[i]	1[j]
8 total courses, %	0.5[g]	0.2[h]	0	0.6[j]
ARR over Y3–9 (95 % CI)	0.16 (0.13 to 0.19)	0.18 (0.16 to 0.21)	0.17 (0.14 to 0.21)	0.17 (0.14 to 0.21)
Proportion of patients relapse-free over Y3–9, % (95 % CI)[e]	53 (45 to 61)	51 (46 to 57)	47 (39 to 56)	47 (38 to 56)
Mean EDSS score change over Y0–9 (95 % CI)	0.17 (−0.06 to 0.39)	0.19 (0.04 to 0.34)	0.28 (0.01 to 0.55)	0.33 (0.03 to 0.63)
Proportion of patients with improved or stable EDSS over Y0–9[f], %	72 (improved, 25; stable, 47)	71 (improved, 22; stable, 50)	70 (improved, 22; stable, 48)	66 (improved, 22; stable, 44)
Proportion of patients free of 6-Month CDW over Y0–9, % (95 % CI)	62 (54 to 69)	64 (59 to 69)	59 (51 to 66)	56 (48 to 64)
Proportion of patients with 6-Month CDI over Y0–9, % (95 % CI)	50 (41 to 59)	46 (40 to 52)	49 (40 to 59)	51 (42 to 60)

Proportion of patients free of MRI disease activity in Y9, % (95 % CI)[e]	69 (61 to 77)	69 (63 to 74)	57 (48 to 66)	69 (60 to 78)
Proportion of patients with NEDA in Y9, % (95 % CI)[e]	62 (54 to 70)	61 (56 to 67)	53 (44 to 63)	59 (49 to 69)
Proportion of patients with cumulative NEDA over Y3–9, % (95% CI)[e]	18 (11 to 25)	20 (15 to 26)	9 (3 to 15)	10 (3 to 17)
Median BPF change over Y0–9, % (95 % CI)	−2.15 (−2.65 to −1.78)	−1.77 (−2.02 to −1.55)	−2.40 (−2.80 to −2.05)	−1.80 (−2.20 to −1.35)

[a] Primary HAD definition: ≥ 2 relapses in the year prior to baseline and ≥ 1 Gd-enhancing lesion at baseline (number of evaluable patients ranged from 112–208); [b] alternative HAD definition 1: ≥ 2 relapses in year prior, independent of Gd-enhancing lesion count at baseline (number of evaluable patients ranged from 211–438); [c] alternative HAD definition 2: ≥ 1 relapse in year prior and ≥ 3 Gd-enhancing lesions at baseline (number of evaluable patients ranged from 98–174); [d] alternative HAD definition 3: ≥ 1 relapse and ≥ 1 Gd-enhancing lesion in the year prior to baseline while on therapy with another DMT (number of evaluable patients ranged from 79–180); [e] CIs were calculated using the Wald method; [f] values may not sum appropriately due to rounding; [g] N=191; [h] N=401; [i] N=159; [j] N=161

Improved EDSS score: ≥ 1.0-point decrease from core study baseline; stable EDSS score: ≤ 0.5-point change in either direction from core study baseline.
CDW: ≥ 1.0-point EDSS increase from core study baseline (or ≥ 1.5 points if baseline EDSS = 0) confirmed over 6 months
CDI: ≥ 1.0-point EDSS decrease from core study baseline confirmed over 6 months (assessed only in patients with baseline EDSS score ≥ 2.0)
Freedom from MRI disease activity: no new Gd-enhancing T1 lesions on current MRI and no new/enlarging T2 hyperintense lesions since last MRI
NEDA: absence of relapse, 6-month CDW, and MRI disease activity
ARR annualized relapse rate, *BPF* brain parenchymal fraction, *CARE-MS* Comparison of Alemtuzumab and Rebif Efficacy in Multiple Sclerosis, *CDI* confirmed disability improvement, *CDW* confirmed disability worsening, *CI* confidence interval, *DMT* disease-modifying therapy, *EDSS* Expanded Disability Status Scale, *Gd* gadolinium, *HAD* highly active disease, *MRI* magnetic resonance imaging, *N/A* not applicable, *NEDA* no evidence of disease activity, *Y* year

Table 3 AE incidence over Years 0–9 in the HAD alemtuzumab-treated populations from CARE-MS I and II (Primary definition)

	Alemtuzumab-Treated HAD Patients Over Years 0−9	
AEs, n (%)	**CARE-MS I (*N* = 105)**	**CARE-MS II (*N* = 103)**
Any AE	104 (99.0)	103 (100.0)
Serious AEs	40 (38.1)	41 (39.8)
Infections	89 (84.8)	93 (90.3)
Serious infections	7 (6.7)	9 (8.7)
Autoimmune AEs[a]		
Thyroid AEs	51 (48.6)	45 (43.7)
Serious thyroid AEs	5 (4.8)	7 (6.8)
ITP	3 (2.9)	0
Nephropathies	1 (1.0)	1 (1.0)
Malignancies	3 (2.9)	0
Deaths	1 (1.0)	2 (1.9)

[a] First occurrence of AE within the time period

Primary HAD definition: ≥ 2 relapses in the year prior to baseline and ≥ 1 Gd-enhancing lesion at baseline

AE adverse event, *CARE-MS* Comparison of Alemtuzumab and Rebif Efficacy in Multiple Sclerosis, *Gd* gadolinium, *HAD* highly active disease, *ITP* immune thrombocytopenia

FIGURE LEGENDS

Fig. 1 HAD definitions and HAD patient disposition: (a) primary and alternative definitions of HAD used in this post hoc analysis; (b) CARE-MS I and II alemtuzumab-treated HAD patient disposition through Year 9 defined by the primary HAD definition
CARE-MS Comparison of Alemtuzumab and Rebif Efficacy in Multiple Sclerosis, *DMT* disease-modifying therapy, *Gd* gadolinium, *HAD* highly active disease, *SC IFNB-1a* subcutaneous interferon beta-1a

Fig. 2 Clinical efficacy in CARE-MS I alemtuzumab-treated HAD patients through Year 9: (a) ARR; (b) percentage of patients with improved or stable EDSS scores over time; may not sum appropriately due to rounding; (c) percentage of patients free of 6-month CDW; (d) percentage of patients achieving 6-month CDI
* $p = 0.01$ vs. SC IFNB-1a over Years 0–2
Primary HAD definition: ≥ 2 relapses in the year prior to baseline and ≥ 1 Gd-enhancing lesion at baseline
Improved EDSS score: ≥ 1.0-point decrease from core study baseline; stable EDSS score: ≤ 0.5-point change in either direction from core study baseline
CDW: ≥ 1.0-point EDSS increase (or ≥ 1.5 points if baseline EDSS = 0) confirmed over 6 months
CDI: ≥ 1.0-point EDSS decrease from baseline confirmed over 6 months (assessed only in patients with baseline EDSS score ≥ 2.0)
ARR annualized relapse rate, *CARE*-MS Comparison of Alemtuzumab and Rebif Efficacy in Multiple Sclerosis, *CDI* confirmed disability improvement, *CDW* confirmed disability worsening, *CI* confidence interval, *EDSS* Expanded Disability Status Scale, *HAD* highly active disease, *SC IFNB-1a* subcutaneous interferon beta-1a, *Y* year

Fig. 3 Clinical efficacy in CARE-MS II alemtuzumab-treated HAD patients through Year 9: (a) ARR; (b) percentage of patients with improved or stable EDSS scores over time; may not sum appropriately due to rounding; (c) percentage of patients free of 6-month CDW; (d) percentage of patients achieving 6-month CDI
* $p = 0.004$ vs. SC IFNB-1a over Years 0–2
Primary HAD definition: ≥ 2 relapses in the year prior to baseline and ≥ 1 Gd-enhancing lesion at baseline
Improved EDSS score: ≥ 1.0-point decrease from core study baseline; stable EDSS score: ≤ 0.5-point change in either direction from core study baseline
CDW: ≥ 1.0-point EDSS increase (or ≥ 1.5 points if baseline EDSS = 0) confirmed over 6 months
CDI: ≥ 1.0-point EDSS decrease from baseline confirmed over 6 months (assessed only in patients with baseline EDSS score ≥ 2.0)
ARR annualized relapse rate, *CARE-MS* Comparison of Alemtuzumab and Rebif Efficacy in Multiple Sclerosis, *CDI* confirmed disability improvement, *CDW* confirmed disability worsening, *CI* confidence interval, *EDSS* Expanded Disability Status Scale, *HAD* highly active disease, *SC IFNB-1a* subcutaneous interferon beta-1a, *Y* year

Fig. 4 MRI outcomes and NEDA in CARE-MS I alemtuzumab-treated HAD patients through Year 9: (a) percentage of patients free of MRI disease activity; (b) percentage of patients achieving annual NEDA; (c) cumulative BVL, change from baseline in median BPF over time
* $p = 0.0007$ vs. SC IFNB-1a in Year 2
Primary HAD definition: ≥ 2 relapses in the year prior to baseline and ≥ 1 Gd-enhancing lesion at baseline
Freedom from MRI disease activity: no new Gd-enhancing T1 lesions on current MRI and no new/enlarging T2 hyperintense lesions since last MRI
NEDA: absence of relapse, 6-month CDW, and MRI disease activity
BPF brain parenchymal fraction, *CARE-MS* Comparison of Alemtuzumab and Rebif Efficacy in Multiple Sclerosis, *CI* confidence interval, *Gd* gadolinium, *HAD* highly active disease, *MRI* magnetic resonance imaging, *NEDA* no evidence of disease activity, *SC IFNB-1a* subcutaneous interferon beta-1a, *Y* year

Fig. 5 MRI outcomes and NEDA in CARE-MS II alemtuzumab-treated HAD patients through Year 9: (a) percentage of patients free of MRI disease activity; (b) percentage of patients achieving annual NEDA; (c) cumulative BVL, change from baseline in median BPF over time
* $p < 0.0001$ vs. SC IFNB-1a in Years 1 and 2
Primary HAD definition: ≥ 2 relapses in the year prior to baseline and ≥ 1 Gd-enhancing lesion at baseline
Freedom from MRI disease activity: no new Gd-enhancing T1 lesions on current MRI and no new/enlarging T2 hyperintense lesions since last MRI
NEDA: absence of relapse, 6-month CDW, and MRI disease activity
BPF brain parenchymal fraction, *CARE-MS* Comparison of Alemtuzumab and Rebif Efficacy in Multiple Sclerosis, *CI* confidence interval, *Gd* gadolinium, *HAD* highly active disease, *MRI* magnetic resonance imaging, *NEDA* no evidence of disease activity, *SC IFNB-1a* subcutaneous interferon beta-1a, *Y* year

FIGURES

Fig. 1 Definitions of HAD used in this study and HAD patient disposition

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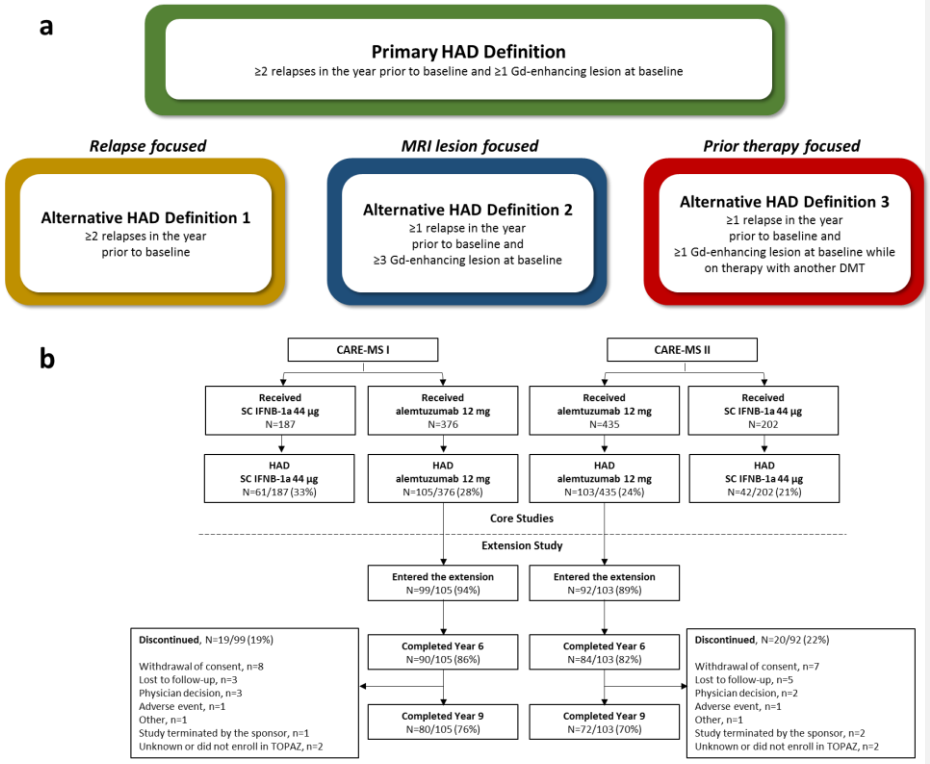


Fig. 2 Clinical efficacy in CARE-MS I alemtuzumab-treated HAD patients through Year 9: (a) ARR; (b) percentage of patients with improved or stable EDSS scores over time; (c) percentage of patients free of 6-month CDW; (d) percentage of patients achieving 6-month CDI

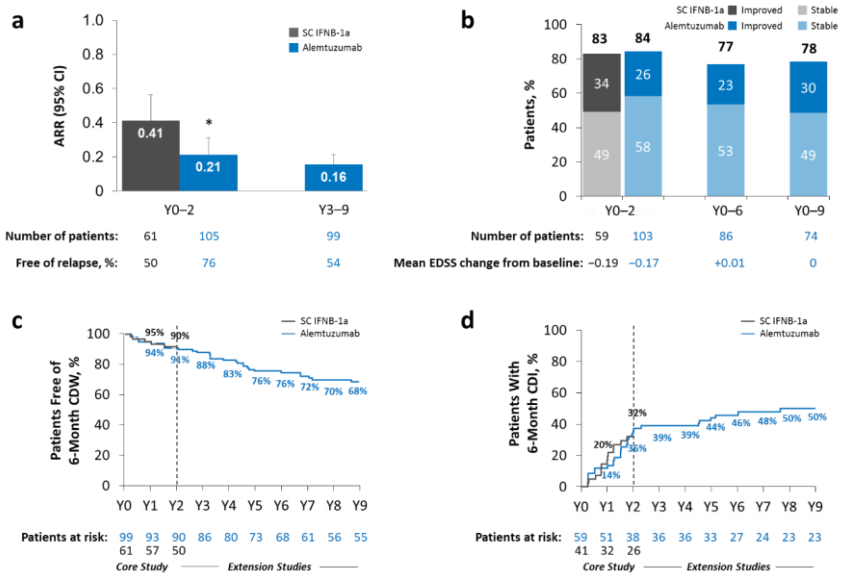


Fig. 3 Clinical efficacy in CARE-MS II alemtuzumab-treated HAD patients through Year 9: (a) ARR; (b) percentage of patients with improved or stable EDSS scores over time; (c) percentage of patients free of 6-month CDW; (d) percentage of patients achieving 6-month CDI

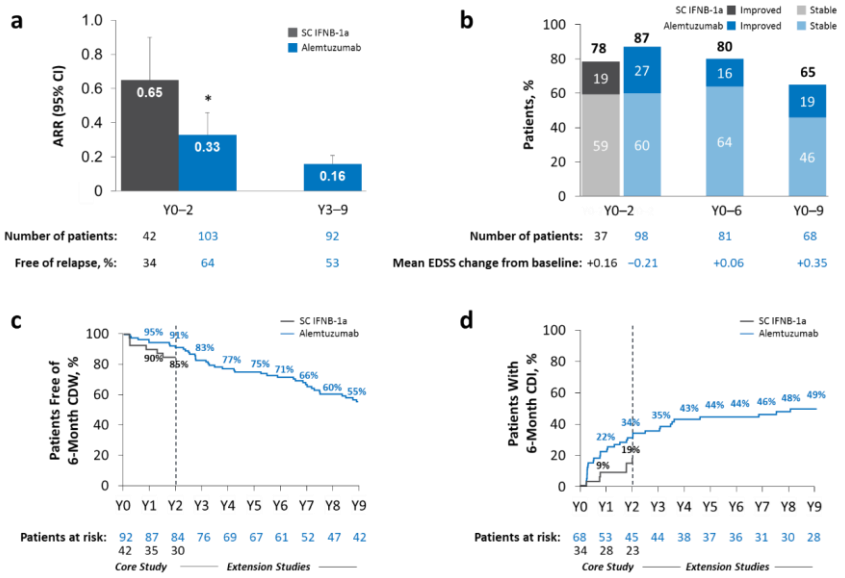


Fig. 4 MRI outcomes and NEDA in CARE-MS I alemtuzumab-treated HAD patients through Year 9: (a) percentage of patients free of MRI disease activity; (b) percentage of patients achieving annual NEDA; (c) cumulative change in median BPF over time

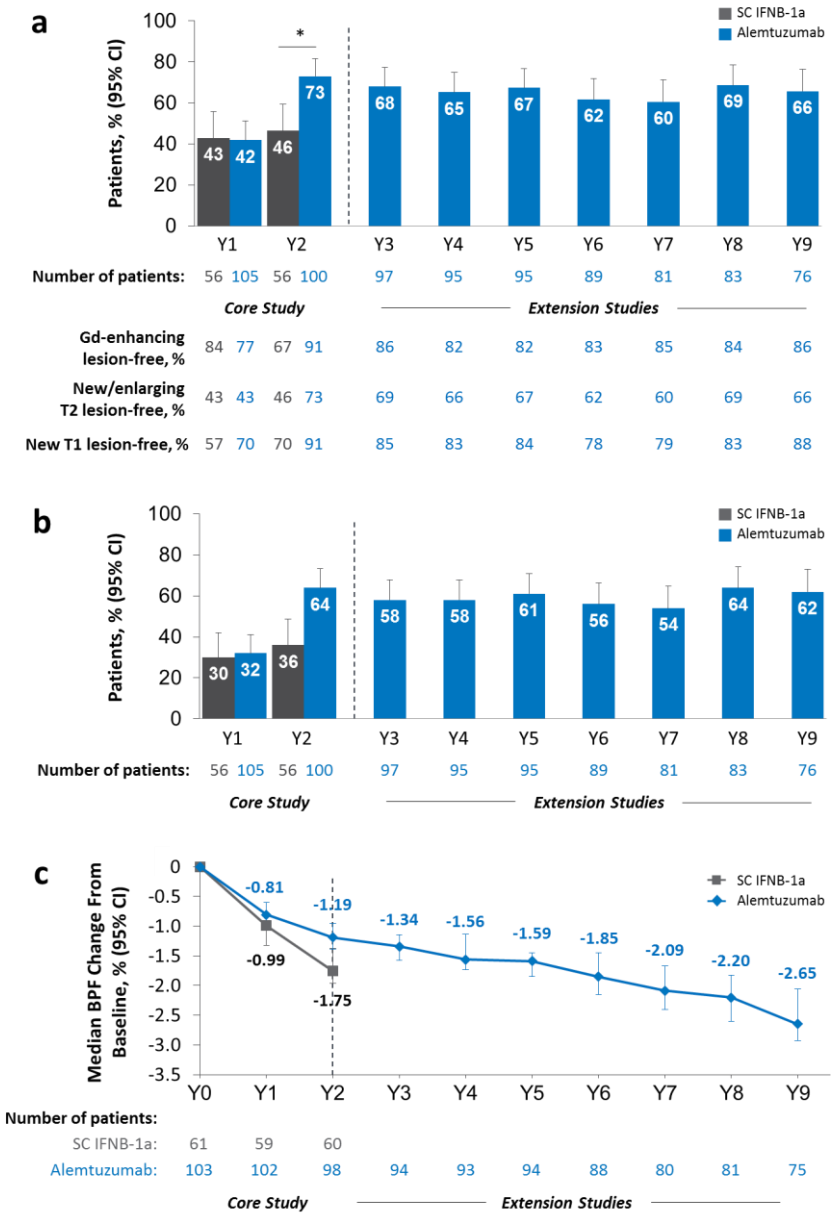
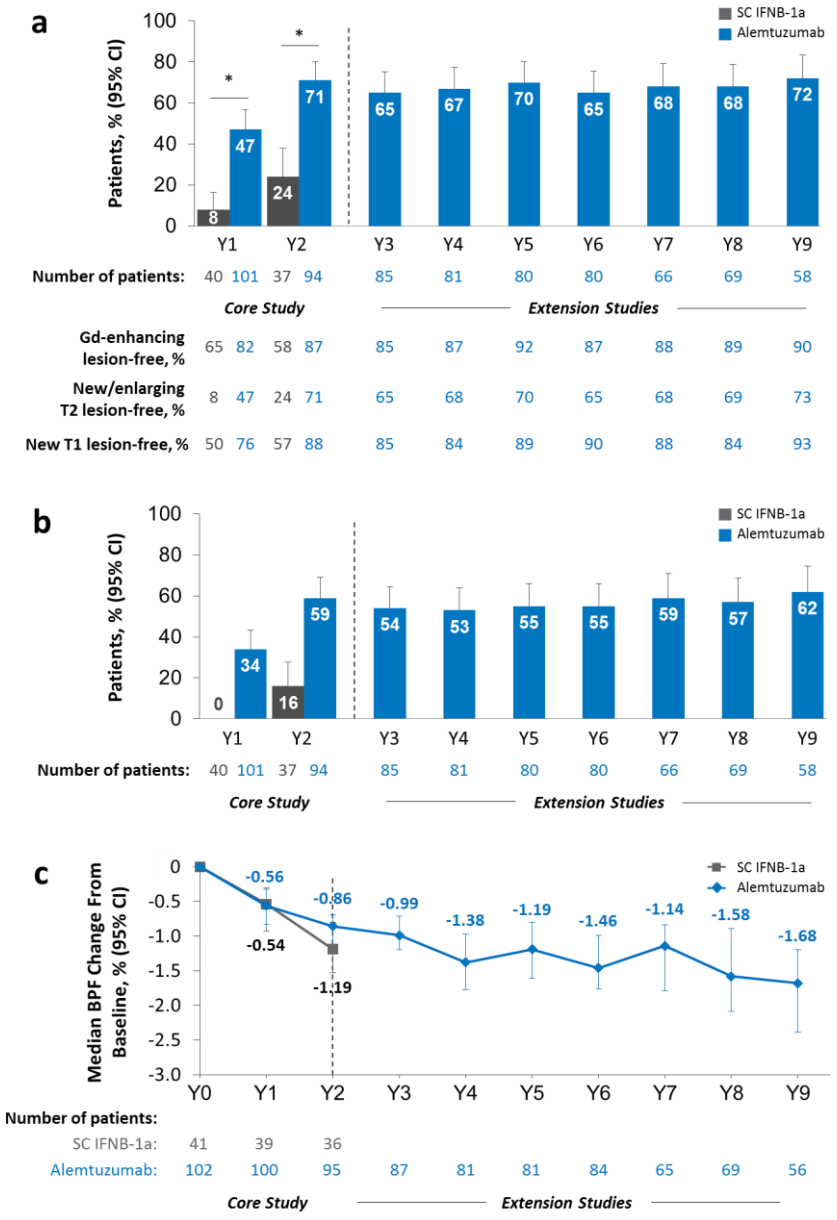


Fig. 5 MRI outcomes and NEDA in CARE-MS II alemtuzumab-treated HAD patients through Year 9: (a) percentage of patients free of MRI disease activity; (b) percentage of patients achieving annual NEDA; (c) cumulative median BPF change over time



SUPPLEMENTAL TABLES

Supplementary Table 1 Total follow-up time through Year 9 in CARE-MS I, CARE-MS II, and pooled CARE-MS I and II alemtuzumab-treated HAD patients

	HAD Patients		
	CARE-MS I (*N* = 105)	**CARE-MS II (*N* = 103)**	**Pooled CARE-MS I and II (*N* = 208)**
Total follow-up time, patient-years	927.1	823.2	1750.3
Mean (SD)	8.8 (2.4)	8.0 (2.6)	8.4 (2.6)
Median (range)	9.8 (1.3–11.1)	9.2 (1.1–10.6)	9.5 (1.1–11.1)

CARE-MS Comparison of Alemtuzumab and Rebif Efficacy in Multiple Sclerosis, *HAD* highly active disease, *SD* standard deviation

Supplementary Table 2 Efficacy through 9 years in alemtuzumab-treated patients from the pooled CARE-MS I and II studies

	Alemtuzumab-Treated Patients
Parameter	**CARE-MS Overall (Pooled Studies) (*N* = 811)**
Proportion of patients who received neither additional alemtuzumab nor another DMT in the extensions, %	48
Proportion of patients who received additional alemtuzumab, %	49[c]
3 total courses, %	26[c]
4 total courses, %	14[c]
5 total courses, %	6[c]
6 total courses, %	2[c]
7 total courses, %	0.5[c]
8 total courses, %	0.1[c]
ARR over Y3–9 (95 % CI)	0.17 (0.15 to 0.19)
Proportion of patients relapse-free over Y3–9, % (95 % CI)[a]	50 (46 to 54)
Mean EDSS score change over Y0–9 (95 % CI)	0.21 (0.09 to 0.33)
Proportion of patients with improved or stable EDSS over Y0–9[b], %	72 (improved, 22; stable, 51)
Proportion of patients free of 6-Month CDW over Y0–9, % (95 % CI)	64 (60 to 67)
Proportion of patients with 6-Month CDI over Y0–9, % (95 % CI)	45 (41 to 50)
Proportion of patients free of MRI disease activity in Y9, % (95 % CI)[a]	70 (66 to 74)

Proportion of patients with NEDA in Y9, % (95 % CI)[a]	62 (58 to 67)
Proportion of patients with cumulative NEDA over Y3–9, % (95% CI)[a]	18 (14 to 21)
Median BPF change over Y0–9, % (95 % CI)	−1.65 (−1.81 to −1.48)

[a] CIs were calculated using the Wald method; [b] values may not sum appropriately due to rounding; [c] N=742
Improved EDSS score: ≥ 1.0-point decrease from core study baseline; stable EDSS score: ≤ 0.5-point change in either direction from core study baseline.
CDW: ≥ 1.0-point EDSS increase from core study baseline (or ≥ 1.5 points if baseline EDSS = 0) confirmed over 6 months
CDI: ≥ 1.0-point EDSS decrease from core study baseline confirmed over 6 months (assessed only in patients with baseline EDSS score ≥ 2.0)
Freedom from MRI disease activity: no new Gd-enhancing T1 lesions on current MRI and no new/enlarging T2 hyperintense lesions since last MRI
NEDA: absence of relapse, 6-month CDW, and MRI disease activity
ARR annualized relapse rate, *BPF* brain parenchymal fraction, *CARE-MS* Comparison of Alemtuzumab and Rebif Efficacy in Multiple Sclerosis, *CDI* confirmed disability improvement, *CDW* confirmed disability worsening, *CI* confidence interval, *DMT* disease-modifying therapy, *EDSS* Expanded Disability Status Scale, *Gd* gadolinium, *MRI* magnetic resonance imaging, *NEDA* no evidence of disease activity, *Y* year

Supplementary Table 3 AE incidence over Years 0–9 in the overall alemtuzumab-treated populations from CARE-MS I and II

	Alemtuzumab-Treated Patients Over Years 0−9	
AEs, n (%)	**CARE-MS I Overall (*N* = 376)**	**CARE-MS II Overall (*N* = 435)**
Any AE	370 (98.4)	434 (99.8)
Serious AEs	144 (38.3)	195 (44.8)
Infections	313 (83.2)	382 (87.8)
Serious infections	25 (6.6)	52 (12.0)
Autoimmune AEs[a]		
Thyroid AEs	174 (46.3)	190 (43.7)
Serious thyroid AEs	24 (6.4)	23 (5.3)
ITP	6 (1.6)	16 (3.7)
Nephropathies	1 (0.3)	2 (0.5)
Malignancies	9 (2.4)	8 (1.8)
Deaths	7 (1.9)	9 (2.1)

[a] First occurrence of AE within the time period
AE adverse event, *CARE-MS* Comparison of Alemtuzumab and Rebif Efficacy in Multiple Sclerosis, *Gd* gadolinium, *ITP* immune thrombocytopenia