Supplementary Methods

General: This international, phase 2b, multicenter, randomized, double-blind, placebo-controlled study was conducted at 51 clinical research facilities in 10 countries per the International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use Good Clinical Practice, applicable national and local laws and regulations, patient privacy requirements, and ethical principles outlined in the Declaration of Helsinki 2008. The study, registered at ClinicalTrials.gov (NCT01457924), was started in November 2011 and completed in June 2015.

Detailed Eligibility Criteria

Inclusion Criteria:

- Patients with RRMS, ages 18 to 55 years, who were otherwise healthy, were eligible for the study if they had a diagnosis of definite MS (revised McDonald Criteria)(1) with a relapsing and remitting disease course as evidenced (or historically confirmed) by: at least 1 confirmed relapse within the previous year, *or* at least 2 confirmed relapses within the previous 2 years, *or* at least 1 relapse in the previous 2 years *and* a GdE brain lesion on MRI within the past year.
- Patients were required to have an Expanded Disability Status Scale (EDSS) score at Screening between 0 and 5.5.
- Other eligibility criteria included not having any manifestation of another type of MS other than RRMS, and to be neurologically stable with no evidence of relapse for at least 30 days prior to start of Screening and during the Screening phase.
- Female patients had to be of non-childbearing potential, or if of childbearing potential, not be pregnant and using an acceptable method of birth control (including abstinence) for the period from consent to the study until 6 months after the last dose of investigational product.
- In France, a patient was eligible for inclusion in this study only if either affiliated to, or a beneficiary of, a social security category.

Exclusion Criteria

- Prior use of disease-modifying therapies (DMTs) was allowed, but patients were excluded if they had previously used: experimental agents for treatment of MS; mAbs (other than natalizumab); immunosuppressive agents (including but not limited to mitoxantrone, azathioprine, cyclosporine, cyclophosphamide, or tacrolimus); any lymphocyte-depleting therapies; particular pre-Screening exposures (within

  1 month for systemic glucocorticoids or adrenocorticotrophic hormone; within

  6 weeks for live vaccines; within 3 months for glatiramer acetate or interferon-β; within 6 months for any other immunomodulatory therapies).
- Patients could not have used any investigational drug/therapy for an indication other than MS within at least 4 weeks.
- Patients could not be participating in another interventional clinical study.
- Patients were excluded if they had a prior history of medically significant adverse effects due to cetirizine, paracetamol/acetaminophen, or corticosteroids; were unable to undergo MRI scans; had any clinically significant brain abnormality other than MS found on MRI; exhibited neurological findings consistent with progressive multifocal leukoencephalopathy; had a history of clinically significant central nervous system trauma; or experienced a relapse during Screening.
- Patients could not be positive for John Cunningham virus, hepatitis B, tuberculosis, or HIV, or have known history of drug or alcohol abuse or attempted suicide.

Randomization, Blinding and Monitoring: Randomization was computer-generated by the project statistician (RAG) and stratified on the basis of entry into a pharmacokinetic (PK) sub-study or presence or absence of GdE lesions on the Screening MRI performed no more than 2 weeks prior. The PK sub-study was used as a stratification factor to ensure a balance across treatment groups entering the sub-study. A Registration and Medication Ordering System Interactive Voice Response System (provided by the sponsor) served as a central system to sequentially allocate and maintain randomization numbers for each subject as they were randomized by Investigators. An unblinded pharmacist at the investigational site prepared the ofatumumab injections and made the placebo to match the ofatumumab doses using normal

saline. Administration of the investigational product was limited to the investigator and authorized site staff who were blinded to the treatment. An unblinded hematologist at GlaxoSmithKline, who was completely independent of the study team, reviewed the hematology data, including B-lymphocyte counts. Investigators did not receive unblinded data on the depletion of B lymphocytes until after the Week 48 visit had been reached for all patients in the study. An independent data monitoring committee reviewed safety data during the conduct of the study.

Patients receiving ofatumumab were monitored in clinic for a minimum of 7 hours post-injection at Weeks 0 and 1. Patients were contacted every day for 4 days following ofatumumab administration at Weeks 0, 1, and 12 to monitor any post-injection systemic reactions.

Study Endpoints: Efficacy was primarily based on MS lesions detected by GdE MRI scans, which were conducted every 4 weeks during the 24-week Treatment phase and every 12 weeks during the 24-week Follow-Up (FU) phase. Because the placebo-control period implemented here was shorter than other phase 2 studies in RRMS (decision made to limit potential risks with exposure to placebo), we employed a double-dose of Gd (0.2 mmol/Kg) contrast medium in a single injection to improve lesion detection. Such a double-dose of Gd has been shown to provide the best balance in terms of sensitivity, time, costs, and the detection of active MS lesions.(2) The scanning protocol consisted of axial dual echo PD/T2 weighted, axial fluid attenuated inversion recovery, axial T1 pre-contrast and axial T1-Gd sequences, the latter obtained approximately 10 minutes after the double dose (0.2 mmol/kg) of Gd contrast. Other MRI endpoints were the cumulative number of new GdE lesions at Week 24 and cumulative number and total volume of new and new plus persisting GdE lesions, new and/or newly enlarging T2 lesions, and T1 hypointense lesions at Weeks 12 and 24. While the primary efficacy outcome was pre-defined as the mean cumulative number of GdE T1 lesions between Weeks 0–12, pre-baseline MRI activity continued to be observed through the Week 4 MRI scan for all treatment groups (see Results) and, in keeping with analyses conducted in other studies reporting a similar phenomenon,(3, 4) additional statistical analyses of MRI endpoints were conducted using a post-hoc defined assessment window of Weeks 4–12.

Assessment of clinical efficacy was based on the proportion of patients who were relapse-free from Week 0 to Week 12. Other clinical endpoints assessed included EDSS (at Screening, Week 4, and every visit

thereafter), the Multiple Sclerosis Functional Composite and component scores (at Screening, every 4 weeks during the treatment phase, and every 6 weeks during FU), the Modified Fatigue Impact Scale (at Screening and Weeks 12 and 24) and questioning regarding suicidality, leading to six patients reporting suicidal ideations (2 in the placebo, and 4 in the ofatumumab groups), but none with suicidal behavior. During the 24-week FU and IFU phases, patients did not receive study medication, and no statistical assessment of efficacy endpoints was conducted. The primary population for efficacy analyses was the modified intent-to-treat (mITT) Population (all patients randomized to treatment who took at least 1 dose of placebo or ofatumumab and who had at least 1 post-Screening MRI assessment). To conservatively account for protocol violations, a per-protocol (PP) Population of 157 patients was also considered in the efficacy analyses based on exclusion of any patient with a protocol violation at any time during the Treatment phase. The most common reasons were patients receiving an incorrect Gd contrast dose (n=30; 13%), and dosing errors (n=22; 10%). Twelve patients received incorrect doses at least once, including 11 during the placebo-control period, 6 of them having been randomized to placebo but receiving ofatumumab (which would serve to dilute rather than exaggerate the efficacy assessments in the mITT population).

Ofatumumab PK and Immunogenicity: Blood samples (~3 mL each) for determination of ofatumumab trough concentrations were collected from all patients at Weeks 12 and 24, with additional sampling (~3-3.5 mL each) at 1, 2, 3, 4, 7, 14 and 21 days post-dose in the PK subpopulation (n=28). Ofatumumab was measured in plasma using a validated ELISA, with an LLQ of 100 ng/mL. Immunogenicity was assessed based on measurement of incidence, titer, and type of HAHA immune responses at Weeks 0, 4, 12, 24, 36, and 48 using the second-generation Meso Scale Discovery electrochemiluminescence assay.

Further Statistical Details: A sample size of 196 patients was estimated to provide 90% power to detect a 63% reduction between the highest ofatumumab dose group (60 mg q4w) and to detect a significant dose response at the 5% significance level for the primary endpoint. A greater number of patients (n=232) were recruited to the study than planned due to an unexpected bolus of patients being screened towards the end of the study, all of whom were eligible and were allowed to participate. The over-recruitment was not considered to have significantly impacted on the overall power or interpretation of the study.

The dose-response analysis on the primary endpoint, GdE lesions, considered four candidate dose-response models (generalized linear models [GLMs] fitting cumulative dose as a categorical/continuous variable with underlying negative binomial distribution; E_{max} model, Sigmoid-Hill model). The primary model for analysis of the primary efficacy endpoint was selected as the one that best fit the data (specified as lowest Bayesian Information Criterion). This was the E_{max} model, which took the following form:

$$E(Yi)=\exp(E0 +\gamma\ GdE_{ind}+ E_{max}\ x_i / (ED_{50}+x_i))$$

where $Yi \sim NB(\mu, p)$ with $1/\mu=E(Y)$, $p=1/(1+k\mu)$ and GdE_{ind} is an indicator variable for presence/absence of GdE lesions on Screening MRI; ED_{50} is the effective dose in 50% of patients, NB is negative binomial distribution, and k is the dispersion parameter for the NB distribution. In the model-based (E_{max}) analysis, the estimate of dose-response and its variability is unaffected by individual treatment group sizes.

Secondary MRI endpoints were analyzed using a GLM fitting dose as a categorical variable assuming an underlying NB distribution, with the exception of changes in T2 lesion volume, which were analyzed using an analysis of covariance (ANCOVA) model adjusting for baseline T2 lesion volume and stratum.

PK parameters were estimated using a 1-compartment model with first-order absorption rate equal to elimination rate (PROC NLMMIXED; PC SAS version 9.2). Parameters included apparent volume of distribution (V/F), absorption and elimination rate (K), apparent clearance (CL/F), time to maximum concentration (T_{max}), elimination half-life ($t_{1/2}$), and accumulation ratio (Ro).

Supplementary Results:

IFU: A smaller proportion of patients in the placebo group (24%) entered the IFU phase compared with the ofatumumab groups (50% to 66%), with 112 patients overall entering this phase. Of the 100 patients who did not enter the IFU phase, the majority (84 patients, 84%) had their CD19 counts return to LLN/baseline, thus completing their participation in the study (half of these patients were in the placebo group [42 patients]). Eighty-eight (88/231, 38%) patients completed the IFU phase—the last subject completed at Week 132.

Secondary efficacy imaging measures and PP Population analyses: All secondary MRI endpoints, as well as all analyses in the PP Population, supported the primary analysis with statistically significant reductions in the cumulative number of new/enlarging T2 lesions for Weeks 0–12 and in the cumulative number of new GdE T1 lesions for Weeks 0–24 for all ofatumumab dose groups (**Table 2, Table e-1** and **Table e-2**). Significant reductions favoring ofatumumab were also observed for the number of new/enlarging T2 lesions (Weeks 0–24 and Weeks 4–24) and, for the 30-mg and 60-mg ofatumumab dose groups, the cumulative volume of new and newly enlarging T2 lesions (Weeks 4–24) (**Table 2**). Post-hoc analyses of Weeks 4–12 in the PP Population further supported the primary analyses, with ≥90% suppression in the cumulative number of new GdE lesions noted for all ofatumumab dose groups ≥30 mg per 12 weeks (**Table e-1)**.

The majority of patients in all groups had no new T1 hypointense lesions, and there was no evidence of separation from placebo for any of the measures related to T1 hypointense lesions. The rate ratios for cumulative number of new/persisting hypointense T1 lesions in the ofatumumab groups ranged between 1.0 and 1.68 at Weeks 0–12 ($P \geq 0.209$) and 0.99 and 1.71 at Weeks 0–24 ($P \geq 0.217$).

MRI lesion counts, including the cumulative number of new T1 GdE brain lesions, and lesion volumes appeared to remain stable from the end of 0–24 Week Treatment phase through Week 48 for all but 1 dose group, the placebo group at Week 12, which showed increases during the 24–48-week FU phase.

Clinical efficacy: The absolute risk reduction values for the proportion of patients relapsing during the study were generally small, but favored treatment with ofatumumab relative to placebo, with the exception of the 60 mg q4w during the FU phase (Table-e3). Therefore, this study did not find any strong evidence for reduced number of relapses with ofatumumab versus placebo, which may be because the placebo group did receive 3 mg ofatumumab during Weeks 12–24 and the duration of treatment was relatively short, meaning the study was underpowered for this endpoint.

PK: Prior to treatment, there were no detectable ofatumumab plasma concentrations (ie, above the LLQ of 100 ng/mL). Subsequently, detectable concentrations were measured in a total of 165 plasma samples from

patients receiving the following ofatumumab regimens: 3 mg q12w (5 samples), 30 mg q12w (9 samples), 60 mg q12w (21 samples), and 60 mg q4w (130 samples).

In samples from the 60 mg q4w ofatumumab group, geometric mean (95% CI) trough concentrations (Figure e-4A) were 806.8 ng/ml (229.3, 835.2) at Week 12 and 1325.9 ng/ml (1291.9, 1360.8) at Week 24, with Ro being 1.75-fold (95% CI 1.5, 2.1). The t1/2 necessary to generate this accumulation ratio when a drug is dosed every 4 weeks is 22 days, which is characteristic of mAbs not subjected to target-mediated clearance (5) and much longer than the t1/2 of 3 days estimated after the first dose. In 6 patients, sufficient data were available to analyze 'first-dose PK profiles' (Figure e-4B) with estimated volume of distribution (V/F), 13.8 L; K, 0.23/day; CL/F, 3.2 L/day; T_{max}, 4.3 days; and $t_{1/2}$, 3.0 days.

Potential for unblinding: Given higher rates of injection-related symptoms in the ofatumumab treatment arms than in the placebo arm, there was potential that blinding could have been compromised. However, this would not be expected to influence the primary endpoint in this study, which was based on imaging. Central MRI readers, who had no access to treatment codes or other data, interpreted the images. Similarly, automated assessment of B cells was documented by personnel having no access to patient data.

References

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