MULTIPLE SCLEROSIS JOURNAL | MSJ

Original Research Paper

Dimethyl fumarate treatment shifts the immune environment toward an anti-inflammatory cell profile while maintaining protective humoral immunity

Multiple Sclerosis Journal

2021, Vol. 27(6) 883–894

DOI: 10.1177/1352458520937282

Erin E Longbrake, Yang Mao-Draayer, Mark Cascione, Tomasz Zielinski, Eris Bame, David Brassat, Chongshu Chen, Shivani Kapadia, Jason P Mendoza, Catherine Miller, Becky Parks, Diana Xing and Derrick Robertson

Abstract

Background: Delayed-release dimethyl fumarate (DMF) demonstrates sustained efficacy and safety for relapsing forms of MS. Absolute lymphocyte count (ALC) is reduced initially, then stabilizes on treatment.

Objective: PROCLAIM, a 96-week, prospective, open-label, phase 3b study, assessed lymphocyte subsets and immunoglobulin (Ig) levels during 48 and 96 weeks (W) of DMF treatment.

Methods: Patients received 240 mg DMF BID. Endpoints: lymphocyte subset count changes (primary); Ig isotypes and ALC changes (secondary); adverse events and relationship between ALC changes and ARR/EDSS (exploratory); and neurofilament assessment (ad hoc).

Results: Of 218 patients enrolled, 158 (72%) completed the study. Median ALC decreased 39% from baseline to W96 (BL–W96), stabilizing above the lower limit of normal (baseline: 1.82×10^9/L; W48: 1.06×10^9/L; W96: 1.05×10^9/L). $CD4^+$ and $CD8^+$ T cells correlated highly with ALC from BL–W96 ($p < 0.001$). Relative to total T cells, naive $CD4^+$ and $CD8^+$ T cells increased, whereas $CD4^+$ and $CD8^+$ central and effector memory T cells decreased. Total IgA, IgG, IgM, and IgG1–4 subclass levels remained stable. Adverse event rates were similar across ALC subgroups. ARR, EDSS, and neurofilament were not correlated with ALCs.

Conclusion: Lymphocyte decreases with DMF were maintained over treatment, yet immunoglobulins remained stable. No increase in infection incidence was observed in patients with or without lymphopenia.

Support: Biogen

Keywords: Delayed-release dimethyl fumarate, multiple sclerosis, lymphocytes, B cells, immunoglobulin

Date received: 4 March 2020; revised: 15 May 2020; accepted: 29 May 2020.

Correspondence to:
C Miller
Biogen, 225 Binney Street, Cambridge, MA 02142, USA.
catherine.miller@biogen.com

Erin E Longbrake
Yale University, New Haven, CT, USA

Yang Mao-Draayer
Autoimmunity Center of Excellence, Department of Neurology, University of Michigan Medical School, Ann Arbor, MI, USA

Mark Cascione
First Choice Neurology, Tampa, FL, USA

Tomasz Zielinski
NOVO-MED Zielinski i wspolnicy Sp j. Katowice, Poland

Eris Bame
David Brassat
Chongshu Chen
Shivani Kapadia
Jason P Mendoza
Catherine Miller
Becky Parks
Diana Xing
Biogen, Cambridge, MA, USA

Derrick Robertson
Multiple Sclerosis Division, Department of Neurology, University of South Florida College of Medicine, Tampa, FL, USA

Introduction

Delayed-release dimethyl fumarate (DMF), an approved oral therapy for relapsing forms of MS (RMS), has demonstrated sustained efficacy and a favorable benefit-risk profile.[1–3] As of January 31, 2020, more than 445,000 patients have been treated with DMF worldwide, representing more than 875,000 patient-years of exposure. Of these, 6335 patients (14,241 patient-years) were from clinical trials. (Biogen, data on file). DMF treatment induces absolute lymphocyte count (ALC) reductions, typically in the first year of treatment, followed by stabilization.[4] Mean ALC reduction is ~30% from baseline and most patients remain above the lower limit of normal (LLN) (0.91×10^9/L).[5] Grade 3/severe lymphopenia (ALC $< 0.5 \times 10^9$/L) persisting for ≥ 6 months develops in ~2% of patients.[5] Absolute T-cell counts are more strongly impacted by DMF than B or natural killer (NK) cells, although most cell types are reduced to some degree. Within the T-cell compartment, $CD8^+$ cells are reduced more profoundly than $CD4^+$ cells.[6–12] Despite ALC changes, DMF-treated patients mount an effective immune response to

vaccination, including both T-cell–dependent and T-cell–independent antigens, comparable to interferon (IFN)-treated patients.[13] Although ALC does not appear to directly correlate with clinical response, functional shifts in circulating lymphocyte subsets favoring naive and anti-inflammatory phenotypes likely contribute to the effect of DMF.[1,2,14,15]

Previous immunophenotyping studies have been either cross-sectional[6,9] or longitudinal, with few patients[10] or minimal data-collection time points.[16] PROCLAIM, the first large longitudinal study designed to assess the effects of DMF on lymphocyte subset counts and immunoglobulin (Ig) isotypes, may provide greater insight into DMF's therapeutic benefit and inform the safety profile.

Methods

Study design

PROCLAIM (EUDRA CT 2015-001973-42) was a prospective, open-label, multicenter, phase 3b study assessing changes in immune cell compartments through 2 years of DMF treatment. The study period consisted of a screening visit, a 96-week treatment period, and a final follow-up visit 4 weeks after the last dose of DMF. All patients received 120 mg twice daily (BID) for the first 7 days, followed by a maintenance dose of 240 mg BID thereafter (approved dosing regimen for MS). Temporary dose reduction to 120 mg BID through 4 weeks was permitted for individuals who did not tolerate the maintenance dose due to flushing or gastrointestinal disturbance. Treatment compliance was monitored by study personnel via capsule counting at visits. Blood samples were collected at baseline and Weeks 4, 8, 12, 24, 36, 48, 72, and 96.

Patients

Eligible patients were aged 18–65 years with a confirmed diagnosis of RMS.[17] Key exclusion criteria were positive serology for HIV or hepatitis B/C; history of drug or alcohol abuse within 1 year before screening; clinically significant comorbid disorders or conditions, including infectious illness; leukocytes $< 3.5 \times 10^9$/L; ALC values $\leq$ LLN (0.91×10^9/L); or prior treatment with cladribine, mitoxantrone, total lymphoid irradiation, alemtuzumab, T-cell or T cell–receptor vaccination, or any therapeutic monoclonal antibody (except natalizumab or daclizumab). Concomitant treatment with alternative MS drugs or systemic steroid therapy was not allowed unless approved by the medical monitor for treatment of a protocol-defined relapse.

Study objectives and endpoints

The study objectives were to evaluate the effect of DMF on lymphocyte subset counts (primary) and the pharmacodynamic effect of DMF on ALCs and Ig isotypes (secondary) in patients with RMS during the first 48 weeks of treatment. Exploratory study objectives included evaluation of safety and tolerability over 96 weeks; lymphocyte subset counts through 96 weeks of DMF treatment; the relationship between changes in ALC and lymphocyte subsets and MS disease activity (measured by clinical relapse; annualized relapse rate (ARR)) or sustained clinical disease progression (CDP) (measured by the Expanded Disability Status Scale (EDSS)); and ad hoc evaluations of hypothesis-driven biomarker analyses, including neurofilament light chain(NfL).

ALC, immune cell phenotyping, and Igs

ALC was measured using complete blood cell differential. Changes in lymphocyte subsets were assessed by flow cytometry utilizing cell surface markers (Supplementary Table 1). Serum Ig levels (total IgA, IgG, IgM, and subclasses IgG1, IgG2, IgG3, and IgG4) were measured using an immunoturbidimetric method on the Integra/Plus (Roche Diagnostics, Basel, Switzerland) or by immunologic methods (LOINC®, LabCorp, Burlington, North Carolina).

Clinical assessments

Safety outcomes were monitored throughout the study and safety assessments were performed at the post-treatment follow-up visit. Relapses were defined as new or recurrent neurologic symptoms not associated with fever or infection, lasting $\geq$ 24 hours, accompanied by objective neurological findings and confirmed by a neurologist. Sustained CDP was defined as $\geq$ 1.0-point increase on the EDSS from a baseline score of $\geq$ 1.0, sustained for 24 weeks, or $\geq$ 1.5-point increase on the EDSS from a baseline EDSS score of 0, sustained for 24 weeks.

ALCs were categorized as follows: always $>$ LLN, 0.91×10^9/L; mild lymphopenia, $< 0.91 \times 10^9$/L anytime, excluding patients with ALC $< 0.8 \times 10^9$/L for $\geq$ 6 months; moderate prolonged lymphopenia, $\geq 0.5 \times 10^9$/L to $< 0.8 \times 10^9$/L for $\geq$ 6 months; and severe prolonged lymphopenia, $< 0.5 \times 10^9$/L for $\geq$ 6 months.

ALCs were stratified by age at baseline; younger patients ($<$50 years) versus older patients ($\geq$50 years). This cutoff was selected in order to explore the immune function in patients who may be prone to decreased immune function and immunosenescence based on age, while maintaining a sufficient sample size.[18,19]

Table 1. Patient baseline demographics and disease characteristics.

Characteristic	PROCLAIM $N=218$
Mean ± SD age at enrollment, years	42 ± 11
Age ⩾ 50 years, *n* (%)	59 (27)
Female, *n* (%)	151 (69)
Mean ± SD body mass index	26.8 ± 7
Mean ± SD baseline lymphocyte level ($\times 10^9$/L)	
ALC	1.97 ± 0.71
T cells	1.32 ± 0.54
$CD4^+$ T cells	0.88 ± 0.40
$CD8^+$ T cells	0.42 ± 0.20
B cells	0.24 ± 0.17
NK cells	0.18 ± 0.11
Mean ± SD time since MS diagnosis, years	6.9 ± 6.5
Mean ± SD number of relapses in prior year	0.8 ± 0.9
Median (range) baseline EDSS score	2.5 (0, 7)
Baseline EDSS score > 2.0, *n* (%)	121 (56)
Mean ± SD duration of prior treatment, weeks	82 ± 27
Any prior DMT, *n* (%)	
No prior DMT	69 (32)
At least one prior DMT	149 (68)
Interferon beta-1a	68 (31)
Glatiramer acetate	57 (26)
Interferon beta-1b	30 (14)
Natalizumab	8 (4)
Other[a]	65 (30)

ALC: absolute lymphocyte count; DMT: disease-modifying therapy; EDSS: Expanded Disability Status Scale; NK: natural killer; SD: standard deviation.
[a]Other DMTs used by ⩾ 3 patients: fingolimod (14), investigational drug (14), methylprednisolone (11), blinded therapy (7), dimethyl fumarate (5), interferon beta (5), fampridine (4), peginterferon beta-1a (3), teriflunomide (3).

Ad hoc analysis

Serum NfL (sNfL) levels were measured using single molecule array (Simoa™ NF-light® Advantage assay, Quanterix, Billerica, MA). Two patients with extremely high (> 10 standard deviations (SDs)) baseline sNfL values were excluded.

Statistical analyses

All patients who received ⩾ 1 dose of study treatment were included in safety and relapse assessments. All patients who received ⩾ 1 dose of study treatment and had ⩾ 1 post-baseline pharmacodynamic measurement were included in the immune cell phenotyping analysis; EDSS was assessed in those with ⩾ 1 post-baseline EDSS measurement. For the primary endpoint, the actual value, change, and percent change from baseline (CFB) were descriptively summarized. The Wilcoxon signed-rank test was used to assess if changes were different from zero. For secondary analyses, changes in levels of Ig isotypes and ALCs were descriptively summarized. Mixed-effect model repeated measurement assessed CFB in the Ig isotypes, and ALC CFB to Week 96 as the dependent variable. The model included visit, corresponding baseline value, age, and sex as fixed effects, and an unstructured variance-covariance matrix structure. Least squares mean, standard error, and 95% confidence intervals were reported for each visit.

ARR was estimated from a negative binomial regression model, adjusted for baseline covariates. No imputation was used for missing data; the missing values were skipped. The observed data were used for all analyses. EDSS metrics and the proportion of patients with CDP were descriptively summarized. All adverse events (AEs) were included.

Results

Study population

A total of 218 patients were enrolled from six countries (Table 1 and Supplementary Figure 1); 158 (72%) patients completed the study and 60 (28%) withdrew, primarily due to withdrawn consent ($n=22$) and AEs ($n=17$). Five patients withdrew due to lymphopenia: one with severe lymphopenia and four with moderate. The mean (SD) age in PROCLAIM was 42 (11) years and 69% of patients were female (Table 1). Patients were older in PROCLAIM than in the pivotal phase 3 clinical trials DEFINE/CONFIRM (NCT00420212/NCT00451451): 27% were aged ⩾ 50 years in PROCLAIM (Table 1) compared with 13% in the DMF 240 mg group in DEFINE/CONFIRM.[1,2] Other demographic variables were consistent with the phase 3 studies.[1,2]

DMF treatment reduced lymphocyte subset counts together with temporal changes in ALC

ALC decreases were evident as early as 8 weeks after DMF treatment initiation, continuing to decline for 6–12 months, then stabilizing; median ALC CFB was –41% by Week 48 and –39% by Week 96 (baseline, 1.82×10^9/L; Week 48, 1.06×10^9/L; and Week 96, 1.05×10^9/L) (Figure 1(a)). When stratified by younger patients (< 50 years) versus older patients (⩾ 50 years) at baseline, median ALC CFB was –38%

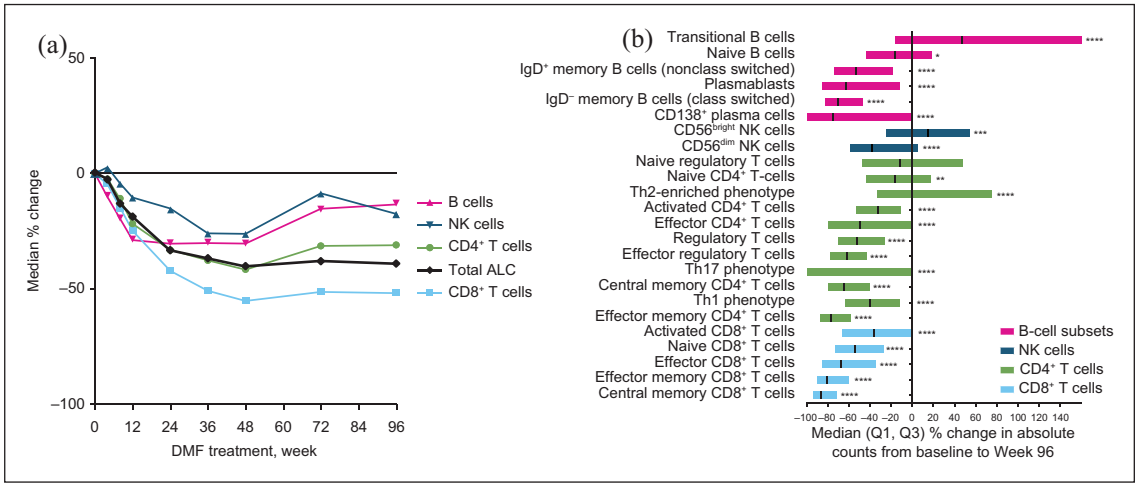

Figure 1. Median percentage change in ALC and major lymphocyte subsets with DMF treatment over time.
For (a), ALC is from the CBC, with unit × 10^9/L. For both (a) and (b), data were collected by flow cytometry. (a) Median ALC is shown for all patients in the analysis population (N=218) and median percent change is shown. (b) Median percent change is shown.
ALC: absolute lymphocyte count; DMF: delayed-release dimethyl fumarate; Ig: immunoglobulin; NK: natural killer; Q: quartile; Th: T helper.
****$p < 0.0001$; ***$p < 0.001$; **$p < 0.01$; *$p < 0.05$.

and –51% by Week 96, respectively. After 2 years of DMF treatment, 110/218 (50%) patients had never developed lymphopenia (all ALCs remained > LLN), 69 (32%) had mild lymphopenia, 32 (15%) had moderate prolonged lymphopenia, and 1 (< 1%) had severe prolonged lymphopenia.

The degree of change in lymphocyte subset counts correlated with changes in ALC; CD4 + and CD8 + T, CD19 + B, and NK cell median CFB to Week 48 were –42%, –55%, –30%, and –26% (all $p < 0.0001$), respectively (Figure 1(b)). The changes were sustained through Week 96 (Figure 1(a)). Using a sequential, cross-sectional analysis of correlations over 2 years, changes in T-cell, B-cell, and NK cell subsets generally correlated with changes in ALC; the strongest correlations were observed in T cells (Pearson's correlation coefficient range, 0.798–0.911; $p < 0.001$), though all correlations reached statistical significance. Among T cells, both CD4 + and CD8 + T cells correlated highly with ALC from baseline through Week 96 (Pearson's correlation coefficient range, 0.559–0.880; $p < 0.001$) (Figure 2(a)). The correlations remained strong when stratified by age < 50 and ⩾ 50 years at baseline (Figures 2(b) and (c)).

DMF drives a general shift toward a naive/anti-inflammatory repertoire

Decreased total CD19 + B-cell counts were observed at Week 4 after treatment initiation (–10% CFB; $p < 0.001$), whereas reductions were observed for ALCs and CD4 + and CD8 + T-cell subsets by Week 8. Total B cells reached nadir at Week 24 (–31% CFB; $p < 0.0001$). After Week 48, a gradual increase in total B cells was observed, though levels did not return to baseline, remaining decreased through Week 96 (–13% CFB; $p < 0.05$). Total CD14 + monocytes appeared stable during DMF treatment, with modest reductions during Year 2 (median CFB at Week 96: –11.6%, $p = 0.005$). Circulating classical monocytes ($CD14^+CD16^-$) remained generally stable during the treatment period (–0.88%, $p = 0.948$ median CFB to Week 96) while the nonclassical ($CD14^{DIM}CD16^+$) monocyte numbers declined beginning at Week 12 (median CFB: –25.5%, $p < 0.0001$) and remained at a lower level for the duration of the treatment period. As measured by flow cytometry, granulocytes slightly increased at Week 96 (median CFB: 13% ($p < 0.001$). When assessed by hematology, neutrophil shifts of potential clinical significance were infrequently reported (< 1.5 cells × 10^9/L: 11/218 patients; ⩽ 1.0 cell × 10^9/L: 0 patients; ⩾ 12.0 cells × 10^9/L: 10/218 patients).

At Week 96, the memory compartment was selectively reduced compared with naive cells for CD4 + and CD8 + T cells and B cells. CFB: $CD4^+CD45RA^-$, –70% ($p < 0.0001$), versus naive $CD4^+CD45RA^+CCR7^+$ T cells, –16% ($p < 0.005$); $CD8^+CD45RA^-$, –85% ($p < 0.0001$), versus naive $CD8^+CD45RA^+CCR7^+$ T cells, –55% ($p < 0.0001$); and $CD27^+IgD^+$ B cells (nonclass switched) –53% ($p < 0.0001$), and $CD27^+IgD^-$ B cells (class switched), –71% ($p < 0.0001$), versus naive $CD27^-IgD^+$ B cells, –16% ($p < 0.05$) (Figure 1(b)).

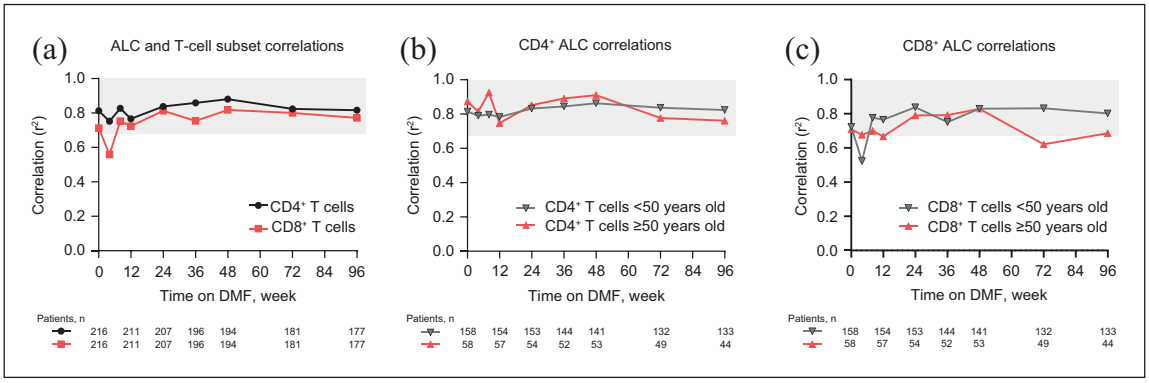

Figure 2. CD4 + and CD8 + T cells are highly correlated with ALC at all time points, regardless of age.
Results from this cross-sectional analysis using time points from baseline to Week 96 are shown. Panel (a) shows the correlation between ALC and either CD4 + or CD8 + T cells; panel (b) shows the correlation between ALC and either CD4 + T cells in patients < 50 years of age, or CD4 + T cells in patients ≥ 50 years of age; panel (c) shows the correlation between ALC and either CD8 + T cells in patients < 50 years of age or CD8 + T cells in patients ≥ 50 years of age. For all panels, $p < 0.001$ at all time points. ALC was determined from the complete blood count with unit $\times 10^9$/L, n = number of patients with both ALC and TBNK subset data at the specified visits. The correlation coefficient was Pearson's rho; p-value = probability of obtaining a sample correlation coefficient more extreme than the value observed, under the null hypothesis that the two variables are not correlated.
ALC: absolute lymphocyte count; DMF: dimethyl fumarate.
A correlation of > 0.7 indicates a strong positive relationship (shaded area).

Only immunoregulatory CD56^{bright} NK and transitional CD24^{hi}CD38^{hi} B-cell numbers increased over 96 weeks: +15% CFB ($p < 0.001$) and +50% CFB ($p < 0.0001$), respectively (Figure 1(b)).

Compartmental relative shifts within lymphocyte subsets

When relative changes were assessed within the total T-cell compartment, naive CD4 + and CD8 + T-cell populations demonstrated a relative increase, whereas CD4 + and CD8 + central memory and effector memory populations decreased (Figure 3). Within T helper (Th) cell subsets, there was a relative decline in pro-inflammatory Th1 and Th17 cells, a relative increase in anti-inflammatory Th2 cells, and stable total regulatory T cells. The relative proportion of regulatory T cells remained stable compared with effector T cells. The relative proportion of the naive and transitional B-cell populations increased, compared with a decrease in the proportion of memory B cells (Figure 3). There was a relative increase in the immunoregulatory CD56^{bright} NK cell population.

Igs remain stable during DMF treatment

At baseline, mean (SD) total IgM, IgA, and IgG levels were 133 (71) mg/dL, 212 (91) mg/dL, and 1051 (233) mg/dL, respectively. Total IgM, IgA, IgG, and IgG1-4 subclass levels remained stable over 2 years of DMF treatment (Figure 4(a)). Ig levels remained stable during the study whether patients experienced relapses ($n = 41$) or not ($n = 170$) while on DMF treatment (Figure 4(b)). Baseline serum IgM, IgA, or IgG levels were below the LLN in 7% (16/218) of patients. Most of these patients had been previously treated with ≥ 1 DMTs, although three had no previous DMT exposure. Seven of the 16 patients with low baseline Ig levels reached a normal value during DMF treatment. In general, when patients were stratified by ALC category, baseline Ig concentrations and median CFB to Weeks 48 and 96 were similar across categories. Data were available for only one patient with severe prolonged lymphopenia and therefore this category was not included in the analysis.

Safety outcomes

AEs and serious AEs were reported in 185/218 (85%) and 26/218 (12%) patients, respectively (Table 2), consistent with the known safety profile of DMF;[1,2] flushing was the most common (46%). Treatment-emergent infections were reported in 89/218 (41%) patients. When stratified by ALC subgroup, a similar percentage of infections were reported across subgroups: 0.5 ≤ ALC < 0.8 $\times 10^9$/L (14/32 (44%) patients); 0.8 $\times 10^9$/L ≤ ALC < LLN (29/69 (42%) and ALC ≥ LLN (43/110 (39%)). No infections were reported in the patient with severe, prolonged lymphopenia.

Serious treatment-emergent infections (nasopharyngitis and cellulitis) were reported in two patients, neither considered related to study treatment. Nasopharyngitis

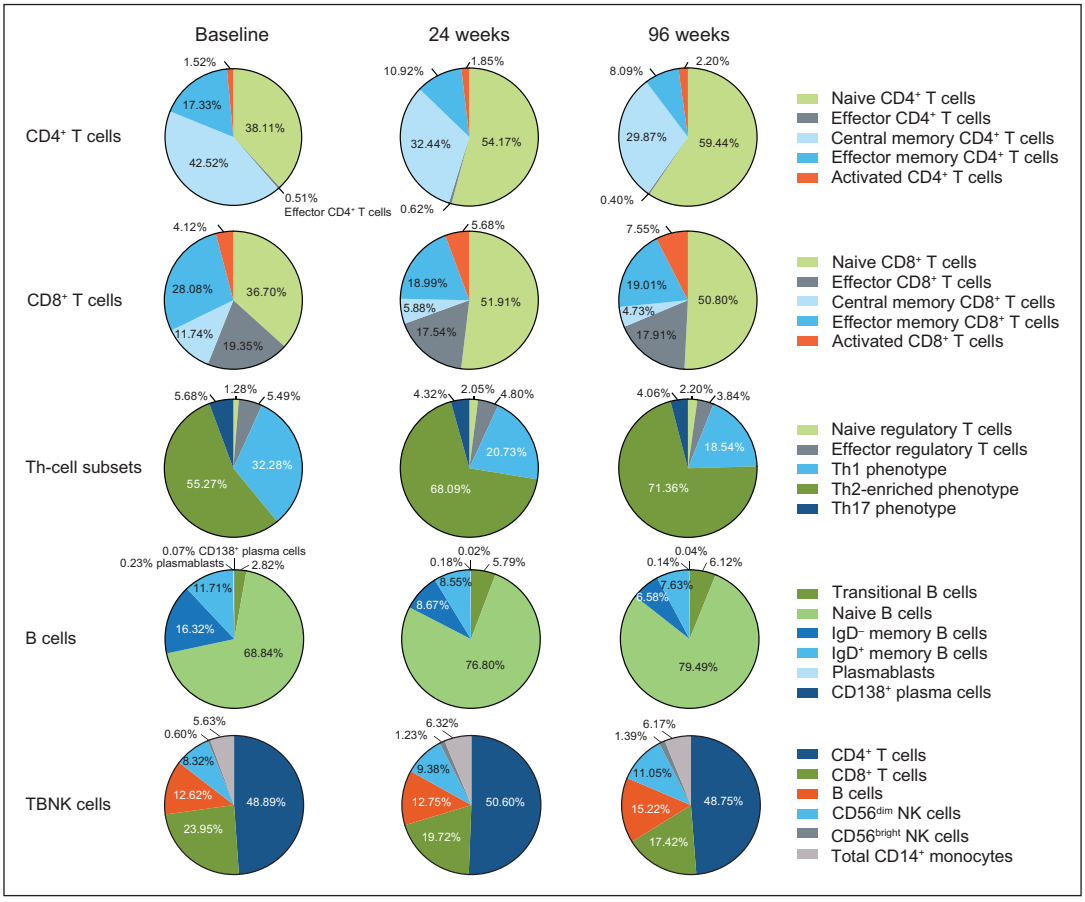

Figure 3. Median percentage change in T-cell subsets, B-cell subsets, NK cells, and monocytes during delayed-release dimethyl fumarate treatment.
Ig: immunoglobulin; NK: natural killer; Th: T helper.

occurred in a patient with mild lymphopenia (ALC 0.8 × 10^9/L to < LLN) and cellulitis occurred in a patient without lymphopenia (ALC ≥ LLN); IgG concentrations were > LLN (700 mg/dL) for both patients. A malignancy, stage 1 breast cancer, was reported in one patient, considered unrelated to study treatment. No deaths or treatment-emergent opportunistic infections were reported.

ALC is not a marker of treatment response

The overall unadjusted ARR was 0.153. When patients were stratified into quartiles by percentage CFB in ALC over 96 weeks, ARR for each quartile was generally consistent, relapse rates were low across all groups, and there were no patterns associating ALC change with relapse rate (Figure 5(a)). Similarly, changes in EDSS score and the proportion of relapse-free patients were not associated with ALC quartiles (Figure 5(b) and (c)). The low rate of disability progression observed in this study (13 patients with CDP events) prevented assessment of CDP by ALC quartile.

Overall mean (SD) percentage change in sNfL from baseline to Week 96 was –19% (34). When stratified by age < 50 and ≥ 50 years at baseline, sNfL mean percentage CFB to Week 96 were –22% (34) and –8% (33), respectively. Mean percentage change in sNfL did not vary significantly based on on-treatment ALCs (always ≥ LLN, –17%; moderate prolonged lymphopenia, –21%; all other lymphopenia, –17%). Similarly, sNfL mean percent CFB to Week 96 were similar regardless of CD4 + and CD8 + levels: CD4 + < 200 cells/mm³ ($n = 24$), –15%, versus CD4 + ≥ 200 cells/mm³ ($n = 141$), –19%, and CD8 + < 100 cells/mm³ ($n = 59$), –20%, versus CD8 + ≥ 200 cells/mm³ ($n = 106$), –18%.

Discussion

The PROCLAIM study results demonstrate that DMF treatment of up to 2 years produced temporal changes in ALC and lymphocyte subsets, consistent with the known effect of DMF treatment. A median 39% ALC reduction from baseline to Week 96 and stabilization ≥ LLN for

Table 2. Adverse events.

Patients, *n* (%)	PROCLAIM *N* = 218
Treatment-emergent adverse events	185 (85)
Treatment-emergent infections	89 (41)
Nasopharyngitis	33 (15)
Upper respiratory tract infection	28 (13)
Sinusitis	10 (5)
Bronchitis	8 (4)
Urinary tract infection	8 (4)
Influenza	7 (3)
Gastroenteritis viral	6 (3)
Pharyngitis	6 (3)
Most common adverse event (⩾ 10%)[a]	
Flushing	100 (46)
MS relapse	48 (22)
Nasopharyngitis	33 (15)
Diarrhea	28 (13)
Upper respiratory tract infection	28 (13)
Fatigue	27 (12)
Abdominal pain	22 (10)
Upper abdominal pain	22 (10)
Nausea	22 (10)
Adverse events leading to study drug discontinuation	28 (13)
Deaths	0
Serious adverse events	26 (12)
Most common serious adverse events (⩾ 1%), *n* (%)	
MS relapse	16 (7)
Adverse events of special interest	
Serious infections	2 (< 1)
Malignancies	1 (< 1)
Opportunistic infections	0
Progressive multifocal leukoencephalopathy	0

[a]Treatment-emergent events by preferred term.

most patients were observed; this decline is slightly higher than that observed in previous phase 3 and extension studies[1,2,5] but is similar to an observational study of patients who were also older at baseline.[20] The number of circulating lymphocytes within most major subsets, including $CD4^+$ and $CD8^+$ T, $CD19^+$ B, and $CD56^+$ NK cells, declined following DMF initiation, consistent with overall ALC. Overall, monocytes were generally stable, which is expected given that classical monocytes, which comprise the majority of the compartment, were not changed with treatment. Interestingly, the nonclassical monocytes, which have been implicated in various inflammatory autoimmune disorders,[21,22] declined and remained low. While likely not a primary driver of DMF efficacy, this change may be another reflection of the mechanism by which DMF shifts circulating cells from an inflammatory to an anti-inflammatory repertoire. Granulocytes, specifically neutrophils, were generally not decreased. These findings are consistent with clinical observations of DMF-treated patients in that the rate of serious and opportunistic infection is generally low. The relative proportion of regulatory T cells remained stable compared with effector T cells, suggesting immune tolerance mechanisms were maintained. Increased immunoregulatory $CD56^{bright}$ NK cells with DMF treatment confirm previous observations.[23,24] Overall, DMF treatment induced a general shift toward a circulating naive/anti-inflammatory cell profile and away from memory/pro-inflammatory phenotypes, in both the T-cell and B-cell compartments. Similar reductions in memory B cells have been previously observed.[9,14] including concomitant reductions in the pro-inflammatory cytokines granulocyte macrophage colony-stimulating factor (GM-CSF), tumor necrosis factor (TNF)-α, and IL-6.[15,25] This shift was established within the first 3–6 months of treatment and maintained over 2 years.

Total IgA, IgG, IgM, and subclass IgG1–4 levels remained stable over 96 weeks of DMF treatment, regardless of ALCs, and were similar to Ig levels in healthy adult and MS populations.[26,27] This is consistent with prior studies demonstrating that DMF-treated patients mount an effective T cell–independent and T cell–dependent immune response to recall and neoantigens comparable with that of IFN-treated patients.[13] Previous IFN studies have shown no decrease in serological response to vaccination when vaccines were coadministered during treatment with IFN;[28,29] therefore, the similar rise in immunoglobulin levels in patients who were vaccinated while treated with IFN or DMF suggests similar seroprotection potential for the two agents. In PROCLAIM, which included patients slightly older at baseline than pivotal trials[1,2] and those previously treated with DMTs, there was no change in serum IgG levels, in contrast to the decrease noted during treatment with other DMTs.[27,30]

These data do not support changes in ALC as a biomarker of treatment efficacy, consistent with previous reports.[5] Although T-cell numbers, specifically $CD8^+$ T cells, are impacted by DMF treatment,[6–12] the magnitude of the reduction does not correlate with relapse rate or changes in EDSS score. This prospective study and prior observational studies agree that relapse status, unadjusted ARRs, and change in EDSS scores were similar across ALC subgroups. Ig isotypes were also not differentially affected by relapse

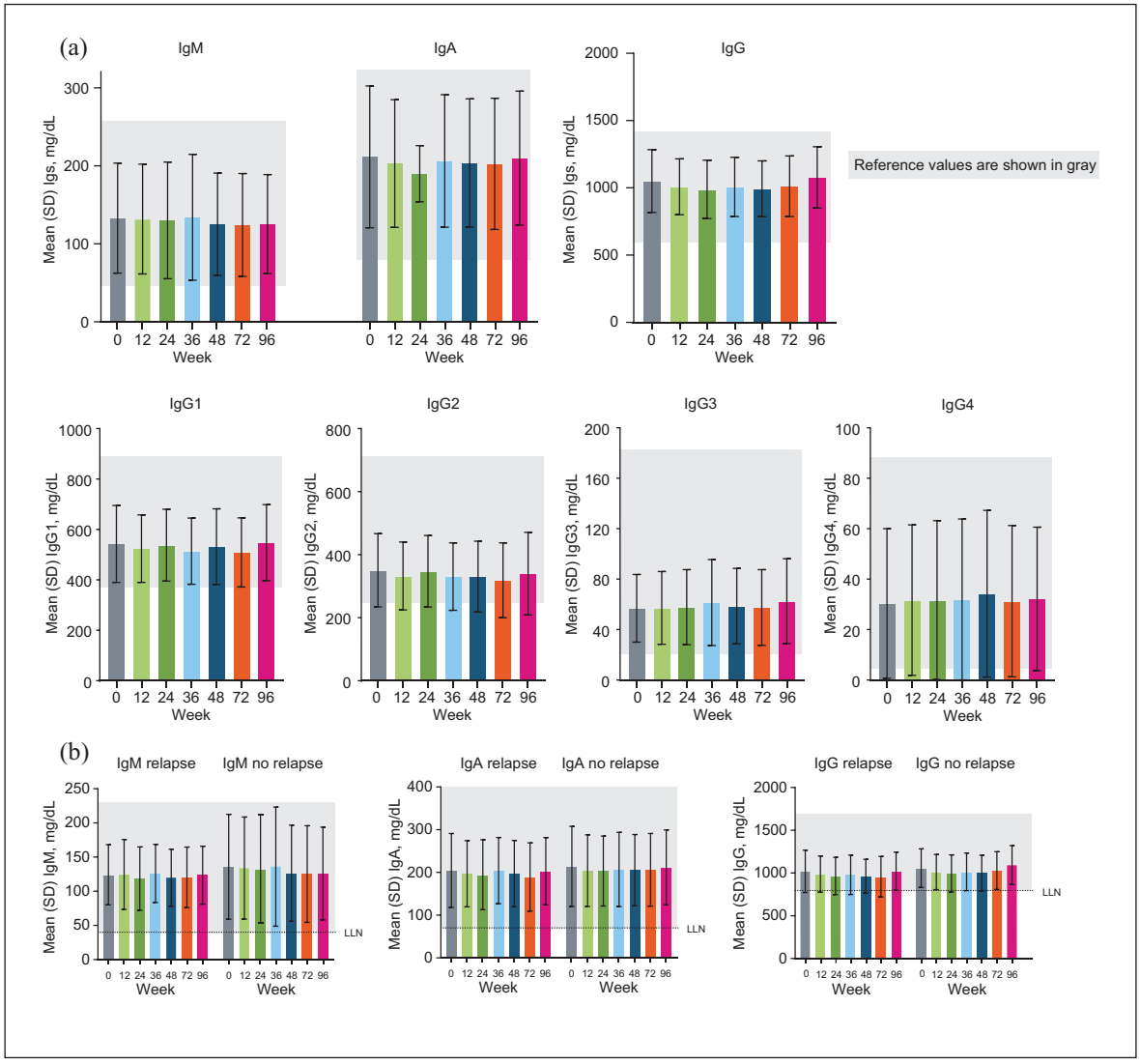

Figure 4. Mean Ig levels (a) over time and (b) in patients with or without relapse during delayed-release dimethyl fumarate treatment.
IgM, primary response; IgA, mucosal response; IgG, main Ig during secondary immune response. IgG subclasses: IgG1, induced by antibody responses to soluble protein antigens and membrane proteins; IgG2, IgG response to bacterial capsular polysaccharide antigens; IgG3, particularly effective in the induction of effector function, typically first response during viral infections; IgG4, induced by allergens, often formed with repeated or long-term exposure.[31,32] The lower limits of normal for serum Ig concentrations were IgM, < 40 mg/dL; IgA, < 70 mg/dL; and IgG, < 700 mg/dL.[26,27] Reference intervals for healthy adults are as follows: IgM, 40–230 mg/dL; IgA, 70–400 mg/dL; IgG, 700–1600 mg/dL;[26] IgG1, 380–930 mg/dL; IgG2, 240–700 mg/dL; IgG3, 20–180 mg/dL; and IgG4, 4–86 mg/dL.[33]
Ig: immunoglobulin; LLN: lower limit of normal; SD: standard deviation.

status or disability progression. The exploratory marker sNfL was decreased by DMF treatment, similar to other studies,[34] but the magnitude of reduction was not associated with ALC. Only younger age was associated with higher baseline ALC.

Despite ALC changes not being indicative of the magnitude of treatment response, it is purported that changes in the peripheral lymphocyte compartment toward an anti-inflammatory phenotype may contribute to reducing disease activity in DMF-treated patients with RMS.[4,11,14,15,35] In addition, a decrease in interleukin-17–producing $CD8^+$ T cells in DMF-treated patients,[36] together with the reduced IFN-gamma production by $CD4^+$ T cells,[37,38] dendritic cells, and monocytes,[37,39,40] and greater reduction of $CD8^+$ T cells,[6–11] suggests DMF impacts MS through multiple mechanisms.

The PROCLAIM dataset provides additional context for the safety implications of T-cell subset changes in

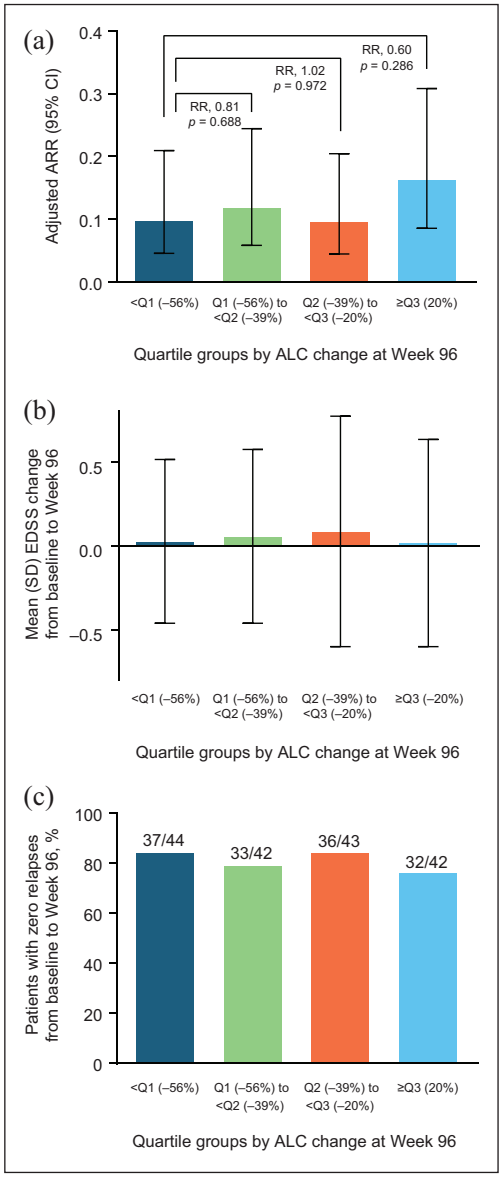

Figure 5. (a) Adjusted ARR, (b) change in EDSS score, and (c) patients with zero relapses by ALC change, from baseline to Week 96. (a) Both protocol-defined and non-protocol–defined relapses are included in the analysis. Based on negative binomial regression, adjusted for baseline EDSS score (⩽2.0 vs >2.0) and baseline age (<40 vs ⩾40 years). If the negative binomial regression model did not converge, a Poisson regression model with the same covariates was used. (b) Includes patients in the clinical assessment population with a baseline EDSS score (n=183). (c) Percentage of patients with 0 relapses from baseline to Week 96.
ALC: absolute lymphocyte count; ARR: annualized relapse rate; CI: confidence interval; EDSS: Expanded Disability Status Scale; Q: quartile; RR: rate ratio; SD: standard deviation.

DMF-treated patients. No deaths or treatment-emergent opportunistic infections were reported over the 2-year study period. Rates of AEs were similar across all ALCs, consistent with the known safety profile of DMF; rates of serious AEs were low. Taken together with the strong correlation between ALC and T-cell subsets, these data continue to support that additional monitoring of T-cell subsets is not required for safety surveillance in routine clinical practice for DMF-treated patients.[11] However, the correlation coefficient is not 1.0; therefore, a small proportion of patients will have lower than expected T-cell subset counts despite normal ALCs.

To our knowledge, PROCLAIM is the first large prospective longitudinal study evaluating immunophenotypic changes among patients treated with DMF. Exclusion criteria for this study were limited, allowing enrollment of patients of a wider age spectrum with prior exposure to DMT. The study population therefore more closely reflects clinical practice, and the data reported here correspond well with the observational data previously reported. The discontinuation rate (28%) in PROCLAIM was higher than the assumption of 10% but in line with the pivotal clinical trials (31% and 30%).

This study was not powered to assess changes that occur in only a small percentage of patients, for example., 2%–3% of patients with severe prolonged lymphopenia; this population has been assessed in other studies.[11,41] Moreover, this open-label prospective study did not include a comparator group, limiting the interpretation of some results. Disease activity at baseline was not controlled for in this study and only 13 patients experienced disability progression, limiting conclusions for changes in ALC in relation to disease activity. The clinical significance of this study is also limited by the lack of MRI data.

Conclusion

DMF modulates a shift in circulating lymphocytes away from memory cells and toward a naive repertoire that does not impair protective humoral immunity. This may contribute to the therapeutic benefits of DMF in MS. Overall, the relative proportion of naive and anti-inflammatory–type (Th2-enriched) cells increase, whereas central and effector memory and pro-inflammatory–type (Th17) cells decrease over 2 years of DMF treatment. These shifts do not affect Ig isotype concentrations. The magnitude of DMF-mediated changes in ALC were not associated with greater efficacy or increases in serious infections, as similarly noted in patients treated with DMF over many years.[4,35] Additional T-cell subset monitoring is not required for safety surveillance in routine clinical practice based on the longitudinal correlation between T cells and ALC, regardless of T-cell subset type or

age. Overall, the lymphocyte subset changes and Ig stability observed, together with the safety profile, indicate that DMF is generally well tolerated as evidenced by 72% of patients remaining on the study at 2 years. Protective humoral immune function is maintained over 96 weeks of treatment.

Acknowledgements

Biogen (Cambridge, MA) provided funding for medical writing support in the development of this paper; Miranda Dixon from Excel Medical Affairs (Horsham, UK) copyedited and styled the manuscript per journal requirements. Biogen reviewed and provided feedback on the paper. The authors had full editorial control of the paper and provided their final approval of all content.

Data Sharing Statement

The datasets used and/or analyzed during the study are available from the corresponding author upon reasonable request. Requests for data supporting this manuscript should be submitted to the Biogen Clinical Data Request Portal (www.biogenclinicaldatarequest.com).

Declaration of Conflicting Interests

The author(s) declared the following potential conflicts of interest with respect to the research, authorship, and/or publication of this article: E.E.L. has received consulting fees from Alexion, Biogen, Celgene, EMD Serono, Genentech, Genzyme, and Teva; research support from the Nancy Davis Center Without Walls and National Institutes of Health K23NS107624. Y.M-D. has received consulting/speaker fees from Bayer, Biogen, Celgene, Chugai, EMD Serono, Genentech, Novartis, Sanofi-Genzyme, and Teva; research support from Biogen, Chugai, National Institute of Neurological Disorders and Stroke R01-NS080821, National Institute of Allergy and Infectious Diseases Autoimmune Center of Excellence UM1-AI110557, Novartis, and Sanofi-Genzyme. M.C. has received consulting fees from Biogen, EMD Serono, Genentech, Novartis, and Sanofi-Genzyme; speaker fees from Acorda, Biogen, EMD Serono, Novartis, and Sanofi-Genzyme; grant support from Adamas, Biogen, Genentech, Novartis, and Sanofi-Genzyme. E.B., D.B., C.C., S.K., J.P.M., C.M., B.P., and D.X. are employees of and hold stock/stock options in Biogen. D.R. has received consulting fees from Alexion, Biogen, Celgene, EMD Serono, Genentech, Novartis, Sanofi-Genzyme, and Teva; speaker fees from Acorda, Alexion, Biogen, Celgene, EMD Serono, Genentech, Mallinckrodt, Novartis, Sanofi-Genzyme, and Teva; grant support from Actelion, Biogen, EMD Serono, Genentech, Mallinckrodt, MedDay, Novartis, Patient-Centered Outcomes Research Institute, Sanofi-Genzyme, and TG. T.Z. has nothing to disclose.

Funding

The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: Biogen provided funding for the study.

Research Ethics and Patient Consent

The PROCLAIM study was conducted in accordance with The International Conference on Harmonization Guidelines on Good Clinical Practice, the ethical principles outlined in the Declaration of Helsinki, and all applicable local laws and regulations. Written informed consent was obtained from each patient prior to eligibility evaluations.

Trial Registration

2015**Clinical trial registration:** EudraCT-001973-42

Supplemental Material

Supplemental material for this article is available online.

References

1. Fox RJ, Miller DH, Phillips JT, et al. Placebo-controlled phase 3 study of oral BG-12 or glatiramer in multiple sclerosis. *N Engl J Med* 2012; 367: 1087–1097.
2. Gold R, Kappos L, Arnold DL, et al. Placebo-controlled phase 3 study of oral BG-12 for relapsing multiple sclerosis. *N Engl J Med* 2012; 367: 1098–1107.
3. Cohan SL, Moses H, Calkwood J, et al. Clinical outcomes in patients with relapsing-remitting multiple sclerosis who switch from natalizumab to delayed-release dimethyl fumarate: A multicenter retrospective observational study (STRATEGY). *Mult Scler Relat Disord* 2018; 22: 27–34.
4. Fox RJ, Chan A, Gold R, et al. Absolute lymphocyte count in patients with relapsing-remitting multiple sclerosis treated with delayed-release dimethyl fumarate: Considerations for patient management. *Neurology* 2017; 88: P5391.
5. Fox RJ, Chan A, Gold R, et al. Characterizing absolute lymphocyte count profiles in dimethyl fumarate-treated patients with MS: Patient management considerations. *Neurol Clin Pract* 2016; 6(3): 220–229.
6. Longbrake EE, Ramsbottom MJ, Cantoni C, et al. Dimethyl fumarate selectively reduces memory T

cells in multiple sclerosis patients. *Mult Scler* 2016; 22: 1061–1070.

7. Spencer CM, Crabtree-Hartman EC, Lehmann-Horn K, et al. Reduction of CD8(+) T lymphocytes in multiple sclerosis patients treated with dimethyl fumarate. *Neurol Neuroimmunol Neuroinflamm* 2015; 2(3): e76.
8. Mills EA, Ogrodnik MA, Plave A, et al. Emerging understanding of the mechanism of action for dimethyl fumarate in the treatment of multiple sclerosis. *Front Neurol* 2018; 9: 5.
9. Longbrake EE, Cantoni C, Chahin S, et al. Dimethyl fumarate induces changes in B- and T-lymphocyte function independent of the effects on absolute lymphocyte count. *Mult Scler* 2018; 24(6): 728–738.
10. Lundy SK, Wu Q, Wang Q, et al. Dimethyl fumarate treatment of relapsing-remitting multiple sclerosis influences B-cell subsets. *Neurol Neuroimmunol Neuroinflamm* 2016; 3(2): e211.
11. Mehta D, Miller C, Arnold DL, et al. Effect of dimethyl fumarate on lymphocytes in RRMS: Implications for clinical practice. *Neurology* 2019; 92: e1724–e1738.
12. Morales FS, Koralnik IJ, Gautam S, et al. Risk factors for lymphopenia in patients with relapsing-remitting multiple sclerosis treated with dimethyl fumarate. *J Neurol* 2020; 267: 125–131.
13. von Hehn C, Howard J, Liu S, et al. Immune response to vaccines is maintained in patients treated with dimethyl fumarate. *Neurol Neuroimmunol Neuroinflamm* 2018; 5(1): e409.
14. Traub JW, Pellkofer HL, Grondey K, et al. Natalizumab promotes activation and pro-inflammatory differentiation of peripheral B cells in multiple sclerosis patients. *J Neuroinflammation* 2019; 16: 228.
15. Li R, Rezk A, Ghadiri M, et al. Dimethyl fumarate treatment mediates an anti-inflammatory shift in B cell subsets of patients with multiple sclerosis. *J Immunol* 2017; 198: 691–698.
16. Wu Q, Wang Q, Mao G, et al. Dimethyl fumarate selectively reduces memory T cells and shifts the balance between Th1/Th17 and Th2 in multiple sclerosis patients. *J Immunol* 2017; 198: 3069–3080.
17. Polman CH, Reingold SC, Banwell B, et al. Diagnostic criteria for multiple sclerosis: 2010 revisions to the McDonald criteria. *Ann Neurol* 2011; 69(2): 292–302.
18. Kennedy RB, Ovsyannikova IG, Haralambieva IH, et al. Immunosenescence-related transcriptomic and immunologic changes in older individuals following influenza vaccination. *Front Immunol* 2016; 7: 450.
19. Grebenciucova E, Reder AT and Bernard JT. Immunologic mechanisms of fingolimod and the role of immunosenescence in the risk of cryptococcal infection: A case report and review of literature. *Mult Scler Relat Disord* 2016; 9: 158–162.
20. Buckle G, Bandari D, Greenstein J, et al. Characterization of baseline lymphocytes and their influence on subsequent ALC and lymphocyte subset decline in patients with relapsing multiple sclerosis treated with delayed-release dimethyl fumarate in a retrospective, multicenter, observational study (REALIZE). *Neurology* 2017; 88: P5376.
21. Gjelstrup MC, Stilund M, Petersen T, et al. Subsets of activated monocytes and markers of inflammation in incipient and progressed multiple sclerosis. *Immunol Cell Biol* 2018; 96(2): 160–174.
22. Narasimhan PB, Marcovecchio P, Hamers AAJ, et al. Nonclassical monocytes in health and disease. *Annu Rev Immunol* 2019; 37: 439–456.
23. Smith MD, Calabresi PA and Bhargava P. Dimethyl fumarate treatment alters NK cell function in multiple sclerosis. *Eur J Immunol* 2018; 48(2): 380–383.
24. Medina S, Villarrubia N, Sainz de la Maza S, et al. Optimal response to dimethyl fumarate associates in MS with a shift from an inflammatory to a tolerogenic blood cell profile. *Mult Scler* 2018; 24(10): 1317–1327.
25. Smith MD, Martin KA, Calabresi PA, et al. Dimethyl fumarate alters B-cell memory and cytokine production in MS patients. *Ann Clin Transl Neurol* 2017; 4(5): 351–355.
26. Gonzalez-Quintela A, Alende R, Gude F, et al. Serum levels of immunoglobulins (IgG, IgA, IgM) in a general adult population and their relationship with alcohol consumption, smoking and common metabolic abnormalities. *Clin Exp Immunol* 2008; 151(1): 42–50.
27. Zoehner G, Miclea A, Salmen A, et al. Reduced serum immunoglobulin G concentrations in multiple sclerosis: Prevalence and association with disease-modifying therapy and disease course. *Ther Adv Neurol Disord* 2019; 12: 1756286419878340.
28. Olberg HK, Cox RJ, Nostbakken JK, et al. Immunotherapies influence the influenza vaccination response in multiple sclerosis patients: An explorative study. *Mult Scler* 2014; 20(8): 1074–1080.
29. Schwid SR, Decker MD, Lopez-Bresnahan M, et al. Immune response to influenza vaccine is maintained in patients with multiple sclerosis receiving interferon beta-1a. *Neurology* 2005; 65: 1964–1966.
30. Outteryck O, Ongagna JC, Brochet B, et al. A prospective observational post-marketing study

of natalizumab-treated multiple sclerosis patients: Clinical, radiological and biological features and adverse events. *Eur J Neurol* 2014; 21(1): 40–48.

31. Schroeder HW Jr and Cavacini L. Structure and function of immunoglobulins. *J Allergy Clin Immunol* 2010; 125: S41–S52.
32. Vidarsson G, Dekkers G and Rispens T. IgG subclasses and allotypes: From structure to effector functions. *Front Immunol* 2014; 5: 520.
33. Oxford University Hospitals NHS and Foundation Trust. IgG subclasses, https://www.ouh.nhs.uk/immunology/diagnostic-tests/tests-catalogue/igg-subclasses.aspx
34. Sejbaek T, Nielsen HH, Penner N, et al. Dimethyl fumarate decreases neurofilament light chain in CSF and blood of treatment naive relapsing MS patients. *J Neurol Neurosurg Psychiatry* 2019; 90: 1324–1330.
35. Fox RJ, Chan A, Gold R, et al. Absolute lymphocyte count and lymphocyte subset profiles during long-term treatment with delayed-release dimethyl fumarate in patients with relapsing-remitting multiple sclerosis. *Mult Scler* 2016; 22: 349.
36. Luckel C, Picard F, Raifer H, et al. IL-17(+) CD8(+) T cell suppression by dimethyl fumarate associates with clinical response in multiple sclerosis. *Nat Commun* 2019; 10: 5722.
37. Schulze-Topphoff U, Varrin-Doyer M, Pekarek K, et al. Dimethyl fumarate treatment induces adaptive and innate immune modulation independent of Nrf2. *Proc Natl Acad Sci U S A* 2016; 113: 4777–4782.
38. Kornberg MD, Bhargava P, Kim PM, et al. Dimethyl fumarate targets GAPDH and aerobic glycolysis to modulate immunity. *Science* 2018; 360: 449–453.
39. Ghoreschi K, Bruck J, Kellerer C, et al. Fumarates improve psoriasis and multiple sclerosis by inducing type II dendritic cells. *J Exp Med* 2011; 208: 2291–2303.
40. Carlstrom KE, Ewing E, Granqvist M, et al. Therapeutic efficacy of dimethyl fumarate in relapsing-remitting multiple sclerosis associates with ROS pathway in monocytes. *Nat Commun* 2019; 10: 3081.
41. Fox RJ, Chan A, Gold R, et al. Lymphocyte decline and reconstitution after discontinuation in patients with severe, prolonged lymphopenia treated with delayed-release dimethyl fumarate. *Mult Scler* 2017; 23: P743.