Evaluation of Potential Drug–Drug Interaction Between Delayed-Release Dimethyl Fumarate and a Commonly Used Oral Contraceptive (Norgestimate/Ethinyl Estradiol) in Healthy Women

Bing Zhu¹, Ivan Nestorov¹, Guolin Zhao¹, Venkata Meka¹, Mark Leahy², Jeanelle Kam³, and Sarah I. Sheikh¹

Abstract
Delayed-release dimethyl fumarate (DMF) is an oral therapy for relapsing multiple sclerosis with anti-inflammatory and neuroprotective properties. This 2-period crossover study was conducted to evaluate the potential for drug–drug interaction between DMF (240 mg twice daily) and a combined oral contraceptive (OC; norgestimate 250 μg, ethinyl estradiol 35 μg). Forty-six healthy women were enrolled; 32 completed the study. After the lead-in period (OC alone), 41 eligible participants were randomized 1:1 to sequence 1 (OC and DMF coadministration in period 1; OC alone in period 2) or sequence 2 (regimens reversed). Mean concentration profiles of plasma norelgestromin (primary metabolite of norgestimate) and ethinyl estradiol were superimposable following OC alone and OC coadministered with DMF, with 90% confidence intervals of geometric mean ratios for area under the plasma concentration–time curve over the dosing interval and peak plasma concentration contained within the 0.8–1.25 range. Low serum progesterone levels during combined treatment confirmed suppression of ovulation. The pharmacokinetics of DMF (measured via its primary active metabolite, monomethyl fumarate) were consistent with historical data when DMF was administered alone. No new safety concerns were identified. These results suggest that norgestimate/ethinyl estradiol–based OCs may be used with DMF without dose modification.

Keywords
delayed-release dimethyl fumarate, drug–drug interaction, multiple sclerosis, oral contraceptives, pharmacokinetic

Multiple sclerosis (MS) is a chronic, disabling, demyelinating, and neurodegenerative disease affecting the central nervous system.¹ Most patients develop the disease between 20 and 40 years of age, with a preponderance of women being affected.²

Delayed-release dimethyl fumarate (DMF) 240 mg twice daily is an oral therapy approved in the United States, the European Union, and other regions for patients with relapsing forms of MS or relapsing-remitting MS. In phase 3 clinical trials, DMF significantly reduced clinical and magnetic resonance imaging disease activity and demonstrated a favorable benefit-risk profile.³,⁴ Because women of childbearing potential are advised by regulatory agencies to use effective contraception during DMF treatment, it is important to evaluate whether hormonal oral contraception is an appropriate option for patients taking DMF.

1 Biogen, Cambridge, MA, USA
2 Covance Laboratories Inc., Madison, WI, USA
3 Covance Clinical Research Unit, Dallas, TX, USA

This is an open access article under the terms of the Creative Commons Attribution-NonCommercial License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited and is not used for commercial purposes.

Submitted for publication 30 August 2016; accepted 7 June 2017.

Corresponding Author:
Bing Zhu, MD, PhD, Biogen, 225 Binney Street, Cambridge, MA 02142 (e-mail: bing.zhu@biogen.com)

Dr. Meka performed the majority of the work while a full-time employee of Biogen, Cambridge, Massachusetts.

Dr. Meka’s current affiliation is with AstraZeneca, Waltham, Massachusetts.
contraception (OC) are reliable when combined with DMF treatment.

DMF is formulated as enteric-coated microtablets in gelatin capsules. Orally administered DMF is rapidly and completely metabolized by esterases to its primary active metabolite, monomethyl fumarate (MMF), in the small intestine. As a result, DMF is not quantifiable in plasma, and all pharmacokinetic (PK) analyses are performed with plasma MMF concentrations. The complex absorption process results in complicated plasma MMF concentration–time profiles with irregular shape and multiple peaks. The terminal half-life (t1/2) of MMF is approximately 1 hour, but the t1/2 is not applied to determine frequency of dosage because of a complicated exposure profile and indirect exposure–response cascade.

The most commonly used hormonal OCs contain a combination of a progestin component (eg, norgestimate) and an estrogen component (eg, ethinyl estradiol), which have been used clinically to assess the

Methods

Study Design

This was a phase 1 open-label, randomized, 2-period crossover study with an additional lead-in period. A schematic of the study design is presented in Figure 1. The duration of study was up to 122 days in total.

All participants received once-daily doses of OC on days 1–28 of the lead-in period to synchronize menstrual cycles. On day 28, participants who were confirmed as eligible were randomized 1:1 to 1 of 2 treatment sequences. In sequence 1, participants received OC once daily and DMF 240 mg twice daily in period 1, then OC alone in period 2. In sequence 2, these regimens were reversed. Each period was 28 days in duration. OCs were administered on days 1–28 of each period. DMF was administered only for the first 21 days during the coadministration period, to be consistent with the active OC treatment period.

Clinical data were collected at scheduled visits to the study site (days -1 and 14, 20, 21, 22, and 28 of the lead-in period; days 14, 20, 21, 22, and 28 of periods 1 and 2; and the follow-up visit). Blood samples for the PK analysis of norelgestromin and ethinyl estradiol were collected predose on day 21 of each period and at multiple times (0.5, 1, 1.5, 2, 3, 4, 5, 6, 8, 10, 12, 16, and 24 hours) postdose. Blood samples for the evaluation of progesterone levels were collected on days 14, 21, 22, and 28 of each period at approximately the same time of day. Blood samples for the PK analysis of DMF (as measured by MMF concentrations) were collected during the period in which DMF was administered, predose, and at multiple times (0.5, 1, 1.5, 2, 3, 4, 5, 6, 7, 8, 10, and 12 hours) post–first dose on day 21. Bioanalysis materials and methods are summarized in the Supplement.

This study was conducted at a single site (Covance Clinical Research Unit, Dallas, Texas) under a single principal investigator (J.K.); institutional review board approval was obtained for the study protocol and consent forms (Schulman Associates IRB, Inc., Cincinnati, Ohio). Written informed consent was obtained from each participant before any protocol-specific procedures. The study was conducted in accordance with International Conference on Harmonisation Guidelines for Good Clinical Practice and the Declaration of Helsinki.

Study Population

Eligible study participants were healthy, nonpregnant, nonlactating women of childbearing potential 18–45 years of age with a body mass index (BMI) of 19.0–30.0 kg/m² who were not smokers. Key exclusion criteria included: (1) any contraindications to
combined OC tablets, including history of thrombosis or any thromboembolic disease, recurrent jaundice, acute or chronic liver disease, hormonally induced migraines, undiagnosed vaginal bleeding, significant hyperlipidemia, or mammary, endometrial, or hepatic carcinoma (known or suspected); (2) administration of injectable contraceptives within 12 months or topical controlled-delivery contraceptives (patch) within 3 months before day -1 of the lead-in period; (3) treatment with drugs or substrates that are known to be an inducer or inhibitor of CYP3A4 within 30 days before day -1 of the lead-in period; and (4) serum concentration of progesterone \( \geq 3 \text{ ng/mL} \) on days 21 or 22 of the lead-in period, which may suggest low adherence to OC administration. Treatment with prescription hormonal OCs was allowed up to and including day -1 of the lead-in period. Participant eligibility was determined during the screening period within 28 days of day -1 of the lead-in period and confirmed before randomization on day 28 of the lead-in period.

**Study Medication**
The OC used in this study was Ortho-Cyclen (Janssen Pharmaceuticals, Inc., Beerse, Belgium). A 28-day supply of OC included 21 active tablets (norgestimate \( 250 \mu \text{g} \), ethinyl estradiol \( 35 \mu \text{g} \); administered on days 1–21) and 7 inactive tablets (administered on days 22–28). Eligible participants started taking daily OCs from day 1 of the lead-in period.

DMF (Biogen, Cambridge, Massachusetts) was formulated as enteric-coated microtablets in gelatin capsules for oral administration and was administered at the standard marketed dose of 240 mg twice daily. During the period in which participants were randomized to receive DMF, the morning dose of DMF was coadministered with OC and the evening dose was administered approximately 12 hours after the morning dose, at approximately the same time each day. On day 21 of each period, the morning dose was preceded by a fast from food of at least 10 hours and followed by a fast from food for at least 4 hours postdose. Consumption of water was prohibited for 2 hours before and 1 hour after the day 21 morning dose, with the exception of water for dose administration. All doses of study medication were self-administered, and an electronic diary was used to remind participants of dosing.

**Study Assessments**
The primary end points were the PK parameters of norelgestromin (the primary metabolite of norgestimate) and ethinyl estradiol, including area under the plasma concentration–time curve over the dosing interval \( (\text{AUC}_{0-\tau}) \) and peak plasma concentration \( (C_{\text{max}}) \). Secondary end points included serum levels of progesterone (a PD measure for ovulation suppression), number and proportion of participants with adverse events (AEs) and serious AEs (SAEs), clinical laboratory parameters, and secondary PK parameters of norelgestromin and ethinyl estradiol, including time to \( C_{\text{max}}(T_{\text{max}}) \) and \( t_{1/2} \). The exploratory end points were the PK parameters of MMF, including AUC from time 0 to infinity \( (\text{AUC}_{0-\infty}) \), \( C_{\text{max}} \), AUC from time 0 to 12 hours postdose \( (\text{AUC}_{0-12}) \), \( T_{\text{max}} \), and \( t_{1/2} \).

**Statistical Analysis**
All statistical analyses were conducted using Statistical Analysis Software (SAS/STAT) version 9.3 (SAS Institute Inc., Cary, North Carolina).

The sample size was calculated using Schuirmann’s two 1-sided \( t \) test of equivalence for crossover design using the natural log-transformed OC PK data (AUC and \( C_{\text{max}} \)). Twenty-four participants were determined to provide 85% power that the 90% confidence interval (CI) of geometric mean ratios of AUC and \( C_{\text{max}} \) fall into the range of 0.8–1.25. Assuming a 33% participant dropout rate, approximately 36 participants were planned to be enrolled.

The demographic analyses and safety analyses included all participants who received \( \geq 1 \) dose of study treatment (OC and/or DMF). The PK population was used in all PK analyses. The PK population was defined as all participants who received \( \geq 1 \) dose of study treatment (OC and/or DMF) and had sufficient measurable PK values. For analyses of the PK of norelgestromin and ethinyl estradiol, sufficient measurable values were \( \geq 50 \text{ pg/mL} \) for norelgestromin and \( \geq 10 \text{ pg/mL} \) for ethinyl estradiol. For analysis of the PK of DMF (measured via MMF), sufficient measurable values of MMF were \( \geq 10 \text{ ng/mL} \). PK measurements that were below the lower limit of quantification were set to 0. All PD analyses were performed on the PD population, defined as all participants who had received \( \geq 1 \) dose of study
treatment (OC and/or DMF) and had sufficient measurable values for progesterone (≥0.21 ng/mL).

Demographic data and PK parameters were summarized using descriptive statistics. The primary end points \(\text{AUC}_{0-\tau}\) and \(C_{\text{max}}\) were used to show the PK comparability of OC with and without DMF. A mixed-effects analysis of variance model including treatment, sequence, and period as fixed effects and participant as a random effect was employed to analyze the log-transformed \(\text{AUC}_{0-\tau}\) and \(C_{\text{max}}\) values. The geometric mean ratio (OC + DMF/OC alone [randomized]) and the corresponding 90%CI are presented. The CI was constructed using Schuirmann’s two 1-sided \(t\) test procedure if the normality assumption was valid; otherwise, the distribution-free CI approach based on the Hodges–Lehmann estimator was used.\(^{11}\) If the 90%CIs for the geometric mean ratios were within the limits of 0.8–1.25, there was considered to be no statistical effect of DMF on the PK of OC. In addition, \(T_{\text{max}}\) and \(t_{1/2}\) calculated for norelgestromin and ethinyl estradiol were presented.

Serum concentrations of progesterone were summarized by time and treatment (OC alone in the lead-in period, OC alone [randomized], or OC coadministered with DMF). All AEs were evaluated based on treatment emergence. PK parameters of MMF including \(\text{AUC}_{\tau-\infty}\), \(C_{\text{max}}\), \(\text{AUC}_{0-12}\), \(T_{\text{max}}\), and \(t_{1/2}\) were exploratory, and the results were compared descriptively to historical data.

**Results**

**Participants**

A total of 46 participants were enrolled and dosed in the lead-in period; among them, 41 were randomized to 1 of the 2 treatment sequences, 40 received study treatment in period 1, and 32 completed the study (Supplemental Figure S1). A total of 39 participants received ≥1 dose of OC coadministered with DMF during randomized treatment (18 participants in sequence 1 period 1 and 21 participants in sequence 2 period 2). A total of 39 participants received ≥1 dose of OC alone during randomized treatment (17 participants in sequence 1 period 2 and 22 participants in sequence 2 period 1).

A total of 16 participants in each sequence completed both period 1 and period 2 (total \(n = 32\)) and were eligible for statistical analysis of drug–drug interaction (estimated geometric mean ratios).

Baseline demographic characteristics were similar between the 2 treatment sequences (Table 1). Fifty-seven percent of participants were black/African American, and 35% were white; mean age ± SD was 31.2 ± 7.0 years, and mean BMI ± SD was 25.7 ± 2.6 kg/m².

**PK of Norelgestromin and Ethinyl Estradiol**

The mean plasma concentration profiles of norelgestromin and ethinyl estradiol were superimposable in 3 groups: OC alone (lead-in period), OC alone (randomized periods), and OC coadministered with DMF (Figure 2). The exposure of norelgestromin and ethinyl estradiol was similar following OC alone (randomized) and OC coadministered with DMF (Table 2). Estimated geometric mean ratios (OC + DMF/OC alone [randomized]) for \(\text{AUC}_{0-\tau}\) and \(C_{\text{max}}\) were 0.975 and 0.990, respectively, for norelgestromin and 0.937 and 0.972, respectively, for ethinyl estradiol. The 90%CIs of the geometric mean ratios for \(\text{AUC}_{0-\tau}\) and \(C_{\text{max}}\) of norelgestromin and ethinyl estradiol were all contained within the 0.8–1.25 range.

Maximum plasma norelgestromin concentrations were achieved at median \(T_{\text{max}}\) values of 1.5 hours (range, 1.0–4.0 hours) for OC alone (randomized) and 2.0 hours (range, 1.0–4.0 hours) for OC coadministered with DMF, and maximum plasma ethinyl estradiol concentrations were achieved at median \(T_{\text{max}}\) values of 1.5 hours (range, 0.5–2 hours) for OC alone (randomized) and 1.1 hours (range, 0.5–2.1 hours) for OC coadministered with DMF (Table 2). After reaching \(C_{\text{max}}\), plasma concentrations of norelgestromin and ethinyl estradiol declined in a multiphasic manner, with the elimination phase nearly the same across all 3 treatment periods, indicating similar \(t_{1/2}\) for both norelgestromin and ethinyl estradiol when OC was coadministered with DMF compared with OC alone (randomized).

**Serum Progesterone Levels**

Median serum progesterone levels were comparable across the times assessed and were comparable following OC alone in the lead-in period, OC alone (randomized), and OC coadministered with DMF, with values on day 21 of 0.325, 0.410, and 0.330 ng/mL, respectively (Figure 3). Because normal serum progesterone levels in adult women during the luteal phase of the menstrual cycle are 3–25 ng/mL, the data suggest that OC coadministered with DMF suppressed ovulation as effectively as OC alone. Seven participants in the OC alone (randomized) treatment period and 5 participants in the OC + DMF treatment period had serum progesterone levels above 3 ng/mL at any point, suggesting that these occurrences were not associated with DMF treatment. In addition, all values above 1.5 ng/mL were higher than \(Q_3 + 1.5 \times \text{IQR}\) and therefore considered outliers (Figure 3).

**Exploratory Analysis: PK of DMF**

DMF (measured via MMF) was rapidly absorbed following oral administration. \(\text{AUC}_{\tau-\infty}\) (mean ± SD) was 3.81 ± 1.08 mg·h/L, \(\text{AUC}_{0-12}\) (mean ± SD) was 3.80 ± 1.07 mg·h/L, and \(C_{\text{max}}\) (mean ± SD) was 2.35 ±
Table 1. Baseline Demographic Characteristics

|                        | Total Enrolled in Lead-in Period | Sequence 1: OC + DMF/OC Alone | Sequence 2: OC Alone/OC + DMFa | Total Randomized |
|------------------------|----------------------------------|--------------------------------|--------------------------------|------------------|
| Participants, n        | 46                               | 18                             | 23                             | 41               |
| Age (years), mean ± SD | 31.2 ± 7.0                       | 32.7 ± 6.1                     | 30.3 ± 7.9                     | 31.3 ± 7.2       |
| Race, n (%)b           |                                  |                                |                                |                  |
| American Indian or     | 1 (2)                            | 1 (6)                          | 0                              | 1 (2)            |
| Alaska Native          |                                  |                                |                                |                  |
| Black or African       | 26 (57)                          | 10 (56)                        | 14 (61)                        | 24 (59)          |
| American White         | 16 (35)                          | 5 (28)                         | 9 (39)                         | 14 (34)          |
| Other                  | 3 (7)                            | 2 (11)                         | 0                              | 2 (5)            |
| Height (cm), mean ± SD | 164.3 ± 6.5                      | 165.1 ± 5.9                    | 164.5 ± 7.1                    | 164.7 ± 6.5      |
| Weight (kg), mean ± SD | 69.5 ± 9.0                       | 69.3 ± 8.3                     | 69.5 ± 9.9                     | 69.4 ± 9.2       |
| BMI (kg/m2), mean ± SD | 25.7 ± 2.6                       | 25.4 ± 3.0                     | 25.6 ± 2.4                     | 25.5 ± 2.6       |

BMI, body mass index; OC, oral contraceptive.
aDMF, delayed-release DMF.

Table 2. Summary of Plasma Norelgestromin and Ethinyl Estradiol PK Parameters

|                        | OC Alone Randomized Treatment | OC Coadministered With DMFa | Estimated Geometric Mean Ratio (90%CI)b |
|------------------------|------------------------------|-----------------------------|----------------------------------------|
| Participants included in analysis, n | 39c                          | 39c                         | 32d                                    |
| Norelgestromin\a        |                              |                             |                                        |
| AUC0–τ, pg·h/mL         | 19 883.9 ± 5863.6            | 19 056.8 ± 5104.2           | 0.975 (0.923–1.030)                   |
| Cmax, pg/mL             | 1925.8 ± 480.6               | 1889.1 ± 449.9              | 0.990 (0.921–1.063)                   |
| Tmax, h                 | 1.5 (1.0, 4.0)               | 2.0 (1.0, 4.0)              | —                                      |
| t1/2, h                 | 15.6 ± 0.7                   | 13.6 ± 3.2                  | —                                      |
| Ethinyl estradiol\a     |                              |                             |                                        |
| AUC0–τ, pg·h/mL         | 1 180.4 ± 501.9              | 1 050.0 ± 403.4             | 0.937 (0.888–0.988)                   |
| Cmax, pg/mL             | 132.2 ± 52.5                 | 123.3 ± 47.0                | 0.972 (0.825–1.119)                   |
| Tmax, h                 | 1.5 (0.5, 2.0)               | 1.1 (0.5, 2.1)              | —                                      |
| t1/2, h                 | 11.8 ± 2.6                   | 11.1 ± 2.2                  | —                                      |

AUC0–τ, area under the plasma concentration–time curve over the dosing interval; CI, confidence interval; Cmax, peak plasma concentration; OC, oral contraceptive; PK, pharmacokinetic; Tmax, time to peak plasma concentration; t1/2, half-life.
aDMF, delayed-release DMF.
bOC coadministered with DMF/OC alone randomized treatment.
cParticipants who received ≥1 dose of study treatment (OC and/or DMF) and had measurable plasma concentration values of norelgestromin and ethinyl estradiol on day 21 that were sufficient to derive PK parameters.
dParticipants who completed both randomized treatment periods and were eligible for the statistical analysis of drug–drug interaction.
eValues are mean ± SD except for Tmax, which is expressed as median (minimum, maximum).

0.76 mg/L. Cmax was achieved at a median Tmax of 2.3 hours postdose (range, 1.0–6.1 hours). After reaching Cmax, plasma MMF concentrations declined rapidly, with a short mean t1/2 of 0.7 hours. These data were consistent with historical data from PK studies with DMF treatment alone.5,6

Safety
Study treatments were well tolerated (Table 3). During the lead-in period (OC alone), 19 participants (41%) experienced a treatment-emergent AE (TEAE). Three participants experienced moderate or severe TEAEs, including 1 participant who experienced a severe AE of pulmonary embolism, which was reported as an SAE. This event, which was the only SAE reported in the study, was considered related to OC and led to withdrawal of the participant from the study.

Of the participants in the randomized periods, 10 (26%) experienced TEAEs during administration of OC alone (randomized) and 26 (67%) experienced TEAEs during coadministration of OC and DMF. One participant each during the OC alone period (randomized) and the OC coadministered with DMF period experienced a TEAE considered moderate. None of the TEAEs were severe.
Overall, 10 participants discontinued treatment and withdrew from the study because of an AE: 2 participants during the lead-in period, 2 participants during a randomized OC alone period, and 6 participants during a randomized OC + DMF period (Supplemental Figure S1). Three participants discontinued because of TEAEs during the randomized OC alone period: 1 participant experienced TEAEs of acne, menorrhagia, and dysmenorrhea, which were considered related to OC; 1 participant experienced TEAEs of contusion and ligament sprain, which were not considered related to OC; and 1 participant experienced a TEAE of urinary tract infection, which was not considered related to OC. Six participants discontinued study treatment because of TEAEs during the randomized OC + DMF period. These TEAEs included vomiting (5 events), decreased appetite (2 events), flushing (3 events), nausea (4 events), abdominal pain (1 event), upper respiratory tract infection (1 event), and otitis media (1 event). The majority of these TEAEs were considered related to DMF, with the exception of upper respiratory tract infection and otitis media.

The most frequently experienced TEAE was flushing. All these events were experienced during coadministration of OC with DMF (18 of 39 participants during OC + DMF randomized treatment [46%]). Gastrointestinal (GI) disorders, mainly nausea and vomiting, were the most frequently reported System Organ Class (15 of 46 total participants [33%]). These events mostly occurred during coadministration of OC and DMF (14 of 39 patients during OC + DMF randomized treatment [36%]), with 10 of 39 participants (26%) experiencing nausea and 9 of 39 participants (23%) experiencing vomiting during this period.

There were no clinically relevant findings in vital sign values and electrocardiogram data during the study.
Figure 3. Box plot summarizing serum progesterone concentrations (ng/mL) over time in participants who received ≥1 dose of study treatment (OC and/or DMF) and had measurable plasma concentration values of progesterone on day 21 that were sufficient to derive PD parameters. Values below the limit of quantification were set to 0 in the calculations. Outliers below 3 ng/mL are presented as solid circles, and those at or above 3 ng/mL are presented as open circles. OC, oral contraceptive. *DMF, delayed-release DMF.

There were no clinically relevant findings in laboratory assessments during periods 1 or 2. One participant presented with abnormal alanine transaminase and aspartate transaminase values during the lead-in and safety follow-up periods.

Discussion
In this study, DMF therapy did not have a clinically relevant effect on the PK of a commonly used OC (norelgestromin/ethinyl estradiol), supporting a lack of drug–drug interaction between DMF and OCs in vivo in healthy female volunteers. In addition, OCs were not observed to have an effect on the PK of MMF. These findings suggest that norgestimate/ethinyl estradiol–based OCs may be used with DMF without dose modification.

The design of this study had several advantages. First, OCs were self-administered by participants in an outpatient setting. To minimize protocol deviation and maximize data validity, participants with progesterone ≥3 ng/mL on days 21 or 22 of the lead-in period (possibly because of low adherence to OC treatment) were excluded from later PK comparison periods. Second, randomization of participants after the lead-in period ensured that sequences 1 and 2 were well balanced in terms of demographics. Third, the crossover design allowed for comparison of OC alone (randomized) and OC + DMF conditions within the same participants. Finally, the choice of a widely used OC extends the generalizability of the findings to agents with similar biotransformation pathways.

OCs are primarily metabolized in the liver by the CYP3A4 and CYP2C subfamilies.9,10 As described earlier, neither DMF nor MMF has been shown to induce CYP2D6, CYP3A4, or P-glycoprotein at clinically relevant concentrations (Biogen, data on file; study P00012-10-04), and the potential of MMF to inhibit these enzymes was found to be low (Biogen, data on file; study P00012-04-13). Furthermore, DMF is metabolized by high-capacity esterases and was shown not to be a substrate of CYP enzymes.5 Hence, DMF was not expected to have a clinically relevant effect on the PK of OC. Consistent with this, mean plasma norelgestromin and ethinyl estradiol concentration profiles were superimposable following treatment with OC alone and OC coadministered with DMF, and there was no statistically significant effect of DMF on the PK parameters of norelgestromin and ethinyl estradiol, with 90%CIs of geometric mean ratios for AUC0– and Cmax contained within the 0.8–1.25 range. Furthermore, median serum levels of progesterone (a PD end point) were below 1 ng/mL between days 14 and 28 in both the randomized OC alone group and the OC coadministered with DMF group, representing the successful suppression of ovulation in
both treatment regimens. Although this study did not examine contraceptives containing other progestogens, an effect of DMF on their exposure is not expected.

The PK of MMF has been extensively characterized under intensive sampling schemes in previous studies. In a single ascending dose study (120, 240, or 360 mg DMF) in healthy volunteers, the exposure demonstrated by C_{max} and partial AUC up to 9 hours postdose (AUC_{0-9}) was generally dose proportional across the tested dose range: for 120, 240, and 360 mg DMF, C_{max} (mean ± SD) was 0.58 ± 0.17, 1.43 ± 0.29, and 1.90 ± 0.57 mg/L, respectively, and AUC_{0-9} (mean ± SD) was 1.21 ± 0.37, 2.41 ± 0.47, and 3.78 ± 1.11 mg·h/L, respectively (Biogen, data on file; study IKP/ID33). Exposure was also generally dose proportional across dosing frequencies when healthy volunteers received DMF 240 mg twice daily or 3 times daily for 4 days. In a multiple-dose study (DMF 240 mg twice daily or 3 times daily for 1 day), the PK of MMF in patients with MS was similar to that observed in healthy volunteers in other studies: MMF C_{max} (mean ± SD) was approximately dose proportional (1.87 ± 1.25 mg/L [twice daily] and 2.46 ± 1.43 mg/L [3 times daily]), with AUC_{0-24} (mean ± SD) of 8.21 ± 3.46 mg·h/L (twice daily) and 12.3 ± 3.07 mg·h/L (3 times daily); t_{1/2} values indicated rapid elimination of MMF (median t_{1/2} of 1.07 for both twice daily and 3 times daily), and no accumulation of MMF exposure was observed (Biogen, data on file; study 109MS101).

In an exploratory analysis of the current study, the PK parameters of DMF (measured via MMF) following coadministration of OC and DMF were similar to those observed in previous studies following administration of DMF alone. These results suggest that the efficacy of DMF is unlikely to be affected by coadministration with OC.

The safety profile of DMF coadministered with OC was consistent with and comparable to safety outcomes from the pivotal phase 3 studies of DMF. In the phase 3 studies, DMF was associated with flushing and GI events, most of which were of mild or moderate severity and self-limiting with continued treatment. In the present study, the most frequently experienced TEAEs during treatment with OC coadministered with DMF were flushing, nausea, and vomiting. TEAEs experienced during treatment with OC alone included known AEs associated with OCs such as oligomenorrhea and headache. Most TEAEs were considered mild.

### Table 3. Overall Summary of TEAEs

| TEAE                  | OC Alone in Lead-in Period | OC Alone Randomized Treatment | OC Coadministered With DMF | Overall |
|-----------------------|----------------------------|-------------------------------|-----------------------------|---------|
| Participants          | 46 (100)                   | 39 (100)                      | 39 (100)                    | 46 (100) |
| Any event             | 19 (41)                    | 10 (26)                       | 26 (67)                     | 31 (67)  |
| Mild                  | 16 (35)                    | 9 (23)                        | 25 (64)                     | 26 (57)  |
| Moderate              | 2 (4)                      | 1 (3)                         | 1 (3)                       | 4 (9)    |
| Severe                | 1 (2)                      | 0                             | 0                           | 1 (2)    |

Events with incidence ≥5% in any period

- Flushing: 0 (0) vs. 18 (46) vs. 18 (39)
- Nausea: 1 (2) vs. 10 (26) vs. 10 (22)
- Vomiting: 0 (0) vs. 9 (23) vs. 9 (20)
- Abdominal pain: 1 (2) vs. 4 (10) vs. 5 (11)
- Headache: 3 (7) vs. 2 (5) vs. 5 (11)
- Oligomenorrhea: 4 (9) vs. 0 vs. 4 (9)
- Decreased appetite: 0 vs. 3 (8) vs. 3 (7)
- Abdominal discomfort: 0 vs. 2 (5) vs. 2 (4)
- Diarrhea: 0 vs. 2 (5) vs. 2 (4)

Discontinuation because of event:

- 2 (4) vs. 3 (8) vs. 6 (15) vs. 10 (22)

Death: 0 vs. 0 vs. 0 vs. 0

AE, adverse event; OC, oral contraceptive; TEAE, treatment-emergent adverse event.

aTEAEs were defined as AEs that (1) started on or after the dosing of study treatment in a period and were before the dosing of study treatment in the subsequent period or (2) were present before the dosing of study treatment and subsequently worsened in severity after receiving study treatment in that period and were before the dosing in any subsequent period.

bDMF, delayed-release DMF.

cOne participant had 2 AEs, both considered leading to treatment discontinuation. One AE was during OC alone (randomized) treatment period, and the second AE was during the OC coadministered with DMF treatment period. In summary, 10 participants had 11 events.
There was 1 SAE of pulmonary embolism—a known complication of OCs—during treatment with OC alone during the lead-in period, and this patient was withdrawn from the study without receiving DMF.

Conclusions
These results suggest there are no relevant effects of DMF on the PK profile of norgestimate and ethinyl estradiol, and therefore norgestimate/ethinyl estradiol-based OCs may be used with DMF without dose modification. In addition, no impact on the PK of DMF (measured via MMF) was seen with the concomitant use of OCs.

Supplement
Bioanalysis Materials
MMF and sodium fluoride were purchased from Sigma-Aldrich (Milwaukee, Wisconsin); the internal standard [13C4] methyl fumarate was purchased from Chemtos (Austin, Texas). Ethinyl estradiol was purchased from USP (Rockville, Maryland); the internal standard (IS) ethinyl estradiol-D4 was purchased from C/D/N Isotopes Inc (Pointe-Claire, Quebec, Canada). Norelgestromin was purchased from Steraoids Inc. (Newport, Rhode Island); the internal standard norelgestromin-D6 was purchased from Covance (Madison, Wisconsin). Blank sodium heparin human plasma was purchased from Bioreclamation, Inc (Hicksville, New York). Blank sodium fluoride/potassium oxalate human plasma was purchased from BioChemed (Winchester, Virginia). All other reagents were purchased from Sigma-Aldrich (Milwaukee, Wisconsin) or EMD Chemicals (Gibbstown, New Jersey).

Bioanalysis of Ethinyl Estradiol, Norelgestromin, and MMF
Determination of plasma concentrations of ethinyl estradiol and norelgestromin was conducted by Covance Laboratories, Inc. (Madison, Wisconsin) using a validated method that followed the US Food and Drug Administration guidelines. The validated analytical ranges were 10.0–500 pg/mL for ethinyl estradiol and 50.0–25 000 pg/mL for norelgestromin. For ethinyl estradiol, the between-run precision and accuracy for the standards were found to be ≤4.4% and -2.9% to 3.2%, respectively. The low, medium, and high QC samples, within-run precision and accuracy were found to be ≤5.4% and -5.3% to -0.6% respectively. For norelgestromin, the between-run precision and accuracy for the standards were found to be ≤4.8% and -7.5% to 5.0%, respectively. The low, medium, and high QC samples, within-run precision and accuracy were found to be ≤7.0% and -5.6% to 4.2%, respectively, whereas between-run precision and accuracy were found to be ≤6.5% and -1.7% to 2.5%, respectively. Ethinyl estradiol, norelgestromin, and IS D4-ethinyl estradiol and D6-norelgestromin were extracted from human plasma using a liquid–liquid extraction procedure and dansyl chloride derivatization method. To aliquots of plasma samples, 1-chlorobutane was added, and the samples were vortexed, centrifuged, and placed on a dry ice/acetone bath. The organic layer was transferred and evaporated to dryness, and the extracts were then reconstituted in a 100 mM sodium bicarbonate solution (pH 11.0). The samples were then derivatized by adding dansyl chloride and incubating for 15 minutes at 60°C. The reaction was quenched by cooling the samples in a room temperature water bath, followed by the addition of 0.2N hydrochloric acid. The samples then underwent an additional liquid–liquid extraction as described above. After evaporation, the final extracts were reconstituted in 0.2% formic acid in acetonitrile:water (50:50). Chromatographic separation and detection were achieved using a Shimadzu Prominence HPLC system (Kyoto, Japan) coupled to a Sciex API 5000 triple quadrupole mass spectrometer. The analytical column was a Phenomenex Onyx Monolithic C18 (3.0 × 100 mm) with a mobile phase consisting of 0.2% (v/v) formic acid in water (mobile phase A) and 0.2%(v/v) formic acid in acetonitrile (mobile phase B). The mass spectrometer was operated in electrospray ionization positive mode optimized as follows: source temperature, 650°C; collision gas, 10; curtain gas, 20; nebulizing gas (gas 1), 70; auxiliary gas (gas 2), 50; ion spray voltage, 5500 V. The collision energy for ethinyl estradiol and ethinyl estradiol-D4 was 51 V, whereas that of norelgestromin and norelgestromin-D6 was 41 V. Ethinyl estradiol, ethinyl estradiol-D4, norelgestromin, and norelgestromin-D6 were detected using multiple reaction mode monitoring transitions set at 530→171, 534→171, 561→170, and 567→170, respectively. The calibration curves were obtained by performing a linear regression weighted 1/X2.

Determination of plasma concentrations of MMF were conducted by InVentiv Health Clinical Lab, Inc (Princeton, New Jersey) using a validated liquid chromatography–tandem mass spectrometry method that followed US Food and Drug Administration guidelines. The validated analytical range was 10–5000 ng/mL. For the calibration curve, the between-run precision and accuracy for the standards were found to be ≤10.4% and -12.6% to 2.0%, respectively. For QC low, medium, and high samples, within-run accuracy and precision were found to be ≤4.48% and -4.40% to 6.00%, respectively, whereas in between-run precision and accuracy were found to be ≤5.03% and
0.00% to 1.00%, respectively. MMF was extracted from human plasma using a protein precipitation extraction procedure as follows. All samples were thawed in an ice water bath to which IS solution containing $^{13}\text{C}4$-methyl fumarate prepared with 100% acetonitrile was added to all samples except the standard blanks, which received blank acetonitrile. The samples were vortexed and centrifuged, and the resulting supernatant was dried and reconstituted with 0.1% formic acid in acetonitrile/water (20/80 v/v). The extracts were separated on a Thermo Aquasil C18 4.6 $\times$ 50 mm, 5-μm column using mobile phase consisting of 0.1% (v/v) formic acid in water (mobile phase A) and 0.1% (v/v) formic acid in acetonitrile (mobile phase B) on an Agilent 1100 series HPLC system (Santa Clara, California) coupled to an API4000 triple quadrupole mass spectrometer (Framingham, Massachusetts). The mass spectrometer was operated in electrospray ionization negative mode optimized as follows: source temperature, 650°C; collision gas, 12; curtain gas, 50; ion spray voltage, -2000; declustering potential, -40 V; entrance potential, -10 V; collision energy, -13; and collision cell exit potential, -10 V. MMF and its IS $^{13}\text{C}_4$-methyl fumarate were detected using multiple reaction mode monitoring transitions set at 129→71 and 133→74, respectively. MMF calibration curves were obtained by performing a linear regression weighted 1/X².

Acknowledgments

Biogen provided funding for medical writing support in the development of this paper. Karyn Myers, PhD, wrote the first draft of the manuscript based on input from authors, and Kristen DeYoung 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.

Declaration of Conflicting Interests

B.Z., I.N., G.Z., and S.I.S. are full-time employees of and hold stock/stock options in Biogen. V.M. has no conflicts to report. M.L. and J.K. are full-time employees of Covance, the contract research organization funded by Biogen for research support for this study.

Funding

This study was sponsored by Biogen (Cambridge, Massachusetts).

References

1. Compston A, Coles A. Multiple sclerosis. Lancet. 2008;372(9648):1502–1517.
2. Tullman MJ. Overview of the epidemiology, diagnosis, and disease progression associated with multiple sclerosis. Am J Manag Care. 2013;19(2 suppl):S15–S20.
3. Fox RJ, Miller DH, Phillips JT, et al, CONFIRM Study Investigators. Placebo-controlled phase 3 study of oral BG-12 or glatiramer in multiple sclerosis. N Engl J Med. 2012;367(12):1087–1097.
4. Gold R, Kappos L, Arnold DL, et al, DEFINE Study Investigators. Placebo-controlled phase 3 study of oral BG-12 for relapsing multiple sclerosis. N Engl J Med. 2012;367(12):1098–1107.
5. Dubey D, Kieseier BC, Hartung HP, et al. Dimethyl fumarate in relapsing–remitting multiple sclerosis: rationale, mechanisms of action, pharmacokinetics, efficacy and safety. Expert Rev Neuroth. 2015;15(4):339–346.
6. Sheikh SI, Nestorov I, Russell H, et al. Tolerability and pharmacokinetics of delayed-release dimethyl fumarate administered with and without aspirin in healthy volunteers. Clin Ther. 2013;35(10):1582–1594.
7. Roy P, Jakate AS, Patel A, et al. Effect of multiple-dose dexloxiuglumide on the pharmacokinetics of oral contraceptives in healthy women. J Clin Pharmacol. 2005;45(3):329–336.
8. Schwartz JI, Liu F, Wang Y-H, et al. Effect of laropiprant, a PGD2 receptor 1 antagonist, on estradiol and norgestimate pharmacokinetics after oral contraceptive administration in women. Am J Ther. 2009;16(6):487–495.
9. Ball SE, Forrester LM, Wolf CR, Back DJ. Differences in the cytochrome P-450 isoenzymes involved in the 2-hydroxylation of oestradiol and 17α-ethinylestradiol. Relative activities of rat and human liver enzymes. Biochem J. 1990;267(1):221–226.
10. Guengerich FP. Oxidation of 17 alpha-ethynylestradiol by human liver cytochrome P-450. Mol Pharmacol. 1988;33(5):500–508.
11. Chow S, Liu J. Design and Analysis of Bioavailability and Bioequivalence Studies. 2nd ed. New York: Marcel Dekker; 2000.
12. Phillips JT, Selmaj K, Gold R, et al. Clinical significance of gastrointestinal and flushing events in patients with multiple sclerosis treated with delayed-release dimethyl fumarate. Int J MS Care. 2015;17(5):236–243.

Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher’s website.