CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

204063Orig1s000

CLINICAL PHARMACOLOGY AND BIOPHARMACEUTICS REVIEW(S)

ADDENDUM TO BIOPHARMACEUTICS REVIEW
Office of New Drug Quality Assessment

Application No.:	NDA 204063	**Reviewer:** Elsbeth Chikhale, PhD	
Submission Date:	February 27, 2012		
Division:	Division of Neurology Products	**Acting Team Leader:** Tapash Ghosh, PhD	
Applicant:	Biogen Inc.	**Acting Supervisor:** Richard Lostritto, PhD	
Trade Name:	(b) (4)	**Date Assigned:**	February 29, 1012
Generic Name:	Dimethyl fumarate (DMF) Also referred to as BG00012	**Date of Addendum to Review:**	February 12, 2013
Indication:	Treatment of Multiple Sclerosis	**Type of Submission:** 505(b)(1) Original New Drug Application	
Formulation/ strengths	(b) (4) delayed release capsules/ 120 mg and 240 mg		
Route of Administration	Oral		

ADDENDUM TO ORIGINAL BIOPHARMACEUTICS REVIEW DATED 11/19/12:

The original Biopharmaceutics review by Elsbeth Chikhale, Ph.D., dated 11/19/12 included the following recommended language for the action letter:

If approved, the AP letter should include the following two comments:

- We have not made a BCS classification determination for your drug, since the data provided in the NDA are inconclusive with regards to the drug's permeability.

- We are reminding you of your commitment to collect 20 minute (buffer stage) dissolution data for all stability samples of all commercial batches to be released post approval for one year in order to evaluate the possibility of tightening the buffer stage dissolution acceptance criterion to Q= (b) (4) at 20 minutes and to submit the data in a prior approval supplement (PAS) one year after approval for our review.

During an ONDQA internal discussion, it was decided that the above comments (with minor revisions) will be sent to the Applicant by ONDQA in a separate communication.

RECOMMENDATION :

It is recommended that ONDQA conveys the following comments to the Applicant in an separate communication after the action letter is issued:

- We would like to remind you of your commitment to collect 20 minute (buffer stage) dissolution data for all stability samples of all commercial batches to be released post approval for one year and to submit these data to FDA as a prior approval supplement (PAS) 15 months after approval in order to determine if the buffer stage acceptance criterion can be tightened to Q= (b) (4) at 20 minutes.

- We would like to inform you that FDA did not make a determination on the BCS classification of your drug (dimethyl fumarate) at this point, because the provided permeability data for your drug are inconclusive.

From the Biopharmaceutics perspective the overall recommendation included in the original Biopharmaceutics Review dated 11/19/12 for this NDA remains the same.

From the Biopharmaceutics perspective, NDA 204063 for dimethyl fumarate delayed release capsules (120 mg/capsule and 240 mg/capsule) is recommended for APPROVAL.

Elsbeth Chikhale, Ph.D.
Biopharmaceutics Reviewer
Office of New Drug Quality Assessment

Tapash Ghosh, Ph.D.
Acting Biopharmaceutics Team Leader
Office of New Drug Quality Assessment

cc: SPope, RLostritto

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/s/

ELSBETH G CHIKHALE
02/12/2013

TAPASH K GHOSH
02/12/2013

BIOPHARMACEUTICS REVIEW Office of New Drug Quality Assessment			
Application No.:	NDA 204063	**Reviewer:** Elsbeth Chikhale, PhD	
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Division:	Division of Neurology Products	**Acting Team Leader:** Tapash Ghosh, PhD	
Applicant:	Biogen Inc.	**Acting Supervisor:** Richard Lostritto, PhD	
Trade Name:	(b) (4)	**Date Assigned:**	February 29, 1012
Generic Name:	Dimethyl fumarate (DMF) Also referred to as BG00012	**Date of Review:**	November 19, 2012
Indication:	Treatment of Multiple Sclerosis	**Type of Submission:** 505(b)(1) Original New Drug Application	
Formulation/ strengths	(b) (4) delayed release capsules/ 120 mg and 240 mg		
Route of Administration	Oral		

SUBMISSION:

This 505(b)(1) New Drug Application is for an (b) (4) delayed release capsule indicated for the treatment of Multiple Sclerosis (MS). The pharmacological properties of BG00012 are proposed to be mediated through activation of the nuclear factor (erythroid-derived 2)-like 2 (NFE2L2 or Nrf2) antioxidant response pathway, which is the primary cellular defense system for responding to a variety of potentially toxic stimuli. DMF is rapidly and completely hydrolyzed to its active metabolite mono-methyl fumarate (MMF) by esterases present in the GI tract, in the gut wall and in blood before DMF reaches the systemic circulation. The drug product was formulated as (b) (4) a size 0 hard gelatin capsule. The design of the drug product formulation was based on the desired gastro-resistant properties and on the physico-chemical properties of the drug substance. The goal was to develop a delayed release formulation that prevents release of the active ingredient in the gastric environment while allowing for rapid release of the active ingredient in the intestine region. A formulation consisting of a capsule (b) (4) was pursued because such systems are designed to achieve the targeted delivery profile (b) (4)

BIOPHARMACEUTICS INFORMATION:

The Biopharmaceutics review for this NDA will be focused on the evaluation and acceptability of 1) the proposed dissolution methodology, 2) dissolution acceptance criteria, 3) the in vitro alcohol dose dumping method and data, and 4) solubility and dissolution data to support the BCS

Composition of the (b) (4)

Process Step	Ingredient	Function	Reference	Amount per capsule (mg)	Amount per capsule (mg)
(b) (4)	Dimethyl fumarate	Active ingredient	Internal specification	120.0	240.0
	Croscarmellose sodium	(b) (4)	USP-NF, Ph. Eur., JP		(b) (4)
	Microcrystalline cellulose		USP-NF, Ph. Eur., JP		
	Magnesium stearate [1]		USP-NF, Ph. Eur., JP		
	Talc		USP-NF, Ph. Eur., JP		
	Colloidal silicon dioxide		USP-NF, Ph. Eur., JP		
			Subtotal		
	Methacrylic acid copolymer, Type A [2]		USP-NF, Ph. Eur., JP		
	Triethyl citrate		USP-NF, Ph. Eur., JP		
	(b) (4)		USP-NF, Ph. Eur.		
			Subtotal		
	Methacrylic acid copolymer dispersion [2]		USP-NF, Ph. Eur., JP		
	Polysorbate 80		USP-NF, Ph. Eur.		
	Sodium lauryl sulfate		USP-NF, Ph. Eur.		
	Triethyl citrate		USP-NF, Ph. Eur., JP		
	(b) (4)		USP-NF, Ph. Eur., JP		
	Simethicone [2]		USP-NF, Ph. Eur.		
	(b) (4)		USP-NF, Ph. Eur.		
			Subtotal		
			TOTAL		

(b) (4)

DISSOLUTION METHOD:

The proposed dissolution method utilizes a two stage approach:

USP Apparatus II (paddle)
Temperature: 37 °C
Rotation speed: 100 rpm
Acid stage for 2 hours: Dissolution medium: 500 mL 0.1 N HCl
Buffer stage after 2 hours: Dissolution medium: 500 mL pH 6.8 phosphate buffer

The dissolution method development was submitted and reviewed under IND 73,061, which is summarized as follows:

8/26/11: Sponsor and FDA met, a dissolution method development report was requested by FDA

9/16/11: Dissolution method development report was submitted to the IND, proposed method has pH 6.8 medium for the buffer stage, the proposed rotation speed is 100 rpm

11/29/11: Dissolution method development report was reviewed by Houda Mahayni, Ph.D. (DARRTS)

12/16/11: Comments from the review by Houda Mahayni, Ph.D. were communicated to the sponsor (DARRTS). It was suggested to try [(b) (4)] for buffer stage with a rotation speed [(b) (4)]

12/29/11: Sponsor responded to the comments. Sponsor provided data and claims that [(b) (4)] . Also at [(b) (4)] incomplete dissolution occurs after 60 minutes [(b) (4)]).

1/25/12: Review of response by Houda Mahayni, Ph.D (in DARRTS). The review acknowledges the [(b) (4)] and agrees to the use of the proposed dissolution method (using pH 6.8 for the buffer stage at 100 rpm) and states that the acceptance criteria will be reviewed during the NDA review. The review states that in the NDA, in addition to the in vitro dose dumping study data for the 120 mg capsules, the in vitro dose dumping data for the 240 mg capsules should also be provided.

1/25/12: The comments from Dr. Houda Mahayni's review were e-mailed to the sponsor.

The dissolution method validation report is provided in the NDA, and the following 3 tables summarize the results:

Table 9: Summary of Dissolution Validation (Acid Stage, 0.1 N HCl)

Validation Parameters	Requirements	Results
Specificity	Analyte signal matches reference $R \geq 1.75$	Match to reference $R_{MHF/DMF} = 10.00$
Linearity	Correlation coefficient, $r \geq 0.99$ Coefficient of variation, $V_{xo} \leq 5$ % No tendency in the plot of the residuals	$r = 1.00$ $V_{xo} = 0.2$ % No tendency
Range	± 20 of specification limits [1]: 0 % - 45 %	0 % - 51 % (0.05-6.09 mg/100ml)
Robustness: Stability of the analysis solutions	Time interval with less than 2 % decrease	27 hours

[1] Specification limits: The average allowable release of dimethyl fumarate is [(b) (4)] of label content, however individual results [(b) (4)] is allowed

Table 10: Summary of Dissolution Validation (Buffer Stage, pH 6.8 Phosphate Buffer)

Validation Parameters	Requirements	Results
Specificity	Resolution, $R \geq 1.75$	$R_{MHF/DMF} = 10.03$
Linearity	Correlation coefficient, $r \geq 0.99$ Coefficient of variation, $V_{x0} \leq 5$ % No tendency in the plot of the residuals	R = 1.00 (0.9999) $V_{x0} = 0.3$ % No tendency
Range	Range is based on results for Linearity	22 % - 111 %
Robustness: Stability of the analysis solutions	Time interval with less than 2 % decrease	140 hours

Table 11:

Validation Parameters	Requirements	Results
Accuracy:		
Acid Stage	Mean recovery $\overline{R} = 100 \pm 5$ % Across a range from 0.8 - 26.6 % [1]	$\overline{R}_{\text{after 5 min}} = 100$ % , $\overline{R}_{\text{after 2 hrs.}} = 99$ % (Recovery $_{0.8\%} = 93$ %) (Recovery$_{12.7\%} = 102$ %) (Recovery$_{26.6\%} = 102$ %)
Buffer Stage	Mean recovery $\overline{R} = 100 \pm 5$ % Across a range from 29 - 102 % [1]	$\overline{R}_{\text{after 5 min}} = 98$ % , $\overline{R}_{\text{after 45 min.}} = 97$ % (Recovery $_{29\%} = 96$ %) (Recovery $_{65\%} = 97$ %) (Recovery$_{102\%} = 97$ %)
Repeatability (Buffer Stage)	$RSD_r \leq 10.0$ %	$RSD_r = 4.6$ %
Intermediate precision (Buffer Stage)	$RSD_{ip} \leq 10.0$ %	$RSD_{ip} = 4.9$ %

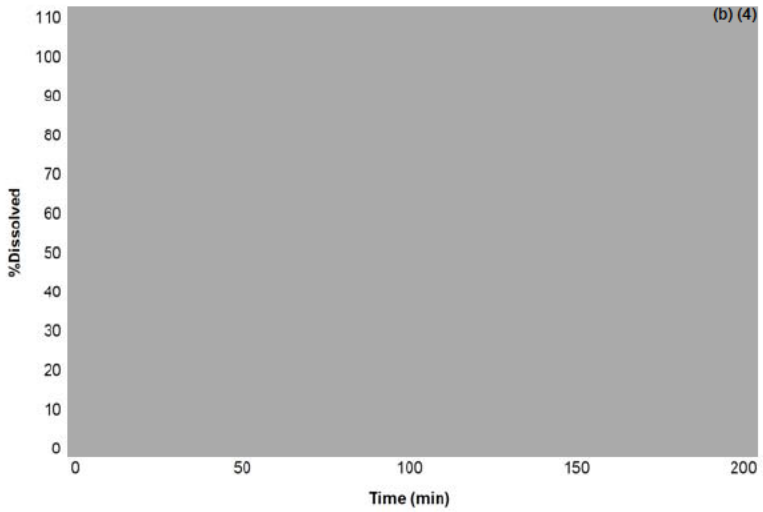
[1] Percentage of label claim (b) (4) = 120 mg

Evaluation of the dissolution method and dissolution method validation:

The proposed dissolution method was reviewed under IND 73,061 and found acceptable.

Based on the provided validation report, the dissolution method has been appropriately validated.

DISSOLUTION ACCEPTANCE CRITERIA:



The proposed dissolution acceptance criteria are:
"Complies with USP <711> for delayed release dosage forms, with Q = (b) (4) in 30 minutes."

The Applicant states that the 30 minute criterion has been determined to be most appropriate time point based on the dissolution data for the drug product with rapid release profile at pH 6.8 buffer stage and that the acceptance criterion Q = (b) (4) is supported by the historical batch analysis data.

Evaluation of the proposed dissolution acceptance criteria:
For the acid stage, the proposed acceptance criteria at 2 hours per USP <711> (copied below) for delayed release dosage forms are acceptable.

Level	Number Tested	Criteria
A_1	6	No individual value exceeds 10% dissolved.
A_2	6	Average of the 12 units (A_1 + A_2) is not more than 10% dissolved, and no individual unit is greater than 25% dissolved.
A	12	Average of the 24 units (A_1 + A_2 + A_3) is not more than 10% dissolved, and no individual unit is greater than 25% dissolved.

Additional dissolution data were needed to evaluate the acceptance criterion for the buffer stage, therefore, the following comment/information request was sent to the Applicant on 7/27/12:

In order to evaluate the proposed dissolution acceptance criteria, provide dissolution profile data (individual, mean, SD, figures) for the pivotal clinical batches and the primary stability/registration batches. For the stability batches, provide the dissolution profile data at release and upon storage during the stability study.

The Applicant responded in an amendment dated 8/10/12:
"Dissolution profile data is available for the 120mg and 240mg registration batches. The data supports the dissolution acceptance criteria. All dissolution profile results for both 120mg and 240mg strength registration batches exhibit similar dissolution profiles with a mean of < 10%

dissolved after 2 hours in the acid stage and a mean of > 90% dissolved within the first 10 minutes in the buffer stage. Based on the consistent and comparable dissolution profile results between strengths and between registration batches the initial registration batch for each strength are presented as representative batches. Dissolution profile data on the 120mg pivotal clinical batches and the primary validation batches is not available. Profile testing was initially not conducted for the 120mg registration batches (b) (4) *prior to the 18 month time point. Profile testing was added to the protocol and initiated at the 18 month time point and will continue through the remainder of the study. Profile data is provided for the available 18 and 24 month time points. A summary of the available dissolution profile data (individual, mean, SD, figures) for representative 120mg and 240mg primary stability / registration batches is provided in Table 1 and Table 2, respectively. The dissolution profile data supports the proposed specification of Q* (b) (4) *at 30 minutes."*

Evaluation of response and provided dissolution profile/stability data:

A representative example of a buffer stage dissolution profile for drug product (registration batch) stored at room temperature for up to 24 months is:

(b) (4)

As stated in the Applicant's response, the provided dissolution data indicate that both 120mg and 240mg strength registration batches exhibit similar dissolution profiles with a mean of < 10% dissolved after 2 hours in the acid stage and a mean of > 90% dissolved within the first 10 minutes in the buffer stage.

Based on the provided data, the acceptance criterion for the buffer stage should be tightened to Q = (b) (4) **at 20 minutes:**

	Proposed dissolution acceptance criterion:	**Recommended dissolution acceptance criterion:**
Acid stage (2 hours)	Stage 1 (n=6): No individual value exceeds 10% dissolved. Stage 2 (n=6): Average of the 12 units is not more than 10% dissolved, and no individual unit is greater than 25% dissolved. Stage 3 (n=12): Average of the 24 units is not more than 10% dissolved, and no individual unit is greater than 25% dissolved.	Stage 1 (n=6): No individual value exceeds 10% dissolved. Stage 2 (n=6): Average of the 12 units is not more than 10% dissolved, and no individual unit is greater than 25% dissolved. Stage 3 (n=12): Average of the 24 units is not more than 10% dissolved, and no individual unit is greater than 25% dissolved
Buffer stage (after 2 hours)	Q= (b) (4) at 30 minutes	Q= (b) (4) at 20 minutes

This recommendation was communicated to the Applicant in an e-mail dated 10/15/12. The Applicant initially accepted the recommendation as is (e-mail dated 10/16/12), but then changed

their response as follows (e-mail dated 10/30/12):
"Biogen Idec agrees with the FDA's recommended acceptance criterion of Q=(b) (4) at 20 min based on the development phase data. However, there is only limited data available on commercial batches at the 20 min time point since a 30 min time point was used to release batches intended for commercial use as required by the current specification. Therefore, we propose that testing at the 20 min time point be performed under a testing protocol post approval on 30 batches in order to assess data on commercial product to ensure that the Q=(b) (4) at 20 min acceptance criterion is supported. It is Biogen Idec's expectation that the data will show that the FDA recommended acceptance criterion is appropriate but we would like to base the decision on a commercial batch dataset. Biogen Idec commits to this as a post approval commitment and will submit the data and the revised specification, if appropriate, in the Annual Report."
FDA responded by e-mail on 11/2/12 as follows:
"We agree that the Applicant can change the acceptance criterion to Q=(b) (4) at 30 minutes on an interim basis for one year. During this period, they need to collect dissolution data both at 20 and 30 minutes for all stability samples of all commercial batches to be released post approval. They need to submit these data for the Agency's evaluation to justify the Agency's recommendation to tighten the dissolution acceptance criterion to Q=(b) (4) at 20 minutes in a PAS after one year. The Applicant should submit a revised specification sheet and a revised stability protocol."
The Applicant responded as follows (e-mail dated 11/6/12):
"We appreciate very much the FDA's response on dissolution proposal. Biogen Idec agrees with the FDA's recommendation to change the acceptance criterion to Q=(b) (4) at 30 minutes post approval. We will collect dissolution data both at 20 and 30 minutes on commercial batches manufactured post approval and submit the data in a prior approval supplement after one year. As requested, Biogen Idec will amend the application with the revised dissolution specification and stability protocol by COB 11/9/2012."
FDA responded as follows (e-mail dated 11/6/12):
"We like to clarify that you should change the acceptance criterion to Q=(b) (4) at 30 minutes <u>pre-approval</u>, and then, based on additional data, tighten to Q=(b) (4) at 20 minutes if deemed appropriate, <u>post-approval</u> after one year."
The Applicant responded as follows (e-mail dated 11/7/12):
"Thank you for the clarification on dissolution proposal. Biogen Idec agrees with the FDA's recommendation to change the acceptance criterion to Q=(b) (4) at 30 minutes pre-approval. However, due to the already-completed manufacturing activities for commercial launch and the additional activities necessary to implement this change retrospectively, we kindly propose that this criterion be applied pre-approval to all commercial batches manufactured from the date of your acceptance of this response. We also commit to apply this change to all ongoing and future stability studies upon acceptance of this proposal by the Agency. The drug product batches already manufactured and released against the originally filed criterion (Q=(b) (4) at 30 min) will be deemed acceptable for commercial use. The revised criterion will be applied for these batches on stability moving forward. As requested, Biogen Idec will amend the application with the revised dissolution specification and post-approval stability protocol within 3 business days of FDA's acceptance of this response. Furthermore, a post approval supplement will be submitted with Q(b) (4) at 20 min data after one year of the NDA approval."
FDA responded as follows (e-mail dated 11/9/12):
"Distribution of drug product batches that do not meet the approved drug product specifications is not acceptable. All commercial, to-be-marketed batches need to meet the acceptance criterion

of Q= (b) (4) *at 30 minutes. However, if you have batches that do not meet this acceptance criterion at stage 1, you can retest those batches according to stage 2 and/or stage 3 testing."*

On 11/14/12, the Applicant submitted revised drug product specifications, including a buffer stage dissolution acceptance criterion of Q= (b) (4) at 30 minutes, and a revised stability protocol with a buffer stage dissolution acceptance criterion of Q= (b) (4) at 30 minutes and with a footnote stating that 20 minute dissolution data will be collected for information only.

Evaluation of response:

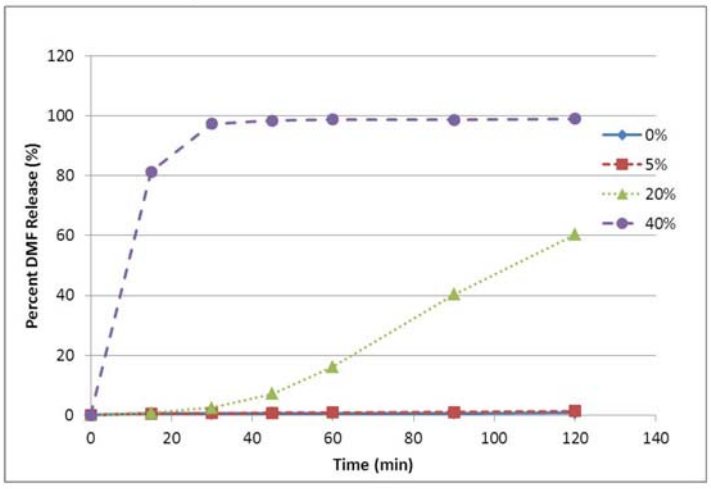
The buffer stage dissolution acceptance criterion of Q= (b) (4) at 30 minutes, with a commitment to collect and submit (as PAS) buffer stage dissolution data at 20 minutes for one year at release and on stability for all commercial batches is acceptable, based on the fast dissolution observed in the buffer stage, and based on previous regulatory actions, where the Agency has allowed other Applicants to collect additional dissolution data on their commercial batches for one year. The possibility of tightening the buffer stage dissolution acceptance criterion to Q= (b) (4) at 20 minutes will be evaluated when the PAS is reviewed. A reminder of the Applicant's commitment to study Q= (b) (4) at 20 minutes should be noted in the AP letter if the NDA is approved. This commitment is not intended to be an official post marketing commitment (PMC).

IN VITRO ALCOHOL DOSE DUMPING:

The Applicant conducted an in vitro dose dumping study using 3 batches of 120 mg strength drug product in 0.1 N HCl (acid stage) containing 0, 5%, 20%, and 40% ethanol. Representative dissolution data and profiles for drug product batch 43664 are shown here: (Similar results were obtained for the other two drug product batches (batch 43665 and 43666))

	Percent DMF Dissolved			
Time (min)	0% Ethanol	5% Ethanol	20% Ethanol	40% Ethanol
0	0.00	0.00	0.00	0.00
15	0.49	0.55	0.75	81.29
30	0.56	0.68	2.46	97.22
45	0.60	0.77	7.13	98.39
60	0.62	0.89	16.19	98.71
90	0.70	1.07	40.33	98.65
120	0.79	1.42	60.30	98.97

Figure 4: DMF Dissolution Profiles of BG00012 Capsules (Batch 43664) in Acid Stage Containing 0%, 5%, 20%, and 40% Ethanol



Evaluation: These dissolution data indicate that alcohol dose dumping is occurring in vitro. The OCP reviewer (Jagan Parepally, Ph.D.) was informed by e-mail on 7/16/12, in order to alert him of the possibility of in vivo alcohol induced dose dumping based on the provided in vitro data. This issue should be further addresses by the OCP reviewer by either request and/or review of additional in vivo alcohol dose dumping studies, or by drug product labeling. The e-mail from the Biopharmaceutics reviewer (this reviewer) to the OCP reviewer (Jagan Parepally, Ph.D.) stated: *"I am sending this e-mail to let you know that the in vitro alcohol dose dumping study for this drug product indicates that dose dumping occurs in vitro. I understand that this issue can be addressed by additional in vivo alcohol dose dumping studies, or by drug product labeling."* According to communications with the OCP reviewer, Dr. Parepally (see also his review dated 11/18/12), it was determined that there is no need for an in vivo alcohol dose dumping study or any labeling statements with regards to alcohol use.

BCS CLASSIFICATION:

Although no specific claims were made based on the BCS class, the Applicant stated in the original NDA that dimethyl fumarate is a BCS class 1 drug. The CMC lead made the following information request, which was sent to the Applicant on 5/8/12:

You state in Module 3.2.S.1.3 that dimethyl fumarate is classified as BCS classification I. Provide data to support this classification or identify the location of the data in the NDA submission.

The Applicant responded in an amendment dated 6/8/12:

As per the guidance by the FDA, there are three criteria for determining the BCS class of a drug substance (FDA Guidance for Industry, Waiver of In Vivo Bioavailability and Bioequivalence Studies for Immediate-Release Solid Oral Dosage Forms Based on a Biopharmaceutics Classification System, August 2000).

For BCS class I, the following criteria need to be met:

- *Solubility – the highest dose strength should be soluble in less than 250 mL or less in aqueous media from pH range pH 1-7.5.*
- *Permeability – the drug substance should be permeable in in vitro assay or have greater than 90% absorption in humans based on the radiolabeled mass balance study.*
- *Dissolution – the IR drug product should rapidly dissolve when no less than 85% is released in 30 min.*

The Applicant provided solubility, permeability, human absorption, and dissolution data for dimethyl fumarate.

Evaluation of response:

Biopharmaceutics, ONDQA (this reviewer): The provided dissolution and solubility data indicate that DMF has a high solubility over the pH range and exhibits a rapid dissolution.

Clinical Pharmacology, OCP: The permeability data were reviewed by Jagan Parepally, Ph.D. from OCP, and his review of the permeability data (e-mailed to the Biopharmaceutics ONDQA reviewer on 9/26/12) concluded that the current available data are inconclusive and DMF cannot be considered as a highly permeable drug (see also his review dated 11/18/12) . Since the Applicant did not make any claim based on the BCS class, the inability to classify this drug as a BCS 1 will not affect the approval of this NDA. If approved, the AP letter should include a comment to that effect.

RECOMMENDATION:

- The applicant's dissolution methodology, as summarized below is acceptable by the Agency:
  USP Apparatus II (paddle)
  Temperature: 37 °C
  Rotation speed: 100 rpm
  Acid stage for 2 hours: Dissolution medium: 500 mL 0.1 N HCl
  Buffer stage after 2 hours: Dissolution medium: 500 mL pH 6.8 phosphate buffer

- Based on the dissolution data provided, agreement was reached on the following (interim) dissolution specification:

	Proposed dissolution acceptance criterion:	**Recommended dissolution acceptance criterion:**
Acid stage (2 hours)	USP <711> for delayed release dosage forms: Stage 1 (n=6): No individual value exceeds 10% dissolved. Stage 2 (n=6): Average of the 12 units is not more than 10% dissolved, and no individual unit is greater than 25% dissolved. Stage 3 (n=12): Average of the 24 units is not more than 10% dissolved, and no individual unit is greater than 25% dissolved.	USP <711> for delayed release dosage forms: Stage 1 (n=6): No individual value exceeds 10% dissolved. Stage 2 (n=6): Average of the 12 units is not more than 10% dissolved, and no individual unit is greater than 25% dissolved. Stage 3 (n=12): Average of the 24 units is not more than 10% dissolved, and no individual unit is greater than 25% dissolved
Buffer stage (after 2 hours)	Q=(b) (4) at 30 minutes	Q=(b) (4) at 30 minutes

  The Applicant committed to collect dissolution data at 20 minutes and submit these data to FDA as a PAS one year after approval in order to determine if the buffer stage acceptance criterion can be tightened to Q=(b) (4) at 20 minutes.

- Although alcohol dose dumping was shown to occur in vitro, it was determined by the OCP reviewer that there is no need for an in vivo alcohol dose dumping study or any labeling statements with regards to alcohol use.

- No determination will be made on the BCS classification of dimethyl fumarate at this point.

From the Biopharmaceutics perspective, NDA 204063 for dimethyl fumarate delayed release capsules (120 mg/capsule and 240 mg/capsule) is recommended for **APPROVAL**. If approved, the AP letter should include the following two comments:

- We have not made a BCS classification determination for your drug, since the data provided in the NDA are inconclusive with regards to the drug's permeability.
- We are reminding you of your commitment to collect 20 minute (buffer stage) dissolution data for all stability samples of all commercial batches to be released post approval for one year in order to evaluate the possibility of tightening the buffer stage dissolution acceptance criterion to Q=(b) (4) at 20 minutes and to submit the data in a prior approval supplement (PAS) one year after approval for our review.

The commitment in the second comment is not intended to be an official post marketing commitment (PMC).

Elsbeth Chikhale, Ph.D.	**Tapash Ghosh, Ph.D.**
Biopharmaceutics Reviewer	Acting Biopharmaceutics Team Leader
Office of New Drug Quality Assessment	Office of New Drug Quality Assessment

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/s/

ELSBETH G CHIKHALE
11/19/2012

TAPASH K GHOSH
11/19/2012

CLINICAL PHARMACOLOGY REVIEW

NDA:	**204063**
Brand Name:	(b) (4)
Generic Name:	Dimethyl Fumarate (BG00012)
Dosage Form & Strength:	(b) (4) Gelatin Capsule (120 and 240 mg)
Indication:	Treatment of patients with relapsing multiple sclerosis (b) (4)
Applicant:	Biogen Idec
Submission:	505(b)(1), Standard
Submission Date:	2/27/2012
OND Division:	OND-1/Division of Neurology Drug Products
OCP Divisions:	Office of Clinical Pharmacology /DCP-1
Primary Reviewer:	Jagan Mohan Parepally, Ph.D.,
Acting Team Leader:	Xinning Yang, Ph.D.

The OCP office level briefing was held on November 13th, 2012.

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1. Executive Summary

The sponsor is seeking approval of [redacted] (dimethyl fumarate, BG00012) for the treatment of patients with relapsing multiple sclerosis (MS) [redacted] It is proposed that dimethyl fumarate (DMF) acts through activating the nuclear factor (erythroid-derived 2)-like 2 (Nrf2) transcriptional pathway, reducing inflammatory responses in both peripheral and central cells, and promoting cytoprotection of central nervous system cells. The proposed dosing regimen is starting at 120 mg twice a day and after 7 days increased to a recommended dose of 240 mg twice a day orally.

To support the approval of the application, one Phase 2 and two pivotal efficacy studies and one extension study were conducted to evaluate the efficacy and long-term safety of DMF in MS subjects. Clinical pharmacology program consists of single- and multiple-dose studies evaluating pharmacokinetics (PK) of mono-methyl fumarate (MMF), the active metabolite of DMF. DMF is not detectable in systemic circulation, since it is rapidly hydrolyzed to MMF by esterases present in gastroenteral (GI) tract, gut wall and blood. The dose proportionality, effects of food and potential for drug-drug interactions were studied based on plasma concentrations of MMF. The proposed dosing regimen is supported by a dose-response relationship from a Phase 2 dose ranging study and two Phase 3 studies.

1.1 Recommendation

The Office of Clinical Pharmacology/ Division of Clinical Pharmacology 1 (OCP/DCP-1) has reviewed the submission and finds NDA 204063 acceptable from an OCP perspective provided that an agreement is reached between the Sponsor and the Agency regarding the revised labeling language.

1.2 Phase IV Commitment

None

1.3 Summary of Important Clinical Pharmacology and Biopharmaceutics Findings

Pharmacokinetics

DMF is rapidly and completely hydrolyzed to its active metabolite MMF. DMF was not quantifiable in plasma by a HPLC-UV assay (lower limit of quantification: 100 ng/mL). Thus, all the PK analyses were performed based on plasma concentrations of MMF. The concentration-time profiles of MMF displayed high inter-individual variability. The maximum concentration (C_{max}) was more variable compared to AUC. The AUC and C_{max} of MMF increased approximately in a dose-proportional manner over a dose range of 120-360 mg following single- and multiple-administrations of DMF (twice daily/BID or thrice daily/TID).

Absorption:

Median T_{max} of MMF was about 2-2.5 hours after single-dose administration of BG00012, and was delayed by normal diet or high fat-meal. A mass-balance study showed that about 1% of the radiolabeled DMF was recovered in feces, implying that most of the administered dose had been absorbed. However, DMF was unstable in (porcine) intestinal fluid because of the presence of esterases, suggesting a potential of DMF to be degraded also in human GI tract

before absorption. Therefore, it cannot be concluded that DMF is absorbed in its intact form in humans.

Distribution:
The mean plasma protein binding of MMF was low (27-45%). The apparent volume of distribution of MMF varied across studies with mean values ranging from 53 - 73 L in healthy subjects.

Metabolism:
DMF is extensively metabolized by esterases present in GI tract, gut wall and blood before DMF reaches systemic circulation. DMF is hydrolyzed to MMF and further metabolism occurs through tricarboxylic acid (TCA) cycle. The metabolism does not involve cytochrome P450 system. The major metabolites identified in plasma were glucose, citric acid, fumaric acid, and MMF. The most abundant metabolites in urine were cysteine and N-acetylcysteine conjugates.

Elimination:
The major elimination route of DMF is exhalation as CO_2 which accounts for approximately 60% of the dose. Renal and fecal elimination are minor routes, accounting for 15.5% and 1% of the administered dose, respectively. Only trace amount (0.23% of dose) of MMF was recovered in urine.

The elimination half-life ($t_{1/2}$) of MMF was 0.5 to 1.4 hours. Thus, no accumulation was observed after multiple-dosing. The apparent clearance (CL/F) of MMF varied from 60 to 96 L/hour depending on the studies.

Dose-Response relationships:
In the Phase 2 dose ranging study, three dosing regimens (120 mg QD, 120 mg TID and 240 mg TID) along with placebo were evaluated in 257 subjects with relapsing-remitting MS. At 240 mg TID (720 mg/day) dose, there was a significant effect of DMF on MRI measurements. The lower doses, 120 mg QD and 120 mg TID (360 mg/day), did not demonstrate a statistically significant effect on any of the efficacy endpoints.

The efficacy and safety of 240 mg BID and 240 mg TID of DMF versus placebo were evaluated in two Phase 3 pivotal studies. The efficacy achieved with the two dosing regimens was comparable. Since TID dosing did not provide additional benefit, 240 mg BID was proposed as the recommended dosing regimen (details in Section 2.2.3.1).

Intrinsic factors:
Age, gender, race:
Body weight was identified as a major covariate that affected MMF exposure in MS patients. Age and gender did not have a statistically significant effect on MMF PK. Based on data from Phase 3 studies (Studies 301 and 302), age, gender, and body weight had no significant effect on the efficacy of BG00012 in MS patients. PK and efficacy of MMF have not been studied in elderly subjects.

Renal and Hepatic impairment:
Impact of renal or hepatic impairment on PK of MMF was not studied. Renal and fecal elimination are minor routes of elimination for DMF as described above. DMF is hydrolyzed to MMF and then undergoes further metabolism through TCA cycle which does not involves CYP enzymes.

Extrinsic factors:

Drug-Drug Interaction (DDI)
In Vitro studies:
MMF did not significantly inhibit CYP1A2, 2B6, 2C8, 2C9, 2C19, 2D6, 2E1, and 3A4. The IC_{50} values were greater than 50 μM. DMF did not inhibit CYP3A4 at concentrations up to 50 μM.

MMF did not induce CYP1A2, 2B6, 2C8, 2C9, 2C19, 3A4 or P-gp at its clinically relevant plasma concentrations.

MMF is not an inhibitor of P-glycoprotein (P-gp).

In Vivo studies:
Effect of co-administered drugs on BG00012:
When co-administered with BG00012 240 mg TID to healthy volunteers, single dose of Interferon (IFN) β-1a or glaterimar actetate had no effects on the PK of MMF.

When administered approximately 30 minutes before BG00012 dosing of 240 mg BID, 240 mg TID and 360 mg BID, aspirin (325 mg) had no significant effect on the PK of MMF.

Effect of BG00012 on co-administered drugs:
No studies have been conducted to evaluate the effect of BG00012 on co-administered drugs. Based on *in vitro* studies, the potential of drug-drug interaction by MMF is low.

Food Effect
After a single dose of 240 mg BG00012 following a normal diet (continental breakfast, 12 healthy subjects), there was no change in AUC and Cmax of MMF. However, Tmax of MMF was delayed from 2.25 hours to 4.5 hours.

In another study (33 healthy subjects), a high-fat meal did not affect AUC of MMF but decreased its Cmax by 40%. The T_{max} was delayed from 2.0 hours to 5.5 hours. Intake of DMF with food showed some extents of improvement in flushing (94% of subjects in fasted state compare to 68% in fed state) and GI disorders (8% of subjects in fasted state compare to 6% in fed state).

In Phase 3 studies patients were instructed to take BG00012 with food. As observed in the high-fat meal study, Cmax of MMF was 60% higher under fasted condition with earlier Tmax compared to fed state, and more subjects experienced flushing in fasted state. Though it is unknown whether there is a relationship between Cmax of MMF and incidence of flushing, a single-dose escalation study (IKP-ID33) showed dose-dependent increase of flushing in a dose range from 120 mg to 360 mg. Overall, considering the dosing instruction in the pivotal trials and the potential benefit of food intake to alleviate flushing, we recommend BG00012 be taken preferably with food.

On the other hand, it is not necessary to restrict administration of BG00012 only with food due to the following reasons: first, since PK samples were not collected in Phase 3 studies, exposure-response relationship in terms of safety and efficacy is not available. Therefore, no definite conclusion can be derived for the impact of earlier T_{max} and higher C_{max} of MMF under fasted condition on safety; secondly, the status of food intake (high-fat meal or norm diet) was

not recorded in the Phase 3 studies. AUC and Cmax of MMF were similar between fasted state and normal diet; lastly, a multiple-dose study 109HV106 documented decreasing flushing scores for BG00012-treated subjects at Day 4 compared to Day 1, suggesting that flushing side-effects were alleviated along with time.

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2. Question Based Review

2.1 General Attributes

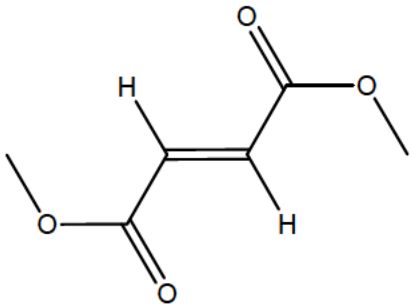
2.1.1 What are therapeutic indication(s) and the proposed mechanisms of action of [redacted]?

[redacted] (Dimethyl Fumarate) is developed for the treatment of patients with relapsing multiple sclerosis (MS) [redacted]

The presumed mechanisms of action are activation of the nuclear factor (erythroid-derived 2)-like 2 (Nrf2) transcriptional pathway, reducing inflammatory responses in both peripheral and central cells, and promoting cytoprotection of central nervous system cells against toxic oxidative insults.

2.1.2 What are the highlights of physico-chemical properties of the drug substance?

Dimethyl fumarate (DMF) is the active ingredient of [redacted] chemically known as dimethyl (E)-butenedioate or fumaric acid dimethyl ester. Its molecular formula is $C_6H_8O_4$ and the molecular weight is 144.13. DMF is a white to off-white powder that is highly soluble in water. The structure for DMF is provided in the Figure below.



2.1.3 What are the proposed dosage(s) and route(s) of administration?

The sponsor proposes that the starting dose of DMF should be 120 mg twice a day orally administered and after 7 days increased to a recommended dose of 240 mg twice a day. The available strengths of [redacted] capsules [redacted] are 120 and 240 mg.

2.2 General Clinical Pharmacology

2.2.1 What are the design features of the clinical pharmacology and clinical studies used to support dosing or claims?

The sponsor conducted 12 clinical pharmacology and biopharmaceutics studies in healthy adults and MS patients. The efficacy and safety of BG00012 was supported by one Phase 2b study, two pivotal efficacy studies and one extension study.

The clinical pharmacology and biopharmaceutics studies include:

- Study IKP/ID33, an open-label, 3-period, single-ascending-dose Phase 1 study, evaluated the PK characteristics of MMF following oral administration of 3 different dose levels of BG00012. BG00012 was administered PO to 12 healthy male subjects (N=12 per dose group). Subjects received a single dose of 120, 240, or 360 mg BG00012.
- Study 109HV101 was conducted to evaluate the potential for BG00012 to prolong the QTc interval. This was a single-center, randomized, double-blind, placebo- and active-controlled (moxifloxacin) crossover study. Fifty-four subjects were randomized to 1 of 4 treatment sequences and received, in random order, placebo, 240 mg BG00012, 360 mg BG00012, and 400 mg moxifloxacin.
- Studies FG-PK-03/04 and 109HV106, were multiple dose studies ranging from 2 days of dosing to 4 days of dosing with varied dose levels and schedules.
- Study 109MS101 was conducted to evaluate the PK after BG00012 in MS patients following administration of either 240 mg BID or 240 mg TID for one day. In addition, the effect of 5% alcohol consumption on BG00012 exposure was examined.
- Study 109HV102 was conducted to determine mass-balance and metabolic profiling following single dose ^{14}C-BG00012.
- Study 109HV103 was conducted to assess potential interaction of Avonex® (IFN β-1a) 30 µg IM when co-administered with BG00012 240 mg PO TID in healthy adult volunteers.
- Study 109HV104 was conducted to assess potential interaction of GA 20 mg SC when co- administered with BG00012 240 mg PO TID in healthy adults.
- Study 109HV106 was conducted to assess potential interaction of aspirin co-administration with BG00012 in healthy adult volunteers.
- Two studies were performed to evaluate the effect of food on BG00012 PK. Subjects in Study FG-PK-02/02 were fed a low fat diet, whereas subjects enrolled in Study C-1903 were fed a high fat diet to test for food effects.
- Study 109HV105 was a relative BA study with 240 mg (standard formulation) 240 mg (API formulation)
- Study 109HV107 was BE study between two dosage strengths i.e., 240 mg and two 120 mg formulations

The Phase 2 and Phase 3 studies in MS patients include:

- Study C-1900: a Phase 2b, randomized, double-blind, placebo-controlled, dose-ranging study in 257 subjects with relapsing-remitting MS (RRMS). In Part 1, the double-blind placebo-controlled portion of the study, subjects received BG00012 (120 mg QD, 120 mg TID, or 240 mg TID) or placebo for 24 weeks. In Part 2, the uncontrolled, dose-blinded extension portion of the study, subjects who had received placebo in Part 1 switched to BG00012 240 mg TID, while the remaining subjects continued on their same BG00012 dose regimen for an additional 24 weeks.
- Studies 109MS301 and 109MS302 were pivotal Phase 3, randomized, double-blind, placebo-controlled studies that evaluated the efficacy and safety of 2 dose regimens of BG00012 versus placebo. In Study 301, subjects were randomized in a 1:1:1 ratio to BG00012 240 mg BID, BG00012 240 mg TID, or matching placebo. In Study 302, subjects were randomized in a 1:1:1:1 ratio to BG00012 240 mg BID, BG00012 240 mg TID, BG00012-matching placebo, or glatiramer acetate (GA; Copaxone®) 20 mg SC injection QD (an active reference comparator). The duration of blinded study treatment

in both studies was to be 96 weeks, with clinic visits every 4 weeks. A total of 1237 RRMS subjects were enrolled into Study 301 and 1430 subjects into Study 302.

- Study 109MS303 is a Phase 3, randomized, dose-blind, extension study to evaluate the long-term efficacy and safety of BG00012.

2.2.3 Dose-Response

2.2.3.1. Is there any significant dose-response relationship? And does the relationship support the proposed dosing regimen?

Yes. There was a dose-efficacy relationship for DMF. In the Phase 2 study (Study C-1900), three dosing regimens (120 mg QD, 120 mg TID, or 240 mg TID) along with placebo were evaluated in 257 subjects with relapsing-remitting MS. The results showed that 240 mg TID (720 mg/day) BG00012 dose was the only effective dose on MRI measurements. The lower doses, 120 mg QD and 120 mg TID (360 mg/day), did not demonstrate a statistically significant effect on any of the efficacy endpoints.

The efficacy and safety of 240 mg BID and 240 mg TID of BG00012 versus placebo were evaluated in two Phase 3 pivotal studies 109MS301 and 109MS302. The treatment effects on primary and secondary efficacy endpoints are summarized in the table below.

Table 1. Comparison of Primary and Secondary Efficacy Endpoints of BG00012 Relative to Placebo

	Pooled Data (Studies301 and302)		
	Placebo	**BG00012 240 mg BID**	**BG00012 240 mg TID**
Number of ITT subjects (n)	771	769	761
Annualized relapse rate			
Adjusted relapse rate (95% CI)	0.371 (0.326, .423)	0.191 (0.164, 0.224)	0.191 (0.163, 0.224)
Rate Ratio (95% CI)		0.515 (0.427, 0.621)	0.515 (0.427, 0.622)
Percentage risk reduction relative to placebo (95% CI)		48.5 (37.9, 57.3)	48.5 (37.8, 57.3)
Proportion of subjects relapsed at 2 years			
Estimated proportion	0.437	0.280	0.251
Percentage reduction relative to placebo (95% CI)		42.5 (31.2, 52.0)	47.4 (36.6, 56.4)
Sustained (12-week) progression of disability at 2 years			
Estimated proportion who progressed	0.222	0.146	0.155
Percentage risk reduction relative to placebo (95%CI)		32.1 (12.1, 47.6)	30.3 (9.9, 46.0)
Number of subjects in MRI Cohort (n)	347	345	354
Number of new or newly enlarging T2 lesions over 2 years			
Adjusted mean (95% CI)	16.8 (14.0, 20.1)	3.7 (3.0, 4.4)	4.5 (3.7, 5.4)
Percentage reduction relative to placebo (95% CI)		78.2 (72.0, 83.1)	73.4 (65.8, 79.3)
Number of new T1 hypointense lesions over 2 years			
Adjusted mean (95% CI)	6.3 (5.3, 7.5)	2.2 (1.8, 2.7)	2.3 (1.9, 2.8)
Percentage reduction relative to placebo (95% CI)		65.0 (55.3, 72.5)	63.7 (53.7, 71.5)
Number of Gd-enhancing lesions at 2 years			
Mean	1.9	0.3	0.4
Percentage odds reduction relative to placebo (95% CI)		82.7 (73.1, 88.8)	69.8 (55.5, 79.5)

(Primary Endpoints: Proportion of subjects relapsed, Annualized relapse rate)

The efficacy achieved with BG00012 240 mg BID and 240 mg TID were comparable, indicating that the TID dose regimen does not provide any additional benefit over the BID dose regimen. Therefore, DMF 240 mg BID is recommended as the dose to be approved.

2.2.3.2 Does this drug prolong the QT or QTc interval?

No, BG00012 did not produce a significant QTc prolongation effect in healthy subjects who received single doses of BG00012 240 mg and 360 mg (supratherapeutic dose). The mean MMF Cmax values after doses of 240 mg and 360 mg were 2.15 μg/mL and 2.74 μg/mL, respectively. In comparison, the mean Cmax of MMF after 240 BID dosing of BG00012 in MS patients was 1.87 mg/L (Study 109MS101). See the thorough QT study review documented by Dr. Qianyu Dang for details.

2.2.4 What are the PK characteristics of the drug and its major metabolite?

DMF is rapidly and completely hydrolyzed to its active metabolite, MMF. DMF was not quantifiable in plasma by a HPLC-UV assay (lower limit of quantification: 100 ng/mL). The PK analyses were performed with plasma MMF concentrations in all clinical studies.

2.2.4.1 What are the single and multiple dose PK parameters?

PK characteristics of MMF following single- and multiple-dose administration of DMF were evaluated in several studies IKP/ID33, PK-02-02, 109HV101, 109HV103, 109HV104, C-1903, 109HV106, FGPK0304 and 109MS101.

The MMF exposure profiles displayed high inter-subject variability. The variability expressed as CV% was 33 to 67% for Cmax and around 30% for AUC. The elimination half-life ($t_{1/2}$) of MMF was 0.5 to 1.4 hours. The MMF concentration levels fell below the limit of detection by 8 to 12 hours post dose for all dose levels tested. Because of short half-life, no accumulation of MMF was observed following multiple dosing.

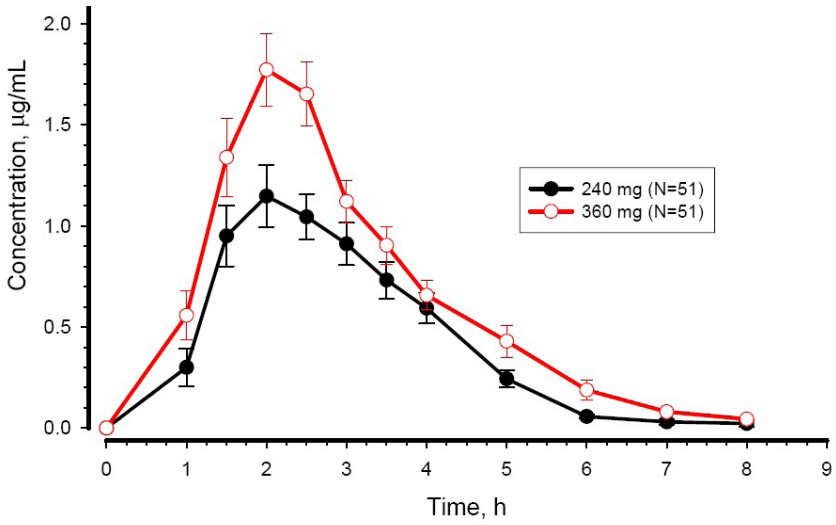
Table 2. Summary of PK parameters of MMF from two studies conducted in healthy volunteers and MS patients, respectively.

Dose	Study Subjects (N)		T_{lag} (hr)	$T_{1/2}$ (hr)	T_{max} (hr)	C_{max} (mg/L)	$AUC_{0\text{-}t}$ or $AUC_{0\text{-}24hr}$ (hr·mg/L)	AUC_{inf} (hr·mg/L)	Food Status
240 mg Single	109HV101 51 healthy	Mean	N.C.	0.57	2.50	2.15	3.35	3.37	fasted
		CV (%)	N.C.	21.1	39.5	44.2	30.1	30.0	
360 mg Single	109HV101 51 healthy	Mean	N.C.	0.63	2.00	2.74	4.96	5.00	fasted
		CV (%)	N.C.	30.2	45.8	39.1	28.6	28.6	
240 mg BID	109MS101 22 patients	Mean	1.00	1.30	5.0	1.87	8.21	N.C.	fed
		CV (%)	115	61.5	77.8	66.8	42.1	N.C.	
240 mg TID	109MS101 26 patients	Mean	0.90	1.39	7.50	2.46	12.4	N.C.	fed
		CV (%)	127	69.1	46.0	58.1	24.8	N.C.	

(Median values of Tmax are listed.)

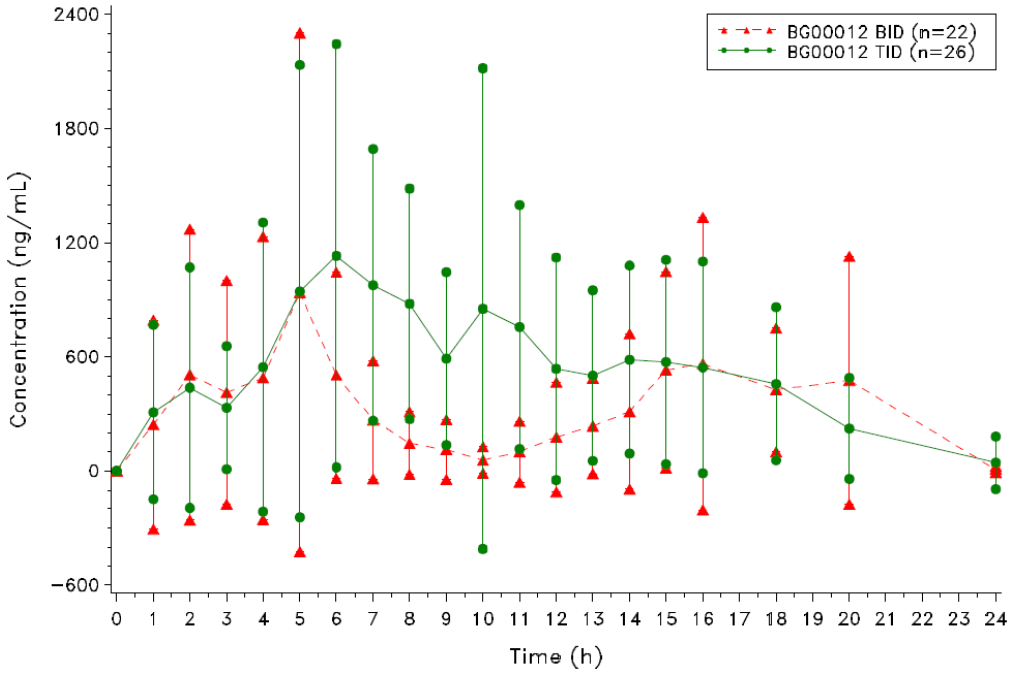
The figure below illustrates the mean plasma MMF concentrations after administration of single doses of BG00012 in healthy subjects.

Figure 1. Mean Plasma MMF ± *Standard Error* of the Mean after Administration of Single Doses of BG00012 240 mg and 360 mg, Study 109HV101



The following figure presents the PK profile of MMF when BG00012 was administered BID or TID in MS patients.

Figure 2. Mean ± Standard Deviation Concentration versus Time of MMF in Plasma, Study 109MS101



2.2.4.2 What are the characteristics of drug absorption?

There was a short lag time about 0.5 hour for MMF after administration of BG00012, (b) (4) [redacted]. Median Tmax of MMF was about 2-2.5 hours under fasting administration, whereas with food intake the Tmax was prolonged to about 5 hours.

A mass-balance study showed that about 1% of the radiolabeled BG00012 dose was recovered in feces. This may imply that most of the administered dose had been absorbed. However, DMF was unstable in (porcine) intestinal fluid because of the presence of esterases, suggesting

a potential degradation of DMF also in human GI tract before absorption (Werdenberg, D *et al.* in *Biopharm. Drug Dispos.* 2003, 24:259-273). Therefore, it cannot be concluded that DMF is absorbed in its intact form in humans.

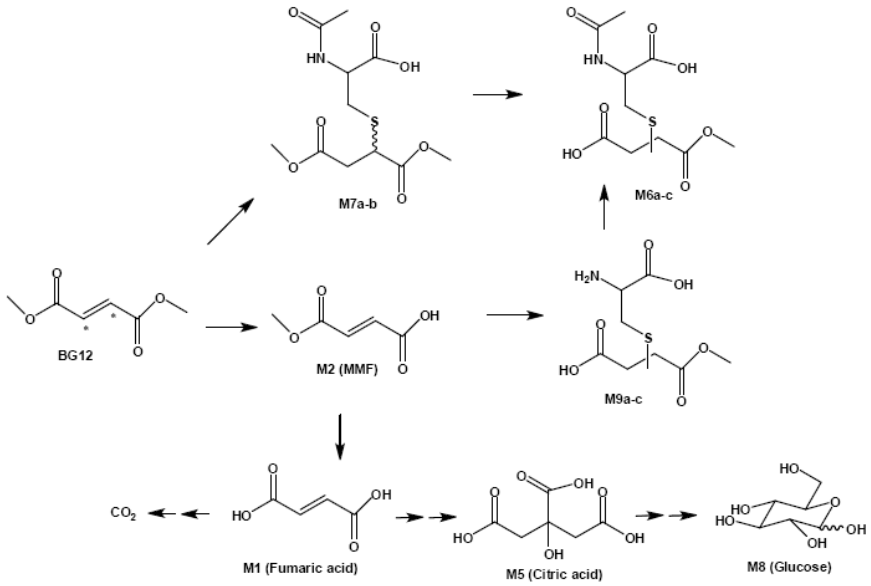
2.2.4.3 What are the characteristics of drug distribution?

The mean plasma protein binding of MMF was determined to be 27-29% across a concentration range from 9.6 to 77 µM using ultrafiltration technique, while the protein binding was higher (40-45%) in another study using equilibrium dialysis method (concentration range studied: 0.05 – 5 µM). Nonetheless, these studies indicated that MMF has low protein binding. MMF bound with human serum albumin but not to alpha l-acid glycoprotein. The blood to plasma (B/P) ratio of MMF was 0.32 – 0.62. The mean apparent volume of distribution of MMF varied from 53 to 73 L in healthy subjects depending on the studies.

2.2.4.4 What are the characteristics of drug metabolism?

BG00012 is extensively metabolized by esterases, which are present in GI tract, gut wall and blood, before DMF reaches systemic circulation. DMF is hydrolyzed to MMF and further metabolism occurs through TCA cycle. DMF and MMF metabolism does not involve cytochrome P450 (CYP) system. The major metabolites identified in plasma were MMF, fumaric acid, citric acid and glucose. MMF constitutes a small fraction of the total circulating radioactivity exposure (4.9% based on samples analyzed until 24 hours). Fumaric acid and citric acid together accounted for 27.5% of total exposure, while glucose was the predominant one (60%). The apparent clearance (CL/F) of MMF varied from 60 to 96 L/hour depending on the studies.

Figure 3. Proposed Metabolism Pathways of BG00012



2.2.4.5 What are the characteristics of drug elimination?

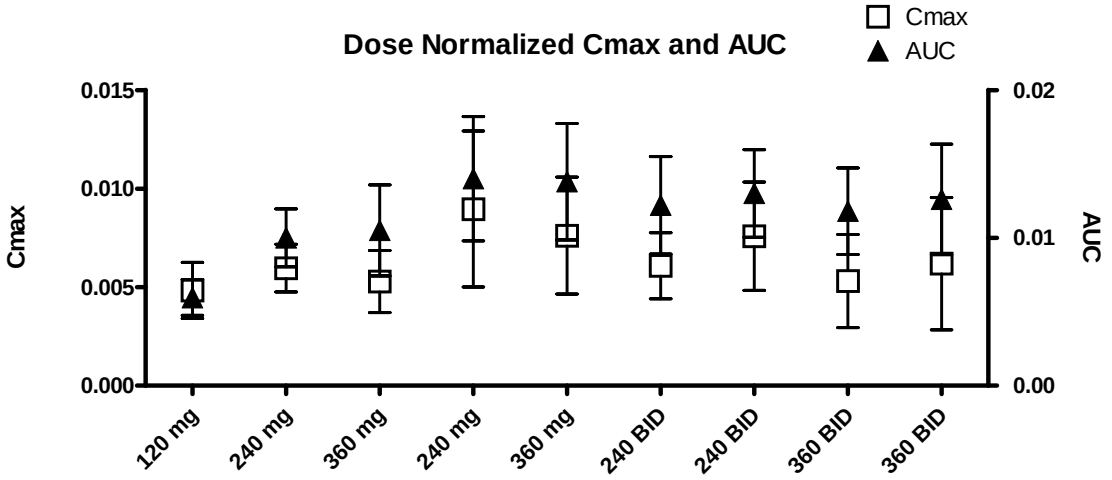
The major route of elimination of BG00012 is exhalation as CO_2 which accounted for approximately 60% of the dose. Renal and fecal elimination are minor routes of elimination,

accounting for 15.5% and 1% of the dose, respectively. Only trace amounts of DMF and MMF (0.06% and 0.23% of dose administered, respectively) were recovered in urine. The most abundant metabolites in urine were cysteine and N-acetylcysteine conjugates.

2.2.4.6 Based on MMF PK parameters, what is the degree of linearity in the dose-concentration relationship?

The figure below shows that Cmax and AUC of MMF increased approximately in a dose-proportional manner over the dose range of 120 -360 mg.

Figure 4. Dose-normalized PK parameters of MMF in single- and multiple-dose studies



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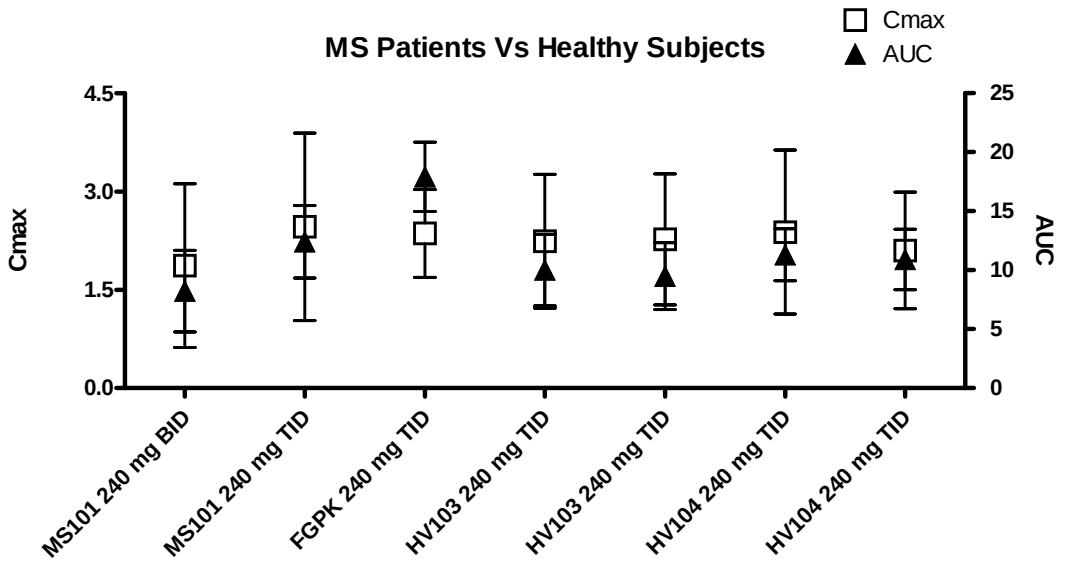
Reviewer's comment:
Dose-linearity for Cmax and AUC of MMF was also demonstrated following administration of 120 mg and 240 mg TID for 2 days (Study FG-PK-0304).

2.2.4.7 How does the PK of MMF in healthy subjects compare to that in patients?

Study 109MS101 was conducted to characterize PK of MMF in MS patients. Cmax and AUC appeared similar between MS patients and healthy subjects.

Figure 5. PK Parameters of MMF in MS Patients and Healthy Subjects



2.2.4.8 What is the inter-subject variability of PK parameters in healthy subjects and patients?

The variability of MMF parameters is moderate to high. The inter-subject variability of Cmax in healthy subjects and MS patients was 33-67%, and the variability for AUC was in the range of 20 to 40% in single- and multiple-dose studies. The inter-subject variability of T_{max} and $T_{1/2}$ was high (ranged from 20% and 80%).

2.3 Intrinsic Factors

2.3.1 What intrinsic factors influence exposure and/or response and what is the impact of any differences in exposure on efficacy or safety of BG00012?

Body weight was identified as a major covariate that affected MMF PK in MS patients (Study 109MS101). The AUC of MMF decreased by about 2% and Cmax decreased by about 1.4% with each 1 kg increase in weight (45 – 114 kg). Age (21-51 years old) and gender did not show a statistically significant effect on MMF PK after correcting for weight. However, based on data from pivotal trials (Studies 301 and 302), age (18-56 years old), gender and body weight had no significant effect on the efficacy of BG00012 in MS patients. The treatment effects in general were comparable across the subgroups based on age (<40 and ≥40 years), gender and weight (quantiles, ≤59, >59 to ≤69, >60 to ≤82, ≥82 kg) at baseline. PK and efficacy of MMF have not been studied in elderly subjects and pediatric patients.

2.3.1.1 Renal impairment

The effect of renal impairment was not assessed, since BG00012 was mainly (about 60% of dose) eliminated as CO_2 in the expired air, and only 15.5% of the dose administered was recovered in urine with trace amount of MMF (0.23% of dose). Therefore, impaired renal function is not expected to alter MMF exposure considerably.

2.3.1.2 Hepatic impairment

The effect of hepatic impairment was not assessed. DMF is pre-systemically hydrolyzed to MMF which is further metabolized by enzymes linked to the TCA cycle. Oxidative metabolism (e.g, CYP enzymes) does not contribute to the elimination of DMF and MMF. Impaired hepatic function is not expected to affect MMF exposure considerably.

2.4 Extrinsic Factors

2.4.1 Is the drug and/or the major metabolite a substrate, inhibitor or inducer of CYP enzymes on an in vitro basis?

Metabolism: The *in vitro* data indicate that DMF and MMF are not the substrates of CYP or FMO enzymes. Metabolic stability of DMF and MMF was evaluated in human hepatic microsomes and cDNA-expressed CYP2D6 or CYP3A4. Though DMF was hydrolyzed to MMF in the presence of hepatic microsomes, conversion to MMF occurred with similar degree in the absence of NAPDH, suggesting that DMF was not a substrate for CYP or FMO enzymes. MMF was essentially stable in these incubation systems (<5% decrease in concentration after 60-minute incubation).

Inhibition potential: MMF did not significantly inhibit CYP1A2, 2B6, 2C8, 2C9, 2C19, 2D6, 2E1, and 3A4 in human liver microsomes. The IC_{50} values were greater than 50 μM. In several other studies using cDNA-expressed CYPs, MMF did not exhibit any inhibition effect on the above mentioned CYP isoforms at concentrations of 50 μM. DMF did not inhibit CYP3A4 at concentrations up to 50 μM. Though DMF inhibited CYP2D6 with an IC_{50} of 27.6 μM, DMF is not detectable in systemic circulation and thus such inhibition effect is not expected to have clinical impact.

Induction potential:
MMF did not significantly induce CYP1A2, 2B6, 2C8, 2C9, 2C19, 3A4 or P-gp. MMF at concentrations up to 200 μM did not significantly induce CYP1A2, CYP2B6 or CYP3A4 measured by enzyme activities. Though MMF induced CYP2C9 and CYP2C19 in one liver donor at a concentration of 200 μM, this level is much higher than the therapeutic concentration of MMF in MS patients (Cmax around 14.4 μM after 240 mg BID dosing of DMF, Study 109MS101). In another study, MMF did not significantly induce mRNA of CYP2B6, CYP2C8 and P-gp or enzyme activity of CYP2B6 at concentrations up to 100 μM.

2.4.2 Is the drug and/or the major metabolite a substrate and/or an inhibitor of P-glycoprotein transport processes or any other transporter system?

The apparent permeability (Papp) values of DMF for transport from basal to apical and apical to basal directions in Caco2 assay were comparable, indicating that DMF may not be a substrate for P-gp. DMF showed high *in vitro* permeability in Caco2 monolayer study, with Papp values of 64.8 (apical to basolateral) and 78.7 (basolateral to apical) x 10^{-6} cm/sec (Werdenberg, D *et al.* in *Biopharm. Drug Dispos.* 2003, 24:259-273). The Papp for MMF was about 10 fold lower than that of DMF, with apical to basolateral Papp of 5.57 ± 0.71x 10^{-6} cm/sec and basolateral to apical Papp of 8.07 ± 0.77 x 10^{-6} cm/sec.

MMF did not inhibit P-gp at concentrations of 5 μM and 50 μM, and DMF did not inhibit P-gp at the concentrations of 50 μM and 500 μM. These findings were consistent with another study

showing that DMF and MMF did not affect P-gp mediated digoxin transport at concentrations up to 300 μM. In vivo significant inhibition of P-gp by DMF or MMF is not expected.

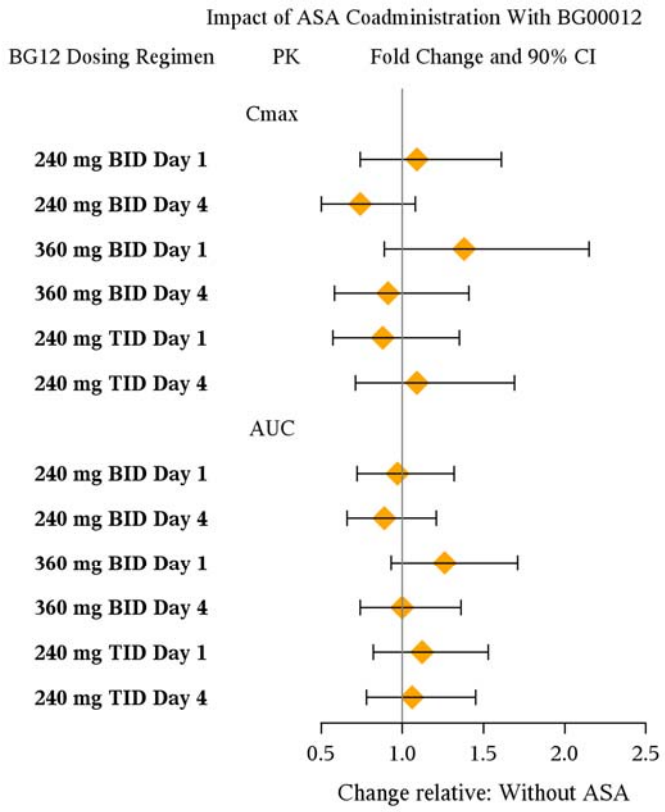
2.4.3 Are there any in vivo drug-drug interaction studies that indicate the exposure alone and/or exposure-response relationships are different when drugs are co-administered? If yes, is there a need for dosage adjustment?

2.4.3.1 Effect of co-administered drugs on BG00012

When co-administered with BG00012 240 mg TID to healthy volunteers, single dose of IFN β-1a (Avonex® 30 μg, intramuscular injection, Study 109HV103) or glaterimar actetate (Copaxone®, 20 mg, subcutaneously injection, Study 109HV104) had no effect on PK of MMF (see Figure 5).

When administered approximately 30 minutes before BG00012 dosing of 240 mg BID, 240 mg TID and 360 mg BID, oral dose of 325 mg aspirin (ASA) had no significant effect on PK of MMF as shown by the Forest plot below.

Figure 6. Effects of Aspirin co-administration on Cmax and AUC of MMF in plasma



2.4.3.2 Effect of BG00012 on co-administered drugs

No studies have been conducted to evaluate the effect of BG00012 on co-administered drugs. Based on *in vitro* findings, BG00012 has low drug-drug interaction potential.

2.5 General Biopharmaceutics

2.5.1 Based on the biopharmaceutics classification system (BCS) principles, in what class is this drug?

The Sponsor submitted information for formal BCS classification and claimed DMF as a BCS class 1 drug.

Reviewer's Comment:
DMF cannot be classified as BCS Class 1 (highly soluble and highly permeable) drug. The current available permeability data are inconclusive for DMF to be considered as highly permeable drug because of the following limitations.

- DMF was unstable in (porcine) intestinal fluid due to the presence of esterases, suggesting a potential degradation of DMF also in human GI tract before absorption. Although the fecal recovery data (less than 1% of dose) may suggest that most of the dose has been absorbed, such absorption does not necessarily reflect the fraction of dose absorbed as intact DMF.
- The Caco2 assay used to determine the *in vitro* permeability of DMF was not validated with probe substrates recommended by the BCS guidance (Werdenberg, D *et al.* in *Biopharm. Drug Dispos.* 2003, 24:259-273).

2.5.2 What is the composition of 120 mg formulation and the 240 mg formulation? Are these formulations compositionally proportional?

The following table summarizes compositions of 120 mg and 240 mg strengths. The strengths are not compositionally similar, because (b) (4)

(b) (4)

Nonetheless, the *in vitro* dissolution results showed that these two strengths had overlapping dissolution profiles. A BE study demonstrated the bioequivalence of single 240 mg BG00012 capsule to two 120 mg BG00012 capsules (see section 2.5.3). Therefore, this study confirms that there is no influence by changed excipients.

Table 3. Composition (b) (4)

Process Step	Ingredient	Function	Amount per capsule (mg) in 120 mg strength	Amount per capsule (mg) in 240 mg strength
(b) (4)	Dimethyl fumarate	Active ingredient	120.0	240.0
	Croscarmellose sodium	(b) (4)		
	Microcrystalline cellulose			
	Silicified microcrystalline cellulose			
	Magnesium stearate			
	Talc			
	Colloidal silicon dioxide			
	Subtotal			
	Methacrylic acid copolymer, Type A			

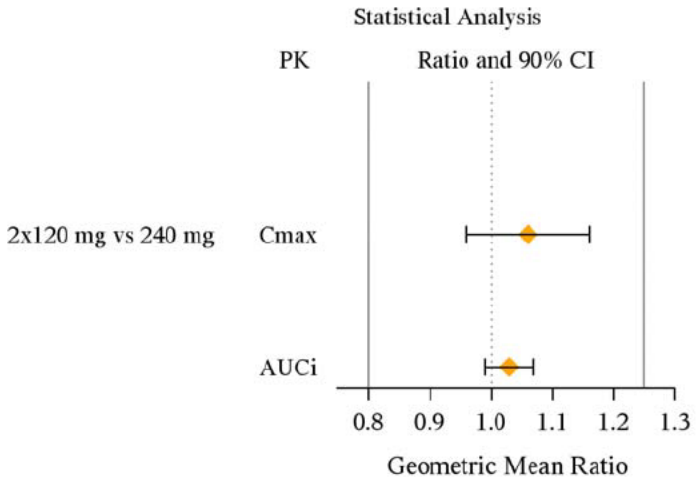
	Triethyl citrate	(b) (4)
	(b) (4)	
	Subtotal	
(b) (4)	Methacrylic acid copolymer dispersion	
	Polysorbate 80	
	Sodium lauryl sulfate	
	Triethyl citrate	
	(b) (4)	
	Simethicone	
	(b) (4)	
	Subtotal	
TOTAL		

2.5.3 What is the relative bioavailability of the proposed to-be-marketed formulation and the formulation used in clinical trials?

BG00012 drug product is formulated as (b) (4) a size 0 hard gelatin capsule. Two dosage strengths (120 mg and 240 mg) are proposed for commercial use. In all the clinical studies 120 mg strength was used, and the clinical formulation is the same as the to-be marketed formulation.

A BE study in healthy volunteers (Study 109HV107) demonstrated the bioequivalence of single 240 mg BG00012 capsule to two 120 mg BG00012 capsules (reference product).

Figure 7. Comparison of PK Parameters between 240 mg Strength and 2 x 120 mg Strength of BG00012.



Office of Scientific Investigations Audit:
At the request of Division of Neurology Products, the Office of Scientific Investigations conducted audit of the bioequivalence study (Study # 109HV107). The clinical and analytical portions of the studies were conducted at Prism Clinical Research (Saint Paul, MN) and (b) (4) respectively. Following the inspection (b) (4) no objectionable conditions were observed and Form FDA 483 was not issued. However, for Prism Clinical Research, Form 483 (Inspectional Observations) was issued. The clinical and analytical audit was based on 100% audit of source data.

OSI evaluated the Prism's response to the Form 483 and associated exhibits related to objectionable observations and recommended that the clinical and bioanalytical portions of Study 109HV107 be accepted for agency review, subject to evaluations by the OCP reviewer of MHF stability in plasma samples without detailed records of handling and preservation.

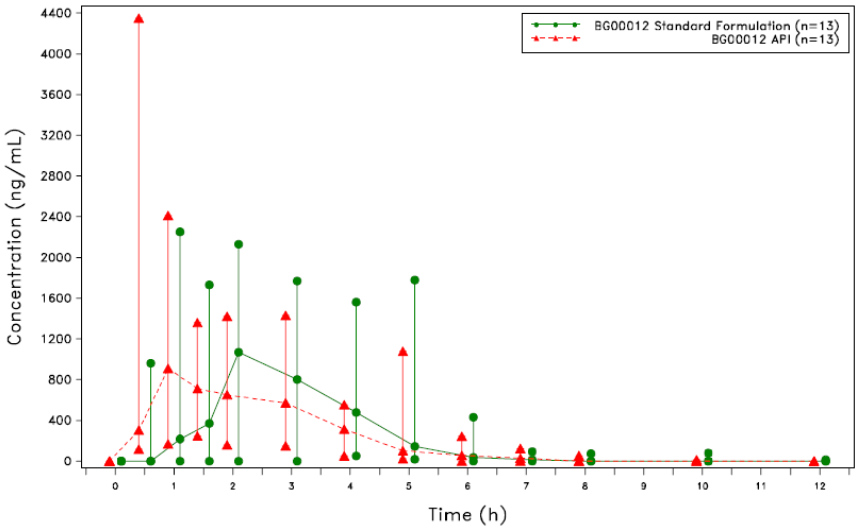
Reviewer's Comments: Methyl hydrogen fumarate (MHF) is the other name for MMF. In vitro human plasma stability studies indicate that MMF has a half-life around 70 hours.

The plasma concentration time profile of MMF (2 x 120 mg capsule group) obtained from this (Study 109HV107) was similar to the PK profiles obtained from several other PK studies (Studies 109-HV-101, FG-PK-02 and C-1903) using same dose (also 2 x 120 mg capsule) under fasting conditions. Studies C-1903 and FG-PK-02 were conducted in (b) (4) *respectively. Thus, the lack of detailed records of handling and preservation of plasma samples at the clinical site (Saint Paul, MN) for the current study did not impact the study results.*

2.5.4 What is the relative bioavailability of the BG00012 formulation and the other dosage forms/route of administrations?

The absolute bioavailability BG00012 was not determined. A relative bioavailability study (109HV105) was conducted to compare the PK profiles of BG00012 120 mg administered as active pharmaceutical ingredient (API) in a gelatin capsule (b) (4) . The PK profile of the API formulation was characterized by absence of lag time, earlier Tmax, and a lower Cmax (reduced by 30%) with respect to the standard formulation. However, the overall exposure (AUC) was similar for both products.

Figure 8. Median and Range of Plasma Concentration vs. Time for MMF after Administration of BG00012 API (Red Color) and Standard Formulation (Green Color)

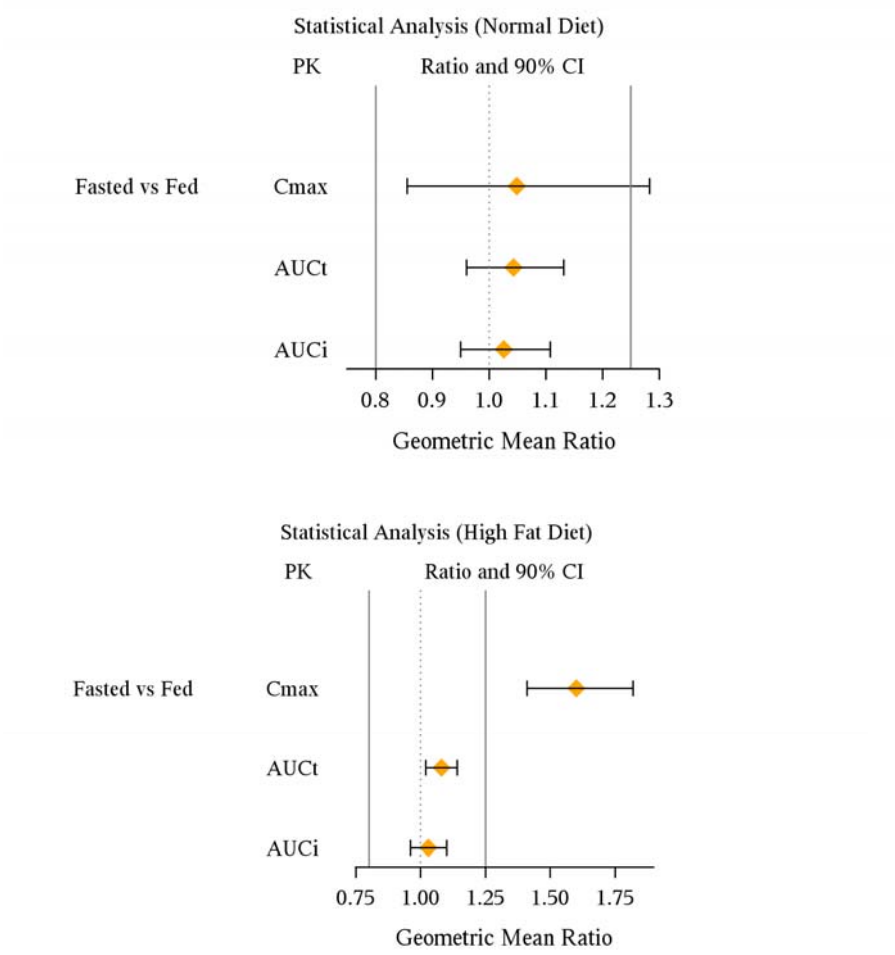


2.5.5. What is the effect of food on the bioavailability (BA) of the drug from the dosage form? What dosing recommendation should be made, if any, regarding administration of the product in relation to meals or meal types?

The effect of food on BA of BG00012 was evaluated in 2 single-dose studies with a normal diet (36% of calories derived from fat, FG-PK-02/02) and standard high-fat diet (>50% of calories derived from fat, C-1903).

A normal diet had no significant effect on AUC and C_{max} of MMF (see figure below) but delayed its T_{max} from 2.25 hours to 4.5 hours. A high-fat meal did not affect AUC of MMF but reduced its C_{max} by 40% (see figure below). The T_{max} was delayed from 2 hours to 5.5 hours bya high-fat meal.

Figure 9. Food Effect on MMF PK (Upper Panel: Normal Diet, fasted state as reference; Lower Panel: High-Fat Meal, fed state as reference)



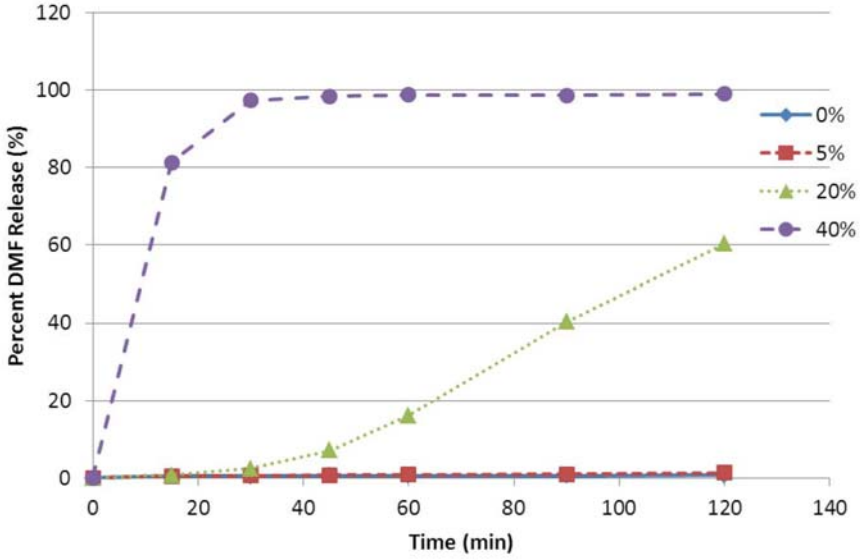
In Phase 3 studies patients were instructed to take BG00012 with food. As observed in the high-fat meal study, Cmax of MMF was 60% higher under fasted condition with earlier Tmax compared to fed state, and more subjects experienced flushing in fasted state. Though it is unknown whether there is a relationship between Cmax of MMF and incidence of flushing, a single-dose escalation study (IKP-ID33) showed dose-dependent increase of flushing in a dose range from 120 mg to 360 mg. Overall, considering the dosing instruction in the pivotal trials and the potential benefit of food intake to alleviate flushing, we recommend BG00012 be taken *preferably* with food.

On the other hand, it is not necessary to restrict administration of BG00012 only with food due to the following reasons: first, since PK samples were not collected in Phase 3 studies, exposure-response relationship in terms of safety and efficacy is not available. Therefore, no definite conclusion can be derived for the impact of earlier T_{max} and higher C_{max} of MMF under fasted condition on safety; secondly, the status of food intake (high-fat meal or norm diet) was not recorded in the Phase 3 studies. AUC and Cmax of MMF were similar between fasted state and normal diet; lastly, a multiple-dose study 109HV106 documented decreasing flushing scores for BG00012-treated subjects at Day 4 compared to Day 1, suggesting that flushing side-effects were alleviated along with time.

2.5.6. What is the effect of concomitant alcohol ingestion with BG00012 formulation on bioavailability of MMF?

In vitro dissolution studies indicated that at 5% (v:v) ethanol in the acid stage had no effect on the dissolution profile of BG00012. However, at higher alcohol concentrations (20% (v:v) and 40% (v:v) ethanol) DMF release exceeded the acceptance criterion indicating dose dumping from the formulation. Nonetheless, relative BA study indicates similar AUC and 30% lower Cmax for API formulation compared to the (b) (4) formulation (see section 2.5.4). The API formulation can be considered as the worst scenario for dose dumping caused by higher concentrations of alcohol. Thus, these results suggest that alcohol at higher concentration will have minor impact on PK profile of MMF and will not result in significant increase of Cmax of MMF.

Figure 10. DMF Dissolution Profiles of BG00012 Capsules in Acid Stage Containing 0%, 5%, 20%, and 40% Ethanol



In Study 109MS101 a subset of MS patients (4 males and 4 females) received 125 mL of wine with their evening dose of BG00012 240 mg BID or TID. Analysis of this small group of subjects did show any difference in PK parameters compared to other patients not drinking wine at the time of drug administration.

2.6 Analytical section

2.6.1 What analytical method was used to determine drug concentrations and was the analytical assay method adequately validated?

A validated LC/MS/MS method using [redacted] (b) (4) as an internal standard was used to quantitate MMF in plasma for majority of the PK studies. A similar method using ^{13}C4-MMF as an internal standard was used to analyze MMF plasma concentrations for Studies 109HV106 and 109HV107. Summary of the former bioanalytical assay is provided in the Table below.

Parameter	Analyte (MMF)
Method	LC/MS/MS
LLOQ	10 ng/mL
Linear range	10 - 5000 ng/mL
QC sample	10, 30, 500, 4000 ng/mL
Inter-day accuracy and precision	% Bias is < 3.8% for three levels and < 2.7% for LLOQ. % CV is < 6.0% for three levels and < 5.4% for LLOQ.
Intra-day accuracy and precision	% Bias is < 9.3% for three levels and < 10.6% for LLOQ. % CV is < 8.1% for three levels and < 15.7% for LLOQ.
Freeze-thaw stability	5 cycles
Benchtop stability at RT	24 hours
Long-term stability at -70 °C	155 days
Cross-validation results Intra-day accuracy and precision	% Bias is < 7.0% for three levels and < 15.0% for LLOQ. % CV is < 4.1% for three levels and < 14.5% for LLOQ.

A validated HPLC/UV method was used to quantitate MMF in plasma from Studies IKP/ID33, FAG-201-FG-PK-02-02 and FAG-201-FG-PK-03/04 using [redacted] (b) (4) as an internal standard. Summary of bioanalytical assay for MMF is provided in the Table below.

Parameter	Analyte (MMF)
Method	HPLC-UV
LLOQ	0.1 mg/L
Linear range	0.1 to 5.27 mg/L
QC sample	0.21, 2.12, 4.24 mg/L
Inter-day accuracy and precision	% Bias was 0.1 to 5.27 mg/L % CV was -5.36 to -0.54 mg/L
Intra-day accuracy and precision	% Bias was -3.37% to 1.91% % CV was 7.37% to 10.6%
Freeze-thaw stability	5 cycles
Benchtop stability at RT Autosampler stability	24 hours 72 hours
Long-term stability at -80 and 4 °C	90 days

3. Detailed Labeling Recommendations

The Office of Clinical Pharmacology has reviewed the proposed labeling for (b) (4) (dimethyl fumarate) capsules and found it acceptable provided that the recommended revisions are made to the labeling language.

Labeling recommendation to be sent to the Sponsor:
The following describes the proposed changes: the underlined text is the proposed change to the label language; the ~~Strikethrough text~~ is recommendation for deletion from the perspective of OCP.

2 DOSAGE AND ADMINISTRATION

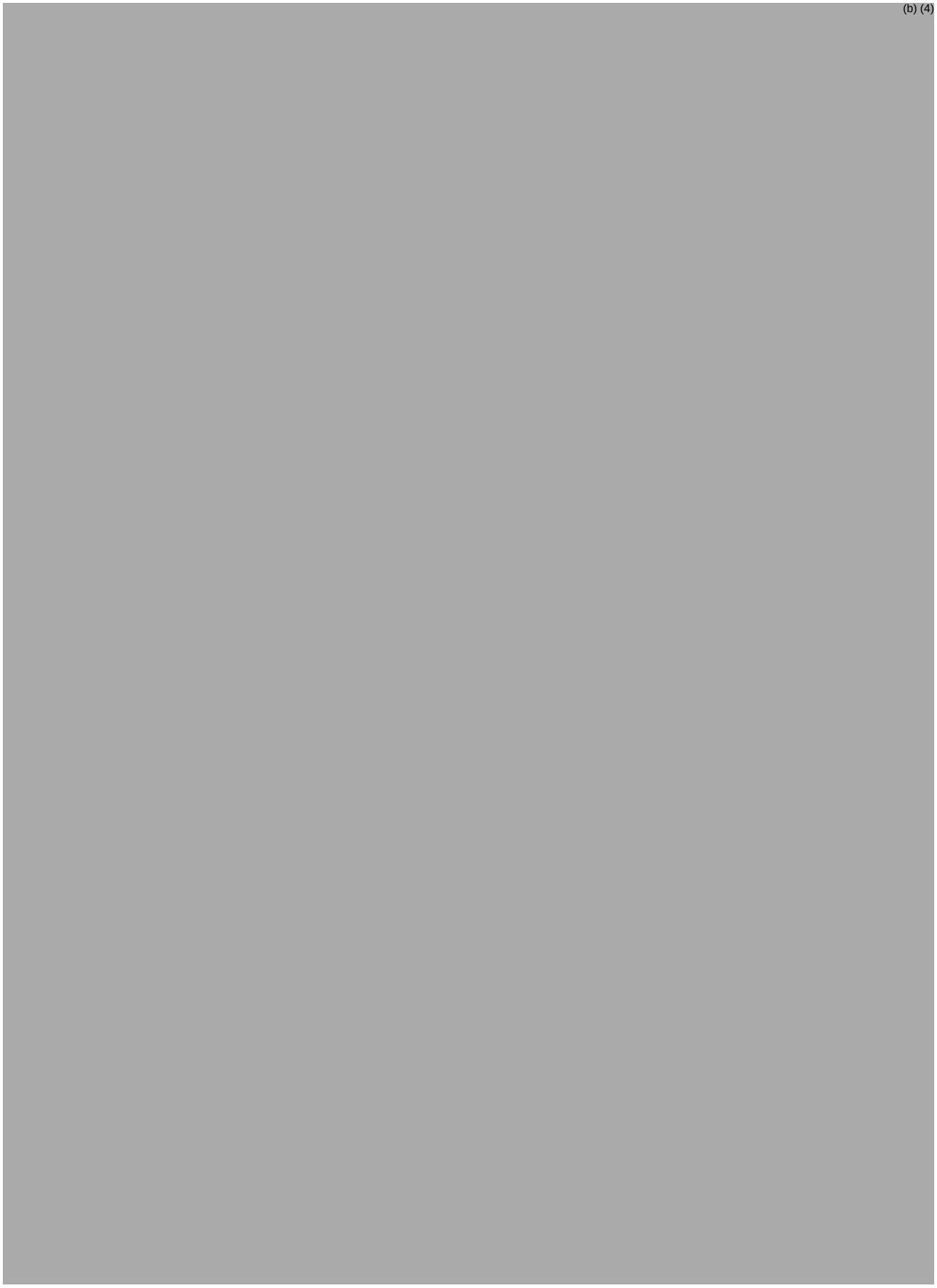
2.1 Dosing Information

The starting dose for TRADENAME is 120 mg twice a day orally. After 7 days, increase to the recommended dose of 240 mg twice a day orally. TRADENAME should be swallowed whole and intact. Do not crush, chew, or sprinkle capsule contents on food. TRADENAME can be taken with or without food. Administration with food may reduce the incidence of flushing [See *Clinical Pharmacology* (12.3)].

(b) (4)

12.3 Pharmacokinetics

(b) (4)


(b) (4)

4. Appendices

4.1 Individual Study Reviews

IKP-ID33: Open four treatment, four period , single ascending dose study to evaluate the pharmacokinetic characteristics especially the dose linearity of dimethyl fumarate following per oral administration of four different dose levels together with a continental breakfast in n=12 healthy male subjects.

Objective:

To evaluate pharmacokinetic (PK) characteristics, especially the dose linearity, of dimethyl fumarate (DMF) following single dose administrations of 120 mg, 240 mg, 360 mg, and 480 mg respectively, administered together with a continental breakfast.

<table>
<tr><td>Study Design</td><td>The study design was an open-label, single-ascending-dose study, evaluated the PK characteristics of monomethyl fumarate (MMF) following oral administration of 4 different dose levels of BG00012. Wash-out period between the administrations was one week.</td></tr>
<tr><td>Study Population</td><td>Healthy male
Age: 18-40 years
BMI: 18-28 kg/m^2
15 subjects included. 12 subjects analyzed for PK per dose group.</td></tr>
<tr><td>Treatment Groups</td><td>Cohort 1: 120 mg DMF
Cohort 2: 240 mg DMF
Cohort 3: 360 mg DMF
Cohort 4: 480 mg DMF
Administered with continental breakfast.

Note: After the review of safety profiles, particularly flushing, the sponsor decided not to proceed with 480 mg dose group.</td></tr>
<tr><td>Dosage and Administration</td><td>The study drug was administered in the multiples of 120 mg DMF (b) (4)</td></tr>
<tr><td>Sampling: Blood</td><td>Blood samples (5 mL) were obtained during each study period at the following times: predose, 1, 1.5, 1.75, 2, 2.5, 3, 3.5, 4, 4.5, 5, 6, 7, and 9 hours postdose.</td></tr>
<tr><td>Analysis</td><td>Two different assays were utilized for sample measurement; a simultaneous HPLC-UV assay for determination of MMF and fumaric acid and a HPLC-UV for determination of DMF concentrations.

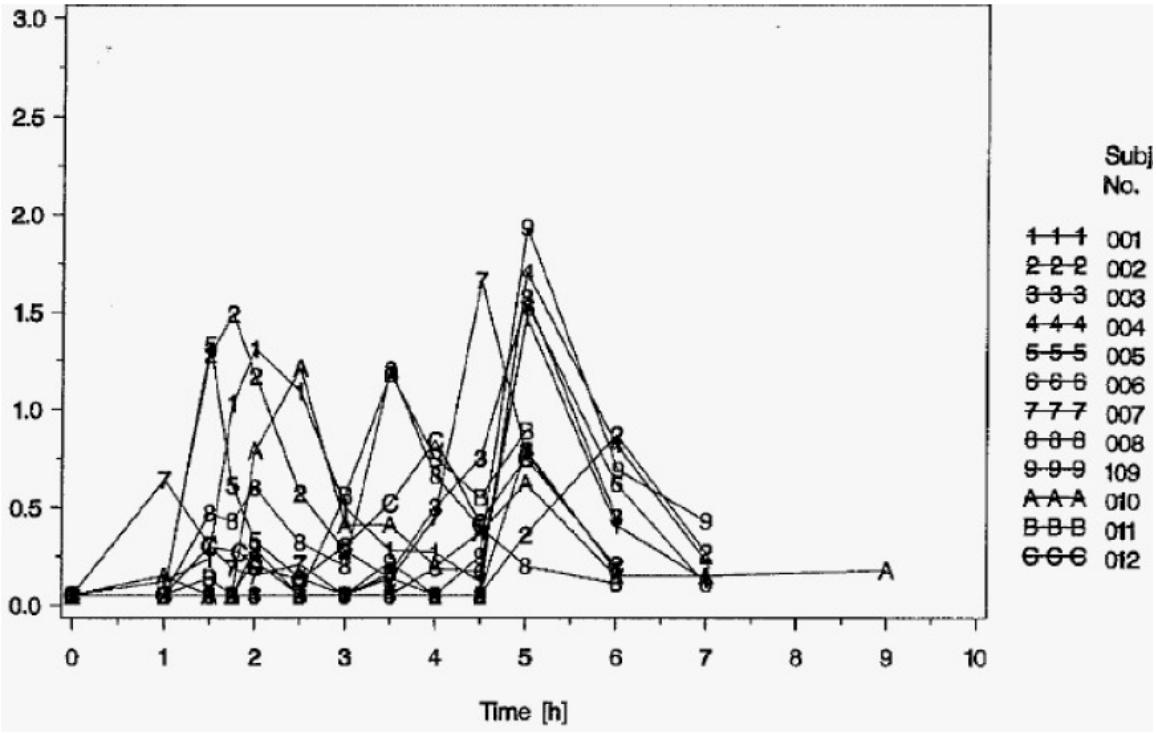
MMF: (b) (4) was used as an internal standard.
<table>
<tr><th>Parameter</th><th>Quality Control Samples</th><th>Standard Curve Samples</th></tr>
<tr><td>Quality Control or Standard Curve Concentration (mg/L)</td><td>0.21, 2.12, and 4.24</td><td>0.1, 0.18, 0.42, 1.05, 3.35, 5.27</td></tr>
<tr><td>Between Batch Precision (%CV)</td><td>6.46 to 7.29</td><td>4.79 to 9.11</td></tr>
<tr><td>Between Batch Accuracy (%RE)</td><td>-4.09 to -0.01</td><td>-1.56 to 1.06</td></tr>
<tr><td>Linearity</td><td colspan="2">Weighted linear equation (1/X^2), mean r= 0.996</td></tr>
<tr><td>Linear Range (mg/L)</td><td colspan="2">0.1 to 5.27</td></tr>
<tr><td>Sensitivity (LLOQ, mg/L)</td><td colspan="2">0.1</td></tr>
</table>
</td></tr>
</table>

<table>
<tr><td></td><td>
DMF: [redacted] (b) (4) was used as an internal standard.
<table>
<tr><th>Parameter</th><th>Quality Control Samples</th><th>Standard Curve Samples</th></tr>
<tr><td>Quality Control or Standard Curve Concentration (mg/L)</td><td>0.29, 3.01, and 4.78</td><td>0.1, 0.25, 0.82, 1.57, 3.14, 5.04</td></tr>
<tr><td>Between Batch Precision (%CV)</td><td>8.57 to 9.12</td><td>5.32 to 8.16</td></tr>
<tr><td>Between Batch Accuracy (%RE)</td><td>-4.26 to 1.74</td><td>-3.30 to 0.61</td></tr>
<tr><td>Linearity</td><td colspan="2">Weighted linear equation ($1/X^2$), mean r= 0.994</td></tr>
<tr><td>Linear Range (mg/L)</td><td colspan="2">0.1 to 5.04</td></tr>
<tr><td>Sensitivity (LLOQ, mg/L)</td><td colspan="2">0.1</td></tr>
</table>
Fumaric Acid: [redacted] (b) (4) was used as an internal standard.
<table>
<tr><th>Parameter</th><th>Quality Control Samples</th><th>Standard Curve Samples</th></tr>
<tr><td>Quality Control or Standard Curve Concentration (mg/L)</td><td>0.37, 2.21, and 4.26</td><td>0.27, 0.36, 0.60, 1.25, 3.51, 5.56</td></tr>
<tr><td>Between Batch Precision (%CV)</td><td>5.68 to 7.40</td><td>3.98 to 8.19</td></tr>
<tr><td>Between Batch Accuracy (%RE)</td><td>1.43 to 3.02</td><td>-4.05 to 2.14</td></tr>
<tr><td>Linearity</td><td colspan="2">Weighted linear equation ($1/X^2$), mean r= 0.992</td></tr>
<tr><td>Linear Range (mg/L)</td><td colspan="2">0.27 to 5.56</td></tr>
<tr><td>Sensitivity (LLOQ, mg/L)</td><td colspan="2">0.27</td></tr>
</table>
The endogenous concentration of fumaric acid quantificated in the human plasma pool used for preparation of calibration standards was found to be 0.17 mg/L.
</td></tr>
<tr><td>PK Assessments</td><td>The pharmacokinetic parameters C_{max}, T_{max}, AUC_{0-t}, $AUC_{(0-inf)}$, t_{lag} and $t_{1/2}$ were calculated from the plasma MMF concentration-time data using noncompartmental analysis.</td></tr>
</table>

RESULTS:

After a single oral dose of BG00012, no parent drug (DMF) or fumaric acid was detected above the LLOQ in plasma. The active metabolite, MMF, showed highly variable concentration-time profiles.

Figure. Plasma MMF Concentration (mg/L) by Subject and Time Following BG00012 240 mg Administration in Study IKP/ID33.



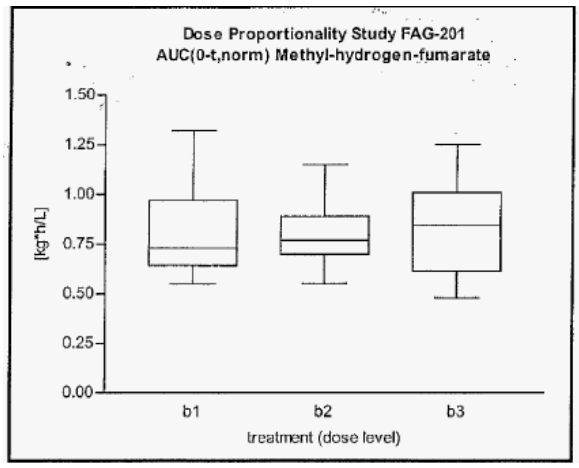
The following table summarizes PK parameters of MMF when single doses of 120 mg, 240 mg and 360 mg BG00012 were administered.

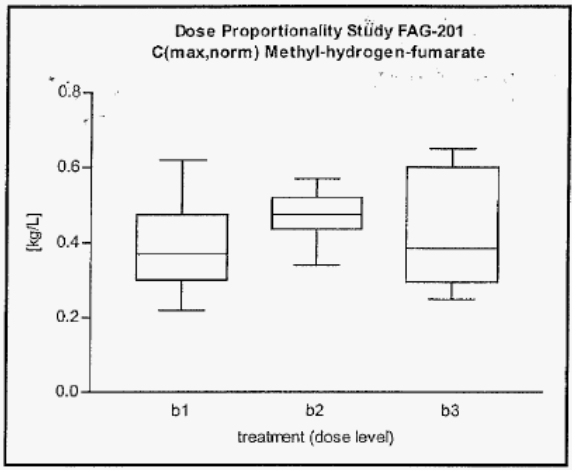
Table. Summary of MMF Pharmacokinetic Parameters in Study IKP/ID33

PK Parameters [Unit] Analyte MMF	Dose Groups (n=12)		
	120 mg	240 mg	360 mg
AUC_{0-t} [mg*h/L]			
Geometric mean (SD)	1.17 (1.34)	2.35 (1.26)	3.62 (1.36)
Median	1.21	2.16	3.85
C_{max} [mg/L]			
Geometric mean (SD)	0.56 (1.31)	1.40 (1.25)	1.82 (1.37)
Median	0.55	1.48	1.88
T_{lag} [h]			
Geometric mean (SD)	2.54 (1.45)	2.04 (1.34)	2.34 (1.23)
Median	2.00	1.50	2.00
T_{max} [h]			
Geometric mean (SD)	4.23 (1.52)	3.86 (1.32)	4.67 (1.13)
Median	4.75	4.25	4.75

Reviewer's Comment: The HPLC assay used to quantitate plasma MMF concentration is less sensitive compared to the LC-MS/MS method used in majority of the PK studies. The LLOQ (0.1 mg/L) of the HPLC assay is close to Cmax of MMF at lower dose (120 mg).

The figures below illustrates box and whisker plots of dose-normalized AUC (left) and Cmax (right) of MMF in this study. It should be noted that the doses used here refer to body weight-adjusted doses (expressed as mg/kg).





Dose proportionality of the AUC and Cmax was tested by comparing the dose per body weight-normalized MMF exposure using ANOVA. Point estimate with 90% confidence intervals (90% CI) was calculated for the ratio of exposure between each pair of doses (see table below).

Tale. Results of Statistical Analysis

PK Parameters (normalized by dose per body weight)	**Ratio**	**Point Estimate (%)**	**90% Confidence Intervals (%)**
AUC_{0-t} (Kg*h/L)	D1/D2	99.11	84.60-116.11
	D1/D3	97.69	83.39-114.45
	D2/D3	95.57	84.13-115.48
Cmax (Kg/L)	D1/D2	80.71	65.83-98.93
	D1/D3	93.91	76.61-115.11
	D2/D3	116.36	94.93-142.62

D1: 120 mg, D2: 240 mg, D3 360 mg

Safety:
The most frequently reported AE was flushing. Four subjects in 120 mg dose group, nine subjects in 240 mg dose group and eleven subjects in 360 mg dose group experienced flushing. Based on these dose-dependent flushing symptoms, the sponsor decided not to proceed with the next dose of 480 mg BG00012 as planned in the protocol, and discontinued the study after the third treatment period (360 mg dose group).

CONCLUSIONS:

1. The peak (Cmax) and overall exposure (AUC) of MMF increased in a dose-proportional manner characterized by high variability.

2. There was a dose-dependent increase for the incidence of flushing.

109-HV-101: A Single-Center, Randomized, Blinded, Placebo- and Active-Controlled Study to Evaluate the QTc Interval Prolongation Potential of BG00012 When Administered to Healthy Volunteers

Objectives:

To evaluate whether BG00012 prolongs the QTc interval when administered to healthy volunteers. The primary endpoint of this study was the time-matched differences between BG00012 and the placebo with respect to change from baseline QTc value.
To evaluate the safety and tolerability of BG00012.
To estimate the pharmacokinetic (PK) parameters of single doses of BG00012.

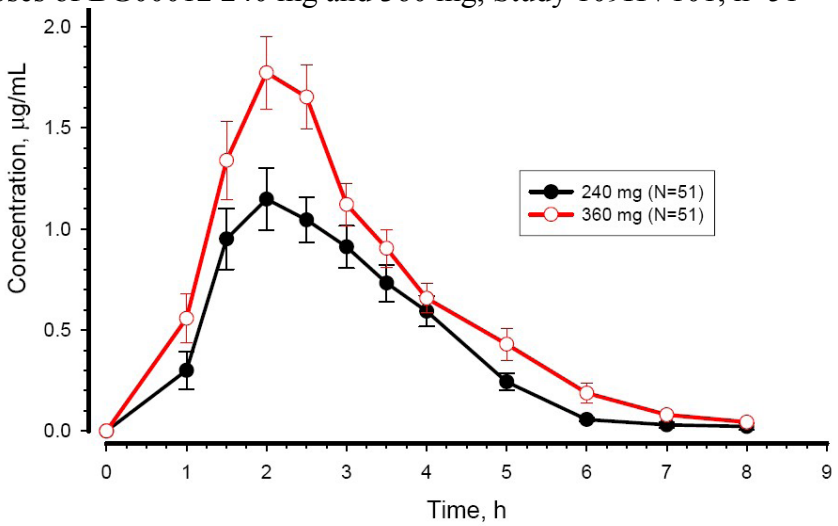
<table>
<tr><td>Study Design</td><td>The study design was single-center, randomized, blinded, placebo- and active-controlled, four-way, crossover study</td></tr>
<tr><td>Study Population</td><td>Healthy male and female
Age: 18-45 years; BMI: 19-30 kg/m^2
54 subjects were analyzed.</td></tr>
<tr><td>Treatment Groups</td><td>
<table>
<tr><th>Treatment Group</th><th>Study Treatment and Dose</th><th>Total Dose</th></tr>
<tr><td>SD1</td><td>240 mg of BG00012</td><td>2 capsules of 120 mg of BG00012,
1 capsule of placebo</td></tr>
<tr><td>SD2</td><td>360 mg of BG00012</td><td>3 capsules of 120 mg of BG00012</td></tr>
<tr><td>PBO</td><td>Placebo for BG00012</td><td>3 capsules of placebo</td></tr>
<tr><td>AC</td><td>400 mg of moxifloxacin</td><td>1 tablet of 400 mg of moxifloxacin</td></tr>
</table>
</td></tr>
<tr><td>Dosage and Administration</td><td>Subjects were randomized to 1 of 4 treatment sequence groups stratified by sex. Each subject received a single dose of 240 mg of BG00012 (study drug; SD1), 360 mg of BG00012 (SD2), placebo for BG00012 (PBO), and 400 mg of moxifloxacin (active control; AC) in 4 separate treatment periods under fasting conditions. Each treatment period was separated by 7 to 12 days.</td></tr>
<tr><td>Sampling</td><td>Blood samples (5 mL) were obtained during each study period at the following times: at predose (-1 hour), 1, 1.5, 2, 2.5, 3, 3.5, 4, 5, 6, 7, and 8 hours after dosing for measurements of MMF concentrations.

To exclude the potential risk of methanol and formic acid exposure that might occur through the metabolism of DMF, blood samples were taken before and 2 hours after administration of BG00012 and were evaluated for methanol and formic acid.</td></tr>
<tr><td>Analysis</td><td>The plasma samples were analyzed for the concentration of MMF by using LC-MS/MS method. The lower limit of quantification (LLOQ) was 10 ng/mL for MMF.
<table>
<tr><th>Parameter</th><th>Quality Control Samples</th><th>Standard Curve Samples</th></tr>
<tr><td>Quality Control or Standard Curve Concentration (ng/mL)</td><td>30, 500, and 4000</td><td>10, 25, 50, 150, 500, 2000, 5000</td></tr>
<tr><td>Between Batch Precision (%CV)</td><td>4.8 to 10.2</td><td>4.8 to 10.2</td></tr>
<tr><td>Between Batch Accuracy (%RE)</td><td>-7.0 to 3.3</td><td>-7.0 to 3.3</td></tr>
<tr><td>Linearity</td><td colspan="2">Weighted linear equation (1/X^2), mean r= 0.988</td></tr>
<tr><td>Linear Range (ng/mL)</td><td colspan="2">10 to 5000</td></tr>
<tr><td>Sensitivity (LLOQ, ng/mL)</td><td colspan="2">10</td></tr>
</table>
</td></tr>
</table>

PK Assessments	The PK parameters C_{max}, T_{max}, $AUC_{0\text{-}t}$, $AUC_{(0\text{-}inf)}$, $t_{1/2}$, apparent clearance (CL/F) and volume of distribution (Vd/F) were calculated using noncompartmental analysis.
Safety Assessments	Physical examination (including vital signs), body weight, 12-lead ECG, adverse event monitoring, hematology, blood chemistry, urinalysis, pregnancy testing, and concomitant medications.

RESULTS:
The figure below illustrates the mean plasma MMF concentrations for each of the two treatments in this study.

Figure: Mean Plasma MMF ± *Standard Error* of the Mean after Administration of Single Doses of BG00012 240 mg and 360 mg, Study 109HV101, n=51



The following table summarizes PK parameters of MMF after administration of single doses of BG00012 240 mg and 360 mg.

Table: Summary of MMF Pharmacokinetic Parameters in Study 109HV101, n=51

	BG00012 240 mg	BG00012 360 mg
AUC_{inf} (h*µg/mL)		
Mean (SD)	3.371 (1.0109)	5.006 (1.4295)
Median	3.340	5.185
C_{max} (µg/mL)		
Mean (SD)	2.153 (0.9498)	2.740 (1.0662)
Median	1.910	2.780
AUC_{last} (h*µg/mL)		
Mean (SD)	3.354 (1.0095)	4.960 (1.4203)
Median	3.330	5.165
T_{max} (h)		
Mean (SD)	2.56 (1.013)	2.60 (1.192)
Median	2.50	2.00
$T_{½}$ (h)		
Mean (SD)	0.5742 (0.12109)	0.6311 (0.19088)
Median	0.5550	0.5780
Apparent Clearance (L/h)		
Mean (SD)	78.39 (26.871)	80.06 (32.274)
Median	71.90	69.50
Apparent Volume of Distribution (L)		
Mean (SD)	64.07 (23.870)	72.69 (43.521)
Median	59.80	58.90

After administration of 240 mg or 360 mg BG00012, increase of MMF Cmax was slightly less than dose-proportional, while its AUC increased dose proportionally.

Methanol was not measurable in any sample. The majority of study subjects had no measurable formic acid concentrations either before or after dosing. Formic acid did not increase after administration of 240 mg or 360 mg BG00012.

Pharmacokinetic/Pharmacodynamic Analyses

PK/PD analysis was conducted using the plasma MMF concentrations and QTcI changes from baseline. BG00012 did not produce a significant QTc prolongation effect in healthy subjects who received BG00012 240 mg and 360 mg (supratherapeutic dose). The QTc data with respect to MMF concentration will be reviewed as a part of QT-IRT review.

CONCLUSIONS:

- The increase in MMF overall exposure (AUC) from 240 mg to 360 mg was dose-proportional, while increase of Cmax was slightly less than dose-proportional..
- The apparent clearance, T_{max} and $T_{½}$ values were similar between the two doses.
- BG00012 did not produce a significant QTc prolongation effect in healthy subjects who received BG00012 single doses of 240 mg and 360 mg
- Formic acid did not increase after administration of 240 mg or 360 mg BG00012.

FG-PK-0304: A Phase 1, open-label, two-period trial to investigate the pharmacokinetic characteristics of FAG-201 after multiple oral dosing in healthy, male, Caucasian subjects

Objectives:
To determine the pharmacokinetics of mono-methyl fumarate (MMF, the active metabolite of dimethyl fumarate) and fumaric acid by estimation of PK parameters from plasma concentrations.

<table>
<tr><td>Study Design</td><td>The study was a Phase 1, open-label, two-period trial with ascending dose design trial to investigate the effect of multiple oral dosing on the PK characteristics of BG00012.</td></tr>
<tr><td>Study Population</td><td>Healthy male and female
Age: 18-45 years
BMI: 19-30 kg/m^2
18 subjects were analyzed.</td></tr>
<tr><td>Treatment Groups</td><td>Treatment A: 120 mg TID, 3 x 1 capsule (120 mg) of FAG-201 on Day 1 and Day 2;
Treatment B: 240 mg TID, the subjects received 3 x 2 capsules (120 mg) of FAG-201 on Day 1 and Day 2.
Each subject received 6 doses of BG00012 within 2 Days at times 0, 4h, 10h, 24h, 28h and 34h.

Food was given approximately 30 minutes before the drug administration (see below). Between the two treatment periods, there was a wash-out period of at least 7 days.

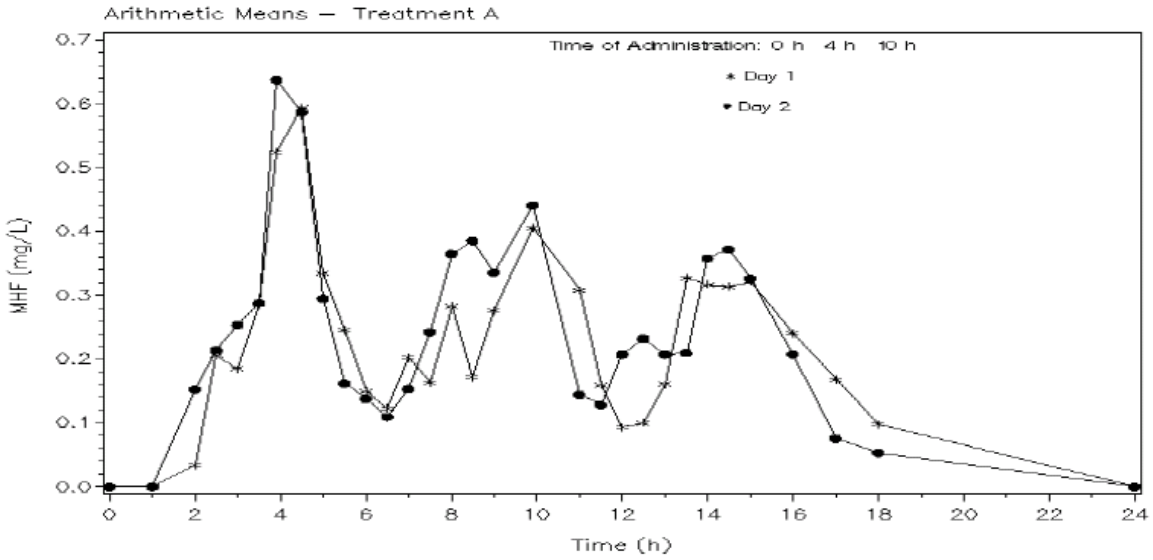
Continental Breakfast
Approximately 30 minutes prior to the first administration in the morning, a standardized continental breakfast (about 2900 – 3300 KJ) was served on Day 1 and on Day 2.

Other Standardized Meals
All subjects received a light lunch (about 2800 – 3100 KJ) approximately 30 minutes prior to the second administration and a standard dinner (about 2400 – 2600 KJ) approximately 30 minutes prior to the third administration on Day 1 and on Day 2.</td></tr>
<tr><td>Sampling</td><td>Blood samples (4.5 mL) were obtained during each study period at the following times on Day 1 and Day 2: predose, 1, 2.5, 3, 3.5, predose, 4.5, 5.5, 6, 6.5, 7, 7.5, 8, 8.5, 9, 9.5, predose, 10, 11, 11.5, 12, 12.5, 13, 13.5, 14, 14.5, 15, 16, 17, 18 hours</td></tr>
<tr><td>Analysis</td><td>The plasma samples were analyzed for the concentration of MMF by using HPLC method. The LLOQ was 0.1 mg/L.
<table>
<tr><th>Parameter</th><th>Quality Control Samples</th><th>Standard Curve Samples</th></tr>
<tr><td>Quality Control or Standard Curve Concentration (mg/L)</td><td>0.26, 2.45, and 4.32</td><td>0.1, 0.27, 0.57, 1.25, 2.38, 3.60, 5.26</td></tr>
<tr><td>Between Batch Precision (%CV)</td><td>6.20 to 18.36</td><td>4.59 to 7.14</td></tr>
<tr><td>Between Batch Accuracy (%RE)</td><td>0.19 to 4.18</td><td>-1.81 to 2.32</td></tr>
<tr><td>Linearity</td><td colspan="2">Weighted linear equation (1/X^2), mean r= 0.996</td></tr>
</table></td></tr>
</table>

	Linear Range (mg/L)	0.1 to 5.26
	Sensitivity (LLOQ, mg/L)	0.1
PK Assessments	The PK parameters C_{max}, T_{max}, AUC_{0-t}, $AUC_{(0-inf)}$, and $t_{1/2}$ were calculated from the plasma MMF concentration-time data using noncompartmental analysis.	
Safety Assessments	Physical examination (including vital signs), body weight, 12-lead ECG, adverse event monitoring, hematology, blood chemistry, urinalysis, pregnancy testing, and concomitant medications.	

RESULTS:
The figure below illustrates the mean plasma MMF concentrations on Day 1 and Day 2 following 120 mg TID dose.

Figure: Mean Plasma Concentration of MMF for Treatment A (BG00012 120 mg TID for 2 Days)



The table below summarizes the mean PK parameters of MMF on Day 1 and Day 2 following 120 mg TID dose. There was high variability for AUC and Cmax.

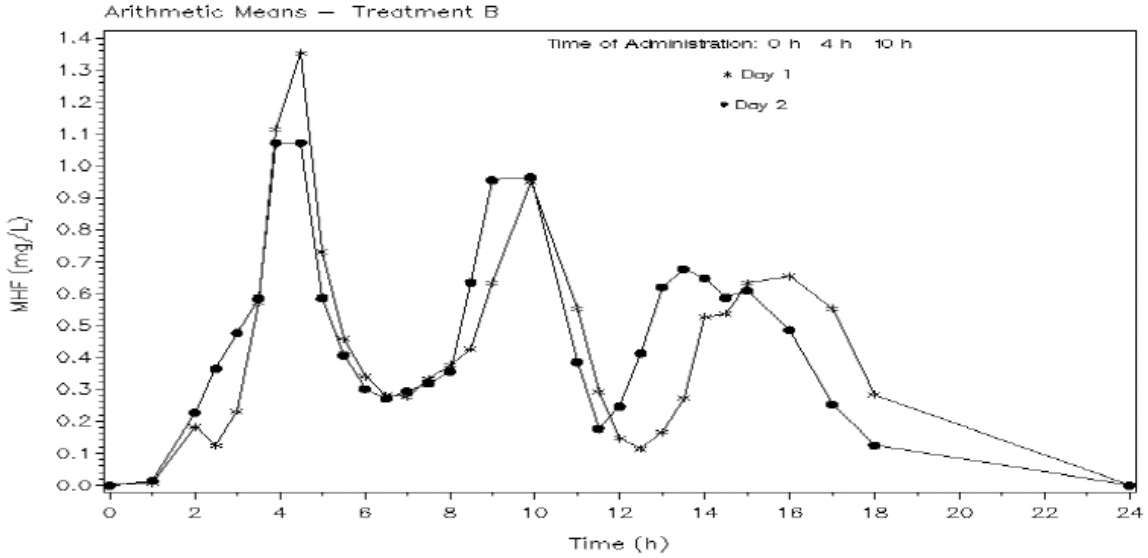
Table: PK Parameters of MMF after administration of BG00012 120 mg TID for 2 Days, Study FG-PK-03/04

Day Treatment Administration (Time After Dose)	Pharmacokinetic Parameter [Unit]			
	AUC_{0-t} [mg*h/L]	C_{max} [mg/L]	T_{max} [h]	$T_{max-admin}$ [h][2]
Day 1				
Administration 1 (0 hours)				
Mean[1]	0.200	0.524	3.709	3.709
SD[1]	8.543	2.970	0.490	0.490
Administration 2 (+4 hours)				
Mean[1]	1.506	0.970	6.129	2.129
SD[1]	1.712	1.455	2.327	2.327
Administration 3 (+10 hours)				
Mean[1]	1.766	0.614	13.112	3.112
SD[1]	2.460	1.733	2.413	2.413
Day 2				
Administration 4 (0 hours)				
Mean[1]	0.355	0.693	27.682	3.682
SD[1]	5.117	2.135	0.581	0.581
Administration 5 (+4 hours)				
Mean[1]	1.775	0.989	30.404	2.404
SD[1]	1.365	1.421	2.370	2.370
Administration 6 (+10 hours)				
Mean[1]	1.787	0.781	36.722	2.722
SD[1]	1.398	1.507	1.849	1.839
Days 1 and 2				
Administrations 1 to 6				—
Mean[1]	8.549	1.293	16.359	
SD[1]	1.249	1.310	13.220	

[1]. Geometric mean and SD for AUC(0-t), Cmax
[2]. Tmax-admin: Tmax relative to most recent dose

The figure below illustrates the mean plasma MMF concentrations on Day 1 and Day 2 following 240 mg TID dose.

Figure: Arithmetic Mean Plasma Concentration of MMF for Treatment B (240 TID for 2 days)



The table below summarizes the mean PK parameters of MMF on Day 1 and Day 2 following 240 mg TID dose.

Table: PK Parameters of MMF after administration of BG00012 240 mg TID for 2 Days,

Day Treatment Administration Number (Time After Dose)	Pharmacokinetic Parameter			
	AUC_{0-t} [mg*h/L]	C_{max} [mg/L]	T_{max} [h]	$T_{max-admin}$ [h][2]
Day 1				
Administration 1 (0 hours)				
Mean[1]	0.509	1.084	3.669	3.669
SD[1]	4.263	2.288	0.585	0.585
Administration 2 (+4 hours)				
Mean[1]	3.186	1.836	6.180	2.180
SD[1]	1.845	1.521	2.522	2.522
Administration 3 (+10 hours)				
Mean[1]	4.448	1.313	13.333	3.333
SD[1]	1.276	1.333	2.931	2.931
Day 2				
Administration 4 (0 hours)				
Mean[1]	0.947	1.163	27.521	3.521
SD	2.216	2.025	0.683	0.683
Administration 5 (+4 hours)				
Mean[1]	3.373	1.764	31.286	3.286
SD[1]	1.482	1.340	2.563	2.563
Administration 6 (+10 hours)				
Mean[1]	3.897	1.458	37.500	3.500
SD[1]	1.346	1.342	2.022	2.022
Days 1 and 2				
Administrations 1 to 6				—
Mean[1]	17.650	2.263	18.322	
SD[1]	1.181	1.362	12.580	

[1]. Geometric mean and SD for AUC(0-t), Cmax
[2]. Tmax-admin: Tmax relative to most recent dose

Plasma concentrations of fumaric acid were not determined, since the concentrations were below the limit of quantification (LLOQ: 0.27 mg/L).

Reviewer's Comment: The HPLC assay used in this study to quantitate plasma MMF concentration is less sensitive compared to the LC-MS/MS method used in majority of the PK studies. The LLOQ (0.1 mg/L) of HPLC assay is close to Cmax of MMF at lower dose (120 mg).

The Sponsor's Conclusions

- The MHF concentration profiles observed on Day 2 were similar to those observed on Day 1 for both 120 mg TID and 240 mg TID
- No accumulation of MHF was observed within the 2-day treatment with 120 mg or 240 mg TID dosing of BG00012.
- MHF concentrations following the 240 mg administrations are in general twice as high as those following the 120 mg administrations.
- The maximum concentrations following the first daily dose were, on average, observed at about 3 to 5 hours after administration.
- For some individual PK profiles, the 1st maximum concentration was observed after the 2nd drug administration and/or the 2nd maximum concentration was observed after the 3rd drug administration.

Reviewer's comment: The sponsor's conclusions are acceptable.

109MS101: A 24-Hour Pharmacokinetic Determination of BG00012 after Single-Day Oral Administration in Subjects with Multiple Sclerosis

Objective:
To establish a pharmacokinetic (PK) profile of monomethyl fumarate (MMF), the primary metabolite of BG00012, during a 24-hour BG00012 dosing period in subjects with relapsing-remitting multiple sclerosis (RRMS).

<table>
<tr><td>Study Design</td><td>This was to be an open-label, multicenter, one-day PK study of two BG00012 dose regimens in MS patients.</td></tr>
<tr><td>Study Population</td><td>MS Patients
Age: 18-55 years
BMI: 24.75 kg/m^2 (median 23.45 kg/m^2) and ranged from 17.6 to 40.1 kg/m^2.
48 subjects were analyzed of which 42 subjects were analyzed for PK.</td></tr>
<tr><td>Treatment Groups</td><td>Group 1: 240 mg BG00012 at approximately 8 AM and 6 PM (BID).
Group 2: 240 mg BG00012 at approximately 8 AM, noon, and 6 PM (TID).
All doses were to be given orally with food. Treatment groups were stratified by weight (10 subjects of light weight [≤59 kg]; 29 subjects of medium weight [>59 to <90 kg]; and 9 subjects of heavy weight [≥90 kg]).

A total of 8 subjects in each treatment group received 1 unit of alcohol (125 mL of wine) with their evening dose (approximately 6 PM). The 8 study subjects were balanced between sexes (4 males, 4 females). There were no restrictions regarding the weight groups from which the subjects were recruited.</td></tr>
<tr><td>Sampling</td><td>A baseline PK blood sample was to be collected 15 minutes prior to the first dose of BG00012.
Following the first dose, PK blood samples for MMF blood level determinations were taken hourly up to 16 hours and again at 18, 20, and 24 hours.</td></tr>
<tr><td>Analysis</td><td>The plasma samples were analyzed for the concentration of MMF by using LC-MS/MS method. The LLOQ was 10 ng/mL for MMF.
<table>
<tr><th>Parameter</th><th>Quality Control Samples</th><th>Standard Curve Samples</th></tr>
<tr><td>Quality Control or Standard Curve Concentration (ng/mL)</td><td>30, 500, and 4000</td><td>10, 25, 50, 150, 500, 2000 and 5000</td></tr>
<tr><td>Between Batch Precision (%CV)</td><td>7.9 to 8.3</td><td>3.6 to 6.9</td></tr>
<tr><td>Between Batch Accuracy (%RE)</td><td>-10.3 to 6.8</td><td>-8.6 to 4.7</td></tr>
<tr><td>Linearity</td><td colspan="2">Weighted linear equation (1/X^2), mean r= 0.994</td></tr>
<tr><td>Linear Range (ng/mL)</td><td colspan="2">10 to 5000</td></tr>
<tr><td>Sensitivity (LLOQ, ng/mL)</td><td colspan="2">10</td></tr>
</table></td></tr>
<tr><td>PK Assessments</td><td>The PK parameters C_{max}, T_{max}, AUC_{0-t}, $AUC_{(0-inf)}$, Apparent volume of distribution, t_{lag} and $t_{1/2}$ were calculated from the plasma MMF concentration-time data using noncompartmental analysis.</td></tr>
<tr><td>Safety Assessments</td><td>Adverse event (AE) and serious adverse event (SAE) monitoring, physical examination and weight, vital signs measurement, clinical laboratory analysis (hematology, blood chemistry, coagulation [PT, PTT], urinalysis, beta-2 microglobulin, microalbumin), 12-lead electrocardiogram (ECG).</td></tr>
<tr><td>Statistical</td><td>Analysis of variance (ANOVA) was performed for overall AUC_{0-24} and C_{max};</td></tr>
</table>

Methods	for AUC_{0-24} and C_{max} with weight as a continuous variable; for C_{max} and T_{max} for the evening dose alone; and for C_{max} and T_{max} for the evening dose with weight as a continuous variable.

RESULTS:

The following table summarizes of MMF PK parameters.

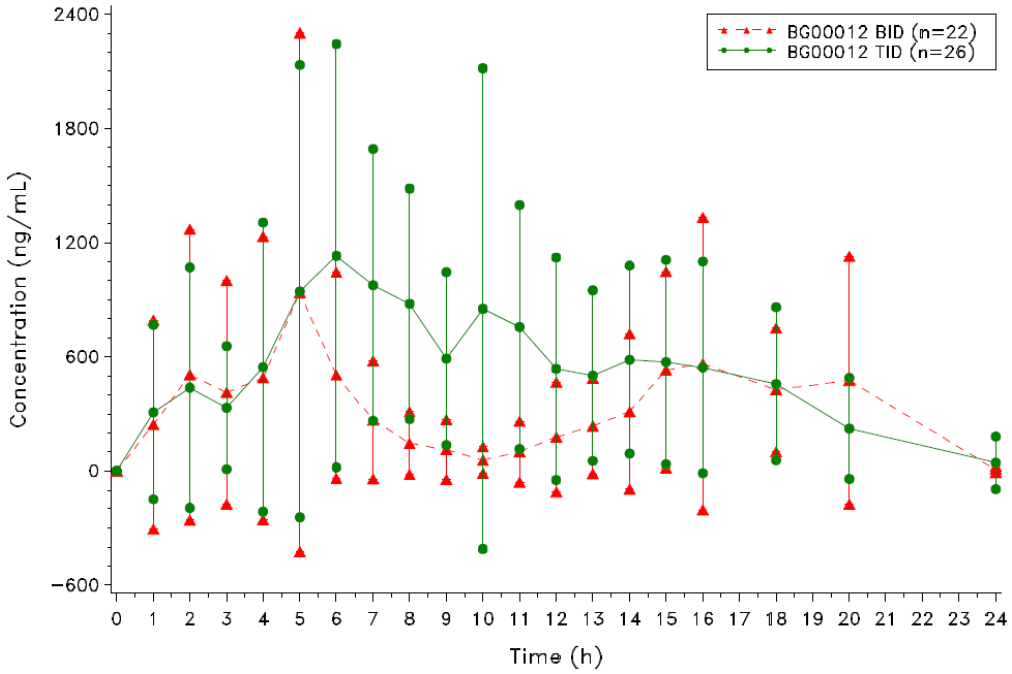
Table: Summary of MMF PK Parameters for BG00012, n=42

	BG0001240 mg BID	BG00012 240 mg TID
AUC(0-24) (h*mg/L)		
Mean	8.205	12.443
SD	3.4637	3.0659
Median	8.015	12.250
Cmax (mg/L)		
Mean	1.8676	2.4601
SD	1.25027	1.43104
Median	1.7150	1.9250
Tmax (h)		
Mean	7.9	8.6
SD	6.15	3.96
Median	5.0	7.5
T1/2 (h)		
Mean	1.2980	1.3915
SD	0.80485	0.95913
Median	1.0650	1.0700
Apparent volume of distribution (L)		
Mean	134.58	117.02
SD	103.171	81.151
Median	93.80	88.05
Apparent clearance (L/h)		
Mean	68.48	59.84
SD	27.322	16.096
Median	57.20	54.70

The PK profiles of BG00012 after both BID and TID administration display large inter-individual variability.

The following figure represents PK profiles of MMF when BG00012 was administered BID or TID.

Figure: Mean ± Standard Deviation Concentration versus Time of MMF in Plasma, n=42



The following table summarizes statistical analysis conducted on PK parameters of MMF based on demographic factors.

Table: Analysis of Variance for MMF AUC and Cmax (Weight as a Continuous Variable, n=42)

Subgroup Factors		Adjusted Values: Mean	Adjusted Values: 95% CI	p-value from ANOVA including all factors
AUC(0-24) h*mg/L				
Treatment group[1]	240 mg BID	7.82	(6.84, 8.94)	< 0.01
	240 mg TID	12.05	(10.67, 13.61)	
Sex	Female	9.15	(8.12, 10.31)	0.259
	Male	10.30	(8.79, 12.06)	
Age group	<=40 yrs	9.03	(7.94, 10.28)	0.126
	>40 yrs	10.43	(9.12, 11.92)	
Weight (a)		0.9802 (regression coefficient) (b)	(0.9742, 0.9862)	< 0.01
Alcohol use	No	9.57	(8.55, 10.71)	0.759
	Yes	9.84	(8.50, 11.40)	
Cmax (mg/L)				
Treatment group	240 mg BID	1.6102	(1.3041, 1.9883)	0.069
	240 mg TID	2.0793	(1.7172, 2.5177)	
Sex	Female	2.0387	(1.6892, 2.4606)	0.191
	Male	1.6423	(1.2813, 2.1049)	
Age group	<=40 yrs	1.9959	(1.6289, 2.4455)	0.235
	>40 yrs	1.6775	(1.3597, 2.0697)	
Weight (a)		0.9858 (regression coefficient) (b)	(0.9764, 0.9953)	< 0.01
Alcohol use	No	1.6714	(1.3995, 1.9962)	0.213
	Yes	2.0031	(1.5906, 2.5226)	

Reviewer's Comment:
Based on ANOVA analysis there was a statistically significant effect of body weight on AUC and Cmax of MMF. The AUC decreased by about 2% and Cmax decreased by about 1.4% with each 1 kg increase in weight. No statistically significant effects on MMF exposure were

identified for other demographic factors explored by the model. In Phase 3 studies (109MS301 and 109MS302), gender, age (<40 and ≥40 years) and weight (quantiles, ≤59, >59 to ≤69, >69 to ≤82 kg, and >82 kg) were examined for their effects on efficacy of BG00012, and no effects of gender and weight on efficacy measures were detected.

CONCLUSIONS:

- The PK profiles show high inter-subject variability.
- There was a statistically significant effect of body weight on MMF exposure (AUC and Cmax), while the effect appear to be clinically insignificant. Gender and age did not show significant effect.
- Alcohol (125 ml wine) intake had no influence on the PK of BG00012 given as BID or TID. However, the data were limited (N=8).

109HV102: An Open-Label Study to Investigate the Absorption, Metabolism, and Excretion of Single Oral Doses of ^{14}C-BG00012 in Healthy Male Subjects

Objective:
To determine the primary route of excretion and metabolism of BG00012, following a single oral 240 mg dose of ^{14}C-DMF [dimethyl (2,3-^{14}C) fumarate] administered to healthy male volunteers and the identification of metabolites in plasma, urine and feces.

<table>
<tr><td>Study Design</td><td>The study was a single-center, open-label study to characterize the absorption, metabolism, and excretion profiles of ^{14}C-BG00012 under fasting conditions.
Subjects were allowed to be discharged prior to 7 days following dosing if 2 consecutive expired air, blood, plasma, urine, and fecal samples reached undetectable levels of radioactivity or if ≥ 90% of the total dosed radioactivity had been recovered in expired air, urine, and feces.</td></tr>
<tr><td>Study Population</td><td>Healthy male subjects
Age: 18-55 years
BMI: median 24.75 kg/m^2 ranged from 17.6 to 40.1 kg/m^2.
Eight subjects enrolled and completed the study.</td></tr>
<tr><td>Treatment Groups</td><td>Up to 8 subjects were to receive a single oral dose of 14C-BG00012 in capsule form (240 mg ^{14}C-BG00012 drug substance and a target radioactivity of 100 microcuries [μCi]) in the fasted state.</td></tr>
<tr><td>Sampling</td><td>Whole blood, plasma, urine, expired air, and fecal samples were obtained pre-dose and post-dose for determination of total radioactivity. In addition, plasma samples were obtained pre-dose and post-dose for determination of BG00012 and MMF concentrations.
Blood Samples for PK and Radioactivity analysis were obtained at the following times predose, 0.5, 1, 1.5, 2, 3, 4, 5, 6, 7, 8, 12, 24, 48, 72, 96, 120, 144, and 168 hours
Urine: Samples for testing were taken from pooled volumes at all time points indicated (Hour 0 to 4, 4 to 8, 8 to 12, 12 to 24, 24 to 48, 48 to 72, 72 to 96, 96 to 120, 120 to 144, and 144 to 168 postdose).
Feces: Samples were collected as available until 168 hours postdose.
Expired air was collected at predose, 0.5, 1, 1.5, 2, 3, 4, 5, 6, 7, 8, 12, 24, 48, 72, 96 hours postdose.

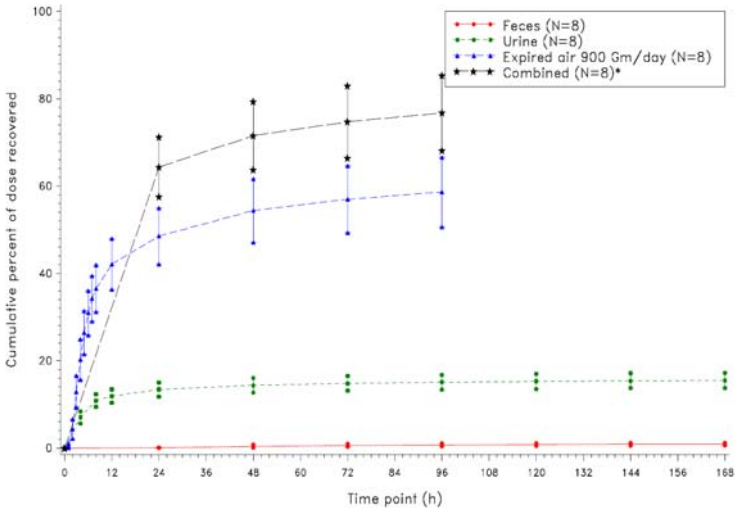
Specific collections of blood (2, 4, 8, and 24 hours after dosing) and pooled urine (0-8, 8-24, and 24-48 hour) were evaluated for metabolite profiling.</td></tr>
<tr><td>Analysis</td><td>The plasma samples were analyzed for the concentration of MMF by using LC-MS/MS method. The LLOQ was 10 ng/mL.
<table>
<tr><th>Parameter</th><th>Quality Control Samples</th><th>Standard Curve Samples</th></tr>
<tr><td>Quality Control or Standard Curve Concentration (ng/mL)</td><td>30, 500, and 4000</td><td>10, 25, 50, 150, 500, 2000 and 5000</td></tr>
<tr><td>Between Batch Precision (%CV)</td><td>2.6 to 9.3</td><td>2.6 to 9.3</td></tr>
<tr><td>Between Batch Accuracy (%RE)</td><td>-2.8 to 9.4</td><td>-3.2 to 1.5</td></tr>
<tr><td>Linearity</td><td colspan="2">Weighted linear equation (1/X^2), mean r= 0.995</td></tr>
<tr><td>Linear Range (ng/mL)</td><td colspan="2">10 to 5000</td></tr>
<tr><td>Sensitivity (LLOQ, ng/mL)</td><td colspan="2">10</td></tr>
</table></td></tr>
</table>

PK Assessments	The PK parameters C_{max}, T_{max}, $AUC_{0\text{-}t}$, $AUC_{(0\text{-}inf)}$, apparent volume of distribution, CL/F, t_{lag} and $t_{1/2}$ were calculated from the plasma MMF concentration-time data using noncompartmental analysis.
PD Assessments	The following Nrf-2 pathway measurements were to be performed as potential biomarkers for BG00012 pharmacologic activity: • NAD(P) H dehydrogenase, quinone 1 (NQO-1) • Heme oxygenase 1 (HO-1) Analysis of serum for candidate biomarkers that may relate to BG00012 PD or MS disease activity was to have been conducted on collected samples. In addition, whole blood samples were to be collected for potential microarray analysis of messenger ribonucleic acid (mRNA) to monitor the patterns of gene expression, and search for candidate PD markers.
Safety Assessments	Adverse event (AE) and serious adverse event (SAE) monitoring, physical examination and weight, vital signs measurement, clinical laboratory analysis (hematology, blood chemistry, coagulation [PT, PTT], urinalysis, beta-2 microglobulin, microalbumin), 12-lead electrocardiogram (ECG).

RESULTS:

Exhalation through expired air constitutes the primary elimination route of total radiolabel, with renal and fecal elimination as minor routes as shown in the figure and table below.

Figure: Mean +/- SD cumulative recovery of total radioactivity in urine, feces, expired air (900 Gm/day) and combined (% of dose) following a single oral administration of 14C-BG00012



* Included 18.7% dose in vomitus from subject 103-002

Table: Total Recovery of Radioactivity in Expired Air, Urine, and Feces after a Single Administration of ^{14}C-BG00012 in Eight Healthy Male Subjects (Study 109HV102)

Excretion Route (0-168 hr)	Extrapolated in Expired Air (% of Dose, 0-96h)	Urine (% of Dose)	Feces (% of Dose)	Total[1] (% of Dose)
Using CO_2 production at 5 mmol/min/m^2	39.7 ± 5.0	15.5 ± 1.7	0.9 ± 0.3	57.8 ± 2.3
Using CO_2 production at 900 g CO_2/day	58.6 ± 8.0	15.5 ± 1.7	0.9 ± 0.3	76.8 ± 3.2

Note: Recovery was approximately 19% greater using 900 gm CO_2/day as a reference when compared to 5 mmol CO_2/m^2 BSA/minute as a reference.

Metabolites identified in plasma

Four metabolites were identified, including MMF, fumaric acid, citric acid, and glucose. MMF was the least predominant and glucose was the most predominant, accounting for < 5% and 60% of total extractable plasma radioactivity, respectively. Together, fumaric acid and citric acid accounted for 27% of extractable radioactivity.

Table: Summary of Abundance of Metabolites

	Urine	Expired Air	Plasma
Total Time Period of Collection	0 to 168 h	0 to 96 h	0 to 168 h
Sample Analyzed for Metabolites	0 to 48 h	0 to 96 h	2 to 24 h
Compound (as % of Dose or % of Sample)	% of Dose	% of Dose	% of Sample
Males:			
% of Total Dose Excreted (0-168 h)	15.5	39.7 to 58.6	NA
Parent (BG00012)	0.06	-	
M1[1]+ M5[2]	-	-	27.5
M2 (MMF)	0.23	-	4.93
M6a[3]	1.77	-	
M6b[3]	0.17	-	
M6c[3]	0.16		
M7a[4]	1.40		
M7b[4]	0.62		
M8[5]	-		60.5
M9a-b[6]	4.64		-
M9c[6]	0.91		-
CO_2	-	39.7 to 58.6	-
Unknown[7]	4.47	-	7.07

[1] Fumarate

[2] Citrate

[3] N-acetylcysteine conjugate of monomethyl succinate

[4] N-acetylcysteine conjugate of dimethyl succinate

[5] Glucose

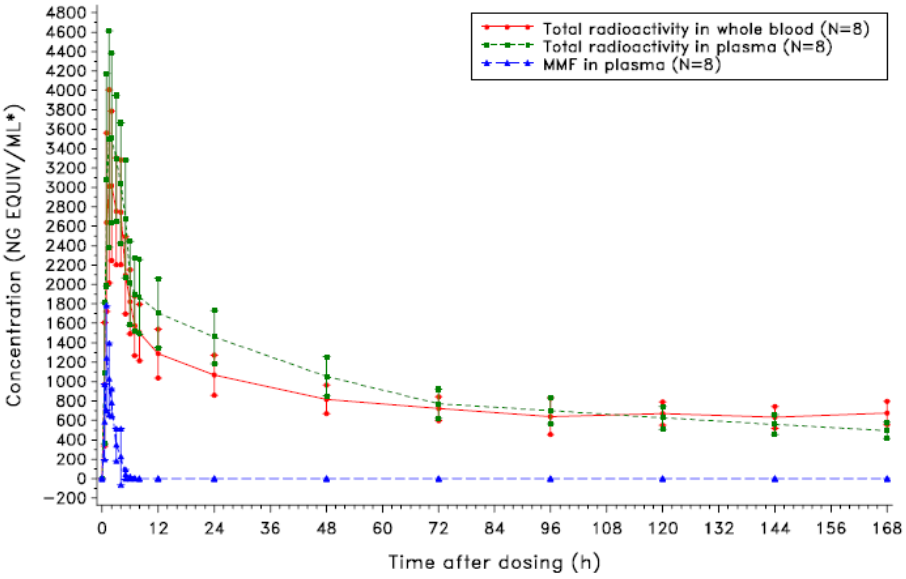
[6] Cysteine conjugates of monomethyl succinate

[7] Including multiple other minor radioactivity peaks

Monomethyl Fumarate (MMF)

The primary metabolite, MMF, constitutes only a fraction (13%) of the total circulating radioactivity exposure (ratio of $AUC_{0\text{-}8hr}$ values) with maximum plasma concentrations approximately one-third of total radioactivity. The MMF concentrations were below levels of quantification by 8 hours after dosing. The figure below illustrates the mean total radioactivity in whole blood and plasma, and MMF in plasma.

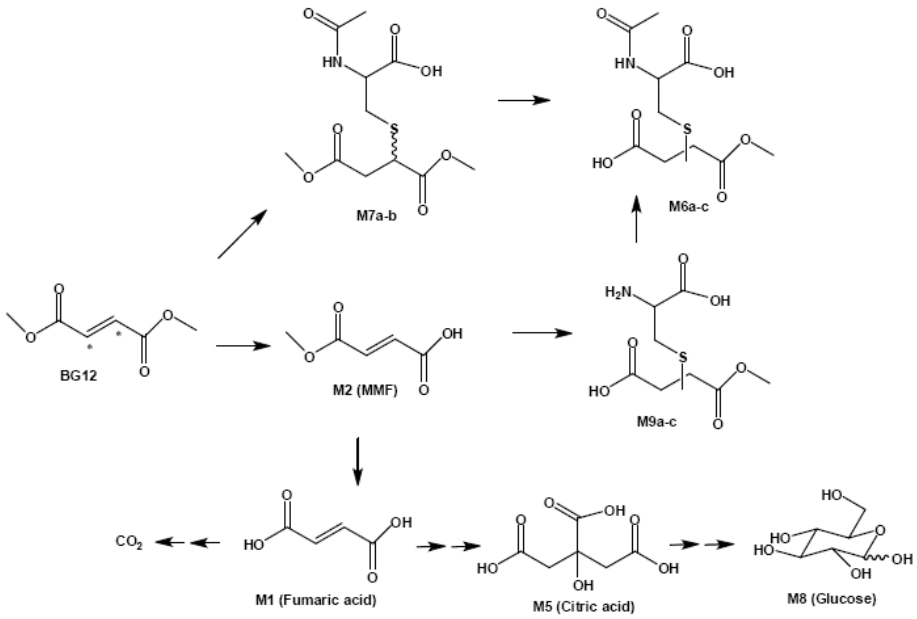
Figure: Mean +/- SD Concentration of Total Radioactivity in Whole Blood and Plasma, and MMF in Plasma Following a Single Oral Administration of ^{14}C-BG00012



Other Metabolites

The majority of drug-related radioactivity found in plasma is respiratory metabolites of MMF. Fumaric acid is a naturally occurring part of the tricarboxylic acid cycle (TCA, Kreb's cycle). DMF and MMF enter the TCA cycle in place of fumaric acid. The production of CO_2 through the TCA cycle is the source of radiolabel in the expired air.

Proposed Metabolism Pathways of BG00012



Metabolites identified in urine

Over 48 hours, an average of 15.5% of the dose was recovered in urine. Unchanged BG00012 was accounted for 0.06% of the dose recovered over 48 hours. MMF was present in very small quantities, accounting for 0.23% of the dose.

The most abundant metabolites were cysteine and N-acetylcysteine conjugates. Cysteine conjugates of monomethyl succinate, N-acetylcysteine conjugates of monomethyl succinate,

and N-acetylcysteine conjugates of dimethyl succinate accounted for 5.55%, 2.0%, and 2.0% of the dose, respectively.

Note: The exploratory PD markers collected during the study were not analyzed to relate BG00012 PK to PD or MS disease activity.

CONCLUSIONS:

- The recovery of the radiolabeled dose was 76-78% by 96 hours after dosing (using 900 gm CO_2/day as a standard reference of CO_2 production), with almost 60% of dose administered recovered in expired air, 15.5% in urine and only 0.9% in feces. Trace amounts of DMF and MMF were recovered in urine.
- DMF and its major metabolite (MMF) are metabolized through the TCA cycle, with exhalation as CO_2 representing a major route of elimination.
- Primary identified metabolites in plasma were glucose, fumaric acid and citric acid, and MMF.
- The most abundant metabolites in urine were cysteine and N-acetylcysteine conjugates of monomethyl-and/or dimethyl succinate.

109HV103: A Single-Center, Open-Label Study to Compare the Pharmacokinetic Effects of BG00012 TID Administered Alone to BG00012 TID Co-administered with a Single Dose of Avonex® (Interferon β-1a) in Healthy Volunteers

Objective:
To assess the potential pharmacokinetic interaction of Avonex® 30 μg intramuscular (IM) injection when co-administered with BG00012 at 240 mg three times daily (TID).
To explore the potential interaction of BG00012 on Avonex pharmacodynamic (PD) effects.

<table>
<tr><td>Study Design</td><td>The study was open-label, single-center, randomized, 2-period, crossover study</td></tr>
<tr><td>Study Population</td><td>Healthy subjects
Age: 18-60 years
BMI: 19 to 30 kg/m^2.
Twenty six subjects enrolled and 24 completed the study.</td></tr>
<tr><td>Treatment Groups</td><td>Sequence 1 (BG00012 followed by BG00012 with Avonex)
First dosing period: Approximately 13 subjects received 3 days of oral BG00012 240 mg TID.
Second dosing period: Subjects were administered 3 days of oral BG00012 240 mg TID. On Day 2 of the second dosing period, subjects received a single dose of Avonex 30 μg IM 15 minutes before the first dose of BG00012.
Sequence 2 (BG00012 with Avonex followed by BG00012)
First dosing period: Approximately 13 subjects received 3 days of oral BG00012 240 mg TID. On Day 2 of the first dosing period, subjects received a single dose of Avonex 30 μg IM.
Second dosing period: Subjects were administered 3 days of oral BG00012 240 mg TID.
BG00012 was administered with food. Dosing periods were separated by at least 7 days.</td></tr>
<tr><td>Sampling</td><td>Blood samples for PK analysis of BG0012 were obtained at the following times predose, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 18, and 20 hours.
Blood samples for neopterin analysis were obtained at the following times predose, 6, 12, 24, 30, 36, 48, 54, 60, 72, 96, 120 hours.
Samples were collected after the first dose on Day 2 for each period.</td></tr>
<tr><td>Analysis</td><td>The plasma samples were analyzed for the concentration of MMF by using LC-MS/MS method. The lower limit of quantification (LLOQ) was 10ng/mL.
<table>
<tr><th>Parameter</th><th>Quality Control Samples</th><th>Standard Curve Samples</th></tr>
<tr><td>Quality Control or Standard Curve Concentration (ng/mL)</td><td>30, 500, and 4000</td><td>10, 25, 50, 150, 500, 2000 and 5000</td></tr>
<tr><td>Between Batch Precision (%CV)</td><td>5.8 to 12.6</td><td>5.0 to 8.2</td></tr>
<tr><td>Between Batch Accuracy (%RE)</td><td>-2.8 to 0.2</td><td>-5.0 to 2.4</td></tr>
<tr><td>Linearity</td><td colspan="2">Weighted linear equation ($1/X^2$), mean r= 0.999</td></tr>
<tr><td>Linear Range (ng/mL)</td><td colspan="2">10 to 5000</td></tr>
<tr><td>Sensitivity (LLOQ, ng/mL)</td><td colspan="2">10</td></tr>
</table></td></tr>
</table>

PK Assessments	The PK parameters C_{max}, T_{max}, AUC_{0-20}, apparent volume of distribution, CL, t_{lag} and $t_{1/2}$ were calculated from the plasma MMF concentration-time data using noncompartmental analysis. The primary PK endpoint was the area under the plasma concentration curve from baseline to 20 hours (AUC_{0-20}).
PD Assessments	Neopterin measurements were collected to assess any potential PD effects that BG00012 may have on Avonex.
Safety Assessments	Adverse event (AE) and serious adverse event (SAE) monitoring, physical examination and weight, vital signs measurement, clinical laboratory analysis (hematology, blood chemistry, coagulation [PT, PTT], urinalysis, beta-2 microglobulin, microalbumin), 12-lead electrocardiogram (ECG).

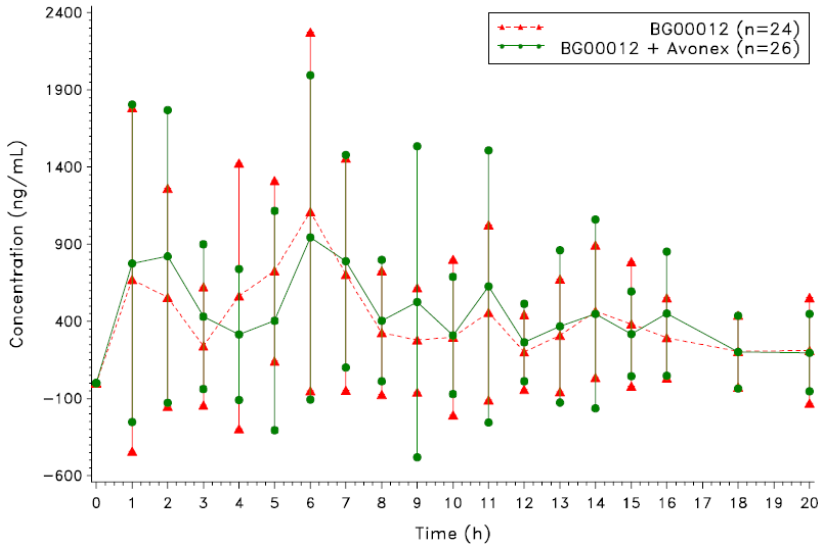
RESULTS:
The following table summarizes PK parameters of MMF when BG00012 was administered alone or co-administered with Avonex.

Table: Summary of MMF Pharmacokinetic Parameters

	BG00012	BG00012 + Avonex
Cmax (ug/mL)		
mean	2.24	2.27
sd	1.018	0.998
median	1.94	1.97
Tmax (h)		
mean	6.07	6.82
sd	3.480	4.613
median	6.00	6.50
AUC(0-20) (h*ug/mL)		
mean	10.00	9.49
sd	3.038	2.835
median	8.73	8.51
T1/2 (h)		
mean	2.05	2.47
sd	1.377	2.671
median	1.60	1.23
AUC(last) (h*ug/mL)		
mean	9.69	9.11
sd	2.972	2.712
median	8.54	8.43
Tlast (h)		
mean	19.83	19.85
sd	0.565	0.543
median	20.00	20.00

The following figure represents PK profiles of MMF when BG00012 was administered alone or co-administered with Avonex.

Figure: Mean ± Standard Deviation Concentration of MMF in Plasma

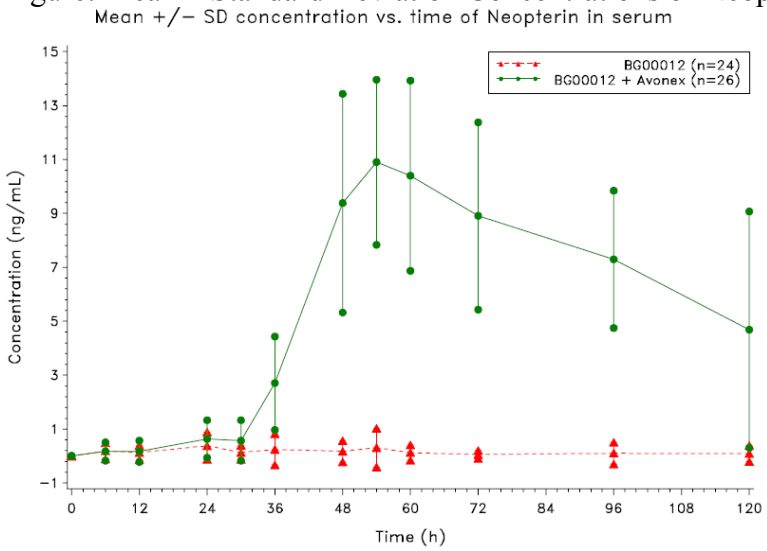


The geometric mean ratio (with Avonex / without Avonex) for $MMFAUC_{(0-20)}$ was 92.3% with a 90% confidence interval (CI) of 83.8% to 101.7%. The geometric mean ratio for Cmax was 99.0% with 90% CI of 81.3% to 120.4%. Both 90% CIs were within the range of 80% to 125%.

Pharmacodynamics:
Nrf2 markers HO-1 and NQO-1 were low or below level of quantitation due to sensitivity of the assay, and did not allow quantitative measurement of the Nrf2 pathway.

Neopterin: The neopterin response was related to Avonex dosing (figure below).

Figure: Mean ± Standard Deviation Concentrations of Neopterin in Serum



CONCLUSION:
PK of MMF was not affected when BG00012 was co-administered with single-dose of Avonex.

109HV104: A Single-Center, Open-Label Study to Compare the Pharmacokinetic Effects of BG00012 TID, Administered Alone, with BG00012 TID Co-Administered with a Single Dose of Glatiramer Acetate in Healthy Volunteers.

Objective:

To assess the potential interaction of oral BG00012 240 mg three times daily (TID) when co-administered with glatiramer acetate (GA) 20 mg subcutaneous (SC) injection. Assessments were to be made by comparing PK profiles of BG00012 240 mg TID when given alone to BG00012 versus when administered with a single dose of GA. The primary PK parameter was AUC_{0-24} from time of first BG00012 dose on Day 2.

<table>
<tr><td>Study Design</td><td>The study was open-label, single-center, randomized, 2-period, crossover study. The start of the dosing periods (Day-1) for each sequence was separated by 7 to 14 days.</td></tr>
<tr><td>Study Population</td><td>Healthy subjects
Age: 18-60 years
BMI: 19 to 30 kg/m^2.
Twenty six subjects enrolled and 25 completed the study.</td></tr>
<tr><td>Treatment Groups</td><td>Sequence 1
First dosing period: Approximately 13 subjects received 2 days of oral BG00012 240 mg TID.
Second dosing period: Subjects were to be administered 2 days of oral BG00012 240 mg TID. On Day 2 of the second dosing period, subjects received a single dose of GA 20 mg SC 15 min prior to first BG00012 dosing.
Sequence 2
First dosing period: Approximately 13 subjects received 2 days of oral BG00012 240 mg TID. On Day 2 of the first dosing period, subjects received a single dose of GA 20 mg SC.
Second dosing period: Subjects were to be administered 2 days of oral BG00012 240 mg TID.
BG00012 was administered with food. Dosing periods were separated by at least 7 days.</td></tr>
<tr><td>Sampling</td><td>Blood Samples were obtained at the following times predose, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 18, 20 and 24 hours.</td></tr>
<tr><td>Analysis</td><td>The plasma samples were analyzed for the concentration of MMF by using LC-MS/MS method. The lower limit of quantification (LLOQ) was 10 ng/mL for MMF.
<table>
<tr><th>Parameter</th><th>Quality Control Samples</th><th>Standard Curve Samples</th></tr>
<tr><td>Quality Control or Standard Curve Concentration (ng/mL)</td><td>30, 500, and 4000 ng/mL</td><td>10, 25, 50, 150, 500, 2000 and 5000</td></tr>
<tr><td>Between Batch Precision (%CV)</td><td>4.5 to 9.0</td><td>4.2 to 7.0</td></tr>
<tr><td>Between Batch Accuracy (%RE)</td><td>-9.8 to -0.6</td><td>-8.8 to 4.7</td></tr>
<tr><td>Linearity</td><td colspan="2">Weighted linear equation ($1/X^2$), mean r= 0.988</td></tr>
<tr><td>Linear Range (ng/mL)</td><td colspan="2">10 to 5000</td></tr>
<tr><td>Sensitivity (LLOQ, ng/mL)</td><td colspan="2">10</td></tr>
</table></td></tr>
<tr><td>PK Assessments</td><td>The PK parameters C_{max}, T_{max}, AUC_{0-24}, AUC_{0-inf} apparent volume of distribution, CL, t_{lag} and $t_{1/2}$ were calculated from the plasma MMF concentration-time data using noncompartmental analysis.</td></tr>
</table>

Safety Assessments	Adverse event (AE) and serious adverse event (SAE) monitoring, physical examination and weight, vital signs measurement, clinical laboratory analysis (hematology, blood chemistry, coagulation [PT, PTT], urinalysis, beta-2 microglobulin, microalbumin), 12-lead electrocardiogram (ECG).

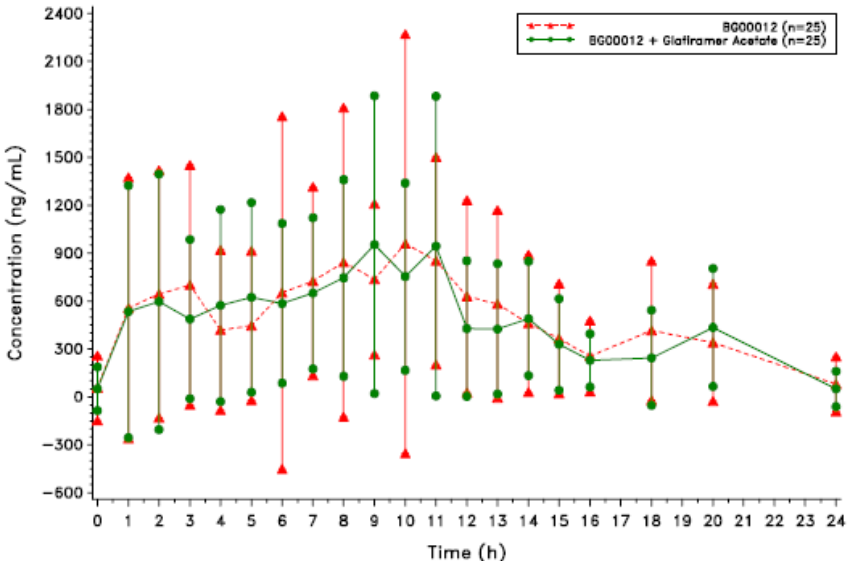
RESULTS:
The following table summarizes PK parameters of MMF when BG00012 was administered alone or co-administered with glatiramer acetate.

Table: Summary of MMF Pharmacokinetic Parameters

	BG00012	BG00012 + Glatiramer Acetat
Cmax (ug/mL)		
mean	2.3804	2.0964
sd	1.24875	0.88939
median	1.9300	1.8500
Tmax (h)		
mean	6.08	6.73
sd	4.102	4.686
median	6.00	6.00
AUC(0-24) (h*ug/mL)		
mean	11.291	10.863
sd	2.2105	2.5605
median	11.150	11.330
T1/2 (h)		
mean	1.3652	1.3328
sd	0.92858	0.83019
median	1.0210	0.9480
AUC(last) (h*ug/mL)		
mean	11.222	10.777
sd	2.2104	2.5429
median	11.100	11.000
Tlast (h)		
mean	22.18	19.84
sd	2.492	0.554
median	23.90	20.00

Following figure represents PK profiles of MMF when BG00012 was administered alone or co-administered with glatiramer acetate.

Figure: Mean ± Standard Deviation Concentration versus Time of MMF in Plasma



There was no effect of GA on PK of MMF. The geometric mean ratio (with GA/ without GA) for MMF AUC $_{(0\text{-}24)}$ was 99.2% with a 90% CI of 93.8% to 104.8%, and the geometric mean ratio for C_{max} was 94.9% with a 90% CI of 80.7% to 111.7%.

The following table summarizes the statistical analysis conducted on PK parameters of MMF when BG00012 was administered alone or coadministered with GA.

Table: Statistical Analysis

	n	Arithmetic Mean	SD	CV	Mean of* Logs	SD of Logs	CV of Logs	Geometric Mean (a)	Geometric Mean Ratio	90% CI of Geometric Mean Ratio (b)
AUC 0-24 (h*ug/mL)										
BG00012	24	11.296	2.2579	19.988	2.404	0.2134	8.879	11.064	99.178%	(93.834%,104.814%)
BG00012+GA	24	11.163	2.1193	18.985	2.395	0.1901	7.937	10.973		
Cmax (ug/mL)										
BG00012	24	2.357	1.2698	53.883	0.759	0.4187	55.137	2.137	94.946%	(80.681%,111.680%)
BG00012+GA	24	2.158	0.8516	39.456	0.707	0.3458	48.883	2.029		

CONCLUSIONS:
Pharmacokinetic profile of MMF did not change when BG00012 was co-administered with single-dose of GA.

109HV106: A Randomized, Double-Blind, Placebo-Controlled Study of the Safety, Tolerability, and Pharmacokinetics of BG00012 Administered With and Without 325 mg Aspirin in Healthy Adult Volunteers

Objective:
To determine if BG00012-induced flushing thought to be mediated by prostaglandin D2 (PGD2) might be affected by administration of this cyclooxygenase inhibitor.

<table>
<tr><td>Study Design</td><td>The study was a single-center, randomized, double-blind, placebo-controlled study in healthy adult volunteers.</td></tr>
<tr><td>Study Population</td><td>Healthy male and female; Age: 18-55 years; BMI: 18-34 kg/m^2
56 subjects were analyzed of which 42 subjects were analyzed for PK.</td></tr>
<tr><td>Treatment Groups</td><td>BG00012 240 mg BID (n=6), BG00012 240 mg TID (n=6), and BG00012 360 mg BID (n=6), or placebo (n=6) without concomitant ASA (referred as “BG00012 alone”)
BG00012 240 mg BID (n=6), BG00012 240 mg TID (n=6), and BG00012 360 mg BID (n=6), or placebo (n=6) with concomitant ASA (referred as “BG00012 with ASA”)
BG00012 (n=6) or placebo (n=2) administered in a modified dosing regimen without concomitant ASA (referred to as the “modified dosing regimen*”)
<u>Modified dosing regimen</u>: BG00012 120 mg (or placebo) every hour for 3 hours in the morning and again in the evening.
1 Capsule of BG12 = 120 mg
<table>
<tr><th></th><th>30 min prior to BG12 or matching PBO</th><th>0800 Hrs (8 AM)</th><th>30 min prior to BG12 or matching PBO</th><th>1200 Hrs (noon)</th><th>30 min prior to BG12 or matching PBO</th><th>1800 Hrs (6 PM)</th><th>Total mg BG12</th></tr>
<tr><td>Group 1: 240 mg BG12 BID plus 325 mg ASA</td><td>1 ASA</td><td>2 BG12
1 PBO</td><td>1 ASA</td><td>3 PBO</td><td>1 ASA</td><td>2 BG12
1 PBO</td><td>480</td></tr>
<tr><td>Group 2: 240 mg BG12 BID plus ASA matching PBO</td><td>1 ASA matching PBO</td><td>2 BG12
1 PBO</td><td>1 ASA matching PBO</td><td>3 PBO</td><td>1 ASA matching PBO</td><td>2 BG12
1 PBO</td><td>480</td></tr>
<tr><td>Group 3: 240 mg BG12 TID plus 325 mg ASA</td><td>1 ASA</td><td>2 BG12
1 PBO</td><td>1 ASA</td><td>2 BG12
1 PBO</td><td>1 ASA</td><td>2 BG12
1 PBO</td><td>720</td></tr>
<tr><td>Group 4: 240 mg BG12 TID plus ASA matching PBO</td><td>1 ASA matching PBO</td><td>2 BG12
1 PBO</td><td>1 ASA matching PBO</td><td>2 BG12
1 PBO</td><td>1 ASA matching PBO</td><td>2 BG12
1 PBO</td><td>720</td></tr>
<tr><td>Group 5: 360 mg BG12 BID plus 325 mg ASA</td><td>1 ASA</td><td>3 BG12</td><td>1 ASA</td><td>3 PBO</td><td>1 ASA</td><td>3 BG12</td><td>720</td></tr>
<tr><td>Group 6: 360 mg BG12 BID plus ASA matching PBO</td><td>1 ASA matching PBO</td><td>3 BG12</td><td>1 ASA matching PBO</td><td>3 PBO</td><td>1 ASA matching PBO</td><td>3 BG12</td><td>720</td></tr>
<tr><td>Group 7: Placebo plus 325 mg ASA</td><td>1 ASA</td><td>3 PBO</td><td>1 ASA</td><td>3 PBO</td><td>1 ASA</td><td>3 PBO</td><td>0</td></tr>
<tr><td>Group 8: PBO plus ASA matching PBO</td><td>1 ASA matching PBO</td><td>3 PBO</td><td>1 ASA matching PBO</td><td>3 PBO</td><td>1 ASA matching PBO</td><td>3 PBO</td><td>0</td></tr>
<tr><td></td><td colspan="7">Modified Dosing</td></tr>
<tr><td></td><td>0800 Hrs (8 AM)</td><td>0900 Hrs (9 AM)</td><td>1000 Hrs (10 AM)</td><td>1800 Hrs (6 PM)</td><td>1900 Hrs (7 PM)</td><td>2000 Hrs (8 PM)</td><td>Total mg BG12</td></tr>
<tr><td>Group 9: 120 mg BG12 (n= 6)</td><td>1 BG12</td><td>1 BG12</td><td>1 BG12</td><td>1 BG12</td><td>1 BG12</td><td>1 BG12</td><td>720</td></tr>
<tr><td>Group 9: PBO (n= 2)</td><td>1 PBO</td><td>1 PBO</td><td>1 PBO</td><td>1 PBO</td><td>1 PBO</td><td>1 PBO</td><td>0</td></tr>
</table>
Aspirin or matching placebo was always administered 30 minutes before BG00012 or its matching placebo. Meals were to be consumed immediately after dosing in Groups 1 to 8. In Group 9, meals were to be provided at Hours 0, 4, and 10 (immediately after dosing when applicable). All the treatments were administered from day 1 through day 4.</td></tr>
</table>

<table>
<tr><td>Sampling</td><td><u>PK Analysis</u> (Day 1 and Day 4): Blood samples (4.5 mL) were obtained during each study period at the following times Day 1 and 4: predose, 1, 1.5, 2, 2.5, 3, 4, 5, 6, 7, 8, 9, and 10 hours
<u>PD Analysis</u>
Plasma samples of both serotonin and histamine were collected at Hours 0, 1, 2, 4, 8, 10, and 12 on Days 1 and 4.
For Prostaglandins Analysis: after dosing at Hours 0.5, 1, 2, 3, 4, 6, 8, 10, and 12.</td></tr>
<tr><td>Analysis</td><td>The plasma samples were analyzed for the concentration of MMF by using LC-MS/MS method. The lower limit of quantification (LLOQ) was 10 ng/mL for MMF.
<table>
<tr><th>Parameter</th><th>Quality Control Samples</th><th>Standard Curve Samples</th></tr>
<tr><td>Quality Control or Standard Curve Concentration (ng/mL)</td><td>30, 500, and 4000 ng/mL</td><td>10, 25, 50, 150, 500, 2000 and 5000</td></tr>
<tr><td>Between Batch Precision (%CV)</td><td>4.71 to 6.20</td><td>2.45 to 5.69</td></tr>
<tr><td>Between Batch Accuracy (%RE)</td><td>-6.41 to -7.73</td><td>-0.71to 0.53</td></tr>
<tr><td>Linearity</td><td colspan="2">Weighted linear equation ($1/X^2$), mean r= 0.998</td></tr>
<tr><td>Linear Range (ng/mL)</td><td colspan="2">10 to 5000</td></tr>
<tr><td>Sensitivity (LLOQ, ng/mL)</td><td colspan="2">10</td></tr>
</table></td></tr>
<tr><td>PK Assessments</td><td>The PK parameters C_{max}, T_{max}, AUC_{0-t}, $AUC_{(0-inf)}$, t_{lag} and $t_{1/2}$ were calculated from the plasma MMF concentration-time data using noncompartmental analysis.</td></tr>
<tr><td>PD Assessments</td><td>The following PD endpoints were evaluated: Flushing scores (GFSS and Flushing Severity Scale [FSS]), GI tolerability assessments (Overall GI Symptom Scale [OGISS], Acute GI Symptom Scale [AGIS], and Bowel Movement Questionnaire [BMQ]). PGD2 metabolite concentrations in plasma and urine, Serotonin concentrations, Histamine concentrations</td></tr>
<tr><td>Safety Assessments</td><td>Adverse event (AE) and serious adverse event (SAE) monitoring, physical examination and weight, vital signs measurement, clinical laboratory analysis (hematology, blood chemistry, coagulation [PT, PTT], urinalysis, beta-2 microglobulin, microalbumin), 12-lead electrocardiogram (ECG).</td></tr>
</table>

RESULTS:

Pharmacokinetics

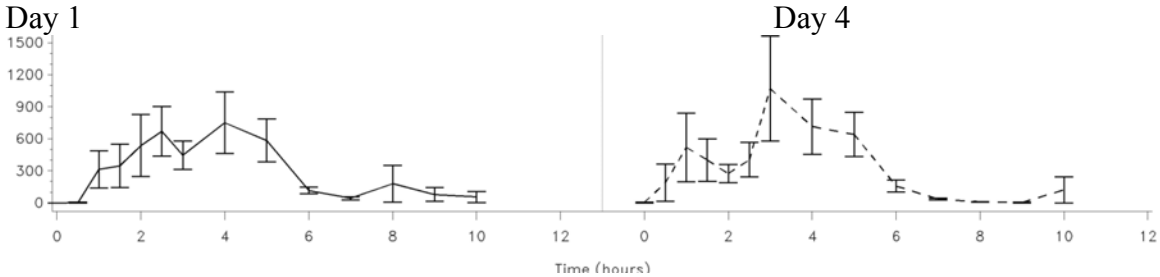
The following table summarizes PK parameters of MMF when BG00012 was administered alone or administration of BG00012 with ASA.

Table: Summary of MMF PK Parameters for BG00012

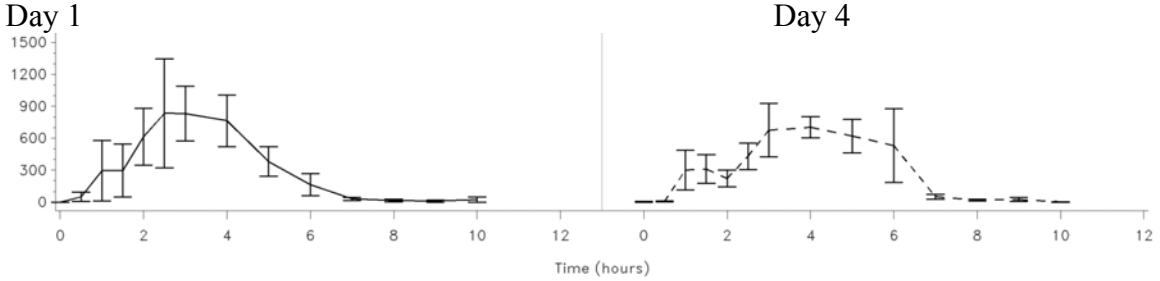
ASA	Dose Group	Day	Median Lag Time hours	Median Tmax hours	Median Cmax mg/L	Median AUC(0-10) h*mg/L	Median t 1/2 hours
Without	240 mg BID	1	0.50	4.0	1.335	2.800	0.81
		4	0.25	3.0	1.730	2.865	0.63
Without	360 mg BID	1	0.50	4.0	1.565	4.350	0.54
		4	0.00	2.8	2.050	4.895	0.76
Without	240 mg TID	1	0.50	6.0	1.935	5.075	0.85
		4	1.00	5.5	2.050	5.815	1.05
With	240 mg BID	1	0.25	2.8	1.625	3.020	0.59
		4	0.25	3.5	1.135	2.590	0.56
With	360 mg BID	1	0.00	4.0	2.780	5.180	0.75
		4	0.50	4.5	1.730	4.055	0.94
With	240 mg TID	1	1.75	5.0	1.970	5.875	0.81
		4	1.00	3.5	1.995	5.885	0.88

Figure. Mean Plasma MMF Concentrations (±SE, ng/mL) Over Time

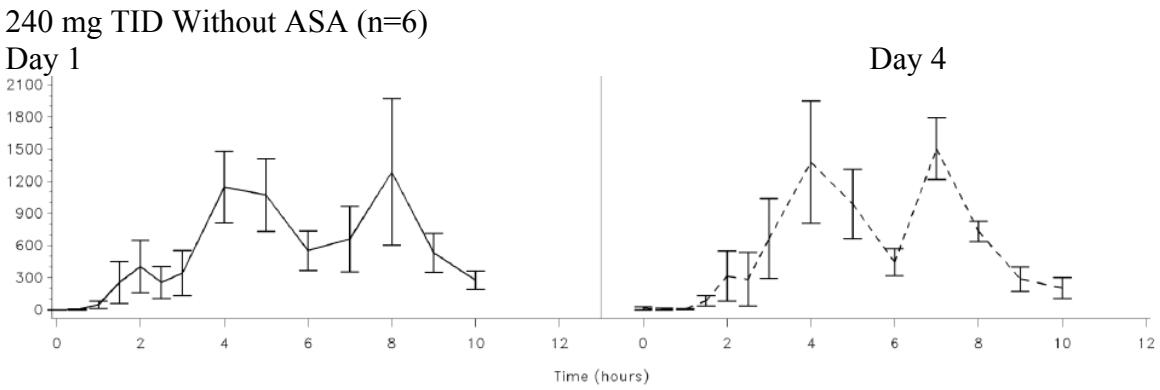
240 mg BID Without ASA (n=6)



240 mg BID With ASA (n=6)

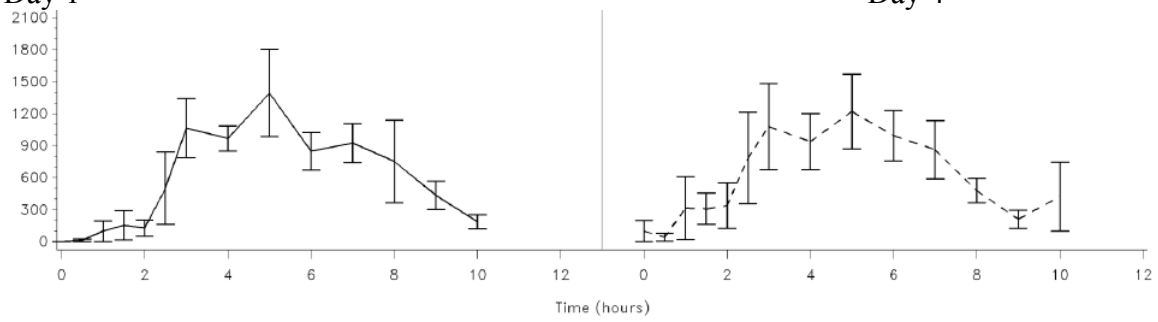


240 mg TID Without ASA (n=6)



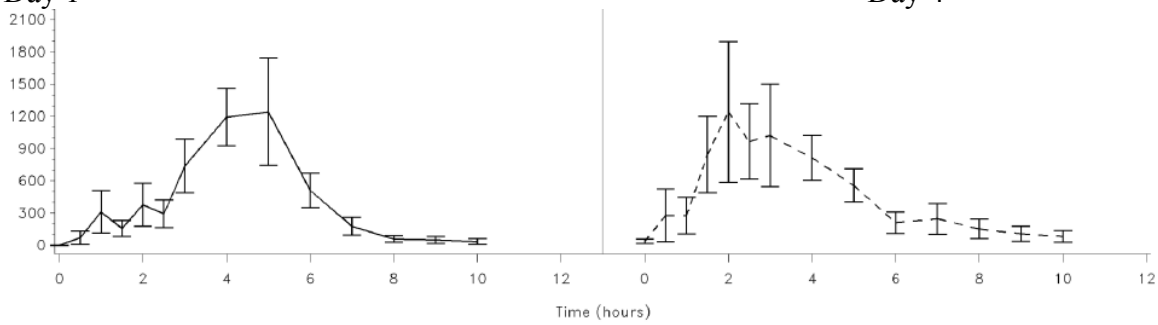
240 mg TID With ASA (n=6)

Day 1 Day 4



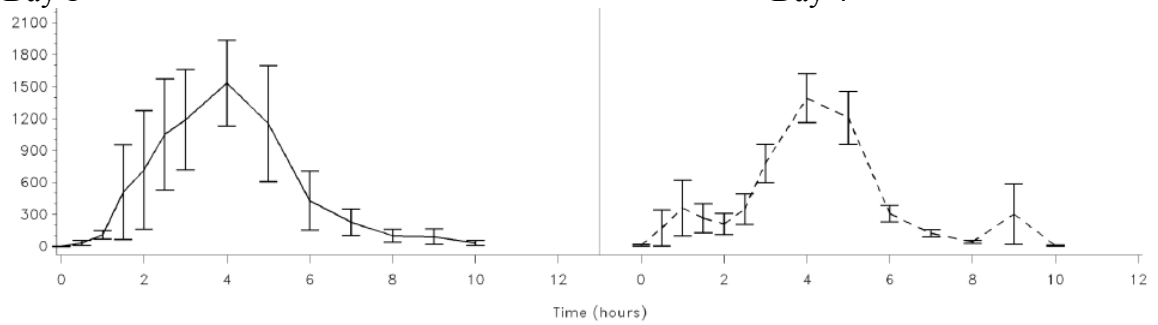
360 mg BID Without ASA (n=6)

Day 1 Day 4



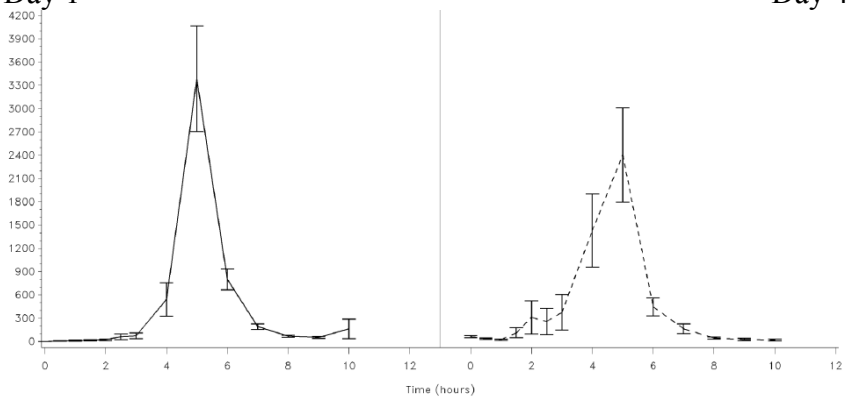
360 mg BID With ASA (n=6)

Day 1 Day 4

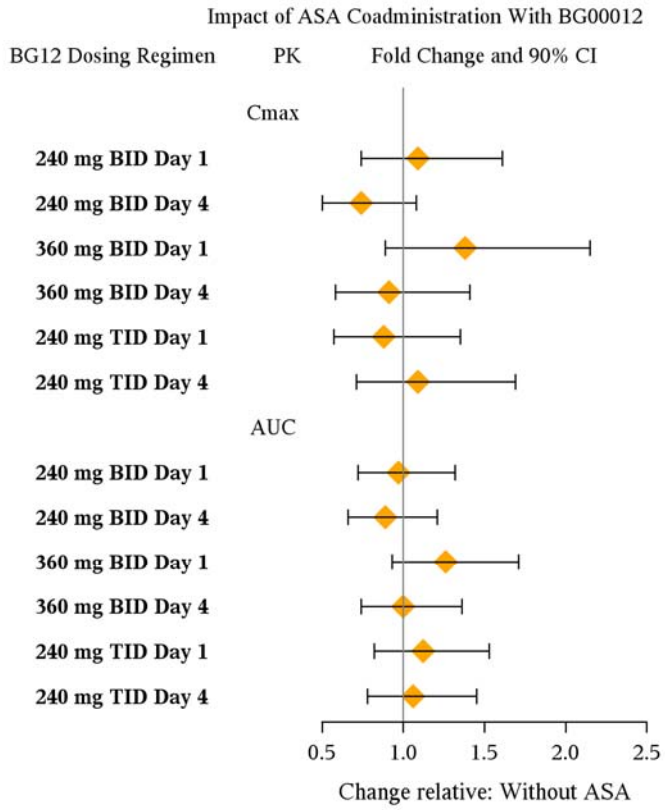


Modified Dosing Regimen (n=6)

Day 1 Day 4



The Forest plot below summarizes the effect of ASA co-administration on Cmax and AUC of BG00012.

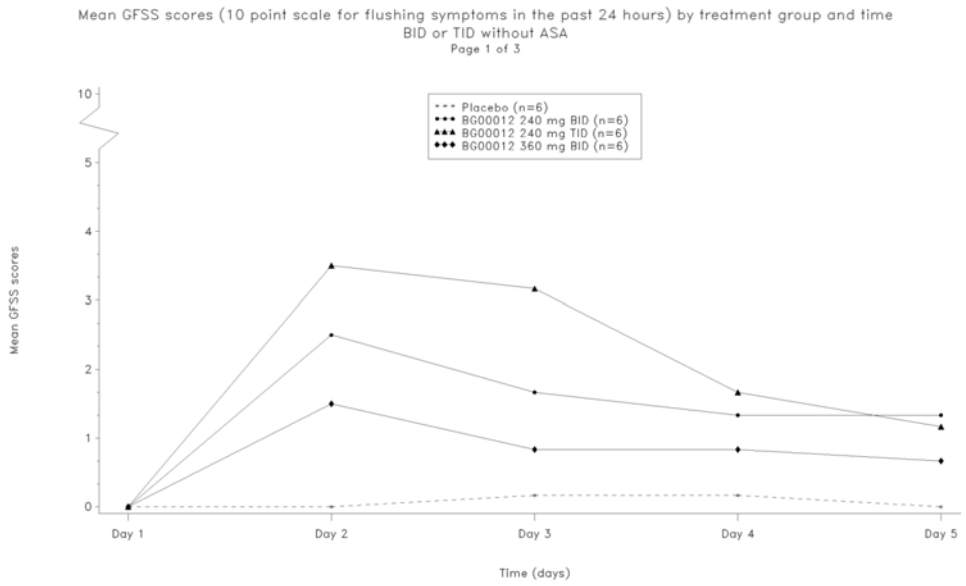


Safety:

Flushing Severity Scale
FSS employed the following scoring for flushing events: 0, no flushing; 1 through 3, mild; 4 through 6, moderate; 7 through 9, severe; and 10, extreme. Scores were related to overall symptoms, redness, warmth, tingling, and itching.

The flushing symptom scores were higher in the 240 mg TID dose group compared to the 240 mg BID and 360 mg BID dose groups. The flushing scores appeared to decrease over time, as shown in the following figure.

Figure. Mean GFSS Scores (10-Point Scale for Flushing Symptoms in the Past 24 Hours) by Treatment Group and Time



Pre-treatment with ASA decreased the intensity and incidence of flushing events in the BG00012 BID or TID treatment groups (see the figure and tables below).

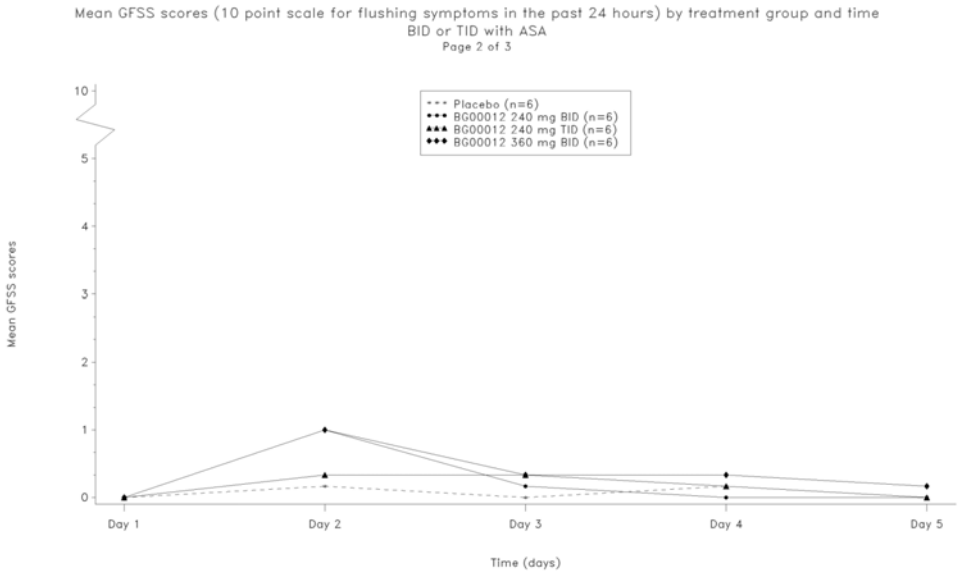


Table: Summary of flushing severity in subjects on BG00012 from baseline to day 4 *without* concomitant ASA.

	BID or TID dosing regimen without concomitant ASA			
	Placebo	BG00012 240 mg BID	BG00012 240 mg TID	BG00012 360 mg BID
Baseline				
0	6 (100)	6 (100)	6 (100)	6 (100)
1 - 4	0	0	0	0
>= 5	0	0	0	0
n	6	6	6	6
Mean	0.0	0.0	0.0	0.0
SD	0.00	0.00	0.00	0.00
Median	0.0	0.0	0.0	0.0
Min, Max	0, 0	0, 0	0, 0	0, 0
Day 2 (pre-dose)				
0	6 (100)	3 (50)	0	2 (33)
1 - 4	0	1 (17)	5 (83)	4 (67)
>= 5	0	2 (33)	1 (17)	0
n	6	6	6	6
Mean	0.0	2.5	3.5	1.5
SD	0.00	3.02	2.07	1.52
Median	0.0	1.5	3.5	1.5
Min, Max	0, 0	0, 7	1, 7	0, 4
Day 3				
0	5 (83)	3 (50)	0	3 (50)
1 - 4	1 (17)	3 (50)	4 (67)	3 (50)
>= 5	0	0	2 (33)	0
n	6	6	6	6
Mean	0.2	1.7	3.2	0.8
SD	0.41	1.97	1.83	0.98
Median	0.0	1.0	3.5	0.5
Min, Max	0, 1	0, 4	1, 5	0, 2
Day 4				
0	5 (83)	3 (50)	2 (33)	3 (50)
1 - 4	1 (17)	3 (50)	4 (67)	3 (50)
>= 5	0	0	0	0
n	6	6	6	6
Mean	0.2	1.3	1.7	0.8
SD	0.41	1.75	1.37	1.17
Median	0.0	0.5	2.0	0.5
Min, Max	0, 1	0, 4	0, 3	0, 3

NOTE 1: Baseline is the pre-dose value closest to dosing on Day 1.
2: Severity scale in a rating of 0 to 10 with 0=did not have and 10=extreme. Moderate is defined as a score of 5 or higher.

Table: Summary of flushing severity in subjects on BG00012 from baseline to day 4 *with* concomitant ASA.

	BID or TID dosing regimen with concomitant ASA			
	Placebo	BG00012 240 mg BID	BG00012 240 mg TID	BG00012 360 mg BID
Baseline				
0	6 (100)	5 (83)	6 (100)	6 (100)
1 - 4	0	0	0	0
>= 5	0	0	0	0
n	6	5	6	6
Mean	0.0	0.0	0.0	0.0
SD	0.00	0.00	0.00	0.00
Median	0.0	0.0	0.0	0.0
Min, Max	0, 0	0, 0	0, 0	0, 0
Day 2 (pre-dose)				
0	5 (83)	3 (50)	4 (67)	2 (33)
1 - 4	1 (17)	3 (50)	2 (33)	4 (67)
>= 5	0	0	0	0
n	6	6	6	6
Mean	0.2	1.0	0.3	1.0
SD	0.41	1.26	0.52	1.10
Median	0.0	0.5	0.0	1.0
Min, Max	0, 1	0, 3	0, 1	0, 3

Day 3				
0	6 (100)	5 (83)	4 (67)	4 (67)
1 - 4	0	1 (17)	2 (33)	2 (33)
>= 5	0	0	0	0
n	6	6	6	6
Mean	0.0	0.2	0.3	0.3
SD	0.00	0.41	0.52	0.52
Median	0.0	0.0	0.0	0.0
Min, Max	0, 0	0, 1	0, 1	0, 1
Day 4				
0	5 (83)	6 (100)	5 (83)	5 (83)
1 - 4	1 (17)	0	1 (17)	1 (17)
>= 5	0	0	0	0
n	6	6	6	6
Mean	0.2	0.0	0.2	0.3
SD	0.41	0.00	0.41	0.82
Median	0.0	0.0	0.0	0.0
Min, Max	0, 1	0, 0	0, 1	0, 2

NOTE 1: Baseline is the pre-dose value closest to dosing on Day 1.
2: Severity scale in a rating of 0 to 10 with 0=did not have and 10=extreme. Moderate is defined as a score of 5 or higher.

Overall GI Symptom Scale (OGISS)
Mean OGISS scores were low (≤1.0) for all treatment groups. No treatment-related differences were seen.

Acute GI Symptom Scale
Mean AGIS scores were low (≤2.0) for all treatment groups. Pre-treatment with ASA did not have an effect on acute GI symptoms.

Reviewer's Comment: All the PD assessments except flushing severity scale were inconclusive in determination of benefits of ASA pretreatment.

CONCLUSIONS:

- When administered approximately 30 minutes before BG00012 dosing of 240 mg BID, 240 mg TID or 360 mg BID, oral doses of 325 mg ASA appeared to have no significant effect on PK of MMF.
- ASA pre-treatment reduced the incidence and severity of flushing in the BG00012 groups.

FG-PK-02: A Phase I, Open-Label, Randomized, Two-Period Cross-Over Trial to Investigate the Possible Food Interaction of FAG-201, Administered as Single Oral Dose in Healthy, Male, Caucasian Subjects

Objective:
To determine the effects of food (continental breakfast approximately 700 kcal) on PK of the major metabolites of dimethyl fumarate including MMF and fumaric acid.

<table>
<tr><td>Study Design</td><td>The study was an open-label, randomized, two-period cross-over trial to investigate food effect on BG00012 (dimethyl fumarate), when administered as single oral dose of two gelatin capsules, (b) (4) (each capsule contained 120 mg dimethyl fumarate).</td></tr>
<tr><td>Study Population</td><td>Healthy males
Age: 18-45 years
BMI: 18 to 28 kg/m^2.
Twelve subjects enrolled and 12 were analyzed for PK.</td></tr>
<tr><td>Treatment Groups</td><td>Subjects were given a dose of 240 mg (2x120 mg) dimethyl fumarate either in fasting condition (treatment A) or after intake of a continental breakfast (treatment B), which contained approximately 700 kcal. The washout period in between two treatment periods was 7 days.

Treatment B: At approximately 30 minutes prior to drug administration, a continental breakfast was served consisting of one rye roll (45 g) and one white roll (45 g); 20 g butter, 25 g jam and 20 g honey; one slice of cheese (45% fat), one slice of ham; 100 ml milk (1.5% fat) and decoffeinated coffee or fruit tea. This meal derived approx. 339, 107 and 254 calories from carbohydrates, proteins and fat, respectively.</td></tr>
<tr><td>Sampling</td><td>Blood Samples were obtained at the following times: predose, 0.5, 0.75, 1, 1.25, 1.5, 1.75, 2, 2.25, 2.5, 2.75, 3, 3.5, 4, 4.25, 4.75, 5, 5.5, 5.75, 6, 6.25, 6.5, 6.75, 7, 7.5, 8, 9, 10, 12, and 24 hours</td></tr>
<tr><td>Analysis</td><td>The plasma samples were analyzed for the concentration of MMF by using HPLC method. The lower limit of quantification (LLOQ) was 0.1 mg/L for MMF.
<table>
<tr><th>Parameter</th><th>Quality Control Samples</th><th>Standard Curve Samples</th></tr>
<tr><td>Quality Control or Standard Curve Concentration (mg/L)</td><td>0.66, 2.54, and 4.35</td><td>0.1, 0.16, 0.48, 1.19, 2.39, 3.601, 5.27</td></tr>
<tr><td>Between Batch Precision (%CV)</td><td>6.34 to 13.87</td><td>2.15 to 3.68</td></tr>
<tr><td>Between Batch Accuracy (%RE)</td><td>-0.56 to 9.56</td><td>-4.69 to 2.13</td></tr>
<tr><td>Linearity</td><td colspan="2">Weighted linear equation (1/X^2), mean r= 0.998</td></tr>
<tr><td>Linear Range (mg/L)</td><td colspan="2">0.1 to 5.27</td></tr>
<tr><td>Sensitivity (LLOQ, mg/L)</td><td colspan="2">0.1</td></tr>
</table></td></tr>
<tr><td>PK Assessments</td><td>The PK parameters C_{max}, T_{max}, AUC_{last}, $AUC_{0\text{-}inf}$, apparent volume of distribution, apparent CL, and $t_{1/2}$ were calculated using NCA analysis.</td></tr>
<tr><td>Safety Assessments</td><td>Adverse event (AE) and serious adverse event (SAE) monitoring, physical examination and weight, vital signs measurement, clinical laboratory analysis (hematology, blood chemistry, coagulation [PT, PTT], urinalysis, beta-2 microglobulin, microalbumin), 12-lead electrocardiogram (ECG).</td></tr>
</table>

Statistical Methods	90% confidence intervals (CI) for ratios were used for the assessment of an effect of food effect on the pharmacokinetics of MMF and fumaric acid. These intervals were calculated based on the residual error of an analysis of variance (ANOVA) for AUC values and Cmax values of MMF and of fumaric acid with treatment, period, sequence and subject within sequence as sources of variation.

RESULTS:
Fumaric acid levels were undetectable in the plasma (LLOQ of the HPLC/UV method was 0.27 mg/L).

When taken with continental breakfast, Tmax of MMF was delayed compared to that under fasted condition.

Figure: Arithmetic Mean Plasma Concentration of Methyl Hydrogen Fumarate (MMF)

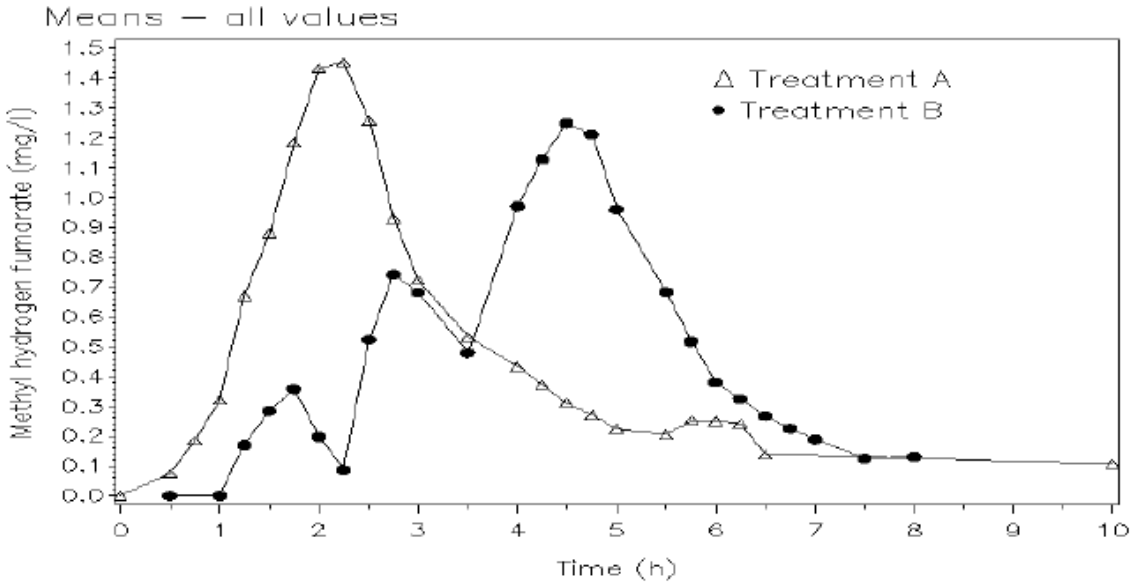
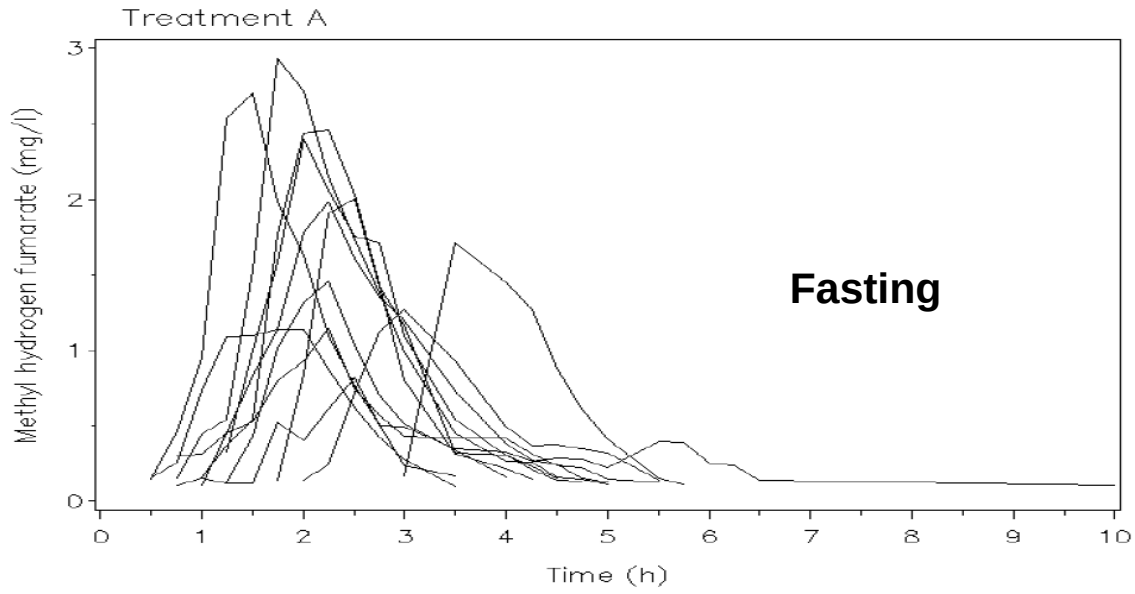
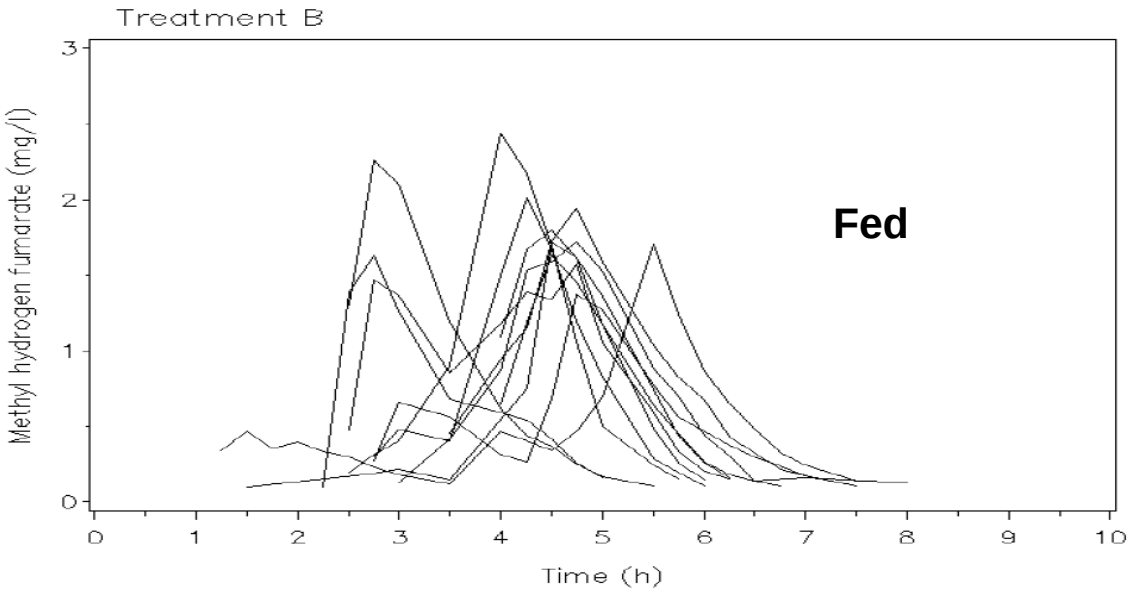


Figure: Individual Plasma Concentration Time Profiles of Methyl Hydrogen Fumarate (MMF), Treatment A: Under Fasting Conditions and Treatment B: Under Fed Conditions.





The following table summarizes PK parameters of MMF when BG00012 was administered under fasted and fed conditions. It should be noted that there was large variability for AUC and especially for Cmax.

Table: Mean PK Parameters for Mono-Methyl Fumarate (MMF) [N=12]

Treatments	Parameter	Unit	Mean[1]	SD[1]	Minimum	Median	Maximum
Treatment A (fasted)	$AUC_{(0-\infty)}$	[mg*h/l]	2.84	1.30	2.07	2.63	4.38
	$AUC_{(0-t)}$	[mg*h/l]	2.71	1.32	1.93	2.41	4.26
	C_{max}	[mg/l]	1.71	1.49	0.82	1.85	2.94
	$t_{½}$	[h]	0.71	0.46	0.38	0.60	2.10
	λ_z	[1/h]	1.19	0.42	0.33	1.16	1.83
	t_{max}	[h]	2.29	0.55	1.50	2.25	3.50
Treatment B (after breakfast)	$AUC_{(0-\infty)}$	[mg*h/l]	2.92	1.19	2.09	2.89	3.82
	$AUC_{(0-t)}$	[mg*h/l]	2.82	1.20	2.02	2.81	3.72
	C_{max}	[mg/l]	1.80	1.17	1.37	1.72	2.45
	$t_{½}$	[h]	0.46	0.12	0.33	0.43	0.74
	λ_z	[1/h]	1.60	0.37	0.94	1.61	2.08
	t_{max}	[h]	4.31	0.81	2.75	4.50	5.50

The following table summarizes statistical analysis conducted on PK parameters of MMF when BG00012 was administered under fasted and fed conditions.

Table: Summary of Pharmacokinetic Parameters, Point Estimates and 90% Confidence Intervals (CI) [Fasted state was used as reference]

Parameter	Unit	Lower 90% CL	Point Estimater	Upper 90% CL	Std. Error	p-Value
$AUC_{(0-\infty)}$	[mg*h/l]	0.9500	1.0259	1.1079	0.04243	0.5603
$AUC_{(0-t)}$	[mg*h/l]	0.9600	1.0427	1.1325	0.04558	0.3804
C_{max}	[mg/l]	0.8569	1.0487	1.2834	0.1114	0.6788

Reviewer's Comment: The HPLC assay used in this study was less sensitive compared to the LC-MS/MS method used in majority of the PK studies. The LLOQ of the HPLC method was only 0.1 mg/L. This may contribute to the large variability observed in this study.

CONCLUSIONS:

A normal diet did not affect overall exposure (AUC) and Cmax of MMF. However, the Tmax of MMF was delayed with food (from 2.25 hours to 4.5 hours).

C-1903: A Single-Center, Randomized, Crossover Study to Investigate Possible Food Effects on BG00012, When Administered as Single Oral Doses in Healthy Volunteers

Objective:
To determine the effect of food (high-calorie and high-fat meal, 800 to 1000 calories, approximately 50% of total calories from fat) on PK of MMF.

<table>
<tr><td>Study Design</td><td>The study was a single-center, randomized, 2-period, crossover study. Randomization to treatment sequence was stratified by gender.</td></tr>
<tr><td>Study Population</td><td>Healthy subjects (21 Male and 15 Female)
Age: 18-55 years
BMI: 18 to 30 kg/m^2.
Thirty six subjects enrolled and 33 were analyzed for PK.</td></tr>
<tr><td>Treatment Groups</td><td>Subjects were given a total of 2 doses of 240 mg of BG00012 orally in the study. Each subject was to receive 1 dose of 240 mg of BG00012 (2x120 mg capsules) in each treatment period (in the fasting and fed states).
Treatment periods were separated by 6 to 10 days.</td></tr>
<tr><td>Sampling</td><td>Blood samples for the plasma BG00012 PK assays in both treatment periods were obtained at -1 hours, 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, 5.5, 6, 6.5, 7, 7.5, 8, 9, 10, 11, and 12 hours after each dose on Day 1 of each treatment period.</td></tr>
<tr><td>Analysis</td><td>The plasma samples were analyzed for the concentration of MMF by using LC-MS/MS method. The lower limit of quantification (LLOQ) was 10 ng/mL for MMF.
<table>
<tr><th>Parameter</th><th>Quality Control Samples</th><th>Standard Curve Samples</th></tr>
<tr><td>Quality Control or Standard Curve Concentration (ng/mL)</td><td>30, 500, and 4000</td><td>10, 25, 50, 150, 500, 2000 and 5000</td></tr>
<tr><td>Between Batch Precision (%CV)</td><td>5.0 to 8.0</td><td>5.0 to 8.0</td></tr>
<tr><td>Between Batch Accuracy (%RE)</td><td>-9.0 to 4.0</td><td>-9.0 to 4.0</td></tr>
<tr><td>Linearity</td><td colspan="2">Weighted linear equation ($1/X^2$), mean r= 0.9998</td></tr>
<tr><td>Linear Range (ng/mL)</td><td colspan="2">10 to 5000</td></tr>
<tr><td>Sensitivity (LLOQ, ng/mL)</td><td colspan="2">10</td></tr>
</table></td></tr>
<tr><td>PK Assessments</td><td>The PK parameters C_{max}, T_{max}, AUC_{last}, $AUC_{0\text{-}inf}$, apparent volume of distribution, apparent CL, and $t_{1/2}$ were calculated from the plasma MMF concentration-time data using noncompartmental analysis.</td></tr>
<tr><td>Safety Assessments</td><td>Adverse event (AE) and serious adverse event (SAE) monitoring, physical examination and weight, vital signs measurement, clinical laboratory analysis (hematology, blood chemistry, coagulation [PT, PTT], urinalysis, beta-2 microglobulin, microalbumin), 12-lead electrocardiogram (ECG).</td></tr>
<tr><td>Statistical Methods</td><td>The 2 one-sided hypotheses at the α=0.05 level were tested by constructing the 90% CI for the geometric mean ratio of BG00012 fasting to BG00012 fed diet for $AUC_{0\text{-}inf}$, C_{max}, and AUC_{last}. The 80 to 125% criterion for log-transformed data was used. The data were transformed using natural logarithms and the log-transformed data were analyzed using an analysis of variance model with factors for sequence, subjects within sequence, period, and diet. The sequence effects were tested using the inter-subject variation and differences between periods or diets were compared using intra-subject variation estimated from the analysis of variance model.</td></tr>
</table>

RESULTS:
The following figure represents PK profiles of MMF when BG00012 was administered under fasted and fed conditions. With a high-fat meal, the overall exposure (AUC) of MMF was not affected, but the Cmax was reduced by approximately 40%. The Tmax was delayed from 2.0 hours to 5.5 hours.

Figure: Mean ± Standard Deviation Concentration versus Time of MMF in Plasma (n=33)

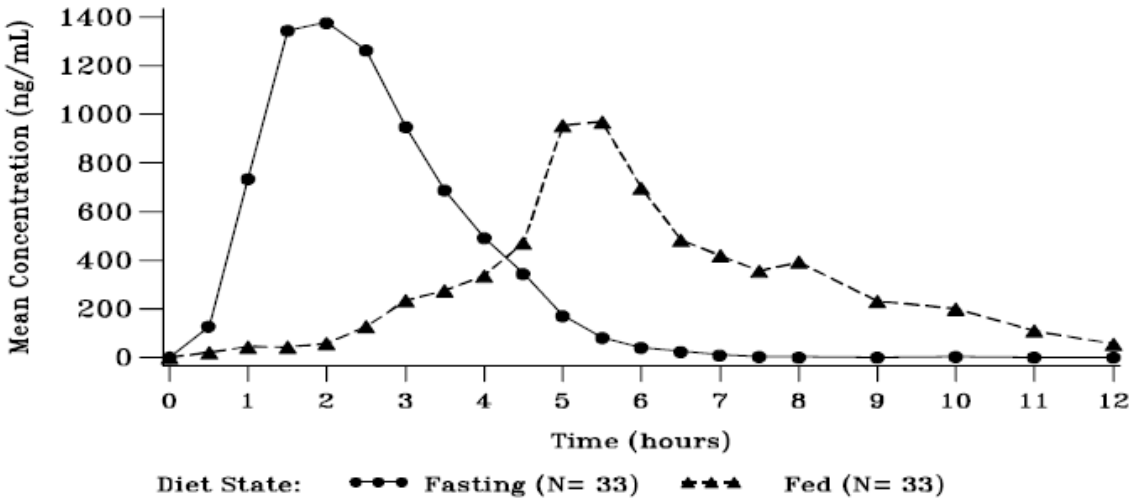
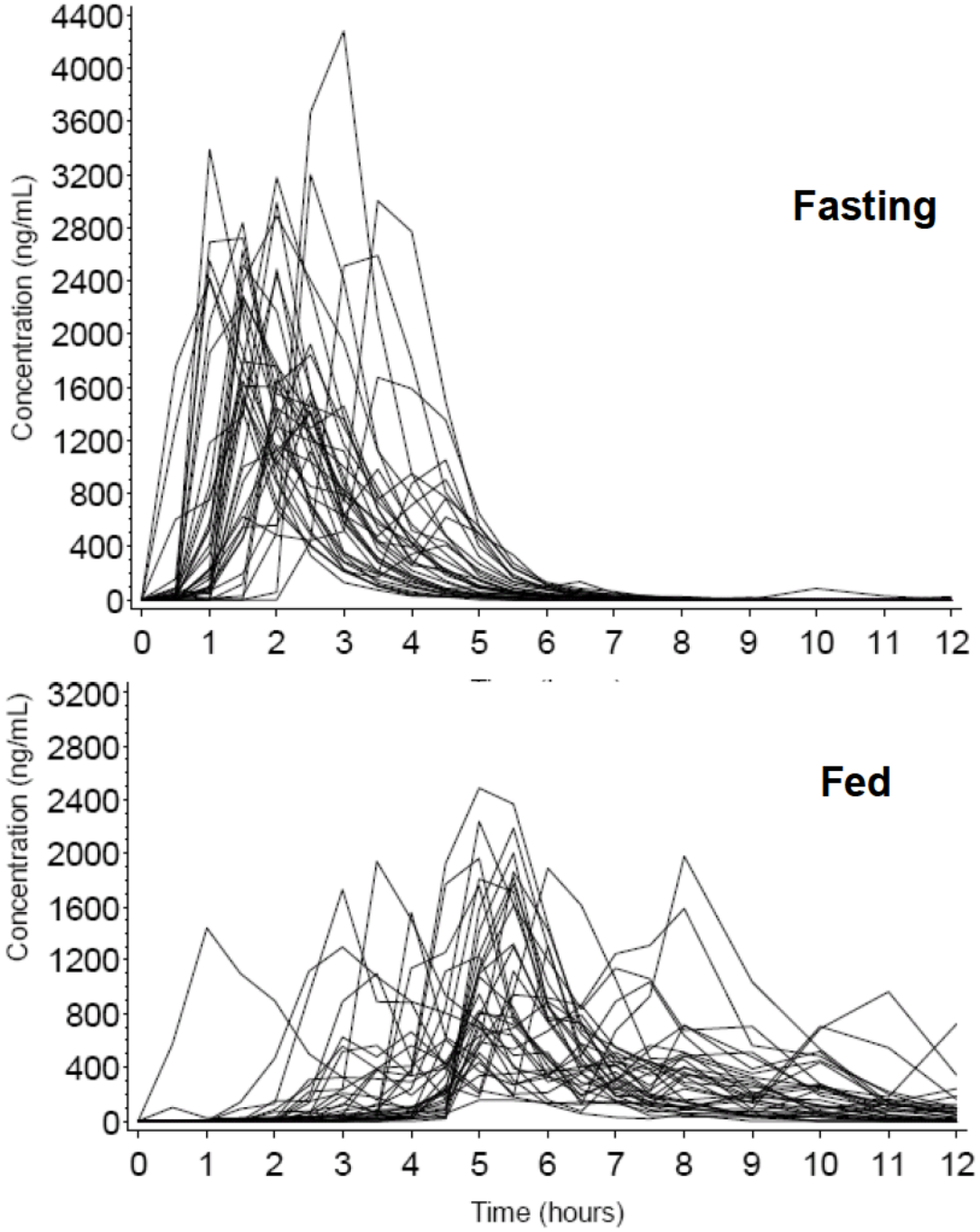


Figure: Individual Plasma Concentration Time Profiles of Methyl Hydrogen Fumarate (MMF), Treatment A: Under Fasting Conditions and Treatment B: Under Fed Conditions.



As shown in the above figures, PK profile of DMF becomes more variable when administered under fed condition (high-fat meal) and there was larger inter-individual variability.

The following table summarizes PK parameters of MMF when BG00012 was administered under fasted and fed conditions.

Table: Mean PK Parameters for Mono-Methyl Fumarate (MMF)

		BG00012 Fasting State	BG00012 Fed State
AUCinf (h*ug/mL)	n	33	33
	Mean	3.93	3.82
	SD	1.179	1.255
	Median	3.61	3.41
	Min, Max	1.88, 7.45	2.65, 8.86
Cmax (ug/mL)	n	33	33
	Mean	2.26	1.45
	SD	0.741	0.532
	Median	2.41	1.56
	Min, Max	1.12, 4.28	0.51, 2.49
AUClast (h*ug/mL)	n	33	33
	Mean	3.92	3.58
	SD	1.180	0.873
	Median	3.60	3.35
	Min, Max	1.87, 7.44	2.37, 5.62
Tmax (h)	n	33	33
	Mean	1.93	5.37
	SD	0.695	1.651
	Median	2.00	5.50
	Min, Max	1.00, 3.50	1.00, 10.00
t 1/2 (h)	n	33	33
	Mean	0.56	1.26
	SD	0.183	1.496
	Median	0.52	0.89
	Min, Max	0.38, 1.49	0.41, 9.04
Cl/F (L/h)	n	33	33
	Mean	66.02	67.41
	SD	18.953	15.609
	Median	66.44	70.35
	Min, Max	32.23, 127.52	27.10, 90.44
Vz/F (L)	n	33	33
	Mean	52.82	109.70
	SD	22.730	74.045
	Median	49.77	94.37
	Min, Max	28.38, 150.26	30.03, 353.52

The following table summarizes statistical analysis conducted on PK parameters of MMF when BG00012 was administered under fasted and fed conditions.

Table: Summary of Pharmacokinetic Parameters, Point Estimates and 90% Confidence Intervals (CI) [Fed state was used as reference]

Population (n= 33)	Arithmetic Mean	SD	CV	Geometric Mean (a)	Mean of Logs	SD of Logs	CV for Logs	Bioequivalence Estimate (b)	Bioequivalence 90% CI (c)
AUCinf (h*ug/mL)									
BG00012 Fasting State	3.9	1.2	30.0	3.8	1.3	0.3	21.5	103	96 - 110
BG00012 Fed State	3.8	1.3	32.8	3.7	1.3	0.3	20.8		
Cmax (ug/mL)									
BG00012 Fasting State	2.3	0.7	32.8	2.1	0.8	0.3	44.1	160	141 - 182
BG00012 Fed State	1.5	0.5	36.6	1.3	0.3	0.4	143.9		
AUClast (h*ug/mL)									
BG00012 Fasting State	3.9	1.2	30.1	3.8	1.3	0.3	21.6	108	102 - 114
BG00012 Fed State	3.6	0.9	24.4	3.5	1.2	0.2	18.4		

This study also showed that, with high-fat meal, there was some extent of improvement in flushing (94% of subjects in fasting compare to 68% in fed state) and GI disorders (8% of subjects in fasting compare to 6% in fed state).

CONCLUSIONS:

With a high-fat meal, the overall exposure (AUC) of MMF was not affected, but the Cmax was reduced by approximately 40%. The Tmax was delayed from 2.0 hours to 5.5 hours. Incidence of flushing decreased by some extent when BG00012 was administered under fed condition compared to fasted state.

109HV105: A Pharmacokinetics Profile Determination of BG00012 Standard Formulation and the BG00012 Active Pharmaceutical Ingredient (API) After a Single Oral Dose Administered to Healthy Male Volunteers

Objective:
To determine the PK profiles of the BG00012 standard formulation and the BG00012 API formulation in healthy volunteers.

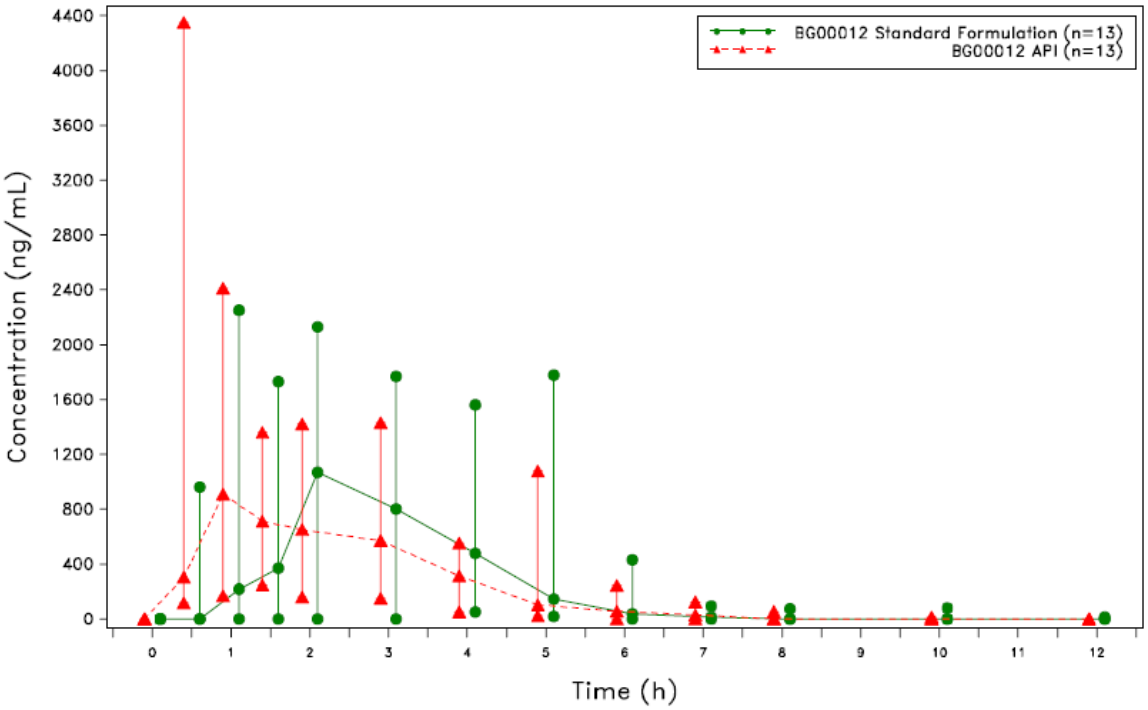
<table>
<tr><td>Study Design</td><td>The study was an open-label, single-center, 2-period crossover, 2-formulation, PK profile study. Approximately 14 subjects were enrolled in this study. Each subject was to be randomized to 1 of 2 dosing sequences.</td></tr>
<tr><td>Study Population</td><td>Healthy male subjects
Age: 18-55 years
BMI: 19 to 30 kg/m^2.
Fourteen subjects enrolled and 12 completed the study.</td></tr>
<tr><td>Treatment Groups</td><td>Sequence 1:
• Dosing Period 1: Approximately 7 subjects were to receive oral 240 mg BG00012 standard formulation [redacted] (b) (4) gelatin capsules).
• Dosing Period 2: Following a washout period of up to 8 days, subjects were to return to the clinic and receive oral 240 mg BG00012 API [redacted] (b) (4) hard gelatin capsules).

Sequence 2:
• Dosing Period 1: Approximately 7 subjects were to receive oral 240 mg BG00012 API.
• Dosing Period 2: Following a washout period of up to 8 days, subjects were to return to the clinic and receive oral 240 mg BG00012 standard formulation
All the treatments were administered under fasting conditions.</td></tr>
<tr><td>Sampling</td><td>Blood Samples were obtained at the following times predose, 0, 0.5, 1, 1.5, 2, 3, 4, 5, 6, 7, 8, 10, and 12 hours</td></tr>
<tr><td>Analysis</td><td>The plasma samples were analyzed for the concentration of MMF by using LC-MS/MS method. The LLOQ was 10 ng/mL.
<table>
<tr><th>Parameter</th><th>Quality Control Samples</th><th>Standard Curve Samples</th></tr>
<tr><td>Quality Control or Standard Curve Concentration (ng/mL)</td><td>30, 500, and 4000</td><td>10, 25, 50, 150, 500, 2000 and 5000</td></tr>
<tr><td>Between Batch Precision (%CV)</td><td>7.8 to 11.4</td><td>5.4 to 8.1</td></tr>
<tr><td>Between Batch Accuracy (%RE)</td><td>-5.8 to 2.8</td><td>-5.2 to 3.3</td></tr>
<tr><td>Linearity</td><td colspan="2">Weighted linear equation (1/X^2), mean r= 0.999</td></tr>
<tr><td>Linear Range (ng/mL)</td><td colspan="2">10 to 5000</td></tr>
<tr><td>Sensitivity (LLOQ, ng/mL)</td><td colspan="2">10</td></tr>
</table></td></tr>
<tr><td>PK Assessments</td><td>The PK parameters C_{max}, T_{max}, AUC_{0-12}, $AUC_{(0-inf)}$, apparent volume of distribution, CL, t_{lag} and $t_{1/2}$ were calculated from the plasma MMF concentration-time data using noncompartmental analysis.</td></tr>
<tr><td>Safety Assessments</td><td>Adverse event (AE) and serious adverse event (SAE) monitoring, physical examination and weight, vital signs measurement, clinical laboratory analysis (hematology, blood chemistry, coagulation [PT, PTT], urinalysis, beta-2 microglobulin, microalbumin), 12-lead electrocardiogram (ECG).</td></tr>
</table>

Statistical Methods	The 90% confidence intervals (CI) of the geometric mean ratio of $AUC_{0-\infty}$ and C_{max} values between the two BG00012 treatments were calculated. The log-transformed data was to be analyzed using an analysis of variance model with factors for sequence, subjects within sequence, period, and treatment groups. The sequence effects were tested using the inter-subject variation and differences between periods or treatments were compared using intra-subject variation estimated from the analysis of variance model.

RESULTS:
The concentration-time profile of the API formulation was characterized by immediate absorption (i.e, no T_{lag}), earlier T_{max} (2 hours vs. 3 hours), and a lower C_{max} (decreased by 30%) with respect to the standard formulation. The overall exposure AUC was similar for both the formulations.

Figure: Median and range of concentration vs. time for MMF in plasma



The following table summarizes PK parameters of MMF following administration of BG00012 standard formulation and the BG00012 API.

Table: Summary of MMF Pharmacokinetic Parameters

	BG00012 Standard Formulation	BG00012 API
AUC(0-inf) (h*ng/mL)		
n	13	13
Mean	3050.7	3021.7
SD	775.95	687.89
Median	3009.0	2874.0
Min, Max	2005, 4938	2132, 4336
T1/2 (h)		
n	13	13
Mean	0.92200	1.02132
SD	0.860956	0.469724
Median	0.60550	0.85730
Min, Max	0.4221, 3.5860	0.6044, 2.3050
Cmax (ng/mL)		
n	13	13
Mean	1747.7	1410.0
SD	335.71	969.31
Median	1770.0	1100.0
Min, Max	1230, 2250	647, 4350
Tmax (h)		
n	13	13
Mean	2.66	1.92
SD	1.079	1.222
Median	3.00	2.00
Min, Max	1.0, 5.0	0.5, 5.0
Tlag (h)		
n	13	13
Mean	0.73	0.00
SD	0.780	0.000
Median	0.50	0.00
Min, Max	0.0, 3.0	0.0, 0.0

The following table summarizes statistical analysis conducted on PK parameters of MMF.

Table: Relative BA of BG00012 standard formulation (reference) vs. BG00012 API (test), summary of 90% CI assessment, (N = 12).

.	Arithmetic Mean	SD	Geometric Mean	Geometric Mean Ratio	90% CI of Geometric Mean Ratio
AUC(0-inf) (h*mg/L)					
Standard Formulation	3.14	0.741	3.06	97.4%	84.8 -112.0%
BG00012 API	3.06	0.704	2.99		
Cmax (mg/L)					
Standard Formulation	1.79	0.312	1.76	70.2%	51.9 - 94.8%
BG00012 API	1.44	1.01	1.24		

CONCLUSIONS:

The [redacted] (b) (4) standard formulation resulted in a lag time for absorption and delayed T_{max} (by approximately 1 hr) compared to API.

The mean Cmax of API was 30% lower compared to standard formulation. The overall exposure (AUC) and elimination half-lives were similar for both formulations.

109HV107: A Randomized, Two-Period Crossover Study in Healthy Volunteers to Establish the Bioequivalence of BG00012 Given as a Single Capsule and Given as Two Capsules

Objective:
To establish bioequivalence of the reference product (two BG00012 120 mg capsules) and test product (a single BG00012 240 mg capsule)

<table>
<tr><td>Study Design</td><td>The study was a single-center, 2-period crossover study in healthy adult volunteers. The two dosing periods were separated by a washout interval of 3 to 7 days.</td></tr>
<tr><td>Study Population</td><td>Healthy male and female
Age: 18-55 years
BMI: 19-30 kg/m^2</td></tr>
<tr><td>Treatment Groups</td><td>ference Product: two BG00012 120 mg capsules
t Product: a single BG00012 240 mg capsule
the treatments were administered under fasting conditions.</td></tr>
<tr><td>Number of Subjects</td><td>Eighty subjects were planned, and 81 subjects were enrolled and dosed. Seventy-seven subjects dosed with reference product, and 81 subjects dosed with test product had measureable MMF concentrations and were analyzed for PK.</td></tr>
<tr><td>Sampling</td><td>Blood samples for PK analysis were obtained during each study period at the following times predose, 30, 60, 90 minutes 2, 3, 4, 5, 6, 7, 8, 10 and 12 hours</td></tr>
<tr><td>Analysis</td><td>The plasma samples were analyzed for the concentration of MMF by using LC-MS/MS method. The lower limit of quantification (LLOQ) was 10 ng/mL
<table>
<tr><th>Parameter</th><th>Quality Control Samples</th><th>Standard Curve Samples</th></tr>
<tr><td>Quality Control or Standard Curve Concentration (ng/mL)</td><td>30, 500, and 4000</td><td>10, 25, 50, 150, 500, 2000 and 5000</td></tr>
<tr><td>Between Batch Precision (%CV)</td><td>3.50 to 7.03</td><td>-1.4 to 1.2</td></tr>
<tr><td>Between Batch Accuracy (%RE)</td><td>-1.33 to 0.0</td><td>2.8 to 8.2</td></tr>
<tr><td>Linearity</td><td colspan="2">Weighted linear equation (1/X^2), mean r= 0.997</td></tr>
<tr><td>Linear Range (ng/mL)</td><td colspan="2">10 to 5000</td></tr>
<tr><td>Sensitivity (LLOQ, ng/mL)</td><td colspan="2">10</td></tr>
</table></td></tr>
<tr><td>PK Assessments</td><td>The PK parameters C_{max}, T_{max}, AUC_{0-t}, AUC_{0-inf}, t_{lag} and $t_{1/2}$ were calculated from the plasma MMF concentration-time data using NCA analysis.</td></tr>
<tr><td>Safety Assessments</td><td>Safety was monitored by vital signs, physical examinations, 12-lead ECG, hematology, blood chemistry, urinalysis, recording concomitant therapy and procedures, and monitoring for adverse events (AEs).</td></tr>
<tr><td>Statistical Methods</td><td>$AUC_{0-\infty}$ and C_{max} were the primary endpoints used to show the PK comparability of the reference and test products. The two one-sided hypotheses at the $\alpha = 0.05$ level were tested by constructing a 90% confidence interval for the geometric mean ratio of the test product to the reference product. The standard 80% to 125% equivalence criterion was used as the bioequivalence limit.</td></tr>
</table>

RESULTS:
The estimated ratio of geometric means $AUC_{0-\infty}$ for test to reference was 103% (90% CI of 99% to 107%), and for Cmax the ratio was 106% (90% CI of 96% to 116%).

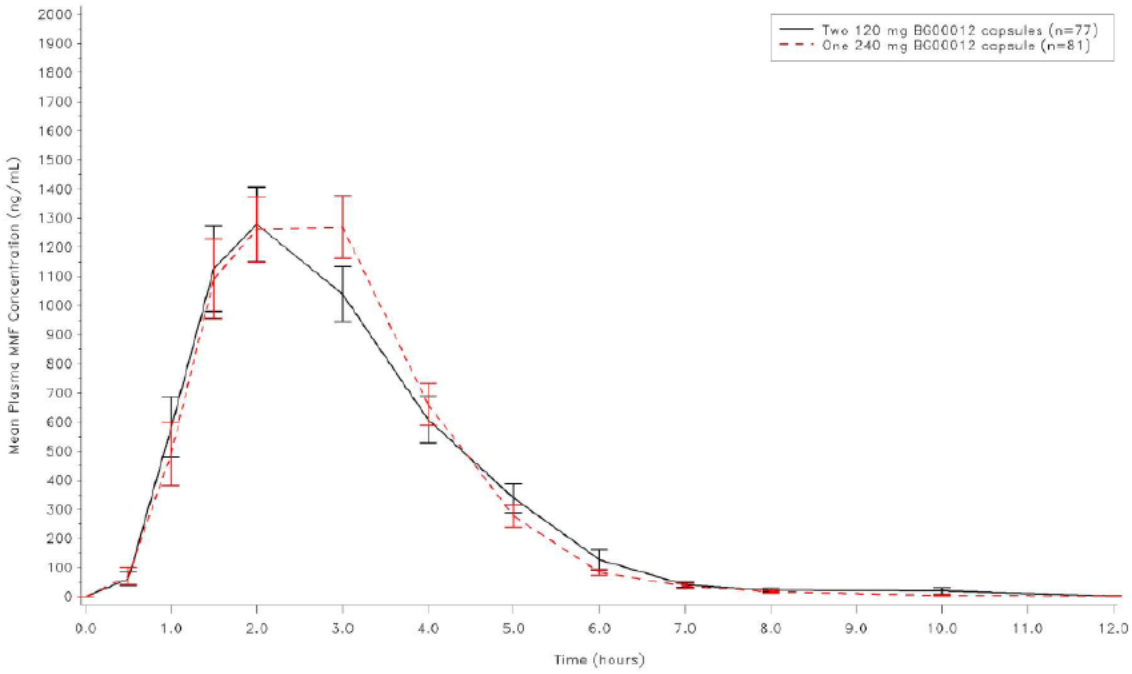
The following table summarizes PK parameters $AUC_{0-\infty}$, C_{max} and T_{max}. Other PK parameters including CL/F, T_{lag} and $T_{½}$, were also similar between the reference and test products.

Table: Summary of MMF PK-Parameters

	Two 120 mg BG00012 capsules	**One 240 mg BG00012 capsules**
Number of subjects dosed	77	81
AUC(inf) (h*ug/L)		
n	77	81
Mean	3866.2	3975.8
SD	1235.75	1153.37
Median	3633.0	3839.0
Min, Max	2031, 7968	1815, 7720
Geometric mean	3701.9	3823.2
CV (%)	32	29
Cmax (µg/L)		
n	77	81
Mean	2339.9	2421.2
SD	1125.01	950.44
Median	2050.0	2210.0
Min, Max	633, 6270	814, 5000
Geometric mean	2101.0	2241.2
CV (%)	48	39
Tmax (h)		
Mean	2.41	2.48
SD	1.149	1.075
Median	2.00	2.00
Min, Max	0.5, 6.0	0.5, 5.0
Geometric mean	2.16	2.24
CV (%)	48	43

The PK profiles of reference and test products were comparable for male and female subjects.

Figure: Mean Plasma MMF Concentration (± *Standard Error*) Time Profile



CONCLUSIONS:

A single BG00012 240 mg capsule was bioequivalent to two BG00012 120 mg capsules. The other PK parameters estimated were similar for both the treatments.

Office of Scientific Investigations Audit:
At the request of Division of Neurology Products, the Office of Scientific Investigations conducted audit of the bioequivalence study (Study # 109HV107). The clinical and analytical portions of the studies were conducted at Prism Clinical Research (Saint Paul, MN) and (b) (4) respectively. Following the inspection (b) (4) no objectionable conditions were observed and Form FDA 483 was not issued. However, for Prism Clinical Research, Form 483 (Inspectional Observations) was issued. The clinical and analytical audit was based on 100% audit of source data.

OSI evaluated the Prism's response to the Form 483 and associated exhibits related to objectionable observations and recommended that the clinical and bioanalytical portions of Study 109HV107 be accepted for agency review, subject to evaluations by the OCP reviewer of MHF stability in plasma samples without detailed records of handling and preservation.

Reviewer's Comments: In vitro human plasma stability of MHF studies indicate that MHF has a half-life around 70 hours.

The plasma concentration time profile of MHF (2 x 120 mg capsule group) obtained from this (Study 109HV107) was similar to the PK profiles obtained from several other PK studies (Studies 109-HV-101, FG-PK-02 and C-1903) using same dose (also 2 x 120 mg capsule) under fasting conditions. Studies C-1903 and FG-PK-02 were conducted in (b) (4) *respectively. Thus, the lack of detailed records of handling and preservation of plasma samples at the clinical site (Saint Paul, MN) for the current study did not impact the study results.*

In Vitro Studies

Study Title	Evaluation of Induction Potential of Cytochrome P450 2B6, 2C8 and P-gp by MMF in Cultured Human Hepatocytes
Study number	P00012-06-05
Study Period	September 2006 to February 2007
Study Director	(b) (4)
Objective	The objective of this study was to evaluate *in vitro* induction potential of MMF for CYP2C8, CYP2B6 and P-gp.

METHODS

In vitro induction potential of Cytochrome P450 2B6, 2C8, and P-gp (P-glycoprotein) by MMF was evaluated using primary cultured human hepatocytes. Hepatocytes were incubated for 3 days with MMF in triplicates at concentrations of 1, 10 and 100 µM of MMF.

Induction was determined by measuring mRNA expression for CYP2B6, 2C8, and P-gp. Real time RT-PCR was used to measure mRNA levels. Induction was also measured by a catalytic activity assay, selective for CYP2B6, which measured S-mephenytoin-N-demethylase activity using HPLC analysis with radiometric detection.

Following positive controls were used for induction assays.

Enzyme	Assay	Positive control inducer	Final concentration
CYP2B6	mRNA/activity	Phenobarbital	2000 µM
CYP2C8	mRNA	Phenobarbital Rifampicin	2000 µM 20 µM
P-gp	mRNA	Phenobarbital Rifampicin	2000 µM 20 µM

RESULTS

The following tables shows the effects of MMF and the positive controls on mRNA expression.

Table. Effect of MMF and the positive control phenobarbital (PB) on CYP2B6 mRNA expression in hepatocytes from 3 donors.

Donor	Treatment	Fold induction	Induction as % of positive control*
	[illegible]	[illegible]	
All three donors	1µM MMF	1.1 ± 0.74	0.5
	10 µM MMF	1.3 ± 1.4	1.5
	100 µM MMF	1.8 ± 1.9	4.0
	Positive controls		
	2000 µM PB	21 ± 2.8	-

Table. Effect of MMF and the positive controls phenobarbital (PB) and rifampicin (RIF) on CYP2C8 mRNA expression in hepatocytes from 3 donors

Donor	Treatment	Fold induction	Induction as % of positive control*

All three donors	1μM MMF	1.32 ± 0.17	6.0
	10 μM MMF	1.19 ± 0.62	3.6
	100 μM MMF	1.1 ± 0.59	1.9
	Positive controls		
	2000 μM PB	6.3 ± 6.8	-
	20 μM RIF	1.5 ± 0.58	-

Table. Effect of MMF and the positive controls phenobarbital (PB) and rifampicin (RIF) on P-gp mRNA expression in hepatocytes from 3 donors

Donor	Treatment	Fold induction	Induction as % of positive control*
All three donors	1μM MMF	1.0 ± 0.43	NA
	10 μM MMF	1.3 ± 0.53	18
	100 μM MMF	1.1 ± 0.54	5.9
	Positive controls		
	2000 μM PB	2.7 ± 0.82	-
	20 μM RIF	1.3 ± 0.47	-

Effect of MMF on CYP2B6 activity
MMF did not exhibit induction of CYP2B6 activity at concentrations of up to 100 μM. In contrast, treatment with 2000 μM PB increased CYP2B6 activity to 6.3, 18 or 38-fold in three livers.

CONCLUSIONS

- No significant induction of CYP2B6, CYP2C8, and P-gp by MMF was observed in mRNA assay.

- No induction of CYP2B6 activity was observed at MMF concentrations up to 100 μM.

Study Title	Methyl Hydrogen Fumarate: Evaluation of CYP450 Induction using Primary Cultures of Human Hepatocytes
Study number	P00012-04-14
Study Period	October 2004
Study Director	(b) (4)
Objective	The objective of this study was to evaluate *in vitro* induction potential of methyl hydrogen fumarate toward specific CYP1A2, 2B6, 2C9, 2C19, and 3A4.

METHODS
In vitro induction potential of methyl hydrogen fumarate (MMF) toward specific CYP1A2, 2B6, 2C9, 2C19, and 3A4 enzymes were determined by standard procedures per Agency's guidance.

Determination of CYP2C9, 2B6, 2C19, and 3A4 Activities
The following Table lists metabolites which were monitored to evaluate CYP enzyme activities using probe substrates.

CYP Isoenzyme	Metabolite	Enzyme	Standard Curve Ranges (µM)
CYP2B6	Hydroxybuproprion	Buproprion hydroxylase	0.0049 to 5
CYP2C9	4-Hydroxydiclofenac	Diclofenac 4′-hydroxylase	0.0244 to 25
CYP2C19	4-Hydroxymephenytoin	Mephenytoin 4′-hydroxylase	0.0003 to 5
CYP3A4	6β-Hydroxytestosterone	Testosterone 6β-hydroxylase	0.0244 to 25

List of Positive Control Inducers

CYP Isoenzyme	Inducer	Solvent	Dose Concentration (µM)
CYP1A2	Omeprazole	0.5% DMSO in HMM	25
CYP2B6	Phenobarbital	HMM	1000
CYP2C9	Rifampicin	0.5% DMSO in HMM	50
CYP2C19	Rifampicin	0.5% DMSO in HMM	50
CYP3A4	Rifampicin	0.5% DMSO in HMM	50

RESULTS

The following table describes the induction potential of MMF at concentrations of 2, 20 and 200 µM after 3-day incubation with hepatocytes.

CYP Isoform	Inducer	Concentration of Inducer (µM)	Fold Induction		
			Donor 1	Donor 2	Donor 3
CYP1A2	MMF	2	1.0	1.0	0.9
	MMF	20	0.9	1.0	1.0
	MMF	200	0.9	0.7	0.6
	Omeprazole	25	3.1	8.2	21
CYP2B6	MMF	2	0.8	1.0	0.8
	MMF	20	1.2	0.9	1.0
	MMF	200	2.7	1.8	2.6
	Phenobarbital	1000	8.8	6.3	11
CYP2C9	MMF	2	0.9	0.9	1.1
	MMF	20	0.8	1.0	1.4
	MMF	200	1.0	1.6	1.6
	Rifampicin	50	2.3	2.0	1.8
CYP2C19	MMF	2	0.8	1.1	1.1
	MMF	20	1.4	1.2	1 3
	MMF	200	1.4	1.6	1.8
	Rifampicin	50	18	2.5	5.1
CYP3A4	MMF	2	1.0	1.1	0.9
	MMF	20	0.9	0.8	1.0
	MMF	200	0.5	0.6	0.6
	Rifampicin	50	2.6	4.7	4.4

Reviewer's Comment: MMF did not have significant induction effects on activities of CYP1A2, CYP2B6 and CYP3A4, as the induction effects were less than 40% of the induction effects of corresponding positive controls. At a concentration of 200 µM, MMF exhibited induction effects on activities of CYP2C9 (in donor 2) and CYP2C19 (in donor 2). However, the mean Cmax of MMF after 240 mg BID dosing in MS patients was around 1.87 mg/L (Study

109MS101), corresponding to 14.4 μM which is more than 10 fold lower than 200 μM. Therefore, in vivo induction of CYP2C9 and CYP2C19 by MMF is unlikely at its therapeutic doses (240 mg BID).

It is noted that CYP2C9 was induced to some extent by MMF at a concentration of 20 μM in donor 3 relative to the positive control. However, per the Drug-Drug Interaction guidance, a negative in vitro result for CYP3A induction eliminates the need for additional in vitro or in vivo induction studies for CYP3A and CYP2C enzymes. As shown in the above table, MMF did not have induction effect on CYP3A4 activity at concentrations up to 200 μM.

CONCLUSIONS
MMF did not significantly induce CYP1A2, CYP2B6, CYP2C9, CYP2C19 or CYP3A4.

Study Title	Evaluation of Inhibition of the Catalytic Activities of Human Recombinant Cytochromes P450 2B6 and 2C8 by MMF and DMF
Study number	P00012-06-04
Study Period	November 2006
Study Director	(b) (4)
Objective	The objective of this study was to evaluate *in vitro* inhibitory potential of MMF and DMF toward specific 2B6 and 2C8 using cDNA-expressed enzymes.

METHODS
In vitro IC50 values to predict the in-vivo drug-drug interaction potential was determined using cDNA-expressed enzymes. Final MMF and DMF concentrations used in the incubation studies were 300, 100, 30, 10, 3, 1, 0.3, 0.1, 0.03, 0.01 and 0 μM. Bupropion was used as probe substrate for CYP2B6 and paclitaxel was used as probe substrate for CYP2C8. HPLC assays were used to quantitate the formation of metabolites.

RESULTS
The IC50 values were not determined because the test articles, MMF or DMF, did not inhibit catalytic activities of CYP2B6 (bupropion hydroxylase) and CYP2C8 (paclitaxel 60-hydroxylase) by more than 50% at the highest concentration (300 μM) studied.

CONCLUSIONS
DMF and MMF did not significantly inhibit CYP2B6 or CYP2C8 activities at concentrations of 0.01 to 300 μM.

Study Title	Evaluation of in vitro Cytochrome P450 Inhibition Profile of Dimethyl Fumarate (DMF) and Monomethyl Fumarate (MMF) using High Throughput Inhibitor Screening Kits.
Study number	P05-28
Study Period	January 2005
Study Director	(b) (4)
Objective	The objective of this study was to evaluate *in vitro* inhibition potential of DMF and MMF for towards six major cytochrome P450 (CYP) isoforms (CYP1A2, 2C9, 2C19, 2D6, 2E1, and 3A4) in recombinant systems.

METHODS
Inhibition potential of DMF and MMF (up to concentration of 50 μM) for CYP isoforms 1A2, 2C9, 2C19, 2D6, 2E1, and 3A4 was measured in 96-well plates with cDNA-expressed enzymes. The inhibition study consisted of the determination of a 50% inhibitory concentration (IC_{50}) for DMF and MMF. The following table lists the substrates and the corresponding fluorescent metabolites for each CYP isoforms tested.

List of substrates and the corresponding fluorescent metabolites for each CYP isoforms tested.

CYP Isoform	Substrate	Fluorescent Metabolite
1A2	3-Cyano-7-Ethoxycoumarin(CEC)	3-Cyano-7-Hydroxycoumarin (CHC)
2C9	7-Methoxy-4-Trifluoromethyl coumarin (MFC)	7-Hydroxy-4-Trifluoromethyl coumarin (MFC)
2C19	3-Cyano-7-Ethoxycoumarin(CEC)	3-Cyano-7-Hydroxycoumarin (CHC)
2D6	3-[2-(N,N-diethyl-N-methylamino)ethyl]-7-methoxy-4-methylcoumarin (AMMC)	3-[2-(N,N-diethyamino)ethyl]-7-hydroxy-4-methylcoumarin (AMHC)
2E1	7-Methoxy-4-Trifluoromethyl coumarin (MFC)	7-Hydroxy-4-Trifluoromethyl coumarin (MFC)
3A4	7-Benzyloxy-trifluoromethylcoumarin (BFC)	7-Hydroxy-trifluoromethylcoumarin (HFC)
3A4	7-Benzyloxiquinoline (BQ)	7-Hydroxyquinoline (HQ)

The following table lists the substrates and positive controls for each CYP isoforms tested.

Concentrations of substrates and positive control inhibitors for each CYP isoform

CYP Isoform	Substrate (Final Concentration, μM)	Positive Control Inhibitor (Highest Concentration Tested, μM)
1A2	CEC (5 μM)	Furafylline (100 μM)
2C9	MFC (75 μM)	Sulfaphenazole (10 μM)
2C19	CEC (25 μM)	Tranylcypromine (100 μM)
2D6	AMMC (1.5 μM)	Quinidine (0.5 μM)
2E1	MFC (70 μM)	Diethyldithiocarbamic acid (40 μM)
3A4	BFC(50 μM)	Ketoconazole (5 μM)
3A4	BQ (40 μM)	Ketoconazole (5 μM)

Incubations were initiated by the addition of pre-warmed enzyme. After the incubation the fluorescent metabolites in each of the isoforms/substrate system was measured using an excitation and emission wavelengths.

RESULTS
The following tables presents the IC50 values determined for each CYP isozyme tested.

Summary of IC_{50} values

CYP Isoform/Substrate	DMF	MMF	Positive Control
1A2/CEC	>50 μM	>50 μM	4.05 μM

2C9/MFC	>50 μM	>50 μM	0.15 μM
2C19/MFC	>50 μM	>50 μM	3.6 μM
2D6/AMMC	>50 μM	>50 μM	0.01 μM
2E1/MFC	>50 μM	>50 μM	3.57 μM
3A4/BFC	>50 μM	>50 μM	0.015 μM
3A4/BQ	>50 μM	>50 μM	0.035 μM

CONCLUSIONS
DMF and MMF did not significantly inhibit any of the CYP isoforms (CYP1A2, 2C9, 2C19, 2D6, 2E1, and 3A4) in recombinant CYP enzyme systems, at concentrations up to 50 μM.

Study Title	Reversible CYP Inhibition Potential of Monomethyl Fumarate Determined in vitro Using Human Liver Microsomes
Study number	P00012-10-03
Study Period	December 2010
Study Director	(b) (4)
Objective	The objective of this study was to determine the reversible CYP inhibition potential of MMF using human liver microsomes and CYP-isoform specific probe substrates.

METHODS
Standard procedures for in vitro metabolism studies were used. Microsomes were pooled from at least 10 human donors. The formation of the selective metabolite from its substrate was measured by LC/MS/MS analysis. Microsomes were pre-incubated with 9 serially diluted concentrations: 30 nM to 200 μM MMF in an NADPH regenerating system and liver microsomes. CYP enzyme substrates and inhibitors were obtained from commercial sources. The following table lists the CYP-isoform specific probe substrates, their corresponding metabolites to be measured, and CYP-isoform specific positive controls used in the assay.

CYP Isoform	Substrate (final concentration μM)	Metabolite	Positive Control (Concentration μM)
1A2	Tacrine (2)	4-Hydroxytacrine	Furafylline (100)
2B6	Bupropion (10)	4-Hydroxybupropion	Ticlopidine (10)
2C8	Amodiaquine (0.5)	N-Desyethylamodiaquine	Montelukast (10)
2C9	Tolbutamide (150)	4-Hydroxytolbutamide	Sulfaphenazole (20)
2C19	(S)-Mephenytoin (100)	4-Hydroxymephenytoin	Benzyl nirvanol (20)
2D6	Dextromethorphan (8)	Dextrorphan	Quinidine (10)
2E1	Chlorzoxazone (10)	6-Hydroxychlorzoxazone	DDTC (1000)
3A4	Midazolam (5)	1-Hydroxymidazolam	Ketoconazole (5)
3A4	Testosterone (17)	6-β-Hydroxytestosterone	Ketoconazole (1)

RESULTS
The IC_{50} values of MMF for all eight CYP isoforms (1A2, 2B6, 2C8, 2C9, 2C19, 2D6, 2E1, and 3A4) are greater than 50 μM.

CYP isoform/Substrate	IC50 [μM]	
	MMF	Positive Control
1A2/tacrine	>50	2.52
2B6/bupropion	>50	0.11
2C8/amodiaquine	>50	0.59
2C9/tolbutamide	>50	0.32
2C19/(S)-mephenytoin	>50	0.56
2D6/dextromethorphan	>50	0.06
2E1/chlorzoxazone	>50	39.5
3A4/midazolam	>50	0.09
3A4/testosterone	>50	0.041

CONCLUSIONS
The results indicate that significant inhibition of CYP (1A2, 2B6, 2C8, 2C9, 2C19, 2D6, 2E1, and 3A4) enzymes by MMF is less likely to occur in vivo.

Study Title	Inhibitory Potential of Dimethyl Fumarate and Methyl Hydrogen Fumarate toward Human Hepatic Microsomal Cytochrome P450 Isoenzymes
Study number	P00012-04-13
Study Period	October 2004
Study Director	(b) (4)
Objective	The objective of this study was to characterize the *in vitro* inhibitory potential of dimethyl fumarate and methyl hydrogen fumarate toward specific isozymes of human hepatic cytochrome P450.

METHODS
Recombinant human cytochromes P450 isozymes were used. Inhibition potential of DMF and MMF (0.1 to 50 μM for each) towards CYP2D6 was determined by evaluating bufuralol 1-hydroxylase activity and CYP3A4 activity by evaluating testosterone 6β-hydroxylase and midazolam 1-hydroxylase activity. Quinidine (0.5 μM) and ketoconazole (0.5 μM) were used as inhibitors of CYP2D6 and CYP3A4 (positive control), respectively.

RESULTS
Inhibition of cytochrome P450 isoenzyme-selective activities by DMF and MMF

CYP450 Isoenzyme	**Activity Measured**	**IC50 (μM)**	
		Dimethyl Fumarate	**Methyl Hydrogen Fumarate**
CYP2D6	Bufuralol 1' -hydroxylase	27.6	No Inhibition
CYP3A4	Testosterone 6 β-hydroxylase	No Inhibition	No Inhibition
CYP3A4	Midazolam 1'-hydroxylase	No Inhibition	No Inhibition

CONCLUSIONS
DMF inhibited CYP2D6 activity with an IC50 of 27 μM, but was not an inhibitor of CYP3A4 at concentrations up to 50 μM. MMF was not an inhibitor of CYP2D6 or CYP3A4 at

concentrations up to 50 µM. Since DMF is not detectable in systemic circulation, the inhibition effect of DMF on CYP2D6 does not have clinical relevance.

Study Title	In Vitro Metabolism of Dimethyl Fumarate and Methyl Hydrogen Fumarate by Human Hepatic Microsomes and Human Recombinant Cytochromes P450
Study number	P00012-04-12
Study Period	October 2004
Study Director	(b) (4)
Objective	The objective of this study was to determine the metabolic stability of dimethyl fumarate and methyl hydrogen fumarate in pooled human hepatic microsomes and in human recombinant cytochromes P450 2D6 and 3A4.

METHODS
Human hepatic microsomes (1 mg/mL final incubation concentration), recombinant cytochromes P450 2D6 and 3A4 (0.5 mg/mL final incubation concentration), or vector control microsomes (0.5 mg/mL final incubation concentration) were incubated with NADPH in a 0.1 M potassium phosphate buffer (pH 7.4) containing 1 mM EDTA (assay buffer) for at least three minutes at 37°C. Incubations were initiated by the addition of 50 µL of test article (50-µM final incubation concentration). Reactions were terminated at 0, 15, 30, and 60 minutes of incubation by the addition of 100 µL of the stopping solution (82.5:17.5 10% (v/v) acetic acid:acetonitrile), and precipitated protein was removed by centrifugation, 1,582 x g for 5 minutes at 4°C. Supernatant (100 µL) was transferred to a separate tube and analyzed by HPLC. Control incubations for each test article were incubated for 60 minutes and were conducted in assay buffer with hepatic microsomes only (minus NADPH) or with vector-treated microsomes. All incubations were conducted in duplicate. Samples were analyzed for test article and metabolites using an HPLC method

RESULTS
Methyl hydrogen fumarate (MMF) was stable upon incubation with human hepatic microsomes, recombinant cytochromes CYP2D6 and CYP3A4, and vector control microsomes. MMF decreased by less than 5% for all matrices after 60 minutes of incubation.

Dimethyl fumarate (DMF) was rapidly degraded to one major metabolite, MMF, in human hepatic microsomes with the presence of NADPH. Incubation of DMF with human hepatic microsomes in the absence of NAPDH showed similar biotransformation with no DMF remaining after 60 minutes of incubation.

DMF was decreased by approximately 25% after 60 minutes of incubation with expressed cytochromes (CYP2D6 and CYP3A4). The decrease is likely mediated by unspecified esterases present in human B lymphoblastoid cell line.

CONCLUSIONS
MMF was stable in human liver microsomes. However, DMF was rapidly metabolized to MMF. The degradation of DMF was independent of NADPH, indicating no involvement of cytochromes P450.

Study Title	Di-Methyl Fumarate and Mono-Methyl Fumarate Inhibition Potential on P-Glycoprotein Using Caco2 System
Study number	P00012-10-04
Study Period	July 2010 to October 2010
Study Director	(b) (4)
Objective	The objective of this study was to determine the P-glycoprotein (P-gp) inhibition potential of Dimethyl fumarate (DMF) and Mono-methyl fumarate (MMF) using Caco-2 cells.

METHODS

Standard procedures for in vitro transport studies were used. ^{3}H-Digoxin (5 μM) was used as P-gp probe substrate. DMF at two nominal concentrations, 50 and 500 μM, were added to the apical side and basolateral side. MMF at two nominal concentrations, 5 and 50 μM, were also added to the apical side and basolateral side. Digoxin concentrations were determined using liquid scintillation counting. DMF and MMF samples were collected from both apical side and basolateral side at 60 min and 120 min, and the concentrations were determined with LC/MS/MS using electrospray ionization. The apparent permeability, P_{app}, and percent recovery (mass balance) were calculated.

RESULTS

Transport of Digoxin

DMF: The apical to basolateral (A-B) and basolateral to apical (B-A) apparent permeability of digoxin in the absence of inhibitors were 1.8-2.0 and 11-12 x10-^{6} cm/s, respectively. The A-B and B-A apparent permeability of digoxin were 1.7-1.9 and 11-12 x10^{-6} cm/s in the presence of 50 μM DMF, and 1.7-2.0 and 11-12 x10^{-6} cm/s in the presence of 500 μM DMF, respectively. The efflux ratio of digoxin was constant (6.1-6.4) in the absence or presence of DMF.

MMF: The A-B and B-A apparent permeability of digoxin in the absence of inhibitor were 1.1-1.2 and 12-13 x10^{-6} cm/s, respectively. The A-B and B-A apparent permeability of digoxin were 1.0-1.2 and 12-13 x10^{-6} cm/s in the presence of 5 μM MMF, and 1.0-1.2 and 12-13 x10^{-6} cm/s in the presence of 50 μM MMF, respectively. The efflux ratio was 11-12 under all conditions.

The following table presents the apparent permeability and recovery of Digoxin.

Test Inhibitor	Direction	Digoxin Recovery (%)	P_{app} x 10^6 (cm/s)				Efflux Ratio	Absorption Potential	Significant Inhibition
			R1	R2	R3	AVG			
No Inhibitor	A-to-B	98.4	2.0	1.8	1.8	1.9	6.2	High	
	B-to-A	101.9	11.0	11.7	12.0	11.5			
3 µM PSC833 (Pos. Control)	A-to-B	96.1	4.2	4.1	4.1	4.1	1.0	High	Yes
	B-to-A	99.7	4.2	4.3	4.2	4.3			
50 µM DMF	A-to-B	98.4	1.8	1.7	1.9	1.8	6.4	High	No
	B-to-A	101.1	11.0	11.7	11.6	11.4			
500 µM DMF	A-to-B	98.4	2.0	1.7	1.9	1.9	6.1	High	No
	B-to-A	101.5	10.8	11.5	11.5	11.3			
No Inhibitor	A-to-B	95.9	1.2	1.2	1.2	1.2	10.9	High	
	B-to-A	95.6	12.3	13.1	13.4	12.9			
3 µM PSC833 (Pos. Control)	A-to-B	94.6	4.3	4.0	3.8	4.0	1.0	High	Yes
	B-to-A	95.3	4.2	4.2	4.0	4.2			
5 µM MMF	A-to-B	95.1	1.2	1.0	1.0	1.1	12.2	High	No
	B-to-A	94.9	12.8	13.0	13.2	13.0			
50 µM MMF	A-to-B	96.8	1.2	1.0	1.0	1.1	12.1	High	No
	B-to-A	95.7	12.8	12.9	13.4	13.0			

Recovery fo DMF and MMF

DMF Recovery at 60 and 120 min

DMF	Average Concentration (µM)	Average Concentration (µM)	% Recovered	Average Concentration (µM)	Recovery (%)
Sample	0 min.	60 min.	60 min.	120 min,	120 min.
50 µM Apical	29.9	2.5	8.5	0.6	2.0
50 µM Basolateral	29.9	35.4	118	17.8	59
500 µM Apical	275	45.8	17	10.2	3.7
500 µM Basolateral	275	255	93	135	49

MMF Recovery at 60 and 120 min

MMF	Average Concentration (µM)	Average Concentration (µM)	Recovery (%)	Average Concentration (µM)	Recovery (%)
Sample	0 min.	60 min.	60 min.	120 min,	120 min.
5 µM Apical	4.9	5.5	114	6.3	129
5 µM Basolateral	4.9	4.6	95	5.0	104
50 µM Apical	48.9	44.7	92	47.1	97
50 µM Basolateral	48.9	43.8	90	46.9	96

DMF concentrations decreased rapidly when added to the apical side. Less than 17% and 4% of DMF was remaining after 1 hr and 2 hr incubation, respectively. The low recovery of DMF, at the apical side, is likely due to the rapid hydrolysis by brush-border membrane associated and/or cytosolic esterase(s) expressed in the Caco-2 cell system. In contrast, percentage of MMF remaining on either the apical side or basolateral side was close to 100% during the 2 hr incubation with Caco-2 cells.

CONCLUSIONS
DMF and MMF at concentrations up to 500 µM and 50 µM, respectively, did not inhibit P-gp transport in Caco2 assay.

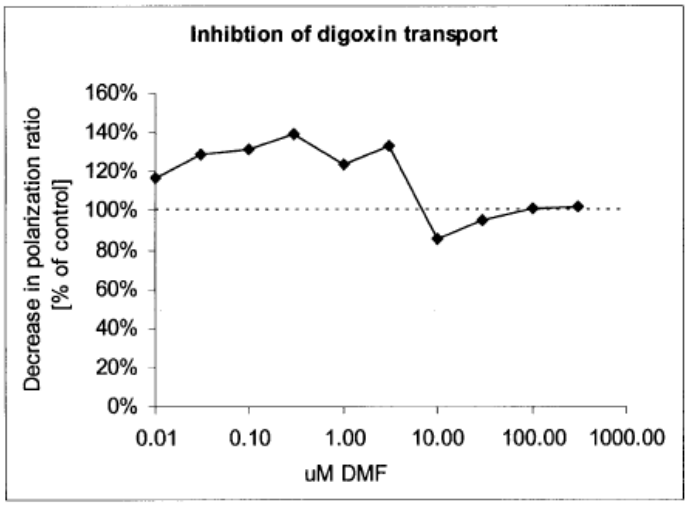
Study Title	Inhibition of P-gp Mediated Transport in LLC-PK1 Cell Monolayers by DMF and MMF
Study number	P00012-06-03
Study Period	December 2006
Study Director	(b) (4)
Objective	The objective of this study was to characterize the inhibition of P-gp mediated transport of digoxin (5 pM) in human P-gp expressing LLC-PK1 cell monolayers by the test compounds DMF and MMF.

METHODS
Porcine kidney-derived, LLC-PK, cells expressing human P-gp cDNA (designated as 22L1) were used. Known P-gp substrate (5 µM [3H]-digoxin) and the P-gp inhibitor ketoconazole (30 µM) were used in the inhibition assay.

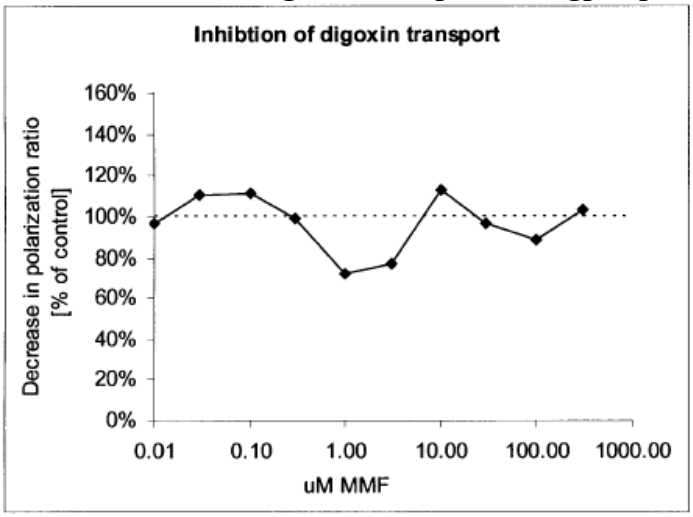
Transport of the P-gp substrate digoxin was determined in the A to B and B to A directions in the presence of increasing concentrations of test article (0.01, 0.03, 0.1, 0.3, 1 .O, 3.0, 10, 30, 100, 300 µM) in the donor and receiver chambers. Digoxin concentrations were measured by liquid scintillation counting.

RESULTS
Inhibition of digoxin transport by increasing concentrations of DMF or MMF (0.01 - 300 pM) was determined in P-gp expressing cells. Concentration dependent inhibition of digoxin transport was not observed for either DMF or MMF. Whereas transport of digoxin was inhibited by the positive control ketoconzole by about 96 to 98%.

Effect of DMF on digoxin transport in P-gp expressing cells



Effect of MMF on digoxin transport in P-gp expressing cells



CONCLUSIONS
MMF and DMF (0.01-300 µM) did not inhibit of digoxin transport in P-gp expressing cells.

Study Title	Determination of DMF Stability in Human Plasma with LC/MS/MS Technique
Study number	P00012-10-09
Study Period	December 2010
Study Director	(b) (4)
Objective	The objective of this study was to assess DMF stability in human plasma

METHODS
DMF at a 50 µM concentration was incubated with pooled human plasma at 37°C. Aliquots of 100 µL were removed from the incubation mixture after 0, 3, 5, 10, 15, and 20 min. Samples were analyzed for MMF using LC/MS/MS assay.

RESULTS
The average DMF plasma concentrations at different incubation timepoints and the corresponding values of the percentage of DMF remaining were summarized in the Table below.

Time, min	Conc, µM	% Remaining
0	58.7	100.0
3	56.9	97.0
5	51.4	87.6
10	47.4	80.7
15	43.7	74.4
20	38.5	65.7

CONCLUSIONS
DMF was unstable in human plasma. The half-life of degradation was estimated as 33.2 min.

Study Title	Plasma Stability of Mono-Methyl Fumarate in Human Plasma Determined In vitro
Study number	P00012-10-06
Study Period	December 2010
Study Director	(b) (4)
Objective	The objective of this study was to determine the stability of MMF in human plasma.

METHODS

MMF at 0.5 and 5 µM concentrations was incubated with human plasma at 37°C. Aliquots of 50 µL were removed from the incubation mixture after 0, 2, 4, 7, 20.5, 30.5, 44.5, 54.5, 68.5, 78.5 and 92.5 hours. Samples were analyzed for MMF using LC/MS/MS assay.

RESULTS

The following table presents the percentage of MMF at different time points over the incubation period.

MMF at different timepoints over the incubation period in human plasma.

	MMF percent remaining					
Sampling time (h)	Incubations with 0.5 µM of MMF			Incubations with 5.0 µM of MMF		
	Sample 1	Sample 2	Sample 3	Sample 1	Sample 2	Sample 3
0	100.0	100.0	100.0	100.0	100.0	100.0
2	90.3	83.8	94.1	88.2	90.6	89.3
4	84.7	85.1	86.0	87.0	86.9	92.6
7	84.3	84.0	90.7	80.3	81.7	92.6
20.5	70.1	69.4	74.0	75.2	75.6	77.3
30.5	63.8	60.4	61.2	64.8	70.0	71.0
44.5	52.8	52.5	53.0	56.3	57.7	64.0
54.5	46.8	47.9	49.9	51.6	53.3	56.8
68.5	49.4	41.1	46.3	47.2	48.8	49.2
78.5	40.5	40.5	42.4	46.4	42.3	45.0
92.5	36.2	39.6	37.9	37.7	37.7	40.8

The following table represents the half-lives of MMF at two concentrations.

Half-lives of MMF determined for three separate human plasma incubations at two concentrations.

	Half-life (h)	
Sample	0.5 µM MMF	5 µM MMF
1	67.6	73.3
2	69.1	70.6
3	66.9	73.0
Mean	**67.9**	**72.3**
Std Dev	1.1	1.5

CONCLUSIONS

MMF is relatively stable in human plasma compared with DMF (see Study P00012-10-09), with half-lives of 67.9 and 72.3 hours at 0.5 and 5.0 µM, respectively in human plasma.

Study Title	Determination of in vitro plasma protein binding of dimethyl fumarate (DMF) and monomethyl fumarate (MMF)
Study number	Pd-05-01
Study Period	January 2005
Study Director	(b) (4)
Objective	The objective of this study was to determine binding characteristics of dimethyl fumarate (DMF) and monomethyl fumarate (MMF) to human plasma proteins by ultrafiltration technique coupled with LC/MS/MS.

METHODS
The binding characteristics of DMF and MMF to human plasma proteins were investigated in human protein fractions (40 mg/ml of albumin or 1 mg/ml of alphal-acid glycoprotein in phosphate buffered saline (PBS) buffer), and in pooled heparinised human plasma with 10 mg/ml of sodium fluoride. The concentrations of DMF and MMF at 1.25, 5 and 10 μg/ml (8.75 to 70 μM for DMF, 9.6 to 77 μM for MMF) in protein fractions or plasma were used and four replicates at each concentration were prepared in this assay to determine DMF and MMF bindings to human plasma proteins.

The ultrafiltration plasma-binding assay was initiated with incubation of test drugs in the plasma or protein fractions at 37°C for 30 min. The separation of free from bound drug was then followed by centrifuging at 4000 rpm for 25 min at 25°C for plasma sample and for 10 min for protein samples. All the samples were analyzed by LC/MS/MS assay.

RESULTS
DMF binding with human plasma ranged from 58% to 68.5% across the range of concentrations tested. DMF binding to human serum albumin ranged from 17% to 23%. Protein binding of DMF was independent of concentration. No significant binding of DMF to alphal-acid glycoprotein was observed.

MMF binding in pooled human plasma was 27.1-29.5% across the range of concentrations tested. MMF to human serum albumin was 35.3-39.5%. Protein binding of MMF was independent of concentration. MMF did not bind to alpha l-acid glycoprotein.

Study Title	Protein Binding of Mono-Methyl Fumarate in Rat, Dog, Monkey and Human Plasma
Study number	P00012-10-05
Study Period	13 September 2010 to 20 September 2010
Study Director	(b) (4)
Objective	The objective of this study was to determine the plasma protein binding (PPB) of mono-methyl fumarate (MMF).

METHODS
MMF equilibrium dialysis was performed using 24-well dialysis device at concentrations of 50, 500 and 5000 nM. Plasma (1.0 mL) and dialysis buffer (0.1 M sodium chloride in 0.1 M potassium phosphate, pH 7.4, 1.0 mL) were added into the plasma and buffer side of the dialysis cells, respectively. Dialysis cells were slowly rotated at a rate of 12 turns/min (setting number 7) in a 370C incubator. After three-hour dialysis, aliquots of 100 μL of plasma and

dialysis buffer samples were removed and transferred into tubes containing, either 100 µL (for the 50 and 5000 nM samples) or 700 µL (for the 5000 nM samples) of dialysis buffer and plasma samples, respectively.

Aliquots of 100 µL of the above (1:1) mixture of plasma and dialysis buffer were analyzed using LC/MS/MS assay.

RESULTS
Unbound fractions of MMF in human plasma at concentrations of 50, 500, and 5000 nM are summarized in the following table.

Table: Unbound Fractions of MMF in Human Plasma

Species	MMF concentration (nM)	fu (%)	Std Dev
Human	50 500 5000	66.1 55.1 58.9	1.8 2.1 7.0

CONCLUSIONS
The plasma protein binding of MMF was concentration independent in the range tested (50-5000 nM). The unbound fraction of MMF in human plasma is approximately 60%. MMF exhibits low plasma protein binding.

Study Title	Red Blood Cell Partitioning of Mono-Methyl Fumarate In Vitro in Human Whole Blood
Study number	P00012-10-07
Study Period	December 2010
Study Director	(b) (4)
Objective	The objective of this study was to study the red blood cell partitioning of MMF in human whole blood.

METHODS
Incubation of MMF with Human Whole Blood
MMF at 0.05, 0.5 and 5 µM concentrations was incubated in human whole blood at 37°C. Aliquots of 50 µL were removed from the incubation mixture after one hour. Samples were analyzed for MMF using LC/MS/MS assay.

RESULTS
The whole blood to plasma partition coefficients ($K_{WB/PL}$) of MMF at concentrations of 0.05, 0.5 and 5.0 µM in human whole blood were 0.83, 0.74 and 0.70, respectively. The red blood cell partition coefficients $K_{RBC/PL}$ of MMF at concentrations of 0.05, 0.5 and 5.0 µM in human whole blood were 0.62, 0.41 and 0.32, respectively.

CONCLUSIONS
MMF does not significantly penetrate into red blood cells (RBCs), since the partition coefficient of RBC to plasma is less than one.

4.2 OCP Filing/Review Form

Office of Clinical Pharmacology and Biopharmaceutics New Drug Application Filing and Review Form			
General Information About the Submission			
	Information		**Information**
NDA Number	204063	**Brand Name**	(b) (4)
OCPB Division (I, II, III)	DCP-1	**Generic Name**	BG00012 (Dimethyl Fumarate)
Medical Division	HFD-120	**Drug Class**	Anti-inflammatory
OCPB Reviewer	Jagan Mohan Parepally	**Indication(s)**	Treatment of Multiple Sclerosis
OCPB Team Leader	Angela Men	**Dosage Form**	Delayed Release Capsules (b) (4)
Date of Submission	2/27/2012	**Dosing Regimen**	240 BID
Estimated Due Date of OCP Review	10/21/2012	**Route of Administration**	Oral
Division Due Date	12/13/2012	**Sponsor**	Biogen Idec.
NDA Number	204063	**Brand Name**	(b) (4)

Clin. Pharm. and Biopharm. Information

Summary: This NDA is to support the marketing approval of BG00012 is an oral fumarate ester drug product contacting the active ingredient dimethyl fumarate (DMF). Pharmacodynamic and functional responses were thought to be mediated through activation of the Nuclear factor (erythroid-derived 2)-like 2 (NFE2L2 or Nrf2) antioxidant response pathway, which is the primary cellular defense system for responding to a variety of potentially toxic stimuli. Orally administered DMF is rapidly and completely hydrolyzed to MMF which is also pharmacologically active, in the gut, gut wall, or during first pass within the portal vein. DMF is generally not quantifiable in plasma in all species tested.

The clinical pharmacology evaluation included assessment of PK, tolerability, relative bioavailability, food effect, ADME, QTc prolongation, drug interaction and bioequivalence, as summarized below:

Single-Dose PK Studies
Study **IKP/ID33**, an open-label, 3-period, single-ascending-dose Phase 1 study, evaluated the PK characteristics of MMF following oral administration of 3 different dose levels of BG00012. BG00012 was administered PO to 12 healthy male subjects (N=12 per dose group). Subjects received a single dose of 120, 240, or 360 mg BG00012.

Study **109HV101** was to evaluate the potential for BG00012 to prolong the QTc interval. This was a single-center, randomized, double-blind, placebo- and active-controlled (moxifloxacin) crossover study. Fifty-four subjects were randomized to 1 of 4 treatment sequences and received, in random order, placebo, 240 mg BG00012, 360 mg BG00012, and 400 mg moxifloxacin.

Multiple-Dose PK Studies
Three studies evaluated MMF PK in subjects treated with multiple doses of BG00012.

Two studies enrolled and treated HV (FG-PK-03/04 and 109HV106), ranging from 2 days of dosing to 4 days of dosing with varied dose levels and schedules.

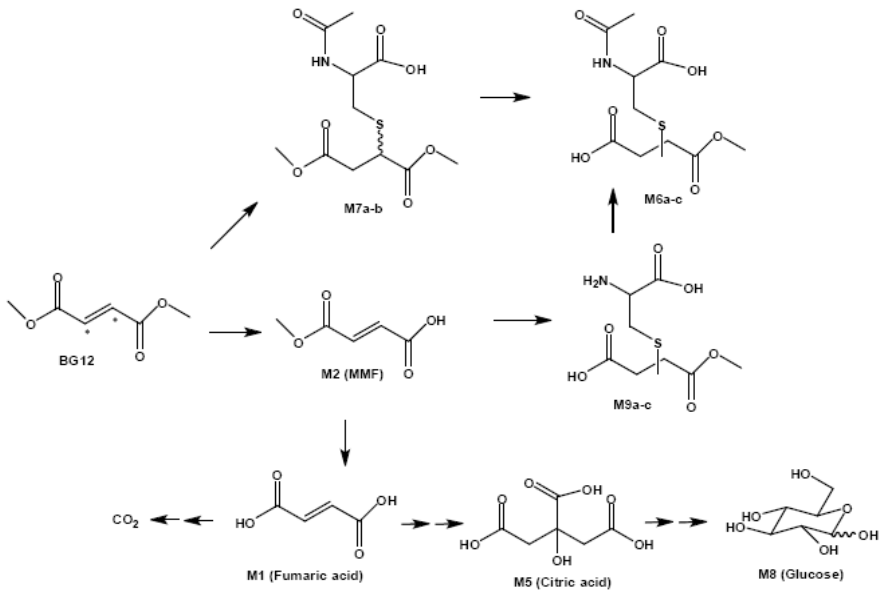
MD study in MS patients

Multiple-dose PK study (109MS101) that specifically enrolled MS patients with a range of demographic characteristics evaluated the PK after BG00012 administration of either 240 mg BID or 240 mg TID for one day. In addition, the effect of alcohol consumption on BG00012 exposure was examined to evaluate whether the (b) (4) BG00012 (b) (4) was robust enough to withstand exposure to alcohol and avoid dose dumping.

Mass Balance study

Study 109HV102: BG00012 was metabolized by esterases and downstream through the tricarboxylic acid (TCA) cycle. The primary route of elimination of ^{14}C-BG00012 derived radioactivity was CO2 exhalation followed by urine (15.5% of the dose) and feces (<1% of the dose). In plasma, glucose was identified as the predominant circulating metabolite (60.5% of the total radioactivity) during the 24-hour post-dose period. Other circulating metabolites included fumaric acid, citric acid and MMF. In urine, cysteine or N-acetylcysteine conjugates of mono- and di-methyl succinate were identified as the major metabolites. DMF and MMF accounted for only 0.06 and 0.23% of the dose, respectively.

(* = ^{14}C label)



Plasma Protein Binding

In plasma, DMF binding ranged from 58.0% to 68.5% across the range of concentrations tested. DMF was less extensively bound to human serum albumin (percentage DMF bound ranged from 17.3% to 23.0%). The binding of MMF to human serum albumin ranged from 35.3% to 39.5%, and no MMF binding to alpha l-acid glycoprotein was observed.

Plasma and Microsomal Stability
The half-life of DMF, calculated by first order decay kinetics was 33.2 minutes, indicating that DMF was unstable in human plasma (P00012-10-09). The stability of MMF in human plasma in vitro was 67.9 hours and 72.3 hours. MMF was stable (<5% decrease in concentration) upon incubation in these systems. MMF was not metabolized by CYP2D6, CYP3A4, or by enzymes endogenously present in human microsomes.

CYP Inhibition
In Study P00012-04-13, potential for inhibition of CYP2D6 and CYP3A4 and Study P00012-06-04 for potential inhibition against human cDNA-expressed CYP2B6 and CYP2C8 by DMF and MMF was determined. In Study PD05-28, inhibition potential of DMF and MMF towards human recombinant CYP isoforms 1A2, 2C9, 2C19, 2D6, 2E1, and 3A4 was tested. The inhibition study consisted of the determination of IC_{50} for DMF and MMF. The results indicated that neither DMF nor MMF was a CYP inhibitor as the IC_{50} values towards these CYP isoforms were all >50µM.

CYP and P-gp Induction
The potential of MMF to induce CYPlA2, 2B6, 2C9, 2C19, and 3A4 activities was evaluated in fresh human hepatocytes (P00012-04-14).

Food Effect Studies
Two studies were performed to evaluate the effect of food on BG00012 PK. Subjects in Study FG-PK-02/02 were fed a low fat diet, whereas subjects enrolled in Study C-1903 were fed a high fat diet to test for food effects. The diets represent the variability in food intake that may affect absorption of BG00012. Based on the results of the two studies, no food-effect was observed.

Special Populations
Studies to address the safety and effectiveness of BG00012 in pediatric or geriatric patients with MS have not been performed. Given the specific metabolism and elimination profile of BG00012, the Sponsor stated that evaluation of PK in individuals with renal and hepatic impairment is not considered necessary.

Effect of Weight
According to the sponsor weight appears to affect PK parameters. However, results from 2 pivotal Phase 3 studies in subjects with MS (109MS301 and 109MS302) indicated that differences in subject body weight did not have a discernable effect on the efficacy of BG00012.

Effect of Alcohol
According to the sponsor, results from in vitro dissolution studies of BG00012 in gastric juice with increasing amounts of alcohol did not affect dissolution. However, in vitro study indicated that dissolution profile of BG00012 changed drastically with 20% and 40% ethanol content (see appendix 1). A subset of MS subjects in Study 109MS101 received 125 mL of wine (standardized by alcohol by volume) with their evening dose of BG00012 at 240 mg BID or TID. Data from this subset indicated there was no effect of alcohol on the PK profile of MMF. Therefore, BG00012 exhibits low potential for dose dumping

Drug-Drug Interactions
BG00012 was pre-systemically metabolized extensively by esterases and had no interaction with the CYP system. Therefore, the potential for CYP-mediated drug-drug interactions at clinically relevant doses was low.
109HV103: Study to assess potential interaction of Avonex® (IFN β-1a) 30 µg IM when co-administered with BG00012 240 mg PO TID in healthy adult volunteers
109HV104: Study to assess potential interaction of GA 20 mg SC when co- administered with BG00012 240 mg PO TID in healthy adult volunteers
109HV106: Study to assess potential interaction of aspirin coadministration with BG00012 in healthy adult volunteers

BA BE studies:
109HV105: Relative BA study with 240 mg (standard formulation) 240 mg (API formulation)
109HV107: BE study with 240 mg (standard formulation) 2 X 120 mg (standard formulation)

Dose rationale for pivotal Phase 3 trials:
The dose regimens were selected for the Phase 3 studies on the basis of the results of the Phase 2 MS study (Study C-1900). A daily dose of BG00012 240 mg TID (720 mg/day) was identified as the efficacious dose in this study. An additional lower BG00012 dose of 240 mg BID (=480 mg/day) was evaluated in the Phase 3 program because it provided higher exposures than the highest non-effective dose in Phase 2 of 120 mg TID (=360 mg/day) without exceeding the maximally well-tolerated single
-dose timepoint of 240 mg (per dose administration) established in a healthy volunteers.

Pivotal Phase 3 Studies 301 and 302
Two well-controlled Phase 3 studies provide the principal efficacy data supporting this application. The design and schedule of efficacy assessments for Study 301 and Study 302.

	"X" if included at filing	Number of studies submitted	Number of studies reviewed	Critical Comments If any
STUDY TYPE				
Table of Contents present and sufficient to locate reports, tables, data, etc.	X			
Tabular Listing of All Human Studies	X			
HPK Summary	X			
Labeling	X			
Reference Bioanalytical and Analytical Methods	**X**	**4**		
I. Clinical Pharmacology				
Mass balance:	**X**	**1**	**-**	**Study 109HV102**

Isozyme characterization:				
Blood/plasma ratio:	**X**	**1**	-	**P00012-10-07**
Plasma protein binding:	**X**	**1**	-	**PD-05-01, P00012-10-05**
Pharmacokinetics (e.g., Phase I) -				
Healthy Volunteers-				
single dose:	**X**	**2**	-	**Study IKP/ID33, Study 109HV101**
multiple dose:	**X**	**2**		**Studies FG-PK-03/04 and 109HV106**
Patients-				
single dose:	-	-	-	
multiple dose:	**X**	**1**	-	**Study 109MS101**
Dose proportionality -				
fasting / non-fasting single dose:	**X**	-	-	**Assessed in SD and MD studies**
fasting / non-fasting multiple dose:	-	-	-	
Drug-drug interaction studies -				
In-vivo effects on primary drug:	**X**	**3**	-	**With Avonex (IFN β-1a) and Copaxone (GA) (109HV103 and 109HV104 With Aspirin (109HV106)**
In-vivo effects of primary drug:	-	-	-	
In-vitro:	**X**	**11**	-	**P00012-10-09, P00012-10-06, P00012-04-12, P00012-04-13, P00012-04-14, PD05-28, P00012-06-04, P00012-06-05, P00012-10-03, P00012-06-03, P00012-10-04**
Subpopulation studies -				
ethnicity:	-	-	-	
gender:	-	-	-	
pediatrics:	-	-	-	
geriatrics:				
renal impairment:	-	-	-	
hepatic impairment:	-	-	-	
PD:				
Phase 1:	**X**	-	-	
Phase 3:	-	-	-	
PK/PD:				
Phase 1 and/or 2, proof of concept:	**X**	-	-	**Phase 2 dose-ranging study (Study C-1900)**
Phase 3 clinical trial:	**X**	-	-	**Studies 301 and 302**
Population Analyses -				
Data rich:	-	-	-	
Data sparse:	-	-	-	
II. Biopharmaceutics				
Absolute bioavailability:	-	-	-	
Relative bioavailability -	-	-		
solution as reference:				
alternate formulation as reference:	**X**	1		**Study 109HV105:**
Bioequivalence studies -				
traditional design; single / multi dose:	X	**1**		**Study 109HV107: BE study 240 mg vs 2 X 120 mg standard formulation**
replicate design; single / multi dose:				
Food-drug interaction studies:	X	**2**		**Low Fat Diet: Study FG-PK-02/02 High Fat Diet: Study C-1903**
Dissolution:	-	-	-	
(IVIVC):				
In vivo alcohol dose dumping	**X**	-	-	
BCS class				
III. Other CPB Studies				

Genotype/phenotype studies:	-	-	-	
Chronopharmacokinetics	-	-	-	
Pediatric development plan	-	-	-	
Literature References	X	-	-	
Total Number of Studies		**12 + 14 in vitro**	**12 in vivo and 14 in vitro**	

Filability and QBR comments		
	"X" if yes	Comments
Application filable?	X	Reasons if the application is not filable (or an attachment if applicable) For example, is clinical formulation the same as the to-be-marketed one?
Comments sent to firm?		
QBR questions (key issues to be considered)	Is final commercial product bioequivalent to the formulation used in clinical studies? Does the mass balance study support waiver for studies in patients with hepatic and renal impairment? Is the Clinical Pharmacology of BG00012 adequately characterized? Is In vivo dose-dumping study necessary to evaluate finds from in vitro studies?	
Other comments or information not included above	BE Study Inspection request 109HV107	
Primary reviewer Signature and Date		
Secondary reviewer Signature and Date		

On **initial** review of the NDA/BLA application for filing:

	Content Parameter	Yes	No	N/A	Comment
Criteria for Refusal to File (RTF)					
1	Has the applicant submitted bioequivalence data comparing to-be-marketed product(s) and those used in the pivotal clinical trials?	X			Electronic data sets available
2	Has the applicant provided metabolism and drug-drug interaction information?	X			
3	Has the sponsor submitted bioavailability data satisfying the CFR requirements?	X			
4	Did the sponsor submit data to allow the evaluation of the validity of the analytical assay?	X			
5	Has a rationale for dose selection been submitted?	X			
6	Is the clinical pharmacology and biopharmaceutics section of the NDA organized, indexed and paginated in a manner to allow substantive review to begin?	X			
7	Is the clinical pharmacology and biopharmaceutics section of the NDA legible so that a substantive review can begin?	X			
8	Is the electronic submission searchable, does it have	X			

	appropriate hyperlinks and do the hyperlinks work?				
Criteria for Assessing Quality of an NDA (Preliminary Assessment of Quality)					
	Data				
9	Are the data sets, as requested during pre-submission discussions, submitted in the appropriate format (e.g., CDISC)?	X			
10	If applicable, are the pharmacogenomic data sets submitted in the appropriate format?			X	
	Studies and Analyses				
11	Is the appropriate pharmacokinetic information submitted?	X			
12	Has the applicant made an appropriate attempt to determine reasonable dose individualization strategies for this product (i.e., appropriately designed and analyzed dose-ranging or pivotal studies)?			X	
13	Are the appropriate exposure-response (for desired and undesired effects) analyses conducted and submitted as described in the Exposure-Response guidance?	X			
14	Is there an adequate attempt by the applicant to use exposure-response relationships in order to assess the need for dose adjustments for intrinsic/extrinsic factors that might affect the pharmacokinetic or pharmacodynamics?	X			
15	Are the pediatric exclusivity studies adequately designed to demonstrate effectiveness, if the drug is indeed effective?			X	
16	Did the applicant submit all the pediatric exclusivity data, as described in the WR?			X	
17	Is there adequate information on the pharmacokinetics and exposure-response in the clinical pharmacology section of the label?			X	
	General				
18	Are the clinical pharmacology and biopharmaceutics studies of appropriate design and breadth of investigation to meet basic requirements for approvability of this product?	X			
19	Was the translation (of study reports or other study information) from another language needed and provided in this submission?			X	

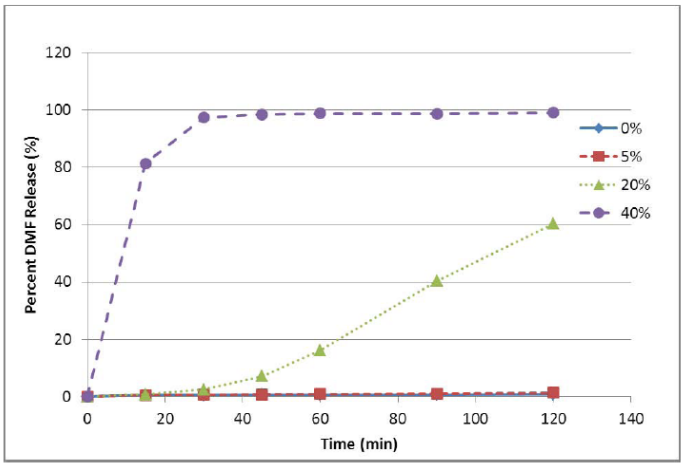
IS THE CLINICAL PHARMACOLOGY SECTION OF THE APPLICATION FILEABLE? ___Yes_

If the NDA/BLA is not fileable from the clinical pharmacology perspective, state the reasons and provide comments to be sent to the Applicant.
Please identify and list any potential review issues to be forwarded to the Applicant for the 74-day letter.

cc: NDA 204063 HFD-850 (Electronic Entry), HFD-120, HFD-860 (Jagan Parepally, Angela Men, Ramana Uppoor, Mehul Mehta)

Appendix 1:

Figure 4: DMF Dissolution Profiles of BG00012 Capsules (Batch 43664) in Acid Stage Containing 0%, 5%, 20%, and 40% Ethanol



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/s/

JAGAN MOHAN R PAREPALLY
11/17/2012

XINNING YANG
11/18/2012

PRODUCT QUALITY - BIOPHARMACEUTICS
FILING REVIEW

NDA Number	204-063
Submission Date	2/27/12
Product name, generic name of the active	Dimethyl fumarate
Dosage form and strength	Delayed Release Capsules – 120 mg and 240 mg/capsule
Applicant	Biogen Idec.
Clinical Division	Division of Neurology Product
Type of Submission	Original NDA – 505(b)(1)
Biopharmaceutics Reviewer	Elsbeth Chikhale, Ph.D.
Biopharmaceutics Supervisory Lead	Angelica Dorantes, Ph.D.

The following parameters for the ONDQA's Product Quality-Biopharmaceutics filing checklist are necessary in order to initiate a full biopharmaceutics review (i.e., complete enough to review but may have deficiencies).

ONDQA-BIOPHARMACEUTICS
A. INITIAL OVERVIEW OF THE NDA APPLICATION FOR FILING

	Parameter	Yes	No	Comment
1.	Does the application contain dissolution data?	x		
2.	Is the dissolution test part of the DP specifications?	x		
3.	Does the application contain the dissolution method development report?		x	The report is provided as part of IND 73,061
4.	Is there a validation package for the analytical method and dissolution methodology?	x		
5.	Does the application include a biowaiver request?		x	
6.	Does the application include a IVIVC model?		x	
7.	Is information such as BCS classification mentioned, and supportive data provided?	x	x	Applicant claims BCS Class 1, but the supportive data as per BCS Guidance were not provided. However, no specific request based on BCS-Class 1 was included.
8.	Is information on mixing the product with foods or liquids included?	x		
9.	Is there any in *vivo* BA or BE information in the submission?	x		A BE study in healthy volunteers to compare two 120 mg capsules with one 240 mg capsule is provided.

PRODUCT QUALITY - BIOPHARMACEUTICS
FILING REVIEW

B. FILING CONCLUSION				
	Parameter	**Yes**	**No**	**Comment**
10.	**IS THE BIOPHARMACEUTICS SECTIONS OF THE APPLICATION FILEABLE?**	x		
11.	If the NDA is not fileable from the product quality-biopharmaceutics perspective, state the reasons and provide **filing** comments to be sent to the Applicant.			
12.	If the NDA is not fileable from the biopharmaceutics perspective, state the reasons and provide **filing** comments to be sent to the Applicant.			
13.	Are there any **potential review** issues to be forwarded to the Applicant for the 74-day letter?		x	

{See appended electronic signature page}

Elsbeth Chikhale, Ph.D. — 4/29/12
Biopharmaceutics Reviewer — Date
Office of New Drug Quality Assessment

{See appended electronic signature page}

Angelica Dorantes, Ph.D. — 4/29/12
Biopharmaceutics Supervisory Lead (acting) — Date
Office of New Drug Quality Assessment

This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

ELSBETH G CHIKHALE
04/29/2012

ANGELICA DORANTES
04/29/2012