BG00012: A 1 Year Chronic Toxicity Study of Dimethyl Fumarate Suspension Administered by Nasogastric Intubation to Cynomolgus Monkeys

Testing Facility Study No. EBA00176
Biogen Idec Study No. P00012-05-08

Final Report

Testing Facility: **Charles River Laboratories**
Preclinical Services Nevada
587 Dunn Circle
Sparks, NV 89431

Study Sponsor: **Biogen Idec**
14 Cambridge Center
Cambridge, MA 02142

Study Completion Date: **June 21, 2007**

I. Abstract

The objectives of this chronic toxicity study were to determine the potential chronic toxicity of Dimethyl Fumarate when administered by nasogastric intubation to cynomolgus monkeys for at least 52 weeks, and to evaluate recovery of animals from any effects of the test article over a treatment-free period of at least 4 weeks.

Dimethyl Fumarate (DMF), and the control article, 0.8% Hydroxypropylmethylcellulose (HPMC), were given orally once daily for 52 consecutive weeks to 48 experimentally naive cynomolgus monkeys at 0 (control), 5, 25, or 75 mg/kg (6/sex/group). One male animal in the 5 mg/kg/day dose group was euthanized on Day 197 because of a dosing accident, and one female monkey in the 25 mg/kg/day dose group was incorrectly transferred from the recovery necropsy to the main study necropsy. Thirty-two animals (4/sex for Groups 1 and 4, 3 males/4 females for Group 2, and 4 males/5 females for Group 3) were euthanized one day after the last dose (Day 365). The remaining 15 animals (2/sex for Groups 1, 2 and 4, 2 males/1 female for Group 3) were continued on study without further treatment, and were terminated approximately 4 weeks after the last dose (Day 393).

There was no test article-related mortality or moribundity, nor were there any test article-associated findings in electrocardiographic evaluations, ophthalmic evaluations, hematological parameters, coagulation parameters, or urinalysis parameters.

Test article-associated findings included increased incidence of low food consumption and initial reduction in mean body weight gain (Week 1 of treatment) in the 75 mg/kg/day treated monkeys. Test article-associated serum chemistry findings consisted of decreased BUN in the 25 and 75 mg/kg/day monkeys of both sexes and decreased creatinine values in a few female monkeys. Reductions in serum phosphorus levels were observed in some monkeys in the 75 mg/kg/day dose group. Reductions in BUN, creatinine, and phosphorus were generally resolved by the end of the recovery period.

Test article-associated pathologic findings at terminal necropsy were limited to the kidney. The macroscopic alterations included pale discoloration, increased size, and watery consistency in the kidneys of some male and female animals in the 25 and 75 mg/kg/day dose groups. Test article-related increases in kidney weight, kidney to brain, and kidney to body weight ratios were noted in females in the 75 mg/kg/day dose group. Histologic alterations consisted of single cell necrosis and regeneration of the cortical tubular epithelial cells in monkeys in the 25 and 75 mg/kg/day dose groups, with a higher incidence and severity in the 75 mg/kg/day dose group. At the recovery necropsy, pale discoloration in a male and female animal in the 75 mg/kg/day dose group, and increased kidney weight, kidney to brain, and kidney to body weight ratios were noted for male animals given 75 mg DMF/kg/day. Microscopic test article-related changes observed at the recovery necropsy in 25 and 75 mg/kg

animals were similar to those seen at the terminal necropsy; however the incidence and/or severity of single cell necrosis and regeneration of the tubule epithelium were reduced in some dose groups, suggesting a trend towards recovery. Additionally, both male monkeys in the 75 mg/kg/day recovery group had mild to moderate interstitial fibrosis, a morphologic indication of irreversible loss of tissue and function, accompanied by tubular atrophy. Renal fibrosis was associated with increased BUN and creatinine in one of these animals at weeks 38, 52 and 56.

After once daily dosing of DMF in cynomolgus monkeys, C_{max} values of MMF increased in a dose proportional manner from 5 to 75 mg/kg. $AUC_{0\text{-}24hr}$ values increased in a dose proportional manner from 5 to 25 mg/kg (Excluding Day 1 values, mean values ranged from 1.99-2.73 µg*hr/mL in the 5 mg/kg group and 12.21-15.58 µg*hr/mL in the 25 mg/kg group), and in a slightly greater than dose-proportional manner from 25 to 75 mg/kg (Excluding Day 1 values, mean values ranged from 43.63-58.41 µg*hr/mL in the 75 mg/kg dose group). There was no statistically significant accumulation of MMF at any tested dose levels. There was no significant gender effect on MMF exposure in any tested dose levels. The terminal half-life was slightly prolonged at higher doses at 25 and 75 mg/kg (mean T1/2 values ranged from 0.46-0.73 hr in the 5 mg/kg group, 0.42-0.89 hr in the 25 mg/kg group and 0.81-1.04 in the 75 mg/kg group, respectively), while the total clearance was slightly decreased.

Levels of methanol in vehicle treated monkeys ranged from near the detection limit of the assay (5 µg/mL) to a value of 39 µg/mL in a male monkey at Week 52. With DMF treatment, there were observed increases in methanol concentration at early time points after dosing, that were comparable to the high level of 39 µg/mL observed in vehicle controls. $AUC_{0\text{-}24\ hr}$ values calculated for DMF and vehicle control groups (178.52 to 233.05 µg*hr/mL in male and 181.6 to 212.84 µg*hr/mL in female control groups) demonstrated a statistically significant increase in the 25 mg/kg males (261.33 to 471.18 µg*hr/mL) and 75 mg/kg treated males (199.92 to 409.34 µg*hr/mL) and females (229.33 to 335.35 µg*hr/mL) at the Week 26 collection interval. At the Week 52 interval, the mean $AUC_{0\text{-}24\ hr}$ ranged from 337.64 to 341.81 µg*hr/mL in vehicle control treated monkeys, with no increase observed after any dose level of DMF. Formic acid $AUC_{0\text{-}24hr}$ levels among different dosing groups were not statistically significant, except on Week 26 in the 75 mg/kg dose group (a 30% decrease). The formic acid $AUC_{0\text{-}24hr}$ in the control group in Week 26 was 57.71 to 109.79 µg*/hr/mL in male animals, and 41.53 to 87.35 µg*/hr/mL in female animals. Formic acid $AUC_{0\text{-}24}$ levels in the 75 mg/kg dose group at Week 26 was 16.36 to 58.19 µg*/hr/mL in males, and 40.27 to 51.15 µg*/hr/mL in females. Formic acid levels, as a product of methanol metabolism, were not changed appreciably at any interval for any dose group during the 1 year DMF treatment

In summary, test article associated clinical observations were limited to low food consumption in animals given 75 mg DMF/kg/day. Initial decreases in body weight gain were observed in both genders during the first week of dosing

at 75 mg/kg. Clinical chemistry changes included decreased BUN in both genders and creatinine in a few females (25 and 75 mg DMF/kg/day). Reductions in serum phosphorus levels were observed in some monkeys in the 75 mg/kg/day dose group. At terminal necropsy, gross and histologic findings related to treatment were limited to the kidney and consisted of pale discoloration, increased size (to include absolute and relative kidney weight increases in the highest dose group), and watery consistency at the 25 and 75 mg/kg/day dose levels. Histologically, this correlated with single cell necrosis and regeneration of tubular epithelial cells. At necropsy following a four week treatment free period, similar findings were noted in the kidney with a trend towards recovery in some dose groups. However, both male monkeys in the 75 mg/kg/day recovery group had mild to moderate interstitial fibrosis accompanied by tubular atrophy and, in one animal, an increase in BUN (42-77% increase from baseline value) and creatinine (22-56% increase from baseline value).

In consideration of these findings, the no-observed-effect level (NOEL) for dimethyl fumarate under the conditions of this study was 5 mg DMF/kg/day.

III. List of Abbreviations

ACVP	American College of Veterinary Pathologists
ANOVA	Analysis of variance
CFR	Code of Federal Regulations
DABT	Diplomate, American Board of Toxicology
DACLAM	Diplomate, American College of Laboratory Animal Medicine
DACVIM	Diplomate, American College of Veterinary Internal Medicine
DACVP	Diplomate, American College of Veterinary Pathologists
DI	Deionized
DMF	Dimethyl Fumarate
ECG	Electrocardiogram
FDA	Food and Drug Administration
GLP	Good Laboratory Practice
HPMC	Hydroxypropylmethylcellulose
IACUC	Institutional Animal Care and Use Committee
ILAR	Institute for Laboratory Animal Resources
MHLW	Ministry of Health, Labor, and Welfare
MSDS	Material Data Safety Sheet
OECD	Organisation for Economic Cooperation and Development
QAU	Quality Assurance Unit
SOP	Standard operating procedures
TK	Toxicokinetic
USDA	United States Department of Agriculture

DOCKET ALARM

Explore Litigation Insights

Docket Alarm provides insights to develop a more informed litigation strategy and the peace of mind of knowing you're on top of things.

Real-Time Litigation Alerts

Keep your litigation team up-to-date with **real-time alerts** and advanced team management tools built for the enterprise, all while greatly reducing PACER spend.

Our comprehensive service means we can handle Federal, State, and Administrative courts across the country.

Advanced Docket Research

With over 230 million records, Docket Alarm's cloud-native docket research platform finds what other services can't. Coverage includes Federal, State, plus PTAB, TTAB, ITC and NLRB decisions, all in one place.

Identify arguments that have been successful in the past with full text, pinpoint searching. Link to case law cited within any court document via Fastcase.

Analytics At Your Fingertips

Learn what happened the last time a particular judge, opposing counsel or company faced cases similar to yours.

Advanced out-of-the-box PTAB and TTAB analytics are always at your fingertips.

API

Docket Alarm offers a powerful API (application programming interface) to developers that want to integrate case filings into their apps.

LAW FIRMS
Build custom dashboards for your attorneys and clients with live data direct from the court.

Automate many repetitive legal tasks like conflict checks, document management, and marketing.

FINANCIAL INSTITUTIONS
Litigation and bankruptcy checks for companies and debtors.

E-DISCOVERY AND LEGAL VENDORS
Sync your system to PACER to automate legal marketing.