# CENTER FOR DRUG EVALUATION AND RESEARCH

***APPLICATION NUMBER:***

**200678Orig1s000**

# PHARMACOLOGY REVIEW(S)

Memorandum

**Pharmacology/Toxicology**
**Center for Drug Evaluation and Research**
**Division of Metabolic & Endocrine Products**

# NDA SECONDARY REVIEW MEMO

| | |
|---|---|
| Date: | 30 September 2010 |
| NDA # | 200678 |
| Sponsor: | Bristol Myers Squibb |
| Drug: | Saxagliptin + metformin XR FDC |
| Primary Reviewer: | Lauren Murphree Mihalcik, Ph.D. |
| Secondary Reviewer: | Todd Bourcier, Ph.D. |

BMS is seeking marketing approval for a fixed-dose combination product of saxagliptin and metformin extended release as a treatment for type 2 diabetes. Both pharmaceutical components are currently approved for the chronic treatment of type 2 diabetes. Saxagliptin is a dipeptidylpeptidase-4 inhibitor approved in 2009 (Onglyza, NDA 22350), and metformin XR is an extended release biguanide (Glucophage XR, NDA 21202). BMS owns all data relevant to saxagliptin but is in part relying the FDA's previous finding of safety and efficacy for metformin.

Dr. Lauren Murphree Mihalcik, the primary pharm/tox reviewer, recommends approval of NDA 200678. *I concur with Dr. Mihalcik's recommendation that the submitted nonclinical information supports approval of the saxa/met XR application.* Our recommendation is based on the available information for saxagliptin and metformin as monotherapies, and on toxicology studies conducted with the drugs in combination to assess general toxicity and embryofetal development.

The toxicology of saxagliptin and metformin in combination was evaluated in a 3-month study in dogs. Experimental groups assessed each drug separately and in combination for comparison. No toxicity unique to the drugs in combination was observed, and the toxicity of each drug separately was reasonably similar to the toxicology profile that supported approval of each drug component.

The applicant also submitted embryofetal development studies in rats and rabbits in support of the saxa/met XR combination and in response to a post-marketing requirement for the saxagliptin monotherapy NDA 22350. During the review cycle for NDA 22350, an embryofetal study conducted in rats with the saxa/met combination yielded a weak signal for neural tube defects (NTD). A relationship specific to the combination could not be excluded due to a study design that lacked separate arms for saxagliptin and metformin. Therefore, the Division imposed a PMR for the saxagliptin monotherapy NDA to conduct embryofetal studies in rats and rabbits

with the drugs alone and in combination. BMS conducted these studies, and as reviewed by Dr. Murphree Mihalcik, these studies did not identify a drug-related neural tube defect as was seen in the original rat study, despite using higher doses of metformin alone or in combination with saxagliptin. Moreover, the 4.5% incidence of NTD observed in the original rat study was shown to be consistent with updated historical control data from the study site for this finding. Therefore, I concur with Dr. Murphree Mihalcik's conclusion that the saxa/met combination is not teratogenic in animals and that the original finding of NTD in rats was incidental to drug treatment. Pregnancy labeling for the saxa/met XR product and the saxagliptin monotherapy will be revised to reflect the most current embryofetal animal data.

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/s/

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TODD M BOURCIER
10/01/2010
Pharm/tox recommends AP

**DEPARTMENT OF HEALTH AND HUMAN SERVICES**
**PUBLIC HEALTH SERVICE**
**FOOD AND DRUG ADMINISTRATION**
**CENTER FOR DRUG EVALUATION AND RESEARCH**

# PHARMACOLOGY/TOXICOLOGY NDA REVIEW AND EVALUATION

| | |
|---|---|
| Application number: | 200678 |
| Supporting document/s: | 1 |
| Applicant's letter date: | 29 December 2009 |
| CDER stamp date: | 29 December 2009 |
| Product: | saxagliptin + metformin extended release (FDC) |
| Indication: | Type 2 Diabetes Mellitus |
| Applicant: | Bristol-Myers Squibb |
| Review Division: | Division of Metabolism and Endocrinology Products |
| Reviewer: | Lauren Murphree Mihalcik, Ph.D. |
| Supervisor/Team Leader: | Todd Bourcier, Ph.D. |
| Division Director: | Mary Parks, M.D. |
| Project Manager: | Mehreen Hai, Ph.D. |

*Template Version: December 7, 2009*

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# 1 Executive Summary

## 1.1 Recommendations

### 1.1.1 Approvability

Pharmacology/Toxicology recommends approval of NDA 200678

### 1.1.2 Additional Non Clinical Recommendations

None

### 1.1.3 Labeling

Reviewer's note: Changes from the sponsor's wording is underlined. Only new language regarding the combination is shown for section 8 below since wording under "saxagliptin" and "metformin" sections are identical to the approved monotherapy labeling and are considered acceptable. The wording in section 13 is acceptable.

8 USE IN SPECIFIC POPULATIONS
8.1 Pregnancy
Pregnancy Category B
There are no adequate and well-controlled studies in pregnant women with TRADENAME or its individual components. Because animal reproduction studies are not always predictive of human response, TRADENAME, like other antidiabetic medications, should be used during pregnancy only if clearly needed.

Coadministration of saxagliptin and metformin, to pregnant rats and rabbits during the period of organogenesis, was neither embryolethal nor teratogenic in either species when tested at doses yielding systemic exposures (AUC) up to 100 and 10 times the maximum recommended human doses (MRHD; saxagliptin 5 mg and metformin 2000 mg), respectively, in rats; and 249 and 1.1 times the MRHDs in rabbits. In rats, minor developmental toxicity was limited to an increased incidence in wavy ribs; associated maternal toxicity was limited to weight decrements of 11-17% over the course of the study, and related reductions in maternal food consumption. In rabbits, coadministration was poorly tolerated in a subset of mothers (12 of 30), resulting in death, moribundity, or abortion. However, among surviving mothers with evaluable litters, maternal toxicity was limited to marginal reductions in body weight over the course of gestation days 21 to 29; and associated developmental toxicity in these litters was limited to fetal body weight decrements of 7% and a low incidence of delayed ossification of the fetal hyoid.

Additional note: Existing pregnancy labeling language in the Onglyza monotherapy NDA will be revised to reflect the additional information on animal teratogenicity (NDA 22350, TSI 000758).

## 1.2 Brief Discussion of Nonclinical Findings

The sponsor has submitted studies using dosing with metformin and saxagliptin including a 3-month study in dogs for general toxicity and embryofetal development studies in rats and rabbits. None of the studies identified a unique or emergent toxicity as a result of co-administration of the two drugs.

Embryofetal development (EFD) studies using the combination were considered crucial for estimating the safety of combination dosing. The original combination study used saxagliptin/metformin doses of 0/0, 5/200, and 25/200 mg/kg/day. In the 25/200 group, two fetuses (from the same litter) displayed craniorachischisis, a rare neural tube defect (NTD). At the time of the study, the litter and fetal incidence were outside the historical control range values for the laboratory. To address this issue, the sponsor used a two-pronged approach. They updated the historical control database, including dose range finding studies, and repeated the study using more animals (n=30) and higher doses of metformin (600 mg/kg), along with appropriate controls (a vehicle control and groups with saxagliptin (25 mg/kg) or metformin alone). The new historical control data showed four instances of craniorachischisis in control animals, bringing the finding in the first EFD study to within the historical control range.

The follow-up rat EFD study had verified exposures to saxagliptin of ~100X MRHD, to BMS-510849 of ~7X MRHD, and to metformin of ~10X MRHD (AUC basis). There were additive decreases in weight gain observed in the drug-treated groups, but there were no malformations observed (other than in one control fetus). The only finding in this study that appeared to be related to treatment was a slight increase in wavy ribs observed in the combination group (a statistically significant increase in fetal incidence). Because wavy ribs are commonly observed in the offspring of dams with reduced body weight gain and because wavy ribs typically resolve during post-natal development in the rat, this finding was not considered an adverse effect. The reviewer does not consider the combination to be teratogenic in rats.

A combination EFD study in rabbits was also requested by the Division as a follow-up to the original craniorachischisis finding in the rat. This study used doses providing ≥ 200X MRHD for saxagliptin and/or ~1X MRHD (AUC basis) for metformin (due to mortality at 2X MRHD). There was high mortality (12/30 dams) in the combination group. Satellite animals evaluated for toxicokinetics and clinical chemistry showed sporadic low levels for bicarbonate, suggesting lactic acidosis may have contributed to some of the deaths. There were no malformations in the nine early decedents with live litters at necropsy. One surviving dam in the combination dose group had a single fetus with malformations, including exencephaly, which is an NTD. This incidence was within the historical control range, however, and was considered incidental (not related to treatment) by both the sponsor and the reviewer. The saxagliptin-only group in this study had a significant increase offspring with small or absent gallbladder. Although this finding could reasonably be attributed to treatment (especially given a similar but not statistiscally significant finding in the monotherapy EFD study), any risk to a developing fetus is very low given the high multiples of exposure involved. This finding was not observed in the combination group.

# 2 Drug Information

## 2.1 Drug

### 2.1.1 CAS Registry Number (Optional)

945667-22-1

### 2.1.2 Generic Name

Saxagliptin/metformin HCL extended release

### 2.1.3 Code Name

BMS-477118-11 (saxagliptin)

### 2.1.4 Chemical Name

Saxagliptin: (1*S*,3*S*,5*S*)-2-[(2*S*)-2-Amino-2-(3-hydroxytricyclo[3.3.1.1$^{3,7}$]dec-1-yl)acetyl]-2-azabicyclo[3.1.0]hexane-3-carbonitrile, monohydrate

Metformin: N,N-dimethylimidodicarbonimidic diamide hydrochloride

### 2.1.5 Molecular Formula/Molecular Weight

Saxagliptin: $C_{18}H_{25}N_3O_2 \cdot H_2O$ / 333.43
Metformin: $C_4H_{11}N_5 \cdot HCl$ / 165.63

### 2.1.6 Structure

**Figure 1. Metformin HCl structure**

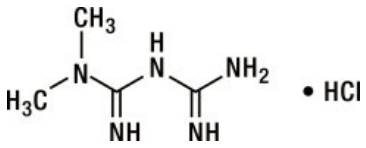
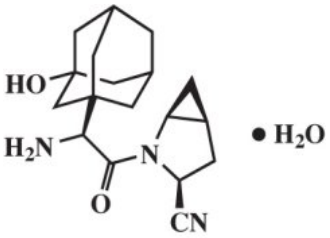


**Figure 2. Saxagliptin structure**



### 2.1.7 Pharmacologic class

Saxagliptin: DPP-4 inhibitor
Metformin: biguanide antihyperglycemic

## 2.2 Relevant IND/s, NDA/s, and DMF/s

Metformin: DMF (b) (4)
Saxagliptin: IND 63634, NDA22350 (Onglyza®)
Combination: IND 76500

## 2.3 Clinical Formulation

### 2.3.1 Drug Formulation

The drug product is manufactured (b) (4) The sponsor proposes the following saxagliptin/metformin combinations: 5/500, 5/1000, and 2.5/1000 mg.

**Table 1. Drug formulation**

**Table 2.3.P.1.T02: Tablet Compositions of Saxagliptin/Extended-Release Metformin Hydrochloride Film-Coated Tablets**

| | Component | Function | Quantity per Unit Dose (mg/tablet) | | |
|---|---|---|---|---|---|
| | | | Saxa 5/ Met XR 500 (mg/mg) | Saxa 5/ Met XR 1000 (mg/mg) | Saxa 2.5/ Met XR 1000 (mg/mg) |
| (b) (4) | Metformin Hydrochloride | Active | 502.5 | 1005.0 | 1005.0 |
| Tablet | (b) (4) | | | | (b) (4) |
| | Carboxymethylcellulose Sodium, USP | | | | |
| | (b) (4) | | | | |
| | Hypromellose 2208, USP, Ph.Eur. | | | | |
| | Hypromellose 2910, USP, Ph.Eur. | | | | |
| | Microcrystalline Cellulose, NF, Ph.Eur. | | | | |
| | Magnesium Stearate, NF, Ph.Eur. | | | | |
| | (b) (4) | | | | |
| Total Tablet Weight | | | Ca. 1180.5 | Ca. 1653.5 | Ca. 1651.0 |

q.s. = quantity sufficient NA = not applicable Ca. = Calculated average

### 2.3.2 Comments on Novel Excipients

There are no novel excipients.

### 2.3.3 Comments on Impurities/Degradants of Concern

An impurity known as (b) (4) . This impurity was seen in Onglyza, as well, and falls below the qualification threshold.

## 2.4 Proposed Clinical Population and Dosing Regimen

The drug is proposed to be given to patients with Type 2 diabetes once daily. The proposed maximum recommended human doses (MRHD) are up to 5 mg saxagliptin (81 and 438 ng.h/mL for saxagliptin and its major metabolite, BMS-510849) and 2000 mg metformin (20451 ng.h/mL)

## 2.5 Regulatory Background

As part of the post marketing requirements for Onglyza®, the sponsor was required to perform additional embryofetal development studies in rats and rabbits using a combination of saxagliptin and metformin, due to a possible signal for neural tube defects in the rat study submitted with the monotherapy application (a study considered inadequate by the Division due to inadequate control groups and dosing levels). This study identified neural tube defects in 2 fetuses (from one litter) in the group receiving 25 mg/kg saxagliptin (~100X) and 200 mg/kg metformin (~3X MRHD).

# 3 Studies Submitted

## 3.1 Studies Reviewed

All submitted studies were reviewed.

## 3.2 Studies Not Reviewed

This NDA references NDA 22350 (Onglyza®). With the exception of the embryofetal development study using saxagliptin and metformin in combination, nonclinical studies included in that submission are not reviewed here but are listed below.

Identification of rat CYP enzymes that generate BMS-510849 (saxagliptin metabolite)
Single dose cyanide formation in rats
Single dose IV cardiovascular study in monkeys
TK study in pregnant rabbits with saxagliptin
TK study in pregnant rats with saxagliptin
Multiple dose saxagliptin-related cyanide release and CNS lesions, part of 2 year rat study
1-3 month cynomolgus monkeys study
3-Month Monkey toxicity study
6-Week comparative study in monkeys with saxagliptin, vildagliptin, and sitagliptin
6-month rat study
12-month dog study
5-Day Toxicology study in rats with active metabolite, BMS-510849
Chronic Investigational Central Nervous System Toxicity Study in Rats
Genotoxicity studies with saxagliptin and active metabolite, BMS-510849
2-Year mouse carcinogenicity study

2-Year rat carcinogenicity study
Fertility studies in male and female rats
Embryo-fetal development study in rats
Embryo-fetal development study in rabbits
Pre- and postnatal development study in rats
Embryo-fetal development study with saxagliptin and metformin combination in rats
One Month Oral In-vivo/In-vitro Cytogenetics Study in Rat Peripheral Blood Lymphocytes
Intermittent Dose Oral Immunotoxicity Study in Monkeys with BMS-477118

## 3.3 Previous Reviews Referenced

Nonclinical data supporting the safety and efficacy of saxagliptin alone is reviewed under NDA 22350 by Dr. Fred Alavi.

# 4 Pharmacology

## 4.1 Primary Pharmacology

No new primary pharmacology information was submitted, so pharmacology information is based on studies submitted in support of NDA 22-350 (saxagliptin) and literature references for both drugs.

The new drug product is a combination of saxagliptin (Onglyza®) and extended release metformin. Saxagliptin is a reversible inhibitor ($K_i$= 1.3 ± 0.3 nM) of dipeptidyl peptidase 4 (DPP4). This enzyme is expressed on the membrane of numerous cell types (e.g., lymphocytes, endothelial cells, and epithelial cells) and has numerous substrates. Although many of the DPP-4 substrates are physiologically important (including neuropeptide Y and growth hormone releasing hormone), the efficacy of DPP4 inhibitors in treatment of diabetes is thought to derive primarily from inhibition of the degradation of glucacon like peptide 1 (GLP-1) and, to a lesser extent, glucose-dependent insulinotropic polypeptide (GIP).

Metformin is an antihyperglycemic agent with a poorly defined mechanism of action but a long history of clinical use. Metformin increases insulin sensitivity in skeletal muscle and reduces gluconeogenesis in the liver.

## 4.2 Secondary Pharmacology

No new secondary pharmacology information was submitted.

Saxagliptin can inhibit other members of the DPP class (especially DPP8 and DPP9) at concentrations 391X and 75X higher than those required for inhibition of DPP4, as shown in the sponsor's table below. Note that clinical $C_{max}$ is approximately 24 ng/mL (70 nM) for saxagliptin and approximately 47 ng/mL (140 nM) for BMS-510849, so although saxagliptin is fairly specific for DPP4, clinical concentrations approach those that could inhibit DPP9. There were no other significant off-target effects identified.

**Table 2. In vitro $K_i$ values for inhibition of DPP subtypes**

| Treatment | DPP4 Ki (nM) mean ± s.d. (n) | DPP8 Ki (nM) mean ± s.d. (n) | DPP9 Ki (nM) mean ± s.d. (n) |
|---|---|---|---|
| Saxagliptin (BMS-477118) | 1.3 ± 0.3 (12) | 508 ± 174 (13) *ratio 391* | 98 ± 44 (11) *ratio 75* |
| BMS-510849 | 2.6 ± 1.0 (12) | 2495 ± 727 (14) *ratio 948* | 423 ± 64 (12) *ratio 163* |
| Vildagliptin (BMS-471211) | 13 ± 3 (12) | 5218 ± 2319 (14) *ratio 401* | 258 ± 93 (12) *ratio 20* |
| Sitagliptin (BMS-730173) | 18 ± 2 (12) | 33780 ± 5532 (12) *ratio 1913* | 55142 ± 19414 (11) *ratio 3063* |

Ratio refers to the selectivity ratio, defined as the ratio of Ki for each peptidase/Ki for DPP4 using gly-pro-pNA substrate. Values are mean ± standard deviation for (n) independent determinations.

## 4.3 Safety Pharmacology

The sponsor did not submit any dedicated safety pharmacology studies. Endpoints relevant to cardiovascular and respiratory safety were incorporated into the 3-month GLP combination toxicology study in dogs. There were no inter-group differences in respiratory parameters (including respiratory rate and $O_2$ saturation) or cardiovascular endpoints (including ECG).

# 5 Pharmacokinetics/ADME/Toxicokinetics

## 5.1 PK/ADME

The sponsor did not submit pharmacokinetics and ADME studies on the combination of metformin and saxagliptin.

## 5.2 Toxicokinetics

Toxicokinetic parameters were measured in the course of the pivotal 3 month toxicity study and in pregnant rats and rabbits. AUC values for saxagliptin, its major metabolite, and metformin are shown in the sponsor's table below with calculated multiples of human exposure. Coadministration of both compounds does not appear to affect the *in vivo* exposures to the individual components. The reviewer concurs with the sponsor's assessment of the NOAEL values. Note that there is not a NOAEL for pregnant rabbits.

**Table 3. Interspecies comparisons of multiples of human exposure for saxagliptin, BMS-510840, and metformin in pivotal studies**

| Species | Dose (mg/kg/day) | | AUC (ng·h/mL) | | | | | | Multiples over Human Exposure (x)[a] | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Saxagliptin | | BMS-510849 | | Metformin | | Saxagliptin | | BMS-510849 | | Metformin | |
| | Saxagliptin | Metformin | M | F | M | F | M | F | M | F | M | F | M | F |
| Dog 3 month | 5 | 0 | 6580 | 6440 | 8140 | 5820 | - | - | 81 | 80 | 19 | 13 | - | - |
| | 0 | 20 | - | - | - | - | 30900 | 33900 | - | - | - | - | 2 | 2 |
| | 1 | 20 | 863 | 639 | 1020 | 1230 | 34100 | 32000 | 11 | 8 | 2 | 3 | 2 | 2 |
| | 5[b] | 20[b] | 5530 | 4790 | 6670 | 7130 | 29600 | 30000 | 68 | 59 | 15 | 16 | 1.4 | 1.5 |
| Pregnant Rat | 5 | 200 | - | 1630 | - | 658 | - | 85200 | - | 20 | - | 2 | - | 4 |
| | 25[b] | 200[b] | - | 8860 | - | 3510 | - | 89300 | - | 109 | - | 8 | - | 4 |
| Pregnant Rat | 25 | 0 | - | 8300 | - | 2410 | - | - | - | 102 | - | 6 | - | - |
| | 0 | 600 | - | - | - | - | - | 172000 | - | - | - | - | - | 8 |
| | 25[c] | 600[c] | - | 8070 | - | 3640 | - | 197000 | - | 100 | - | 8 | - | 10 |
| Pregnant Rabbit | 40 | 0 | - | 17400 | - | 69700 | - | - | - | 215 | - | 159 | - | - |
| | 0 | 50 | - | - | - | - | - | 28600 | - | - | - | - | - | 1.4 |
| | 40 | 50 | - | 20200 | - | 76500 | - | 22000 | - | 249 | - | 175 | - | 1.1 |

"-" = Not applicable

[a] Safety margins are calculated based on 5 mg saxagliptin, QD (81 ng·h/mL; 438 ng·h/mL BMS-510849) and 1000 mg metformin IR, BID (20451 ng·h/mL) Despite being essentially equivalent (2000 mg metformin XR, QD; 20544 ng·h/mL), the lower AUC value was utilized for a more conservative assessment.

[b] NOAEL - no-observed-adverse-effect-level; represents developmental and maternal NOAEL in pregnant rats

[c] Developmental NOAEL

# 6 General Toxicology

## 6.1 Single-Dose Toxicity

**Study title: Saxagliptin and Metformin: Single-dose oral toxicokinetic and tolerability study in dogs**

| | |
|---|---|
| Study no.: | DN08032 |
| Study report location: | EDR, SN000 |
| Conducting laboratory and location: | BMS Drug Safety Evaluation, New Brunswick, NJ |
| Date of study initiation: | Not provided |
| GLP compliance: | No |
| QA statement: | No |
| Drug, lot #, and % purity: | Saxagliptin, 4K85994, 94%<br>Metformin, C13414, 99.4% |

### Key Study Findings

Dogs (2/sex/dose) were given a single dose of either saxagliptin (1, 5, or 10 mg/kg), metformin (10, 20, or 40 mg/kg), or a combination (5 mg/kg saxagliptin and 20 mg/kg metformin). Unformed, mucoid, or bloody feces were reported in groups receiving ≥ 5 mg/kg saxagliptin and/or any dose of metformin...

Toxicokinetics parameters are shown in the sponsor's table below. Females receiving both drugs had slightly lower exposures to both drugs and the saxagliptin metabolite in this study, but TK parameters in males did not appear to be affected by coadministration.

**Table 4. Single dose dog toxicity study: Toxicokinetics**

| Parameter | Saxagliptin (BMS-477118) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Group 1 Saxagliptin: 1 mg/kg | | Group 2 Saxagliptin: 5 mg/kg | | Group 3 Saxagliptin: 10 mg/kg | | Group 7 Saxagliptin: 5 and Metformin: 20 mg/kg | |
| | Male | Female | Male | Female | Male | Female | Male | Female |
| Cmax (ng/mL) | 391 | 419 | 2000 | 2140 | 4560 | 4150 | 2100 | 1380 |
| AUC(0-T)[a] (ngxh/mL) | 1040 | 893 | 4460 | 5130 | 10300 | 8870 | 4660 | 3020 |
| | Saxagliptin Metabolite (BMS-510849) | | | | | | | |
| | Male | Female | Male | Female | Male | Female | Male | Female |
| Cmax (ng/mL) | 205 | 267 | 1340 | 1340 | 2620 | 3090 | 1330 | 992 |
| AUC(0-T)[a] (ngxh/mL) | 942 | 1120 | 6330 | 5620 | 12100 | 14300 | 6810 | 4410 |
| Parameter | Metformin (BMS-207150) | | | | | | | |
| | Group 4 Metformin: 10 mg/kg | | Group 5 Metformin: 20 mg/kg | | Group 6 Metformin: 40 mg/kg | | Group 7 Saxagliptin: 5 and Metformin: 20 mg/kg | |
| | Male | Female | Male | Female | Male | Female | Male | Female |
| Cmax (ng/mL) | 4210 | 3760 | 8020 | 8170 | 14800 | 15000 | 6390 | 3870 |
| AUC(0-24h) (ngxh/mL) | 13400 | 17700 | 26300 | 28500 | 43000 | 55700 | 27200 | 21200 |

[a] In AUC(0-T), T = 4 to 8 hours post dose for BMS-477118; 8 to 24 hours post dose for BMS-510849.

## 6.2 Repeat-Dose Toxicity

**Study title: Saxagliptin and Metformin: Two-week oral investigative combination toxicokinetic and tolerability study in dogs**

| | |
|---|---|
| Study no.: | DN08043 |
| Study report location: | EDR, SN000 |
| Conducting laboratory and location: | BMS Drug Safety Evaluation, New Brunswick, NJ |
| Date of study initiation: | Not provided |
| GLP compliance: | No |
| QA statement: | No |
| Drug, lot #, and % purity: | Saxagliptin, 4K85994, 94%<br>Metformin, C13414, 99.4% |

## Key Study Findings

Dogs (3/sex/dose) were given saxagliptin (5 mg/kg), metformin (20 mg/kg), or a combination (1 or 5 mg/kg saxagliptin and 20 mg/kg metformin) once daily for 2 weeks in this non-terminal study. Unformed, mucoid, or bloody feces were reported in all groups without apparent relationship to treatment. Food consumption and body weight loss were seen in all groups during the first week, again without relationship to treatment. There were saxagliptin-related increases in eosinophils (up to 7.7X pretest) that were not exacerbated by cotreatment with metformin. Females receiving the 5/20 dose combination had 12-19% decreases in RBC parameters.

Toxicokinetics parameters are shown in the sponsor's table below. There were no significant toxicokinetic interactions caused by coadministration of the two drugs.

**Table 5. Two week dog study: Toxicokinetics**

| Analyte | Parameter | Study Day | Saxagliptin/Metformin Dose (mg/kg/day) | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | 5/0 | | 0/20 | | 1/20 | | 5/20 | |
| | | | M | F | M | F | M | F | M | F |
| Saxagliptin | Cmax (ng/mL) | 1 | 2120 | 1870 | NA | NA | 313 | 360 | 2340 | 1660 |
| | | 15 | 1590 | 1650 | NA | NA | 326 | 283 | 1550 | 1600 |
| | AUC(0-8h) (ngxh/mL) | 1 | 5430 | 5140 | NA | NA | 917 | 799 | 4950 | 3380 |
| | | 15 | 4370 | 4180 | NA | NA | 875 | 663 | 3520 | 3930 |
| BMS-510849 | Cmax (ng/mL) | 1 | 780 | 840 | NA | NA | 195 | 174 | 1080 | 1280 |
| | | 15 | 804 | 972 | NA | NA | 179 | 167 | 867 | 1090 |
| | AUC(0-T)* (ngxh/mL) | 1 | 5430* | 4530 | NA | NA | 891 | 850 | 5140 | 5920 |
| | | 15 | 3830 | 4570 | NA | NA | 819 | 691 | 3940 | 5230 |
| Metformin | Cmax (ng/mL) | 1 | NA | NA | 8560 | 8040 | 7220 | 6750 | 8260 | 8020 |
| | | 15 | NA | NA | 7490 | 10000 | 7190 | 4890 | 6470 | 6580 |
| | AUC(0-24h) (ngxh/mL) | 1 | NA | NA | 30000 | 38700 | 33100 | 34000 | 30600 | 35800 |
| | | 15 | NA | NA | 29000 | 40400 | 29700 | 26900 | 27900 | 29600 |

* AUC(2 to 24 h) was calculated for dog 1103.

For AUC(0-T), T= 8 or 24 hours post dose.

NA: Not applicable.

**Study title: Saxagliptin and metformin: Three-month Oral combination toxicity study in dogs**

| | |
|---|---|
| Study no.: | DN08067 (803500) |
| Study report location: | EDR, SN000 |
| Conducting laboratory and location: | (b) (4) |
| Date of study initiation: | 30 September 2008 |
| GLP compliance: | Yes |
| QA statement: | Yes |
| Drug, lot #, and % purity: | Saxagliptin, 4K85994, 93.8% or 93.1%<br>Metformin, C13414, 99.4% |

## Key Study Findings

- Dogs given up to 5 mg/kg saxagliptin with or without 20 mg/kg metformin had exposures to saxagliptin of up to ~70X MRHD (AUC basis), to BMS-510849 of ~16X MRHD, and to metformin of ~1.5X MRHD. There was no vehicle control.
- Tremor and shivering was observed more often in the combination treated group (11 instances vs. ≤ 1 instance in other groups), but the finding did not appear to correlate with hypoglycemia.
- Effects on body weight gain were not statistically significantly different between groups.
- There were no inter-group differences in respiratory parameters or cardiovascular endpoints (including ECG).
- One combination treated female had mild necrosis of the fat in the dermis associated with inflammation.

*Reviewer Comments*: This study provided adequate exposure levels to assess potential interactions of saxagliptin and metformin. There were no findings in the combination group that could be definitively attributed to treatment with the combination. The NOAEL for this study was 5/20 mg/kg of saxagliptin/metformin. Overall exposures were considered adequate for evaluation of possible interactions of saxagliptin and metformin by the Division at the pre-NDA meeting.

### Methods

Doses:

| Group Number Identification | Saxagliptin | | Metformin | | Number of Dogs | |
|---|---|---|---|---|---|---|
| | Dose Level (mg/kg/day) | Conc (mg/mL) | Dose Level (mg/kg/day) | Conc (mg/mL) | Males | Females |
| 1/ Saxagliptin | 5 | 5 | 0 | 0 | 3 | 3 |
| 2/ Metformin | 0 | 0 | 20 | 20 | 3 | 3 |
| 3/ Saxagliptin + Metformin | 1 | 1 | 20 | 20 | 3 | 3 |
| 4/ Saxagliptin + Metformin | 5 | 5 | 20 | 20 | 3 | 3 |

| | |
|---|---|
| Frequency of dosing: | Once daily |
| Route of administration: | Oral gavage |
| Dose volume: | 1 mL/kg per drug or vehicle (2 mL/kg/day total) |
| Formulation/Vehicle: | Saxagliptin: 0.003M HCl or 0.016M HCl (LD & HD) in deionized water<br>Metformin: deionized water |
| Species/Strain: | Beagle dog |
| Number/Sex/Group: | 3/sex/group |
| Age: | 10-11 months |
| Weight: | M: 9.9-11.9 kg; F: 7.2-8.7 kg |
| Satellite groups: | None |
| Unique study design: | None |
| Deviation from study protocol: | None affecting study outcome |

## Observations and Results

### Mortality

Dogs were observed twice daily for signs of mortality, ill health, and reactions to treatment.

There were no unscheduled deaths in this study.

### Clinical Signs

In addition to mortality checks, clinical signs were recorded at a daily detailed examination (2-4h post dose). Physical examination by a veterinarian occurred pretreatment and during W12, including temperature, lung sounds, and respiration rate. Additional cage-side observations were made at 1h post dose during TK sampling.

The sponsor's table below adequately conveys the findings that may be treatment-related in the study. Changes in feces were observed in all groups and did not appear to worsen with combination treatment. Tremor and/or shivering was seen more often in the 5/20 group (but at low incidence).

**Text Table 3.3 Incidence of Clinical Observations Summary**

| Saxagliptin (mg/kg/day) | 5 | | 0 | | 1 | | 5 | |
|---|---|---|---|---|---|---|---|---|
| Metformin (mg/kg/day) | 0 | | 20 | | 20 | | 20 | |
| Sex: | M | F | M | F | M | F | M | F |
| Tremors/shivers | - | - | - | 1 (1) | - | 1 (1) | 4 (3) | 7 (2) |
| Circling | - | - | 7 (1) | - | - | - | - | - |
| Soft/liquid feces | 12 (3) | 27 (3) | 8 (3) | 3 (2) | 20 (3) | 2 (2) | 11 (3) | 11 (3) |
| Feces red | 1 (1) | 2 (1) | - | 1 (1) | - | - | 1 (1) | 1 (1) |
| Mucoid material | 1 (1) | 19 (3) | 2 (1) | - | - | - | 8 (3) | 20 (2) |
| Salivation | - | - | 95 (3) | 16 (3) | 67 (3) | 42 (3) | 51 (3) | 3 (1) |
| Eye discharge | - | 93 (3) | - | - | 2 (1) | 17 (2) | - | 1 (1) |

A dash (-) indicates absence of finding in group
Data are expressed as the total number of occurrences/group (number of dogs affected)

### Body Weights

Body weights were measured weekly and just prior to scheduled necropsy. Because there were no vehicle control groups, dogs treated with both drugs are compared to those given a single drug. In males, all groups given metformin gained less weight than the saxagliptin-only group (1.1-1.9% of body weight versus 5.3%). There did not appear to be a separate effect of the combination. In females, animals in the 5/20 group gained 38% less weight on average compared the saxagliptin-only group and . The effects in both sexes were not statistically significant. Average weights over time are shown in the sponsor's figures below.

**Table 6. Dog combination toxicity study: Body weight gain**

| Body Weight Gain | | | | |
|---|---|---|---|---|
| Sex | Saxa/Met dose | BW gain (kg) over study | % decrement (change from G1) | % BW gained |
| Males | 5/0 | 0.57 ± 0.21 | - | 5.3% |
| | 0/20 | 0.13 ± 0.49 | ↓77% | 1.1% |
| | 1/20 | 0.13 ± 0.23 | ↓77% | 1.2% |
| | 5/20 | 0.20 ± 0.10 | ↓65% | 1.9% |
| Females | 5/0 | 0.60 ± 0.44 | - | 7.2% |
| | 0/20 | 0.67 ± 0.35 | ↑12% | 8.7% |
| | 1/20 | 0.73 ± 0.40 | ↑22% | 9.3% |
| | 5/20 | 0.37 ± 0.31 | ↓38% | 4.7% |

**Figure 3. Dog combination toxicity study: Male body weight**

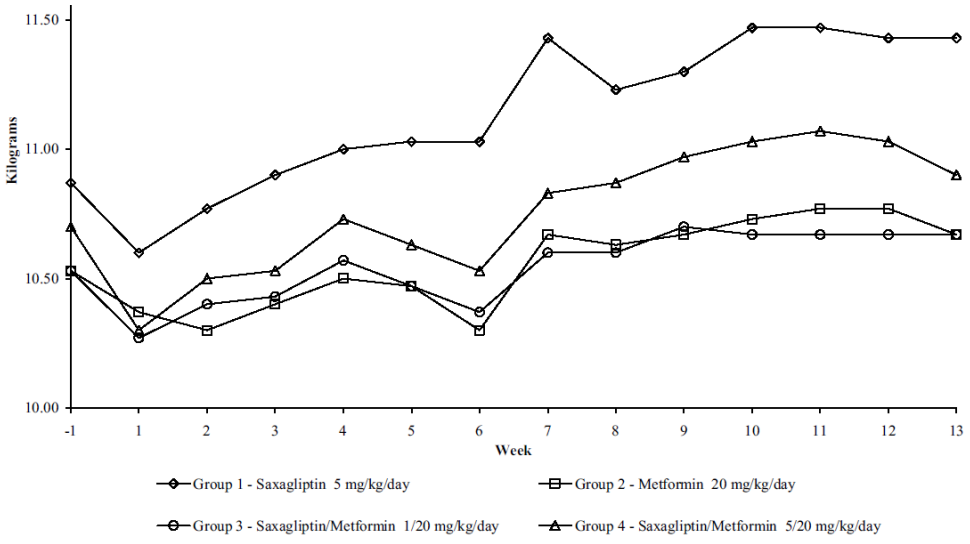



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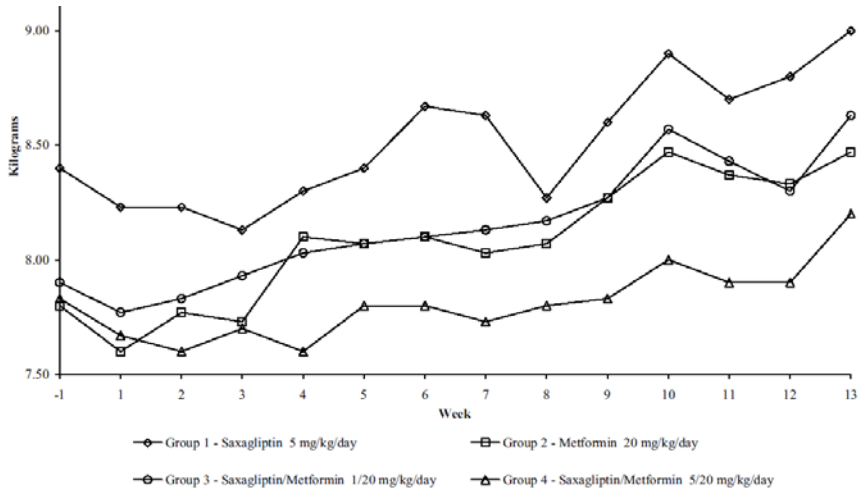
**Figure 4. Dog combination toxicity study: Female body weight**



## Food Consumption

Food intake was recorded daily for individual animals. Water consumption was measured pretreatment and during W4 and W12 (18 hour measurement).

Over then entire study, male animals in Groups 3 and 4 (1/20 and 5/20 mg/kg/d) consumed 7% and 10% less food than those given saxagliptin alone. Metformin treated males were similar to saxagliptin treated males. In the females, only metformin-only dosing (Group 2) led to a reduction in feeding relative to saxagliptin only animals (↓8%). Food consumption over time is displayed for males and females in Figure 5 and Figure 6 below (error bars not shown for clarity).

**Figure 5. Dog combination toxicity study: Male food consumption**

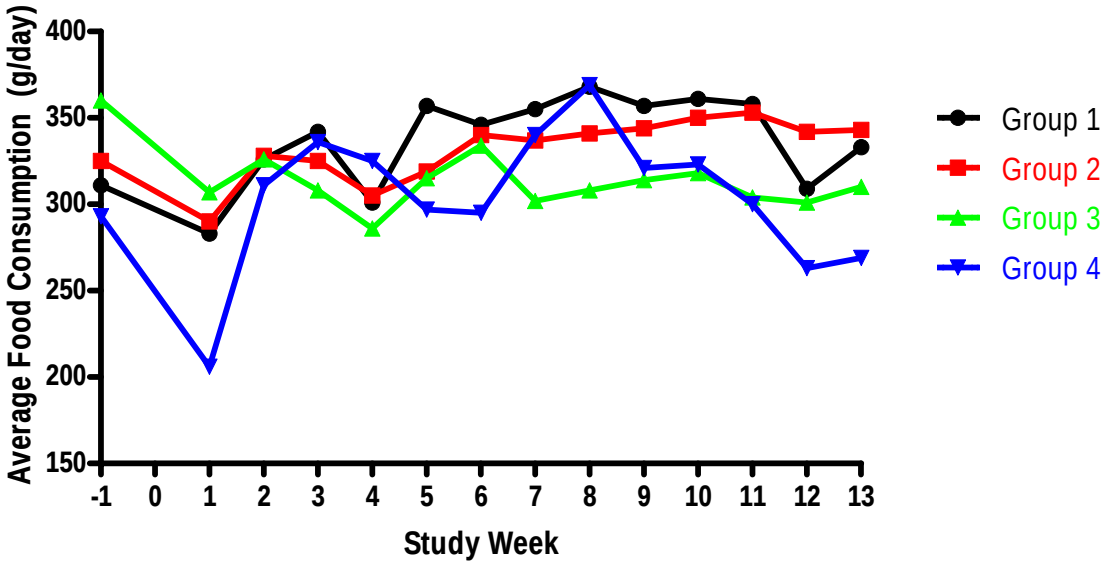
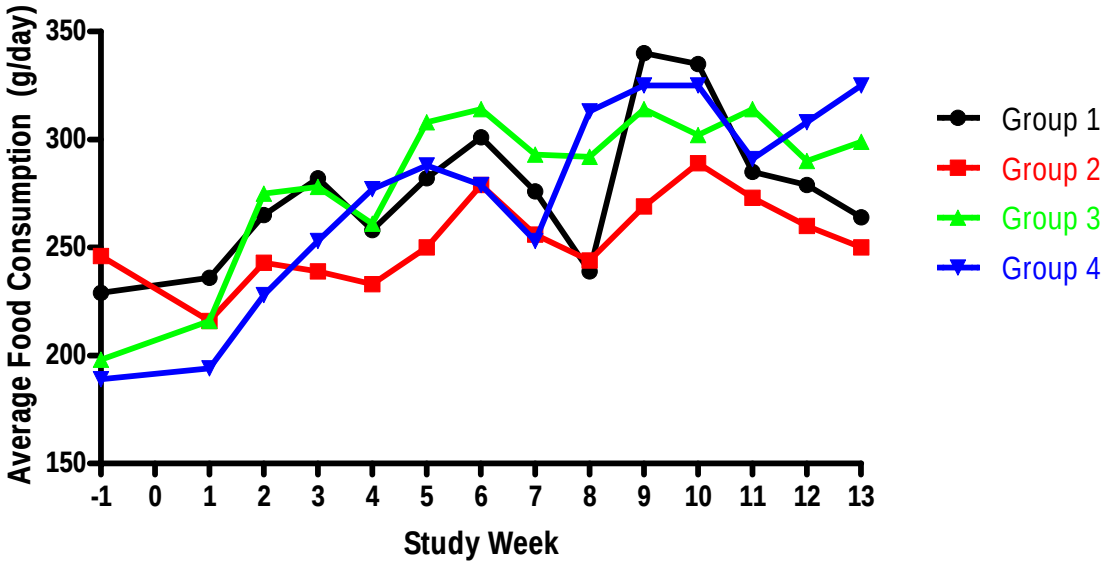



**Figure 6. Dog combination toxicity study: Female food consumption**



## Ophthalmoscopy

Ophthalmologic exams were performed pretreatment and during W12 for all dogs.

There were no treatment-related ocular changes.

## Respiratory Parameters

Arterial oxygen saturation and respiration rate were measured during pretreatment and during W12 for all dogs. There were no treatment-related changes in $O_2$ saturation in any dose group. Respiratory rate was higher during W12 in all groups, as shown in the sponsor's table below, but the effect did not appear to be treatment-related.

**Table 7. Dog combination toxicity study: Respiration rate**

| Males | | | | Females | | | |
|---|---|---|---|---|---|---|---|
| Group | Summary Information | Pretreatment | Week 12 | Group | Summary Information | Pretreatment | Week 12 |
| 1 | Mean | 20.0 | 36.0 | 1 | Mean | 30.7 | 32.0 |
| | SD | 4.0 | 4.0 | | SD | 6.1 | 4.0 |
| | N | 3 | 3 | | N | 3 | 3 |
| 2 | Mean | 26.7 | 34.7 | 2 | Mean | 26.7 | 33.3 |
| | SD | 2.3 | 6.1 | | SD | 2.3 | 2.3 |
| | N | 3 | 3 | | N | 3 | 3 |
| 3 | Mean | 24.0 | 30.0 | 3 | Mean | 28.0 | 32.0 |
| | SD | 4.0 | 0.0 | | SD | 4.0 | 3.5 |
| | N | 3 | 3 | | N | 3 | 3 |
| 4 | Mean | 25.3 | 29.3 | 4 | Mean | 25.3 | 26.0 |
| | SD | 6.1 | 6.1 | | SD | 2.3 | 3.5 |
| | N | 3 | 3 | | N | 3 | 3 |

## ECG

Cardiovascular evaluations were performed pretreatment and during W12 for all dogs using limb leads I, II, III, aVR, aVL, and aVF. Quantitative assessments of ECG parameters were made, including QTc (Van de Water method).

The consulting cardiologist states: “Other than normal, mild variations in values for the measured parameters, all the recordings in all the animals did not show any changes (quantitative or qualitative) that could be attributed to the treatment at any dose level compared to pre-study recordings or to the Control.” The reviewer assumes the cardiologist refers to Groups 1 and/or 2 as the Control group and concurs with this assessment. There does not appear to be a significant effect of co-administration of the two drugs.

## Hematology

Standard hematology and clotting parameters were measured twice pretreatment and during W4 and W13.

There were decreases in hematocrit in all groups receiving metformin between the pretreatment and W4 assessments. In females, the hematocrit values in the combination dosed groups were statistically significantly different from Groups 1 or 2 as shown in the sponsor's table below. Metformin-only dosing caused a decrease in hematocrit from baseline that was also observed in the combination treated groups.

This effect appeared to resolve somewhat by the end of dosing. There did not appear to be an exacerbation of this effect by cotreatment with saxagliptin.

**Table 8. Dog combination toxicity study: Hematocrit**

| | | Males | | | | Females | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | Week | | | | Week | |
| Group | Summary Information | First Pretreatment | Second Pretreatment | 4 | 13 | First Pretreatment | Second Pretreatment | 4 | 13 |
| 1 | Mean | 45.37 | 46.20 | 43.50 | 45.70 | 45.97 | 40.63 | 42.53 | 47.73 |
| | SD | 2.06 | 6.24 | 2.52 | 2.36 | 3.96 | 5.22 | 2.97 | 4.12 |
| | N | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 |
| 2 | Mean | 45.27 | 51.37 | 42.27 | 46.53 | 42.40 | 42.53 | 36.77 | 40.27 |
| | SD | 2.90 | 1.69 | 2.85 | 2.25 | 2.21 | 4.36 | 0.65 | 3.69 |
| | N | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 |
| 3 | Mean | 47.43 | 46.67 | 41.17 | 46.90 | 44.53 | 47.03 | 39.87 ^ | 45.20 |
| | SD | 1.46 | 1.35 | 4.36 | 4.86 | 2.27 | 0.67 | 0.50 | 2.27 |
| | N | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 |
| 4 | Mean | 47.27 | 46.93 | 42.87 | 46.03 | 49.40 | 46.13 | 39.07 * | 41.57 |
| | SD | 2.61 | 6.39 | 3.75 | 7.77 | 0.72 | 2.87 | 1.14 | 3.57 |
| | N | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 |

Significantly different from Saxagliptin control group (group 1) value: * - P ≤ 0.05 ** - P ≤ 0.01 *** - P ≤ 0.001 (*t*-test)
† - P ≤ 0.05 †† - P ≤ 0.01 ††† - P ≤ 0.001 (Wilcoxon)
Significantly different from Metformin control group (group 2) value: ^ - P ≤ 0.05 ^^ - P ≤ 0.01 ^^^ - P ≤ 0.001 (*t*-test)
& - P ≤ 0.05 && - P ≤ 0.01 &&& - P ≤ 0.001 (Wilcoxon)
Group 3 significantly different from Group 4 value: ° - P ≤ 0.05 °° - P ≤ 0.01 °°° - P ≤ 0.001 (*t*-test)
φ - P ≤ 0.05 φ φ - P ≤ 0.01 φ φ φ - P ≤ 0.001 (Wilcoxon)

## Clinical Chemistry

Standard clinical chemistry parameters (plus bicarbonate) were measured twice pretreatment and during W4 and W13 following an overnight fast. On D3 (M) or D2 (F), a predose sample was taken from all dogs. A follow-up sample was taken post dose (from all dogs) if tremors were observed in any dog for measurement of blood glucose.

There was no evidence of treatment-related changes in clinical chemistry parameters. Additionally, there was no evidence of acute post-dose hypoglycemia on D2/3.

## Urinalysis

Standard urinalysis parameters were measured twice pretreatment and during W4 and W13 using a 16h overnight collection. There were no treatment-related changes in urinalysis parameters.

## Gross Pathology

Complete necropsies were performed on all animals (fasted) following euthanization. There were no findings that appeared to be treatment-related.

## Organ Weights

Organ weights were taken for all animals: adrenal, brain, heart, kidneys, liver (with gall bladder), ovaries/testes, pituitary gland, prostate, spleen, thyroid + parathyroid.

There were no treatment-related differences in organ weights.

## Histopathology

Adequate Battery – Yes
Peer Review – Yes
Histological Findings: Findings in the microscopic pathology assessment generally appeared to be sporadic and not related to treatment. Two findings occurred in the skin/subcutis that were possibly related to treatment: moderate fibrosis in the subcutis of a G4 male (corresponding to bilateral thickening of the pinna noted at necropsy) and mild necrosis in the skin of a G4 female (described as necrosis of the fat in the dermis with inflammation). Skin lesions are a known class effect of DPP-4 inhibitors in nonclinical studies, particularly monkeys; however, the low incidence of these findings makes it difficult to definitively attribute them to treatment.

**Table 9. Dog combination toxicity study: Microscopic findings**

| | | MALE | | | |
|---|---|---|---|---|---|
| **DOSE GROUP** | | **1** | **2** | **3** | **4** |
| **NUMBER OF ANIMALS EXAMINED** | | **3** | **3** | **3** | **3** |
| SUBCUTANEOUS TISSUE | EXAMIN: | - | - | 1 | 1 |
| | N.A.D. | - | - | 1 | - |
| - Fibrosis | | - | - | - | 1 |
| | Grade 3 | - | - | - | 1 |
| | | **FEMALE** | | | |
| **DOSE GROUP** | | **1** | **2** | **3** | **4** |
| **NUMBER OF ANIMALS EXAMINED** | | **3** | **3** | **3** | **3** |
| SKIN | EXAMIN: | 3 | 3 | 3 | 3 |
| | N.A.D. | 3 | 2 | 3 | 2 |
| - Granuloma | | - | 1 | - | - |
| | Grade 1 | - | 1 | - | - |
| - Necrosis | | - | - | - | 1 |
| | Grade 2 | - | - | - | 1 |

## Toxicokinetics

Toxicokinetic parameters were measured on D1, D23, and D86. Exposure to metformin was similar between sexes and across all groups receiving 20 mg/kg. Exposure to saxagliptin was slightly lower in groups also receiving 20 mg/kg metformin compared to those receiving saxagliptin alone (74-92% of Group 1 levels), as shown in the sponsor's table below. Exposures to the metabolite BMS-510849 were also lower in Group 4 males but were higher in Group 4 females by the end of the study. Overall exposures were considered adequate for evaluation of possible interactions of saxagliptin and metformin by the Division at the pre-NDA meeting.

**Table 10. Dog combination toxicity study: Toxicokinetics**

| Parameter | Day | Saxagliptin/Metformin Dose (mg/kg) | | | | | |
|---|---|---|---|---|---|---|---|
| | | 5/0 | | 1/20 | | 5/20 | |
| | | Saxagliptin | | | | | |
| | | Male | Female | Male | Female | Male | Female |
| $C_{max}$ (ng/mL) | 1 | 2470 | 2660 | 467 | 456 | 2160 | 2220 |
| | 23 | 3560 | 3130 | 417 | 440 | 2480 | 2580[c] |
| | 86 | 3080 | 2920 | 418 | 373 | 3000 | 2850 |
| $AUC_{(0-T)}$[a] (ng•h/mL) | 1 | 6440 | 5280 | 996 | 804 | 5450 | 4640 |
| | 23 | 6850 | 6140 | 931 | 656 | 5060 | 4100[c] |
| | 86 | 6580 | 6440 | 863 | 639 | 5530 | 4790 |

| Parameter | Day | BMS-510849 | | | | | |
|---|---|---|---|---|---|---|---|
| | | Male | Female | Male | Female | Male | Female |
| $C_{max}$ (ng/mL) | 1 | 1150 | 1410 | 225 | 277 | 1030 | 1500 |
| | 23 | 1370 | 1580 | 256 | 444 | 1570 | 1890[c] |
| | 86 | 1530 | 1280 | 264 | 336 | 1560 | 1720 |
| $AUC_{(0-T)}$[b] (ng•h/mL) | 1 | 6380 | 6580 | 903 | 1140 | 5700 | 6380 |
| | 23 | 7560 | 6980 | 995 | 1470 | 6810 | 7590[c] |
| | 86 | 8140 | 5820 | 1020 | 1230 | 6670 | 7130 |
| Parameter | Day | 0/20 | | 1/20 | | 5/20 | |
| | | Metformin | | | | | |
| | | Male | Female | Male | Female | Male | Female |
| $C_{max}$ (ng/mL) | 1 | 6790 | 6610 | 6830 | 7430 | 6940 | 7810 |
| | 23 | 7900 | 10100 | 6250 | 8260 | 7970 | 9740[c] |
| | 86 | 9560 | 8890 | 9270 | 8150 | 9540 | 8560 |
| $AUC_{(0-24h)}$ (ng•h/mL) | 1 | 26000 | 27600 | 28400 | 31100 | 25000 | 29300 |
| | 23 | 28600 | 32400 | 26400 | 27000 | 26000 | 29300[c] |
| | 86 | 30900 | 33900 | 34100 | 32000 | 29600 | 30000 |

a For $AUC_{(0-T)}$, T = 4 or 8 hours post dose.
b For $AUC_{(0-T)}$, T = 8 or 24 hours post dose.
c N = 2 due to exclusion of Animal No. 452 from mean calculations.

### Stability and Homogeneity

Stability and homogeneity data were acceptable.

# 7 Genetic Toxicology

Saxagliptin (as it is currently manufactured) and metformin were not genotoxic individually when tested in a standard battery of assays. They were not tested in combination.

# 8 Carcinogenicity

Metformin and saxagliptin were each tested in separate two-year carcinogenicity studies in rats and mice. A combination carcinogenicity study was not required. Saxagliptin along did not cause an increase in tumors when given to mice at doses of 50, 250, and 600 mg/kg/d (20-32X, 428-376X, 870-1165X MRHD, AUC basis) or when given to male and female rats at doses of 25, 75, 150, and 300 mg/kg/d (43-108X, 173-380X, 355-1012X, 847-2214X MRHD). Metformin alone caused an increase in benign stromal uterine polyps in female rats given 900 mg/kg (~4X MRHD, AUC basis) but did not cause an increase in tumors in male rats given the same dose or in mice given 1500 mg/kg/d (~4X MRHD).

# 9 Reproductive and Developmental Toxicology

## 9.2 Embryonic Fetal Development

### Metformin: Ten-day oral range finding study in pregnant rats

| | |
|---|---|
| Study no: | DN08049 |
| Study report location: | EDR, SN000 |
| Conducting laboratory and location: | BMS, Department of Reproductive Toxicology, New Brunswick, NJ |
| Date of study initiation: | Not provided |
| GLP compliance: | No |
| QA statement: | No |
| Drug, lot #, and % purity: | Metformin, batch C13414, 99.4% |

**Study summary:**

This non-GLP, range-finding study in pregnant rats (n=8/group) used doses of 0, 100, 300, 500, or 1000 mg/kg, based on previous toxicity studies (overt toxicity expected at the high dose, providing exposures >2X MRHD). Doses were given in 5 mL/kg on GD6-15, and animals were euthanized on GD20 or GD21. Toxicokinetic evaluations, standard clinical monitoring, and cesarean section data were evaluated. Evaluation of fetuses was limited to external examination.

There were no treatment-related clinical signs. HD dams gained 24% less weight (consuming 9% less food) than controls during the dosing period but recovered during GD16-21 so that overall gains were similar. Postimplantation loss was numerically higher in HD dams (11.6±15.7 vs. 4.8 ±7.6), generally due to early resorptions, but was not statistically significant compared to control. Fetuses of dams given ≥ 300 mg/kg weighed 5-7% more than controls, but the difference was not statistically significant. The sponsor considered the NOAEL to be 1000 mg/kg. Toxicokinetic parameters are shown in the sponsor's table below.

**Table 11. Range-finding in pregnant rats: Toxicokinetics**

| Parameter | Metformin (mg/kg/day) | | | |
|---|---|---|---|---|
| | 100 | 300 | 500 | 1000 |
| Cmax (ng/mL) | 5910 | 15100 | 17400 | 16800 |
| AUC(0-24 h) (ngxh/mL) | 40400 | 113000 | 177000 | 204000 |

## Saxagliptin and Metformin: Oral combination study of embryofetal development in rats (I)

| | |
|---|---|
| Study no: | DN08072 / 901689 |
| Study report location: | EDR, SN000 |
| Conducting laboratory and location: | (b) (4) |
| Date of study initiation: | 11 November 2008 |
| GLP compliance: | Yes |
| QA statement: | Yes |
| Drug, lot #, and % purity: | Saxagliptin, benzoate salt<br>Metformin HCl with 0.5% magnesium stearate, 8E35072, 99.5% |

### Key Study Findings

- Sprague-Dawley rats dosed with 5/200 mg/kg (saxagliptin/metformin) or 25/200 mg/kg had exposures to saxagliptin of 20 and 109X MRHD for saxagliptin, 1.5X and 8X BMS-510840, and 4X metformin.
- There were no signs of maternal toxicity.
- In the 25/200 mg/kg group malformations including craniorachischisis and forelimb flexure in 2 fetuses in one litter with fetal and litter incidences of 0.7 and 4.5%, respectively. One of the fetuses had cleft palate and both had absent renal papillae. Additionally, digital malformations were observed in two fetuses from another litter, exceeding the historical fetal incidence.

*Reviewer comments:* This study was submitted as part of the NDA for Onglyza®. As part of the post marketing requirements, the sponsor was required to perform additional studies to evaluate the possibility of treatment-related neural tube defects due to either metformin or the combination of the two drugs. Although the incidence of NTD in this study was low, it was outside the historical control range (as of the writing of the original report), and there is evidence in the literature of metformin-induced NTD at very high concentrations. An updated and expanded review of control groups from the laboratory showed that the fetal and litter incidences of craniorachischisis for control SD rats was up to 1.2% and 16.7%, respectively. The relatively high litter incidence is due to the fact that there were two pilot studies included in which one litter of six had this finding. Excluding range-finding studies from the historical controls gives maximum litter incidence of 5.00%. The reviewer considers it reasonable to conclude that the original finding of craniorachischisis was incidental and not related to drug treatment.

**Methods**

| | |
|---|---|
| Doses: | Saxagliptin/Metformin: 0/0, 5/200, 25/200 mg/kg |
| Frequency of dosing: | Daily on GD6-15 |
| Dose volume: | 5 mL/kg (total) |
| Route of administration: | Oral gavage |
| Formulation/Vehicle: | Saxagliptin: suspension in 1.25% Avicel® (RC-591, FMC Biopolymer in deionized water<br>Metformin: deionized water |
| Species/Strain: | Sprague-Dawley rats (b) (4) |
| Number/Sex/Group: | 22/sex/group (main study) |
| Satellite groups: | 12/sex/group (satellite for clinical pathology and TK) |
| Study design: | Standard Segment 2 study |
| Deviation from study protocol: | None that affected study outcome |

## Observations and Results

## Mortality

Animals were observed twice daily for signs of mortality, ill health, and reactions to treatment.

## Clinical Signs

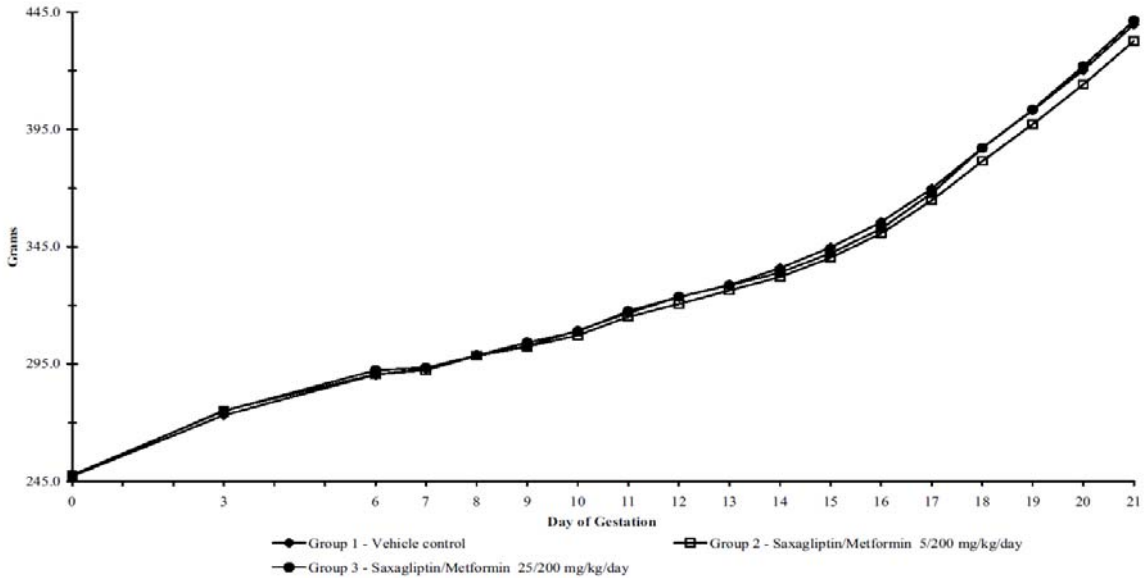
Clinical signs were additionally recorded at a daily detailed examination during non-dosing periods and two detailed examinations during the dosing period (predose and 1-2h post dose).

There were no treatment-related clinical signs.

## Body Weight

Body weights were measured on GD0, 3, and 6-21. There were no treatment-related differences in body weight gains over the course of the study, as shown in the sponsor's figure below.

**Figure 7. Rat combination EFD study (I): Maternal body weight**



## Feed Consumption

Food consumption was measured individually for GD3-6 and daily for GD6-21 for main study animals only.

Drug-treated animals consumed ~10% less food on the first day of dosing but were similar to controls thereafter.

## Hematology and Clinical Pathology

Satellite animals only were sampled on GD14 for standard hematology and clinical chemistry parameters, including bicarbonate.

The hematology assessment in the satellite animals revealed a decrease in platelet number (↓13-16%) and an increase in lymphocytes (16-31%) in drug-treated animals. Serum biochemistry measurements showed 4-6% decreases in albumin in treated animals.

## Toxicokinetics

Toxicokinetic parameters measured on GD14 in the satellite animals are shown in Table 12. Increases in exposure to saxagliptin and the major metabolite were approximately dose proportional, and increases in saxagliptin dose did not appear to affect metformin TK parameters. Both dosing conditions provided drug exposures that exceed the average clinical exposure to the three analytes, but the multiples were markedly lower for metformin and the saxagliptin metabolite than for saxagliptin itself.

**Table 12. Rat combination EFD study (I): Toxicokinetics**

| | 5/200 | | | | 25/200 | | | |
|---|---|---|---|---|---|---|---|---|
| Analyte | $T_{max}$ (h) | $C_{max}$ (ng/mL) | AUC (ng.h/mL) | AUC Multiple of MRHD | $T_{max}$ (h) | $C_{max}$ (ng/mL) | AUC (ng.h/mL) | AUC Multiple of MRHD |
| Saxagliptin | 1 | 513 | 1630 | 20X | 0.5 | 2790 | 8860 | 109X |
| BMS-510840 | 2 | 170 | 658 | 1.5X | 2 | 732 | 3510 | 8X |
| Metformin | 2 | 11400 | 85200 | 4X | 1 | 10500 | 89300 | 4X |

## Stability and Homogeneity

Dosing formulations were acceptable.

## Necropsy

All dams were examined for gross lesions and pregnancy status. Main study dams were euthanized on GD21.

Maternal performance across groups was similar, as shown in the sponsor's table below. There were no treatment-related findings at gross necropsy.

**Table 13. Rat combination EFD study (I): Maternal performance**

Group 1 - Vehicle control
Group 2 - Saxagliptin/Metformin 5/200 mg/kg/day
Group 3 - Saxagliptin/Metformin 25/200 mg/kg/day

| | Group 1 | Group 2 | Group 3 |
|---|---|---|---|
| Number of Rats Mated | 22 | 22 | 22 |
| Number of Pregnant Rats | 22 | 21 | 22 |
| Number of Rats Identified as Pregnant by Ammonium Sulfide | 0 | 0 | 0 |
| Number of Pregnancy Status not Determinable | 0 | 0 | 0 |
| Number of Rats Dying/Euthanized on Study | 0 | 0 | 0 |
| Number of Rats with Total Resorptions | 0 | 0 | 0 |
| Number of Rats Littering Prior to Scheduled Cesarean | 0 | 0 | 0 |
| Pregnancy Rate (%) | 100.0 | 95.5 | 100.0 |

* Excluding satellite animals

## Cesarean Section Data (Implantation Sites, Pre- and Post-Implantation Loss, etc.)

After euthanasia, the ovaries were removed and the corpora lutea counted. The uterus was weighed (gravid and non-gravid); uterine contents were examined, including placenta. The numbers and positions of liver fetuses, dead fetuses, and resorptions were recorded. There were no treatment-related differences in uterine or ovarian parameters, as shown in the sponsor's table below.

**Table 14. Rat combination EFD study (I): Uterine and ovarian parameters**

Group 1 - Vehicle control
Group 2 - Saxagliptin/Metformin 5/200 mg/kg/day
Group 3 - Saxagliptin/Metformin 25/200 mg/kg/day

| Group | Summary Information | Total Number of Corpora Lutea | Total Implantation Sites | Male Fetuses | Female Fetuses | Sex Ratio (% Males) |
|---|---|---|---|---|---|---|
| 1 | Mean | 15.0 | 13.2 | 7.1 | 5.5 | 56.96 |
| | SD | 2.9 | 2.5 | 1.9 | 1.9 | 12.62 |
| | N | 22 | 22 | 22 | 22 | 22 |
| 2 | Mean | 14.4 | 13.1 | 6.3 | 5.6 | 51.53 |
| | SD | 1.9 | 2.2 | 2.7 | 2.0 | 19.58 |
| | N | 21 | 21 | 21 | 21 | 21 |
| 3 | Mean | 14.5 | 13.3 | 6.5 | 6.3 | 50.40 |
| | SD | 2.4 | 2.8 | 2.4 | 2.4 | 15.90 |
| | N | 22 | 22 | 22 | 22 | 22 |

Significantly different from control group (group 1) value: * - $P \leq 0.05$ ** - $P \leq 0.01$ (Dunnett)
† - $P \leq 0.05$ †† - $P \leq 0.01$ (Dunn)

| Group | Summary Information | Live Fetuses | Dead Fetuses | Early Resorptions | Middle Resorptions | Late Resorptions |
|---|---|---|---|---|---|---|
| 1 | Mean | 12.6 | 0.0 | 0.6 | 0.0 | 0.0 |
| | SD | 2.4 | 0.0 | 0.9 | 0.0 | 0.0 |
| | N | 22 | 22 | 22 | 22 | 22 |
| 2 | Mean | 11.9 | 0.0 | 1.1 | 0.05 | 0.0 |
| | SD | 2.6 | 0.0 | 1.5 | 0.22 | 0.0 |
| | N | 21 | 21 | 21 | 21 | 21 |
| 3 | Mean | 12.7 | 0.05 | 0.5 | 0.05 | 0.0 |
| | SD | 2.7 | 0.21 | 0.7 | 0.21 | 0.0 |
| | N | 22 | 22 | 22 | 22 | 22 |

Significantly different from control group (group 1) value: * - P ≤ 0.05 ** - P ≤ 0.01 (Dunnett)
† - P ≤ 0.05 †† - P ≤ 0.01 (Dunn)

| Group | Summary Information | Sum of Resorptions | Preimplantation Loss (%) | Post Implantation Loss (%) | Gravid Uterus Weight (g) |
|---|---|---|---|---|---|
| 1 | Mean | 0.6 | 11.72 | 4.22 | 101.1 |
| | SD | 0.9 | 9.55 | 5.92 | 17.9 |
| | N | 22 | 22 | 22 | 22 |
| 2 | Mean | 1.2 | 9.23 | 9.25 | 96.6 |
| | SD | 1.5 | 11.18 | 10.90 | 19.8 |
| | N | 21 | 21 | 21 | 21 |
| 3 | Mean | 0.5 | 8.51 | 4.82 | 102.2 |
| | SD | 0.7 | 11.68 | 6.65 | 20.0 |
| | N | 22 | 22 | 22 | 22 |

Significantly different from control group (group 1) value: * - P ≤ 0.05 ** - P ≤ 0.01 (Dunnett)
† - P ≤ 0.05 †† - P ≤ 0.01 (Dunn)

## Offspring (Malformations, Variations, etc.)

Each fetus was weighed and examined externally for sex and gross alterations. Euthanized offspring were examined for either visceral changes (~50%) or skeletal alterations (~50%).

There were no treatment-related differences in fetal weights, but major malformations appeared to be higher in drug-treated groups, as shown in the sponsor's table below. Especially concerning were the effects in the 25/200 mg/kg group, including incidence of craniorachischisis (neural tube defect) and forelimb flexure in 2 fetuses in one litter with fetal and litter incidences of 0.7 and 4.5%, respectively. This finding was outside the historical control incidence values available at the time of the original report, but a subsequent investigation found four studies with findings of craniorachischisis in the control groups, providing fetal and litter incidences that exceed those observed in this study (see Table 15 below).

One of the fetuses with NTD had cleft palate, and both had absent renal papillae. Additionally, digital malformations were observed in two fetuses from another litter, exceeding the historical fetal incidence. The noted single incidence of sinus inversus and absent kidney was considered spontaneous and within or slightly greater than historical range of the testing facility.

**Table 15. Updated historical control incidence of craniorachischisis**

INCIDENCE OF CRANIORACHISCHISIS*

| Study Type | F/E | F/A | L/E | L/A | Incidence F/A | Incidence L/A |
|---|---|---|---|---|---|---|
| **Pre- and Postnatal Study** | | | | | | |
| Study 1 (1999) | 269 | 1@ | 20 | 1 | 0.37 | 5.00 |
| **Pilot/Range-finding Embryo-Fetal Development Study** | | | | | | |
| Study 1 (May 2009) | 126 | 1 | 8 | 1 | 0.79 | 12.50 |
| Study 2 (2004) | 83 | 1 | 6 | 1 | 1.20 | 16.67 |
| **Embryo-Fetal Development Study** | | | | | | |
| Study 1 (2006) | 292 | 1@ | 22 | 1 | 0.34 | 4.55 |

* Only studies with a finding of craniorachischisis are listed
( ) = Month/year of occurrence

@ Noted in dead fetus or pup

L/A Litters affected
L/E Litters examined

F/E Fetuses examined
F/A Fetuses affected

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**Table 16. Rat combination EFD study (I): Major malformations**

Group 1 - Vehicle control
Group 2 - Saxagliptin/Metformin 5/200 mg/kg/day
Group 3 - Saxagliptin/Metformin 25/200 mg/kg/day

| | Group 1 | | Group 2 | | Group 3 | |
|---|---|---|---|---|---|---|
| | L/E | F/E | L/E | F/E | L/E | F/E |
| External (EXT) | 22 | 277 | 21 | 250 | 22 | 280[a] |
| Visceral (VIS) | 22 | 138 | 21 | 126 | 22 | 142[b] |
| Skeletal (SKE) | 22 | 139 | 21 | 124 | 22 | 141[a,c] |
| Technique of Wilson (WT) | 22 | 138 | 21 | 126 | 22 | 140 |
| | L/A | F/A | L/A | F/A | L/A | F/A |
| Major Malformations (Total) | 0 | 0 | 2 | 2 | 2 | 4 |
| Head | | | | | | |
| Cleft palate (WT) | 0 | 0 | 0 | 0 | 1 | 1[c] |
| Gross Exam | | | | | | |
| Craniorachischisis (EXT, confirmed by SKE and/or WT) | 0 | 0 | 0 | 0 | 1 | 2[b] |
| General | | | | | | |
| Situs inversus (VIS) | 0 | 0 | 1 | 1 | 0 | 0 |
| Kidneys | | | | | | |
| Kidney(s) absent (VIS) | 0 | 0 | 1 | 1 | 0 | 0 |
| Ureter(s) | | | | | | |
| Ureter(s) absent (VIS) | 0 | 0 | 1 | 1 | 0 | 0 |
| Limbs | | | | | | |
| Digit(s) of forepaws and hindpaws absent (ectrodactyly) (EXT, confirmed by SKE) | 0 | 0 | 0 | 0 | 1 | 2[d] |
| Digit(s) of forepaws shortened (brachydactyly) (EXT, confirmed by SKE) | 0 | 0 | 0 | 0 | 1 | 2[d] |
| Abnormal flexure of forelimb(s) (EXT) | 0 | 0 | 0 | 0 | 1 | 2[b] |
| Tail | | | | | | |
| Tail shortened (microcaudia) (EXT, confirmed by SKE) | 0 | 0 | 0 | 0 | 1 | 2[d] |

L/E = Litters examined L/A = Litters affected F/E = Fetuses examined F/A = Fetuses affected
Significantly different from control group (group 1) value: * - $P \leq 0.05$ ** - $P \leq 0.01$ (Fisher's)
a – excludes dead fetus 3514-3 b – includes fetuses 3522-1 and 3522-4 c – includes fetus 3522-4 d – includes fetuses 3501-2 and 3501-5

**Table 17. Rat combination EFD study (I): Minor external and visceral anomalies**

Group 1 - Vehicle control
Group 2 - Saxagliptin/Metformin 5/200 mg/kg/day
Group 3 - Saxagliptin/Metformin 25/200 mg/kg/day

| | Group 1 | | Group 2 | | Group 3 | |
|---|---|---|---|---|---|---|
| | L/E | F/E | L/E | F/E | L/E | F/E |
| External (EXT) | 22 | 277 | 21 | 250 | 22 | 280[a] |
| Visceral (VIS) | 22 | 138 | 21 | 126 | 22 | 142[b] |
| Skeletal (SKE) | 22 | 139 | 21 | 124 | 22 | 141[a,c] |
| Technique of Wilson (WT) | 22 | 138 | 21 | 126 | 22 | 140 |
| Minor External and Visceral Anomalies (Total) | 4 | 4 | 3 | 4 | 3 | 4 |
| Kidneys | | | | | | |
| Renal papilla(e) absent (VIS) | 0 | 0 | 0 | 0 | 1 | 2[b] |
| Liver | | | | | | |
| Supernumerary liver lobe (VIS) | 0 | 0 | 1 | 1 | 0 | 0 |
| Discoloration pale (VIS) | 0 | 0 | 0 | 0 | 1 | 1 |
| Ureter(s) | | | | | | |
| Ureter(s) dilated (megaureter) (VIS) | 4 | 4 | 2 | 3 | 2 | 2 |
| Convoluted ureter(s) (VIS) | 0 | 0 | 0 | 0 | 1 | 1 |

There was a slight increase in incomplete ossification of the parietal bones in Groups 2 and 3, but the effect was not statistically significant, as shown in the sponsor's table below.

**Table 18. Rat combination EFD study (I): Minor skeletal anomalies**

Group 1 - Vehicle control
Group 2 - Saxagliptin/Metformin 5/200 mg/kg/day
Group 3 - Saxagliptin/Metformin 25/200 mg/kg/day

| | Group 1 | | Group 2 | | Group 3 | |
|---|---|---|---|---|---|---|
| | L/E | F/E | L/E | F/E | L/E | F/E |
| External (EXT) | 22 | 277 | 21 | 250 | 22 | 280[a] |
| Visceral (VIS) | 22 | 138 | 21 | 126 | 22 | 142[b] |
| Skeletal (SKE) | 22 | 139 | 21 | 124 | 22 | 141[a,c] |
| Technique of Wilson (WT) | 22 | 138 | 21 | 126 | 22 | 140 |
| Minor Skeletal Anomalies (Total) | 17 | 42 | 16 | 51 | 16 | 42 |
| Skull | | | | | | |
| Parietal bone(s): Incomplete ossification | 1 | 1 | 5 | 6 | 4 | 4 |
| Frontal bone(s): Incomplete ossification | 0 | 0 | 1 | 1 | 0 | 0 |
| Interparietal bone: Incomplete ossification | 10 | 23 | 12 | 31 | 8 | 17 |
| Supraoccipital bone: Incomplete ossification | 0 | 0 | 2 | 2 | 0 | 0 |
| Hyoid bone: Incomplete ossification | 7 | 11 | 9 | 16 | 5 | 6 |
| Vertebral Column | | | | | | |
| Ossification center(s) on 1st lumbar vertebra or 14th thoracic vertebra | 10 | 19 | 8 | 19 | 12 | 23 |
| Ossification center(s) on 4th lumbar vertebra | 1 | 1 | 0 | 0 | 0 | 0 |
| Lumbar centrum semi-bipartite | 1 | 1 | 0 | 0 | 1 | 1 |
| Sacral vertebral centrum: Fused | 0 | 0 | 0 | 0 | 1 | 2 |
| Thoracic vertebra(e) centrum: Irregular ossification | 0 | 0 | 0 | 0 | 1 | 1 |
| Lumbar vertebral arch(es): Incomplete ossification | 0 | 0 | 0 | 0 | 1 | 1 |
| Ribs | | | | | | |
| Notched rib(s) | 0 | 0 | 2 | 2 | 1 | 1 |
| Rudimentary 14th rib(s) | 0 | 0 | 1 | 1 | 0 | 0 |
| Ossification center(s) on 7th cervical vertebra | 1 | 1 | 0 | 0 | 0 | 0 |
| Rib(s) on 7th cervical vertebra | 1 | 1 | 0 | 0 | 0 | 0 |
| Limbs | | | | | | |
| Femur incomplete ossification | 0 | 0 | 3 | 3 | 1 | 1 |

There were no treatment-related differences in findings among the common skeletal variants.

**Table 19. Rat combination EFD study (I): Common skeletal variants**

Group 1 - Vehicle control
Group 2 - Saxagliptin/Metformin 5/200 mg/kg/day
Group 3 - Saxagliptin/Metformin 25/200 mg/kg/day

| | Mean of % Affected Fetuses/Litters (SD) Group 1 | Group 2 | Group 3 |
|---|---|---|---|
| Common Skeletal Variants | | | |
| Thoracic centrum variants (unossified/incomplete/ semi-bipartite/bipartite) | 36.71 (26.42) | 33.79 (29.54) | 43.45 (24.13) |
| Sternebrae, 1st to 4th (unossified/incomplete/ semi-bipartite/bipartite) | 0.00 (0.00) | 1.36 (6.24) | 0.00 (0.00) |
| Sternebra, 5th and Xiphisternum (unossified/incomplete/ semi-bipartite/bipartite) | 19.43 (18.43) | 9.49 (17.09) | 10.41 (14.68) |

Significantly different from control group (group 1) value: * - $P \leq 0.05$ ** - $P \leq 0.01$ (Dunnett)
† - $P \leq 0.05$ †† - $P \leq 0.01$ (Dunn)

## Saxagliptin and Metformin: Oral combination study of embryofetal development in rats (II)

| | |
|---|---|
| Study no: | DN09018 / 901952 |
| Study report location: | EDR, SN000 |
| Conducting laboratory and location: | (b) (4) |
| Date of study initiation: | 27 April 2009 |
| GLP compliance: | Yes |
| QA statement: | Yes |
| Drug, lot #, and % purity: | Saxagliptin, benzoate salt, 2J66264, 67.6% free base<br>Metformin HCl with 0.5% magnesium stearate, 8E35072, 99.5% (free base content, 78%) |

### Key Study Findings

- Sprague-Dawley rats dosed with saxagliptin/metformin concentrations of 25/0, 0/600, or 25/600 mg/kg had saxagliptin exposures of ~100X, BMS-510849 of ~7X, and/or metformin exposures of ~10X MRHD (AUC basis). There were no PK interactions of the two drugs on AUC, but saxagliptin $C_{max}$ values were ~50% lower in combination dosed animals.
- Weight gain decreased relative to controls for all drug-treated groups. The effects of saxagliptin and metformin appeared to be additive.
- There were no malformations in drug-treated groups (n=29-30 litters/group).
- A slight increase in wavy ribs was seen in the combination and metformin-only treatment groups

*Reviewer comments:* This study provided adequately high exposure and adequate controls to examine possible effects of saxagliptin, metformin, and the combination on potential risk to expectant mothers taking the combination. There does not appear to be a risk of neural tube defects beyond the background incidence.

**Methods**

| | |
|---|---|
| Doses: | Saxagliptin/Metformin: 0/0, 25/0, 0/600, 25/600 mg/kg |
| Frequency of dosing: | Daily on GD6-15 |
| Dose volume: | 5 mL/kg (total) |
| Route of administration: | Oral gavage |
| Formulation/Vehicle: | Saxagliptin: suspension in 1.25% Avicel® (RC-591, FMC Biopolymer in deionized water<br>Metformin: deionized water |
| Species/Strain: | Sprague-Dawley rats (b) (4) |
| Number/Sex/Group: | 30 females/group (main study) |
| Satellite groups: | None |
| Study design: | Standard Segment 2 design (dosing GD6-15); TK performed on main study animals |
| Deviation from study protocol: | Misdosing on GD6 resulted in five saxagliptin-only animals being replaced. |

## Observations and Results

### Mortality

Animals were observed twice daily for signs of mortality, ill health, and reactions to treatment. There were no unscheduled deaths.

### Clinical Signs

Clinical signs were additionally recorded at a daily detailed examination during non-dosing periods and two detailed examinations during the dosing period (predose and 1-2h post dose). There were no treatment-related clinical signs.

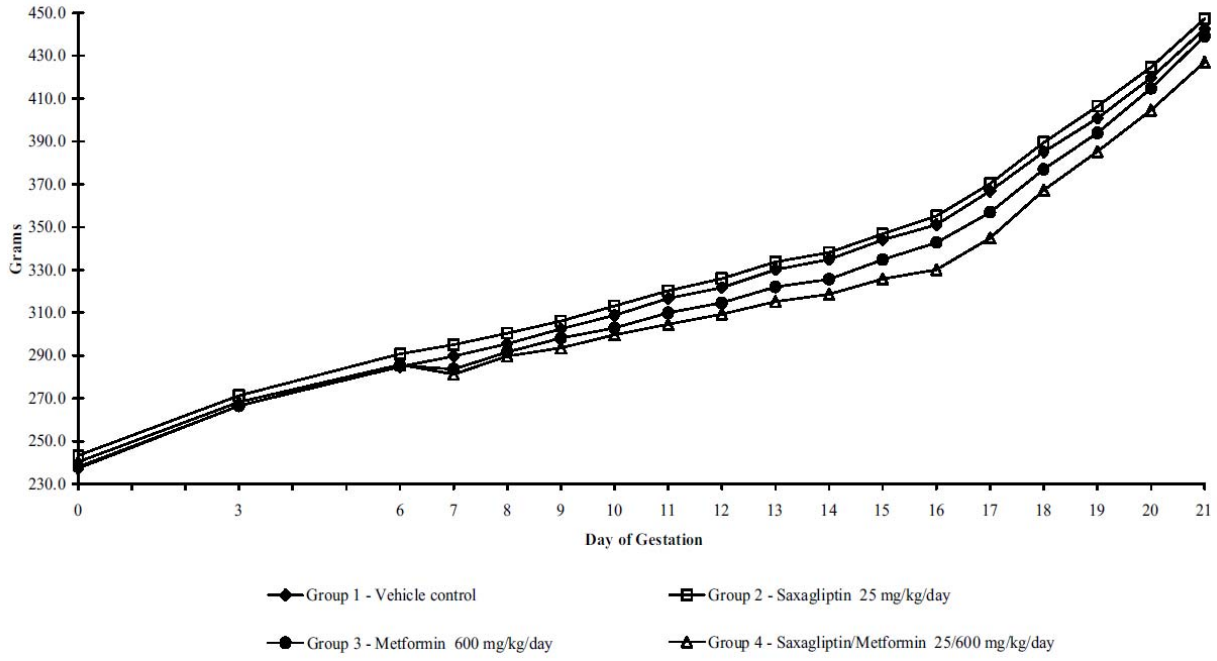
### Body Weight

Body weights were measured on GD0, 3, and 6-21. Animals dosed with saxagliptin or metformin alone had slight decreases in body weight gain, as shown in the table below. During the dosing period, the effects on body weight were slightly greater than additive in the animals receiving both drugs. Over the entire study, the effects generally appeared to be additive when comparing dosing period weight gain and gain corrected for gravid uterine weight.

**Table 20. Rat combination EFD study (II): Maternal body weight**

| Body Weight | | | | |
|---|---|---|---|---|
| Time | Saxa/Met dose | BW gain (g) | % decrement | BW %control |
| Dosing Period (GD6-16) | 0/0 | 66.2 | - | 100% |
| | 25/0 | 64.5 | ↓3% | 102% |
| | 0/600 | 57.4 | ↓13% | 100% |
| | 25/600 | 44.3* | ↓14% | 100% |
| GD6-21 | 0/0 | 157.8 | - | 100% |
| | 25/0 | 150.4 | ↓1% | 101% |
| | 0/600 | 153.8 | ↓3% | 98% |
| | 25/600 | 141.3 | ↓11% | 94% |
| GD6-21 (corrected for gravid uterus) | 0/0 | 51.1 | - | 100% |
| | 25/0 | 49.6 | ↓3% | 101% |
| | 0/600 | 43.9 | ↓14% | 99% |
| | 25/600 | 42.4* | ↓17% | 96% |

**Figure 8. Rat combination EFD study (II): Maternal body weights**




## Feed Consumption

Food consumption was measured individually for GD3-6 and daily for GD6-21.

During the first dosing day, Group 3 and 4 animals consumed significantly less food than controls. Consumption remained low in Group 4 (↓10%) animals throughout the dosing period and was slightly greater than controls (↑10%) during GD18-21 for Groups 3 and 4.

## Hematology and Clinical Pathology

Animals (first 15 per group) were sampled on GD13 for standard hematology and clinical chemistry parameters, including bicarbonate.

G4 animals had decreases in BUN (↓20%), total protein (↓5%), and albumin (↓7%) measured 2h post dose. By 24 hours post dose, these effects were unchanged, and there were additional increases in phospohorus (↑10%) and decreases in sodium (↓1%) and chloride (↓2%) that were statistically significant in Groups 3 and 4. There were no changes in bicarbonate.

## Toxicokinetics

Toxicokinetic parameters measured on GD15 in the second 15 animals per group.

**Table 21. Rat combination EFD study (II): Toxicokinetics**

| Analyte | Saxagliptin/Metformin Dose (mg/kg/day) | $C_{max}$ (ng/mL) | AUC (ng·h/mL) | Human Exposure Multiple[a,b] |
|---|---|---|---|---|
| Saxagliptin | 25/0 | 3660 | 8300 | 102x |
| | 25/600 | 1100 | 8070 | 100x |
| BMS-510849 | 25/0 | 845 | 2410 | 6x |
| | 25/600 | 453 | 3640 | 8x |
| Metformin | 0/600 | 15900 | 172000 | 8x |
| | 25/600 | 15700 | 197000 | 10x |

a - Relative to human AUC values for saxagliptin and BMS-510849 (81 and 438 ng·h/mL, respectively) associated with a therapeutic dose of 5 mg saxagliptin daily.[1]

b - Relative to human AUC values for metformin associated with therapeutic doses of 1000 mg BID or 2000 mg QD metformin (20544 or 20451 ng·h/mL, respectively).[2]

## Stability and Homogeneity

Dosing formulations were acceptable.

## Necropsy

All dams were euthanized on GD21 and examined for gross lesions and pregnancy status.

There were no treatment-related findings at necropsy. Maternal performance is shown in the sponsor's table below.

**Table 22. Rat combination EFD study (II): Maternal performance**

Group 1 - Vehicle control
Group 2 - Saxagliptin 25 mg/kg/day
Group 3 - Metformin 600 mg/kg/day
Group 4 - Saxagliptin/Metformin 25/600 mg/kg/day

| | Group 1 | 2 | 3 | 4 |
|---|---|---|---|---|
| Number of Rats Mated | 30 | 30 | 30 | 30 |
| Number of Pregnant Rats | 30 | 29 | 30 | 29 |
| Number of Rats Identified as Pregnant by Ammonium Sulfide | 0 | 0 | 0 | 0 |
| Number of Pregnancy Status not Determinable | 0 | 0 | 0 | 0 |
| Number of Rats Dying/Euthanized on Study | 0 | 0 | 0 | 0 |
| Number of Rats with Total Resorptions | 0 | 0 | 0 | 0 |
| Number of Rats Littering Prior to Scheduled Cesarean | 0 | 0 | 0 | 0 |
| Pregnancy Rate (%) | 100.0 | 96.7 | 100.0 | 96.7 |

## Cesarean Section Data (Implantation Sites, Pre- and Post-Implantation Loss, etc.)

After euthanasia, the ovaries were removed and the corpora lutea counted. The uterus was weighed (gravid and non-gravid); uterine contents were examined, including placenta. The numbers and positions of liver fetuses, dead fetuses, and resorptions were recorded.

There were no statistically significant differences in uterine and ovarian parameters as shown in the sponsor's tables below. Gravid uterus weight was ~10% lower in Group 4 animals, consistent with their lower body weights and lower food consumption during the dosing period.

**Table 23. Rat combination EFD study (II): Cesarean section data**

Group 1 - Vehicle control
Group 2 - Saxagliptin 25 mg/kg/day
Group 3 - Metformin 600 mg/kg/day
Group 4 - Saxagliptin/Metformin 25/600 mg/kg/day

| Group | Summary Information | Total Number of Corpora Lutea | Total Implantation Sites | Male Fetuses | Female Fetuses | Sex Ratio (% Males) |
|---|---|---|---|---|---|---|
| 1 | Mean | 15.4 | 14.1 | 6.2 | 7.4 | 45.24 |
| | SD | 2.2 | 2.0 | 2.5 | 2.3 | 15.43 |
| | N | 30 | 30 | 30 | 30 | 30 |
| 2 | Mean | 16.4 | 14.4 | 6.7 | 7.1 | 48.19 |
| | SD | 2.4 | 2.1 | 2.4 | 2.2 | 16.09 |
| | N | 27 | 29 | 29 | 29 | 29 |
| 3 | Mean | 15.5 | 14.2 | 7.0 | 6.7 | 51.13 |
| | SD | 1.9 | 1.6 | 2.3 | 2.3 | 14.92 |
| | N | 30 | 30 | 30 | 30 | 30 |
| 4 | Mean | 15.2 | 13.3 | 6.4 | 6.2 | 50.56 |
| | SD | 2.2 | 2.7 | 2.2 | 2.2 | 14.48 |
| | N | 29 | 29 | 29 | 29 | 29 |

Significantly different from control group (group 1) value: * - $P \leq 0.05$ ** - $P \leq 0.01$ (Dunnett)
† - $P \leq 0.05$ †† - $P \leq 0.01$ (Dunn)

| Group | Summary Information | Live Fetuses | Dead Fetuses | Early Resorptions | Middle Resorptions | Late Resorptions |
|---|---|---|---|---|---|---|
| 1 | Mean | 13.6 | 0.0 | 0.4 | 0.0 | 0.0 |
| | SD | 2.2 | 0.0 | 0.6 | 0.0 | 0.2 |
| | N | 30 | 30 | 30 | 30 | 30 |
| 2 | Mean | 13.8 | 0.0 | 0.6 | 0.1 | 0.0 |
| | SD | 2.2 | 0.0 | 0.7 | 0.3 | 0.0 |
| | N | 29 | 29 | 29 | 29 | 29 |
| 3 | Mean | 13.7 | 0.0 | 0.4 | 0.0 | 0.0 |
| | SD | 1.7 | 0.0 | 0.5 | 0.2 | 0.2 |
| | N | 30 | 30 | 30 | 30 | 30 |
| 4 | Mean | 12.6 | 0.0 | 0.8 | 0.0 | 0.0 |
| | SD | 2.7 | 0.0 | 0.9 | 0.0 | 0.0 |
| | N | 29 | 29 | 29 | 29 | 29 |

Significantly different from control group (group 1) value: * - P ≤ 0.05 ** - P ≤ 0.01 (Dunnett)
† - P ≤ 0.05 †† - P ≤ 0.01 (Dunn)

| Group | Summary Information | Sum of Resorptions | Preimplantation Loss (%) | Post implantation Loss (%) | Gravid Uterus Weight (g) |
|---|---|---|---|---|---|
| 1 | Mean | 0.5 | 7.71 | 3.55 | 106.8 |
| | SD | 0.7 | 7.49 | 5.25 | 16.5 |
| | N | 30 | 30 | 30 | 30 |
| 2 | Mean | 0.6 | 11.11 | 4.37 | 107.2 |
| | SD | 0.8 | 9.24 | 5.41 | 17.9 |
| | N | 29 | 27 | 29 | 29 |
| 3 | Mean | 0.5 | 8.27 | 3.28 | 109.9 |
| | SD | 0.5 | 6.92 | 3.62 | 13.6 |
| | N | 30 | 30 | 30 | 30 |
| 4 | Mean | 0.8 | 11.87 | 5.66 | 98.9 |
| | SD | 0.9 | 17.06 | 6.77 | 19.5 |
| | N | 29 | 29 | 29 | 29 |

Significantly different from control group (group 1) value: * - P ≤ 0.05 ** - P ≤ 0.01 (Dunnett)
† - P ≤ 0.05 †† - P ≤ 0.01 (Dunn)

## Offspring (Malformations, Variations, etc.)

Each fetus was weighed and examined externally for sex and gross alterations. Euthanized offspring were examined for visceral changes (~50%) or skeletal alterations (~50%).

Despite lower gravid uterine weights in Group 4 dams, there were no effects on average fetal weights in any group.

**Table 24. Rat combination EFD study (II): Fetal weights**

Group 1 - Vehicle control
Group 2 - Saxagliptin 25 mg/kg/day
Group 3 - Metformin 600 mg/kg/day
Group 4 - Saxagliptin/Metformin 25/600 mg/kg/day

| Group | Summary Information | Males | Females | Total |
|---|---|---|---|---|
| 1 | Mean | 5.901 | 5.652 | 5.758 |
| | SD | 0.310 | 0.255 | 0.260 |
| | N | 30 | 30 | 30 |
| 2 | Mean | 5.819 | 5.534 | 5.667 |
| | SD | 0.355 | 0.396 | 0.377 |
| | N | 29 | 29 | 29 |
| 3 | Mean | 6.025 | 5.679 | 5.855 |
| | SD | 0.316 | 0.333 | 0.321 |
| | N | 30 | 30 | 30 |
| 4 | Mean | 5.884 | 5.616 | 5.768 |
| | SD | 0.493 | 0.295 | 0.337 |
| | N | 29 | 29 | 29 |

Significantly different from control group (group 1) value: * - $P \leq 0.05$ ** - $P \leq 0.01$ (Dunnett)
† - $P \leq 0.05$ †† - $P \leq 0.01$ (Dunn)

The only malformation seen in this study was situs inversus (inverted positions of the internal organs) seen in a single vehicle control animal. There was no evidence of the craniorachischisis, cleft palate, and digital malformations observed in the previous study.

**Table 25. Rat combination EFD study (II): Major malformations**

Group 1 - Vehicle control
Group 2 - Saxagliptin 25 mg/kg/day
Group 3 - Metformin 600 mg/kg/day
Group 4 - Saxagliptin/Metformin 25/600 mg/kg/day

| | Group 1 | | 2 | | 3 | | 4 | |
|---|---|---|---|---|---|---|---|---|
| | L/E | F/E | L/E | F/E | L/E | F/E | L/E | F/E |
| External (EXT) | 30 | 409 | 29 | 401 | 30 | 412 | 29 | 364 |
| Visceral (VIS) | 30 | 203 | 29 | 200 | 30 | 207 | 29 | 184 |
| Visceral and Partial Visceral (VIS) ++ | 30 | 409 | 29 | 401 | 30 | 411 @ | 29 | 364 |
| Skeletal | 30 | 206 | 29 | 201 | 30 | 205 | 29 | 180 |
| Technique of Wilson | 30 | 203 | 29 | 200 | 30 | 207 | 29 | 184 |
| | L/A | F/A | L/A | F/A | L/A | F/A | L/A | F/A |
| Major Malformations (Total) | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 |
| General | | | | | | | | |
| Situs inversus (VIS) | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 |

There was an increase in the incidence of wavy ribs in the combination group that was statistically significant. An increase of similar magnitude was observed in the metformin-only group. Wavy ribs are typically associated with lower fetal weights or lower maternal weight gain and resolve with time. This finding was not considered adverse by the sponsor or the reviewer.

**Table 26. Rat combination EFD study (II): Minor alterations**

Group 1 - Vehicle control
Group 2 - Saxagliptin 25 mg/kg/day
Group 3 - Metformin 600 mg/kg/day
Group 4 - Saxagliptin/Metformin 25/600 mg/kg/day

| | Group 1 | | 2 | | 3 | | 4 | |
|---|---|---|---|---|---|---|---|---|
| | L/E | F/E | L/E | F/E | L/E | F/E | L/E | F/E |
| External (EXT) | 30 | 409 | 29 | 401 | 30 | 412 | 29 | 364 |
| Visceral (VIS) | 30 | 203 | 29 | 200 | 30 | 207 | 29 | 184 |
| Visceral and Partial Visceral (VIS) ++ | 30 | 409 | 29 | 401 | 30 | 411 @ | 29 | 364 |
| Skeletal | 30 | 206 | 29 | 201 | 30 | 205 | 29 | 180 |
| Technique of Wilson | 30 | 203 | 29 | 200 | 30 | 207 | 29 | 184 |
| | L/A | F/A | L/A | F/A | L/A | F/A | L/A | F/A |
| Minor External and Visceral Anomalies (Total) | 1 | 3 | 4 | 6 | 4 | 5 | 3 | 3 |
| Ureter(s) | | | | | | | | |
| Ureter(s) dilated (Moderate) (VIS) | 1 | 3 | 4 | 6 | 4 | 5 | 3 | 3 |
| Minor Skeletal Anomalies (Total) | 27 | 92 | 23 | 73 | 25 | 80 | 24 | 77 |
| Skull | | | | | | | | |
| Parietal bone(s): Incomplete ossification | 9 | 14 | 6 | 10 | 4 | 9 | 6 | 11 |
| Frontal bone(s): Incomplete ossification | 2 | 2 | 1 | 1 | 0 | 0 | 0 | 0 |
| Interparietal bone: Incomplete ossification | 18 | 50 | 13 | 31 | 18 | 49 | 15 | 43 |
| Vertebral Column | | | | | | | | |
| Ossification center(s) on 1st lumbar vertebra or 14th thoracic vertebra | 17 | 30 | 14 | 23 | 13 | 27 | 16 | 32 |
| Lumbar centrum semi-bipartite | 0 | 0 | 0 | 0 | 2 | 2 | 1 | 1 |
| Sternebrae | | | | | | | | |
| Sternebrae misaligned | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 |
| Ribs | | | | | | | | |
| Wavy | 0 | 0 | 1 | 1 | 3 | 4 | 3 | 6 ** |
| Notched | 2 | 2 | 1 | 2 | 1 | 1 | 2 | 3 |
| Rudimentary 14th rib(s) | 0 | 0 | 3 | 3 | 1 | 1 | 4 | 4 |
| Rudimentary 14th rib with contralateral ossification center | 1 | 5 | 2 | 2 | 1 | 1 | 2 | 2 |
| Extra 14th rib(s) | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 |
| Extra 14th rib(s) with contralateral rudimentary rib | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 |
| Ossification center(s) on 7th cervical vertebra | 2 | 2 | 2 | 2 | 3 | 3 | 1 | 1 |
| Rib(s) on 7th cervical vertebra | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 |
| Rib(s) incomplete ossification | 0 | 0 | 1 | 1 | 0 | 0 | 1 | 1 |
| Limb(s) | | | | | | | | |
| Femur: Incomplete ossification | 0 | 0 | 0 | 0 | 2 | 2 | 0 | 0 |

Group 1 - Vehicle control
Group 2 - Saxagliptin 25 mg/kg/day
Group 3 - Metformin 600 mg/kg/day
Group 4 - Saxagliptin/Metformin 25/600 mg/kg/day

| | Affected Fetuses/Litters Mean % (SD) Group 1 | 2 | 3 | 4 |
|---|---|---|---|---|
| Common Skeletal Variants | | | | |
| Thoracic centrum variants (unossified/incomplete/ semi-bipartite/bipartite) | 35.59 (27.24) | 38.91 (25.52) | 35.45 (25.86) | 43.74 (31.21) |
| Sternebrae 1 to 4 (unossified/incomplete/ semi-bipartite/bipartite) | 0.97 (3.74) | 0.49 (2.66) | 0.00 (0.00) | 0.58 (3.10) |
| Sternebra 5 and Xiphisternum (unossified/incomplete/ semi-bipartite/bipartite) | 20.19 (23.19) | 16.38 (17.20) | 15.65 (15.74) | 8.93 (14.10) |

Significantly different from control group (group 1) value: * - P ≤ 0.05 ** - P ≤ 0.01 (Dunnett)
†- P ≤ 0.05 ††- P ≤ 0.01 (Dunn)

## Metformin: Thirteen-day oral toxicokinetic and tolerability study in pregnant rabbits

| | |
|---|---|
| Study no: | DN09019 |
| Study report location: | EDR, SN000 |
| Conducting laboratory and location: | BMS, Drug Safety Evaluation, New Brunswick, NJ |
| Date of study initiation: | Not provided |
| GLP compliance: | No |
| QA statement: | No |
| Drug, lot #, and % purity: | Metformin, batch 8C44395, 99.4% |

### Study summary:

This non-GLP, range-finding study in pregnant rabbits (n=6/group) used doses of 0, 25, 50, 100 or 150 mg/kg (p.o.), based on previous toxicity studies. Doses were given in 2 mL/kg on GD7-19, and animals were euthanized on GD29. Toxicokinetic evaluations, standard clinical monitoring, and cesarean section data were evaluated. Evaluation of fetuses was limited to external observations.

All animals receiving 100 or 150 mg/kg were either found dead or euthanized for humane reasons following low food intake, weight loss and clinical signs including “absent feces, tremors and convulsions, oral or vulva discharge, ataxia, loss of righting reflex, vocalization, decreased activity and labored respiration.” There were no effects on weight gain, food consumption, or clinical signs in the 25 and 50 mg/kg groups, which were the only groups surviving to Cesarean section. There were no treatment-related differences in reproductive endpoints. No malformations or variations were noted in any fetuses examined. TK parameters for the 25 and 50 mg/kg groups are shown in the sponsor's table below. AUC values for these two doses were 0.6X and 1.1X MRHD for metformin.

**Table 27. Metformin TK and tolerability study in pregnant rabbits: Toxicokinetics**

| Parameter | Metformin (BMS-207150) Dose (mg/kg/day) | |
|---|---|---|
| | 25 | 50 |
| Cmax (ng/mL) | 2220 | 3490 |
| AUC(0-4h) (ng•h/mL) | 12300 | 23800 |
| Tmax (h) | 1.0 | 1.0 |

## Saxagliptin and Metformin: Oral combination study of embryofetal development in rabbits

| | |
|---|---|
| Study no: | DN09020 |
| Study report location: | EDR, SN000 |
| Conducting laboratory and location: | Bristol-Myers Squibb, Drug Safety Evaluation, New Brunswick, NJ |
| Date of study initiation: | 21 May 2009 |
| GLP compliance: | Yes |
| QA statement: | Yes |
| Drug, lot #, and % purity: | Saxagliptin, 4K85994, 93.1%<br>Metformin HCl with 0.5% magnesium stearate, 8C44395, 99.4% (free base content, 78%) |

### Key Study Findings

- New Zealand white rabbits given saxagliptin/metformin concentrations of 0/50, 40/0, or 40/50 mg/kg had exposures to saxagliptin ≥ 200X MRHD, to BMS-510849 of ≥ 150X MRHD, and to metformin of ~1X MRHD (AUC basis).
- High mortality in the combination group (12/30 animals) was consistent with metformin-related lactic acidosis. There was only one animal found dead in the metformin-only group.
- Excluding early decedents in the combination group, weight gains were similar to controls. Early decedents tended to lose weight prior to death.
- Offspring of Group 4 weighed 7% less than controls on average.
- One Group 4 fetus had signs of neural tube defect (gastroschisis and exencephaly), but the incidence was within the historical control range.
- The saxagliptin-only group (Group 3) had 7 animals with missing gallbladder and five with small gallbladders.
- Increased incidence of incomplete ossification of the fetal hyoid was noted in combination treated animals.

*Reviewer's comments*: Both gallbladder findings (absent and small) observed in the saxagliptin-only group were outside the historical control range and were observed in no more than 1 fetus in the other groups. In the saxagliptin monotherapy NDA, the rabbit EFD study had 4/165 fetuses with absent gallbladder (200 mg/kg) vs. 1/177 in controls. This result from the monotherapy study was not statistically significant, all incidences were in the 200 mg/kg group (not seen at 40 mg/kg); however, exposures at 40 mg/kg are 40-50% higher for saxagliptin and its major metabolite in this study. It seems reasonable to conclude that small/absent gallbladder is a consequence of very high exposures to saxagliptin alone. The increase in incomplete ossification of the hyoid is likely related to lower body weights in the fetuses in this group and is not considered adverse. Due to the mortality observed in the combination dose group, there was no maternal NOAEL for the combination, but a sufficient number of animals (17) were evaluated for fetal developmental effects. The observed lack of developmental effects in the combination dose group suggests a low risk to the developing fetus.

Methods

| | |
|---|---|
| Doses: | Saxagliptin/Metformin: 0/0, 0/50, 40/0, 40/50 mg/kg |
| Frequency of dosing: | Daily on GD6-15 |
| Dose volume: | 6 mL/kg (total) |
| Route of administration: | Oral gavage |
| Formulation/Vehicle: | Saxagliptin: HCl-acidified reagent grade water (pH 5 ± 0.5)<br>Metformin: reagent grade water |
| Species/Strain: | New Zealand white rabbits, time-mated (b) (4) ) |
| Number/Sex/Group: | 30 females/group (main study) |
| Satellite groups: | 5/group (for TK on GD19 and clinical pathology) |
| Study design: | Standard Segment 2 design (dosing GD7-19) |
| Deviation from study protocol: | |

## Observations and Results

## Mortality

Animals were observed at least once daily for signs of mortality, ill health, and reactions to treatment.

There was high mortality in dams given both saxagliptin and metformin (12/30), one of which was euthanized after abortion (GD25) as shown in the sponsor's table below. There were no deaths in the saxagliptin-only group and only one in the metformin-only group.

**Table 28. Rabbit combination EFD study: Mortality**

| **Metformin Dose (mg/kg/day):** | **0** | **50** | **0** | **50** |
|---|---|---|---|---|
| **Saxagliptin Dose (mg/kg/day):** | **0** | **0** | **40** | **40** |
| Found Dead | - | 1 (GD15) | - | 8 (GD15 to 23) |
| Euthanatized Moribund | - | - | - | 3 (GD 22 to 24) |
| Euthanatized after Abortion | - | - | - | 1 (GD 25) |
| Euthanatized due to Injury | 1 (GD 20) | - | - | - |

A dash (-) indicates absence of finding in group
Data are expressed as the total number of occurrences/group (number of animals affected)

## Clinical Signs

Clinical signs were additionally recorded at a daily detailed examination at ~2h post dose during the dosing period.

Early decedent animals in the combination dose group showed clinical signs including perianal fur soiling and changes to the feces along with decreased body weight and inappetance. Less frequent signs in this group included ataxia, decreased activity, labored respiration, and salivation. Survivors in this group showed only reduced/unformed feces. Other than the single dam found dead in the metformin-only group, there were no treatment-related clinical signs in dams given a single drug.

## Body Weight

Body weights were measured daily.

There were not treatment-related changes in body weight gain in dams surviving to scheduled euthaniasia; however, combination-dosed dams that did not survive lost weight prior to death.

**Table 29. Rabbit combination EFD study: Maternal body weights**

| Body Weight | | | | |
|---|---|---|---|---|
| Time | Saxa/Met dose | BW gain (kg) | % decrement | BW %control |
| Dosing Period (All animals) | 0/0 | 0.17 | - | 100% |
| | 0/50 | 0.12 | ↓29% | 98% |
| | 40/0 | 0.16 | ↓6% | 100% |
| | 40/50 | 0.04** | ↓76% | 97% |
| Dosing Period (Survivors to GD29) | 0/0 | 0.17 | - | 100% |
| | 0/50 | 0.13 | ↓23% | 97% |
| | 40/0 | 0.13 | ↓21% | 99% |
| | 40/50 | 0.17 | - | 99% |

## Feed Consumption

Food consumption was measured individually on a daily basis.

Food consumption was reduced by 27% in the combination group over the dosing period. While this was mostly due to inappetance among early decedents, there was a 10% decrease in food consumption among survivors in this group. There were no treatment-related effects in the groups given a single drug.

## Hematology and Clinical Pathology

Animals (satellite groups) were sampled on GD19 for standard hematology and clinical chemistry parameters, including bicarbonate. Two animals in the combination dose group had increases in triglycerides (2-6X control means), blood urea nitrogen (6-15X), creatinine (3-7X), and phosphorus (2-5X) and decreases in calcium (↓19-27%) and bicarbonate (↓32-55%). In addition, one of the two had increases in serum glucose (~4X), cholesterol (~7X), potassium (↑50%), and globulin (↑30%).

## Toxicokinetics

Toxicokinetic parameters were measured on GD19 in satellite animals only. The results are shown in the sponsor's table below. Exposures to the individual components did not appear to be significantly affected by coadministration of both drugs, so the deaths in the combination group can not be easily attributed to increased drug exposure.

**Table 30. Rabbit combination EFD study: Toxicokinetics**

| Analytes | Saxagliptin/Metformin Dose (mg/kg/day) | Cmax (ng/mL) | AUC (ng·h/mL) | Human Exposure Multiple[a,b] |
|---|---|---|---|---|
| Saxagliptin | 40/0 | 8,530 | 17,400 | 215× |
| | 40/50 | 8,490 | 20,200 | 249× |
| BMS-510849 | 40/0 | 24,900 | 69,700 | 159× |
| | 40/50 | 27,400 | 76,500 | 175× |
| Metformin | 0/50 | 4,240 | 28,600 | 1.4× |
| | 40/50 | 3,210 | 22,000 | 1.1× |

a - Relative to human AUC values for saxagliptin (81 ng·h/mL) and BMS-510849 (438 ng·h/mL) associated with a therapeutic dose of 5 mg daily.[1]

b - Relative to human AUC values for metformin associated with therapeutic doses of 1000 mg BID or 2000 mg QD (20,544 or 20,451 ng·h/mL, respectively).[2]

## Stability and Homogeneity

Dosing formulations were acceptable.

## Necropsy

All surviving dams were euthanized on GD29 and examined for gross lesions and pregnancy status. Early decedents (including euthanasia due to abortion or poor condition) were similarly examined (to the extent possible). Five of 30 combination-dosed dams (including 4 early decedents) had enlarged gallbladders, consistent with decreased food consumption. There were no other findings that appeared to be related to treatment.

## Cesarean Section Data (Implantation Sites, Pre- and Post-Implantation Loss, etc.)

After euthanasia, the ovaries were removed and the corpora lutea counted. The uterus was weighed (gravid and non-gravid); uterine contents were examined, including placenta. The numbers and positions of liver fetuses, dead fetuses, and resorptions were recorded. Similar observations were made for early decedents to the extent possible.

There were no treatment-related differences in C-section parameters, as shown in the sponsor's table below.

**Table 31. Rabbit combination EFD study: C-section data**

| GROUP<br>TREATMENT<br>DAILY DOSE (mg/kg/day) | | 1<br>Saxa/Metfn<br>0/0 | 2<br>Saxa/Metfn<br>0/50 | 3<br>Saxa/Metfn<br>40/0 | 4<br>Saxa/Metfn<br>40/50 |
|---|---|---|---|---|---|
| PREGNANT REPRO RABBITS SURVIVING TO DAY 29 CESAREAN-SECTIONING | N | 28 | 24 | 30 | 17 |
| CORPORA LUTEA | MEAN | 9.4 | 8.4 | 8.8 | 9.2 |
| | SD | 1.6 | 1.4 | 1.5 | 1.5 |
| IMPLANTATIONS | MEAN | 9.0 | 7.8* | 8.2 | 9.0 |
| | SD | 1.6 | 1.7 | 1.7 | 1.3 |
| PREIMPLANTATION LOSS | MEAN% | 3.6 | 6.6 | 6.3 | 1.6 |
| | SD | 6.9 | 11.7 | 8.9 | 3.6 |
| POSTIMPLANTATION LOSS | MEAN% | 5.2 | 6.1 | 5.1 | 8.6 |
| | SD | 9.6 | 7.7 | 8.0 | 10.3 |
| LITTER SIZE (LIVE+DEAD) | MEAN | 8.6 | 7.3 | 7.8 | 8.2 |
| | SD | 1.9 | 1.6 | 1.7 | 1.2 |
| LIVE FETUSES | MEAN | 8.6 | 7.3 | 7.8 | 8.2 |
| | SD | 1.9 | 1.6 | 1.7 | 1.2 |
| DEAD FETUSES | N | 0 | 0 | 0 | 0 |
| RESORPTIONS (EARLY+LATE) | N | 12 | 12 | 12 | 14 |
| | MEAN | 0.4 | 0.5 | 0.4 | 0.8 |
| | SD | 0.8 | 0.7 | 0.6 | 1.0 |
| EARLY RESORPTIONS | N | 11 | 11 | 9 | 12 |
| | MEAN | 0.4 | 0.5 | 0.3 | 0.7 |
| | SD | 0.7 | 0.6 | 0.6 | 0.9 |
| LATE RESORPTIONS | N | 1 | 1 | 3 | 2 |
| DOES WITH ANY RESORPTIONS | N(%) | 7(25.0) | 10(41.7) | 10(33.3) | 8(47.1) |
| DOES WITH NO VIABLE CONCEPTUSES | N(%) | 0(0.0) | 0(0.0) | 0(0.0) | 0(0.0) |

Preimplantation loss calculated as: [(Corpora lutea - implantations)/corpora lutea] x 100.
Postimplantation loss calculated as: [(Dead + resorbed conceptuses)/implantations] x 100.

## Offspring (Malformations, Variations, etc.)

Each fetus was weighed (live fetuses only) and examined externally for sex and gross alterations. Euthanized offspring were examined for both visceral and skeletal alterations. Offspring of Group 4 weighed 7% less than controls on average.

**Table 32. Rabbit combination EFD study: Fetal parameters**

| GROUP<br>TREATMENT<br>DAILY DOSE (mg/kg/day) | | 1<br>Saxa/Metfn<br>0/0 | 2<br>Saxa/Metfn<br>0/50 | 3<br>Saxa/Metfn<br>40/0 | 4<br>Saxa/Metfn<br>40/50 |
|---|---|---|---|---|---|
| LITTERS WITH ONE OR MORE LIVE FETUSES ON DAY 29 OF GESTATION | N | 28 | 24 | 30 | 17 |
| LIVE FETUSES | N | 241 | 176 | 235 | 139 |
| | MEAN | 8.6 | 7.3 | 7.8 | 8.2 |
| | SD | 1.9 | 1.6 | 1.7 | 1.2 |
| LIVE MALE FETUSES | N | 126 | 98 | 130 | 76 |
| % LIVE MALE FETUSES PER LITTER | MEAN% | 52.2 | 56.4 | 55.5 | 55.0 |
| | SD | 19.8 | 18.5 | 20.1 | 16.1 |
| MEAN FETAL BODY WEIGHT(Grams) PER LITTER | MEAN | 43.66 | 45.01 | 43.67 | 40.76* |
| | SD | 3.30 | 3.37 | 3.92 | 3.99 |
| MALE FETUSES | MEAN | 44.30 | 45.49 | 44.64 | 42.03 |
| | SD | 3.53 | 3.09 | 4.37 | 5.16 |
| FEMALE FETUSES | MEAN | 43.44 | 44.55[a] | 42.78[a] | 39.80* |
| | SD | 3.80 | 4.19 | 4.14 | 3.66 |
| % DEAD OR RESORBED CONCEPTUSES PER LITTER | MEAN% | 5.2 | 6.1 | 5.1 | 8.6 |
| | SD | 9.6 | 7.7 | 8.0 | 10.3 |

a -Excludes litters 2201 and 3216 which contained all male fetuses.

Statistical Analysis: Analysis of Variance with Dunnett's procedure was used for continuous data.
Kruskal-Wallis test with Dunn's procedure used for enumeration data.

* Significantly different from the control at P≤ 0.05.

** Significantly different from the control at P≤ 0.01.

The number of fetuses with any alteration was slightly higher in saxagliptin-only animals than in controls, as shown in the sponsor's table below. There were eight fetuses with malformations in this group, compared to one in each of the vehicle control and combination dosed groups.

**Table 33. Rabbit combination EFD study: Fetal alterations**

| GROUP<br>TREATMENT<br>DAILY DOSE (mg/kg/day) | | 1<br>Saxa/Metfn<br>0/0 | 2<br>Saxa/Metfn<br>0/50 | 3<br>Saxa/Metfn<br>40/0 | 4<br>Saxa/Metfn<br>40/50 |
|---|---|---|---|---|---|
| FETUSES EVALUATED | N | 241 | 176 | 235 | 139 |
| Live Fetuses | N | 241 | 176 | 235 | 139 |
| Dead Fetuses | N | 0 | 0 | 0 | 0 |
| LITTERS EVALUATED | N | 28 | 24 | 30 | 17 |
| ALTERATIONS (Malformations + Variations) | | | | | |
| Fetuses with any Alterations | N(%) | 28(11.6) | 13(7.4) | 42(17.9) | 24(17.3) |
| Litter with Fetuses with any Alterations | N(%) | 17(60.7) | 10(41.7) | 21(70.0) | 13(76.5) |
| Percent Fetuses per Litter with Alterations | MEAN | 12.86 | 7.18 | 18.53 | 17.54 |
| | SD | 14.52 | 10.51 | 17.30 | 14.83 |
| | N | 28 | 24 | 30 | 17 |
| VARIATIONS | | | | | |
| Fetuses with any Variations | N(%) | 27(11.2) | 11(6.3) | 36(15.3) | 24(17.3) |
| Litter with Fetuses with any Variations | N(%) | 17(60.7) | 9(37.5) | 20(66.7) | 13(76.5) |
| Percent Fetuses per Litter with Variations | MEAN | 12.35 | 6.20 | 15.67 | 17.54 |
| | SD | 14.20 | 10.06 | 14.75 | 14.83 |
| | N | 28 | 24 | 30 | 17 |
| MALFORMATIONS | | | | | |
| Fetuses with any Malformation | N(%) | 1(0.4) | 2(1.1) | 8(3.4)* | 1(0.7) |
| Litter with Fetuses with any Malformation | N(%) | 1(3.6) | 2(8.3) | 4(13.3) | 1(5.9) |
| Percent Fetuses per Litter with Malformation | MEAN | 0.51 | 0.98 | 4.00 | 0.74 |
| | SD | 2.70 | 3.34 | 10.81 | 3.03 |
| | N | 28 | 24 | 30 | 17 |

The one fetus with malformations in Group 4 exhibited gastroschisis, exencephaly, cutis aplasia, short and flexed limbs, adactyly, and small open eyes. The two most concerning findings (regarding neural tube defects) were near or within the historical control range. When live litters (n=9) from the early

necropsy dams are included (all of which were without gross malformations), the incidence is well below the historical control range, as shown in the table below.

**Table 34.Rabbit combination EFD study: Incidence of NTD-like effects**

| Finding | Current Study (scheduled necropsy) %F/L | Current study (scheduled & live litters from early necropsy) % F/L | Historical % F/L |
|---|---|---|---|
| Gastroschisis | 0.7% / 5.9% | 0.4% / 3.8% | 0.7% / 5.6% |
| Exencephaly | 0.7% / 5.9% | 0.4% / 3.8% | 0.7% / 5.3% |
| Adactyly | 0.7% / 5.9% | 0.4% / 3.8% | 0.7% / 5.6% |

The saxaglipin-only group (Group 3) had 7 animals with missing gallbladder and five with small gallbladders. Both findings were outside the historical control range and were observed in no more than 1 fetus in the other groups.

**Table 35. Rabbit combination EFD study: Incidence of small/absent gallbladder**

| Finding | Group 1 | Group 2 | Group 3 | Group 4 | Historical Control |
|---|---|---|---|---|---|
| Dose (saxa/met) | 0/0 | 0/50 | 40/0 | 40/50 | |
| Absent gallbladder | 0/0 | 0.6/4.2 | 3.0*/13.3 | 0.7/5.9 | 0.6/5.0 |
| Small gallbladder | 0.4/3.6 | 0/0 | 1.7/13.3 | 0/0 | 0.5/4.3 |

Results given as %F/%L

The only other variations that appeared to occur more often in combination treated offspring was an increase in the incidence of incomplete ossification of the hyoid.

**Table 36. Rabbit combination EFD study: Skeletal variations**

| GROUP | | 1 | 2 | 3 | 4 |
|---|---|---|---|---|---|
| TREATMENT | | Saxa/Metfn | Saxa/Metfn | Saxa/Metfn | Saxa/Metfn |
| DAILY DOSE (mg/kg/day) | | 0/0 | 0/50 | 40/0 | 40/50 |
| FETUSES EVALUATED | N | 241 | 176 | 235 | 139 |
| LIVE FETUSES | N | 241 | 176 | 235 | 139 |
| DEAD FETUSES | N | 0 | 0 | 0 | 0 |
| LITTER EVALUATED | N | 28 | 24 | 30 | 17 |
| Frontal , Supernumerary bone ( V ) | | | | | |
| Fetal Incidence | N(%) | 1(0.4) | 1(0.6) | 1(0.4) | 0(-) |
| Litter Incidence | N(%) | 1(3.6) | 1(4.2) | 1(3.3) | 0(-) |
| Hyoid , Incomplete ossification ( V ) | | | | | |
| Fetal Incidence | N(%) | 3(1.2) | 1(0.6) | 9(3.8) | 8(5.8)* |
| Litter Incidence | N(%) | 2(7.1) | 1(4.2) | 6(20.0) | 5(29.4) |

# 11 Integrated Summary and Safety Evaluation

The sponsor has submitted studies using dosing with metformin and saxagliptin including a 3-month study in dogs for general toxicity and embryofetal development studies in rats and rabbits. These studies were designed to address concerns that the combination of saxagliptin and metformin could cause unforeseen toxicities due to overlapping PD effects (addressed by the 3 month dog study) or could cause developmental abnormalities in fetuses of dams given the combination, based on a combination study performed under the Onglyza® NDA. None of the studies identified a unique or emergent toxicity as a result of co-administration of the two drugs.

The three month dog study used doses that provided exposures to saxagliptin of up to ~70X MRHD (AUC basis), to BMS-510849 of ~16X MRHD, and to metformin of ~1.5X MRHD. These dosing levels were considered adequate by the Division at the pre-NDA meeting. Tremor and shivering were observed more often in the combination treated group, but this did not correlate with post-dose hypoglycemia when measured on Day 2 or 3 of dosing. There were no effects specific to the combination on respiratory or ECG parameters, but the reviewer notes that there was no vehicle control group for comparison. Since the safety of both drugs individually has been previously established and the combination of the two drugs did not cause any unique adverse effects, this study was adequate to support the safety of combination dosing.

Embryofetal development (EFD) studies using the combination were considered crucial for estimating the safety of combination dosing. The original combination study used saxagliptin/metformin doses of 0/0, 5/200, and 25/200 mg/kg/day. In the 25/200 group, two fetuses (from the same litter) displayed craniorachischisis, a rare neural tube defect (NTD). At the time of the study, the litter and fetal incidence were outside the historical control range values for the laboratory. To address this issue, the sponsor used a two-pronged approach. They updated the historical control database, including dose range finding studies, and repeated the study using more animals (n=30) and higher doses of metformin (600 mg/kg), along with appropriate controls (a vehicle control and groups with saxagliptin (25 mg/kg) or metformin alone). The new historical control data showed four instances of craniorachischisis in control animals, bringing the finding in the first EFD study to within the historical control range.

The follow-up rat EFD study had verified exposures to saxagliptin of ~100X MRHD, to BMS-510849 of ~7X MRHD, and to metformin of ~10X MRHD (AUC basis). There were additive decreases in weight gain observed in the drug-treated groups, but there were no malformations observed (other than in one control fetus). The only finding in this study that appeared to be related to treatment was a slight increase in wavy ribs observed in the combination group (a statistically significant increase in fetal incidence). Because wavy ribs are commonly observed in the offspring of dams with reduced body weight gain and because wavy ribs typically resolve during post-natal development in the rat, this finding was not considered an adverse effect. The reviewer does not consider the combination to be teratogenic in rats.

A combination EFD study in rabbits was also requested by the Division as a follow-up to the original craniorachischisis finding in the rat. This study used doses providing ≥ 200X MRHD for saxagliptin and/or ~1X MRHD (AUC basis) for metformin (due to mortality at 2X MRHD). There was high mortality (12/30 dams) in the combination group, but sufficient numbers of dams survived to provide an adequate assessment of fetal abnormalities. Satellite animals evaluated for toxicokinetics and clinical chemistry showed sporadic low levels for bicarbonate, suggesting lactic acidosis may have contributed to some of the deaths; however, metformin exposure in the combination and monotherapy group appears similar despite the imbalance in deaths. There were no malformations in the nine early decedents with live litters at necropsy. One surviving dam in the combination dose group had a single fetus with malformations, including exencephaly, which is an NTD. This incidence was within the historical control range, however, and was considered incidental (not related to treatment) by both the sponsor and the reviewer. The saxagliptin-only group in this study had a significant increase of offspring with small or absent gallbladder. Although this finding could reasonably be attributed to treatment (especially given a similar but not statistically significant finding in the monotherapy EFD study), any risk to a developing fetus is very low given the high multiples of exposure involved. This finding was not observed in the combination group.

Overall, the data submitted with this NDA supports the safety of the fixed dose combination of saxagliptin and metformin. This NDA is approvable from a pharmacology/toxicology perspective.

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/s/

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LAUREN M Mihalcik
09/22/2010

TODD M BOURCIER
09/22/2010
I concur

# PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA/BLA or Supplement

**NDA/BLA Number: 200678** **Applicant: Bristol-Myers Squibb** **Stamp Date: 29 Dec 2009**

**Drug Name:** (b) (4) **NDA/BLA Type: 505(b)(1)**

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

| | Content Parameter | Yes | No | Comment |
|---|---|---|---|---|
| 1 | Is the pharmacology/toxicology section organized in accord with current regulations and guidelines for format and content in a manner to allow substantive review to begin? | x | | |
| 2 | Is the pharmacology/toxicology section indexed and paginated in a manner allowing substantive review to begin? | x | | All pdf files are viewable and appropriately bookmarked. |
| 3 | Is the pharmacology/toxicology section legible so that substantive review can begin? | x | | All pdf files for nonclinical studies are created rather than scanned! |
| 4 | Are all required (*) and requested IND studies (in accord with 505 b1 and b2 including referenced literature) completed and submitted (carcinogenicity, mutagenicity, teratogenicity, effects on fertility, juvenile studies, acute and repeat dose adult animal studies, animal ADME studies, safety pharmacology, etc)? | x | | The sponsor has submitted a nonclinical package consistent with our recommendations at the pre-NDA meeting. |
| 5 | If the formulation to be marketed is different from the formulation used in the toxicology studies, have studies by the appropriate route been conducted with appropriate formulations? (For other than the oral route, some studies may be by routes different from the clinical route intentionally and by desire of the FDA). | x | | The to-be-marketed formulation includes extended-release metformin, but bolus oral gavage of metformin was used for nonclinical studies. Use of extended-release formulations in nonclinical studies was not required for the Glucophage XR NDA and are not necessary for this application since the toxicities of metformin are well-characterized. |
| 6 | Does the route of administration used in the animal studies appear to be the same as the intended human exposure route? If not, has the applicant submitted a rationale to justify the alternative route? | x | | Oral dosing was used for nonclinical studies and is the intended route of human exposure. |
| 7 | Has the applicant submitted a statement(s) that all of the pivotal pharm/tox studies have been performed in accordance with the GLP regulations (21 CFR 58) or an explanation for any significant deviations? | x | | |
| 8 | Has the applicant submitted all special studies/data requested by the Division during pre-submission discussions? | x | | Consistent with the pre-NDA meeting minutes, they have submitted a 3m dog study and Seg2 studies in rats and rabbits using saxagliptin and metformin. The |

# PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA/BLA or Supplement

| | Content Parameter | Yes | No | Comment |
|---|---|---|---|---|
| | | | | requested Seg2 studies with saxa only and met only (rats and rabbits) were also included, along with updated historical control data. |
| 9 | Are the proposed labeling sections relative to pharmacology/toxicology appropriate (including human dose multiples expressed in either mg/m2 or comparative serum/plasma levels) and in accordance with 201.57? | x | | |
| 10 | Have any impurity – etc. issues been addressed? (New toxicity studies may not be needed.) | x | | There appears to be a new impurity (b) (4) [redacted] CMC states their specs are below qualification threshold. |
| 11 | Has the applicant addressed any abuse potential issues in the submission? | n/a | | Abuse of either component is not expected. |
| 12 | If this NDA/BLA is to support a Rx to OTC switch, have all relevant studies been submitted? | n/a | | |

**IS THE PHARMACOLOGY/TOXICOLOGY SECTION OF THE APPLICATION FILEABLE? ___yes_____**

If the NDA/BLA is not fileable from the pharmacology/toxicology perspective, state the reasons and provide comments to be sent to the Applicant.
n/a

Please identify and list any potential review issues to be forwarded to the Applicant for the 74-day letter.
n/a

Additional Internal Comments:
The embryofetal toxicity studies in rats and rabbits (BMS #DN09018 & #DN09020) included in NDA 200678 are also intended to satisfy the nonclinical post-marketing requirements described in NDA 22-350 for Onlyza (saxagliptin) monotherapy. By accepting NDA 200678 for filing, pharm/tox also implicitly agrees that these two embryofetal toxicity studies satisfy the 31 July 2010 Final Protocol Submission deadline for PMRs #1493-2 & 1493-3 in NDA 22-350.

Reviewing Pharmacologist Date

Team Leader/Supervisor Date

| Application Type/Number | Submission Type/Number | Submitter Name | Product Name |
|---|---|---|---|
| NDA-200678 | ORIG-1 | BRISTOL MYERS SQUIBB | (b) (4) (saxagliptin + metformin XR) Tablets |

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/s/

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LAUREN M Mihalcik
02/16/2010

TODD M BOURCIER
02/16/2010
Concur; filing acceptable for pharm/tox