

2.6.5 PHARMACOKINETICS TABULATED SUMMARY

Pharmacokinetics: Absorption After a Single Dose (Saxagliptin, Rat)

Study Description or Title: Pharmacokinetics of Saxagliptin and BMS-510849 in Sprague-Dawley Rats following Single Dose Intravenous or Oral Administration of Saxagliptin

Test Article: Saxagliptin  
 Study Type: Non-GLP  
 Location in Dossier:

Study No./Document Control Number: MAP005-477118/930000866

Species/Strain:	Rat/Sprague-Dawley			
Gender (M/F) / Number of Animals:	Male / 2 per treatment		Male / 2 per treatment	
Feeding condition:	Fasted overnight and fed 4 h after dosing		Fasted overnight and fed 4 h after dosing	
Vehicle/Formulation:	Water		Water	
Method of Administration:	IV Bolus		Oral gavage	
Sample (Whole blood, plasma, serum etc.):	Plasma		Plasma	
Analytes:	Saxagliptin and BMS-510849		Saxagliptin and BMS-510849	
Assay:	LC/MS/MS		LC/MS/MS	
Saxagliptin Dose (mg/kg):	10		8	
Route:	IV		PO	
Parameter	Saxagliptin	BMS-510849 <sup>a</sup>	Saxagliptin	BMS-510849 <sup>a</sup>
C <sub>max</sub> (µg/mL)	5.2	0.2	0.5	0.2
AUC(0-infinity) (µg·h/mL)	1.6	0.3	0.9	0.4
T <sub>max</sub> (h)	-	0.3	0.7	1.1
CL <sub>TP</sub> (mL/min/kg)	115	ND	-	-
V <sub>ss</sub> (L/kg)	5.2	ND	-	-
T <sub>1/2</sub> (h)	2.1	ND	-	-
% dose excreted unchanged in urine (0-10 h)	33	-	ND	-
Bioavailability (%)	-	-	75	-

Additional Information: ND = not determined.

Saxagliptin was well, rapidly absorbed, and rapidly cleared in rats. Saxagliptin was excreted in urine as parent. The V<sub>ss</sub> value indicated extravascular distribution of saxagliptin.

<sup>a</sup> The bioanalytical assay that was used to measure concentrations of BMS-510849 in this study may not have been completely specific for BMS-510849. Other mono-hydroxylated metabolites with the same MRM transition as BMS-510849 in the LC/MS/MS (332 → 196) may have co-eluted with BMS-510849 under the conditions employed in the assay. Therefore the reported concentration values for BMS-510849 may include BMS-510849 and other mono-hydroxylated metabolites. The measurement of saxagliptin was not impacted in this method.

**Pharmacokinetics: Absorption After a Single Dose (BMS-510849, Rat)**

Study Description or Title: Pharmacokinetics of BMS-510849 in Sprague-Dawley Rats following Single Dose Intravenous, Subcutaneous, and Oral Administration of BMS-510849

	Test Article: Study Type: Location in Dossier:		BMS- 510849 Non-GLP	
	Study No./Document Control Number:		TSU3725/930023244	
Species/Strain:	Rat/Harlan Sprague-Dawley			
Gender (M/F) / Number of Animals:	Male / 3 per dose	Male / 3 per dose	Male/ 3 per dose <sup>a</sup>	
Feeding condition:	Fasted overnight and fed 4 h after dosing	Fasted overnight and fed 4 h after dosing	Fasted overnight and fed 4 h after dosing	
Vehicle/Formulation:	10 mM citrate buffer, pH 4/solution	10 mM citrate buffer, pH 4/solution	0.5% Methocel A4M (citrate buffered, pH 4)/suspension	
Method of Administration:	IV Bolus	Subcutaneous	Oral gavage	
Sample (Whole blood, plasma, serum etc.):	Plasma	Plasma	Plasma	
Analyte:	BMS-510849	BMS- 510849	BMS- 510849	
Assay:	LC/MS/MS	LC/MS/MS	LC/MS/MS	
BMS-510849 Dose (mg/kg):	75	150	300	600 1200
Route:	<u>IV</u>	<u>SC</u>	<u>PO</u>	
Parameter				
Cmax (µg/mL)	298 ± 26.1	84.5 ± 14.8	136 ± 19.2	2.00, 6.30 6.14, 5.91
AUC(0-infinity) (µg <sup>h</sup> /mL)	52.9 ± 7.38	137 ± 42.3	274 ± 15.6	9.74, 17.9 29.5, 28.1
Tmax (h)	0.0333 ± 0.00	0.583 ± 0.144	0.583 ± 0.144	1.00, 1.00 0.500, 1.00
CLTp (L/h/kg)	1.43 ± 0.186	-	-	-
Vss (L/kg)	0.920 ± 0.203	-	-	-
T1/2 (h)	11.4 ± 2.21	-	-	-
Bioavailability (%)	-	129	129	2.30, 4.23 3.49, 3.32

Additional Information: BMS-510849 was poorly absorbed in rats following oral administration. However, the absorption was approximately complete following subcutaneous administration.

<sup>a</sup> Individual animal values of pharmacokinetic parameters are listed instead of group mean ± SD values; pharmacokinetic parameters were only determined in 2 of 3 rats in the oral dosing groups because incomplete concentration versus time profiles were obtained from 1 rat/group.

**Pharmacokinetics: Absorption After a Single Dose (Saxagliptin, Dog)**

Study Description or Title: Pharmacokinetics of Saxagliptin in Beagle Dogs following a Single Dose Intravenous or Oral Administration of Saxagliptin

	Test Article: Study Type: Location in Dossier:		Saxagliptin Non-GLP	
	Study No./Document Control Number:		MAP005-477118/930000866	
Species/Strain:	Dog/Beagle			
Gender (M/F) / Number of Animals:	Male / 2 per treatment	Male / 2 per treatment		
Feeding condition:	Fasted overnight and fed 4 h after dosing	Fasted overnight and fed 4 h after dosing		
Vehicle/Formulation:	Water	Water		
Method of Administration:	IV infusion (10 min)	Oral gavage		
Sample (Whole blood, plasma, serum etc.):	Plasma	Plasma		
Analyte:	Saxagliptin	Saxagliptin		
Assay:	LC/MS/MS	LC/MS/MS		
Saxagliptin Dose (mg/kg):	5.9	5.2		
Route:	<u>IV</u>	<u>PO</u>		
Parameter				
Cmax (µg/mL)	10.1	2.7		
AUC(0-infinity) (µg <sup>h</sup> /mL)	10.7	7.3		
Tmax (h)	-	1.2		
CLTp (mL/min/kg)	9.3	-		
Vss (L/kg)	1.3	-		
T1/2 (h)	3.0	-		
Amount excreted unchanged in urine (0-24 h, %)	40	ND		
Bioavailability (%)	-	76		

Additional Information: ND = not determined

Saxagliptin was well and rapidly absorbed in dogs. Saxagliptin showed intermediate clearance, and was excreted in urine as parent.

**Pharmacokinetics: Absorption After a Single Dose (Saxagliptin, Monkey)**

**Study Description or Title:** Pharmacokinetics of Saxagliptin in Cynomolgus Monkeys following Single Dose Intravenous or Oral Administration of Saxagliptin

**Test Article:** Saxagliptin

**Study Type:** Non-GLP

**Location in Dossier:**

**Study No./Document Control Number:** MAP005-477118/930000866

<b>Species/Strain:</b>	Monkey/Cynomolgus	
<b>Gender (M/F) / Number of Animals:</b>	Male / 2 per treatment <sup>a</sup>	Male / 2 per treatment
<b>Feeding condition:</b>	Fasted overnight and fed 4 h after dosing	Fasted overnight and fed 4 h after dosing
<b>Vehicle/Formulation:</b>	Water	Water
<b>Method of Administration:</b>	IV infusion (10 min)	Oral gavage
<b>Sample (Whole blood, plasma, serum etc.):</b>	Plasma	Plasma
<b>Analyte:</b>	Saxagliptin	Saxagliptin
<b>Assay:</b>	LC/MS/MS	LC/MS/MS
<b>BMS-477118 Dose (mg/kg):</b>	3.4	3.4
<b>Route:</b>	<b>IV</b>	<b>PO</b>
<b>Parameter</b>		
<b>C<sub>max</sub> (µg/mL)</b>	5.4	1.0
<b>AUC(0-infinity) (µg·h/mL)</b>	3.9	2.0
<b>T<sub>max</sub> (h)</b>	-	1.0
<b>CL<sub>Tp</sub> (mL/min/kg)</b>	14.5	-
<b>V<sub>ss</sub> (L/kg)</b>	1.8	-
<b>T<sub>1/2</sub> (h)</b>	4.4	-
<b>% dose excreted unchanged in urine (0-24 h)</b>	60	ND
<b>Bioavailability (%)</b>	-	51

Additional information: ND = not determined. Saxagliptin was rapidly absorbed in monkeys. The oral bioavailability was approximately 51%. Saxagliptin showed moderate to high clearance and was extensively eliminated in urine.

<sup>a</sup> Pharmacokinetic parameters were determined in only 1 of 2 monkeys assigned to intravenous administration; the second monkey administered saxagliptin intravenously was euthanized 4 hours after drug administration.

**Pharmacokinetics: Organ Distribution (Tissue: Plasma Concentration Ratios)**

**Study Description or Title:** Tissue Distribution of Radioactivity in Male Long-Evans Rats following Oral Administration of [<sup>14</sup>C]Saxagliptin

**Test Article:** [<sup>14</sup>C]Saxagliptin

**Study Type:** Non-GLP

**Location in Dossier:**

**Study No./Document Control No.:** DDBS038/930007588

<b>Species:</b>	Long-Evans Rats		
<b>Gender (M/F) / Number of Animals:</b>	Male / 24 (3 rats per time point)		
<b>Feeding condition:</b>	Fasted overnight and for 4 h after dosing, then fed <i>ad libitum</i> for the remainder of the study		
<b>Vehicle/Formulation:</b>	0.011 M hydrochloric acid in water		
<b>Method of Administration:</b>	Oral		
<b>Dose (mg/kg):</b>	20 mg/kg (100 µCi/kg)		
<b>Radionuclide:</b>	<sup>14</sup> C		
<b>Specific Activity:</b>	5.84 µCi/mg		
<b>Sampling time:</b>	0, 1, 4, 12, 24, 48, 96 and 168 h		
<b>Matrix</b>	<b>Tissue:Plasma Concentration Ratios</b>		
	<b>1 h</b>	<b>4 h</b>	<b>12 h</b>
	<b>Mean ± SD</b>	<b>Mean ± SD</b>	<b>Mean ± SD</b>
Adipose (epididymal)	0.11 ± 0.03	0.08 ± 0.07	0.32 ± 0.55
Adipose (visceral)	0.51 ± 0.18	0.30 ± 0.36	0.95 ± 0.62
Adipose (subcutaneous)	0.30 ± 0.08	0.31 ± 0.05	0.00 ± 0.00

**Pharmacokinetics: Organ Distribution (Tissue: Plasma Concentration Ratios)**

**Study Description or Title:** Tissue Distribution of Radioactivity in Male Long-Evans Rats following Oral Administration of [<sup>14</sup>C]Saxagliptin

**Test Article:** [<sup>14</sup>C]Saxagliptin

**Study Type:** Non-GLP

**Location in Dossier:**

**Study No./Document Control No.:** DDBS038/930007588

Matrix	Tissue:Plasma Concentration Ratios		
	1 h	4 h	12 h
	Mean ± SD	Mean ± SD	Mean ± SD
Adrenal Glands	0.99 ± 0.12	0.91 ± 0.10	2.18 ± 0.27
Blood	0.71 ± 0.05	0.88 ± 0.04	1.55 ± 0.31
Bone (femur)	0.16 ± 0.03	0.13 ± 0.12	0.00 ± 0.00
Bone Marrow (femur)	0.83 ± 0.08	0.85 ± 0.04	2.19 ± 0.12
Brain	0.04 ± 0.01	0.68 ± 1.07	0.34 ± 0.18
Cecum	0.43 ± 0.05	36.51 ± 33.23	95.79 ± 67.45
Duodenum	5.38 ± 1.40	8.72 ± 1.39	3.81 ± 0.21
Eyes	0.34 ± 0.01	0.91 ± 0.24	4.17 ± 1.06
Heart	0.51 ± 0.02	0.55 ± 0.05	1.27 ± 0.26
Ileum	1.68 ± 0.81	245.17 ± 205.25	8.77 ± 1.46
Intestine, Large	0.82 ± 0.17	4.01 ± 2.07	61.23 ± 27.13
Jejunum	37.12 ± 7.41	25.21 ± 29.07	8.05 ± 1.96
Kidneys	4.50 ± 0.31	5.32 ± 0.54	20.22 ± 4.86
Liver	17.18 ± 2.23	32.91 ± 10.34	74.05 ± 9.79
Lungs	0.91 ± 0.07	1.31 ± 0.08	3.65 ± 0.44
Pancreas	0.95 ± 0.03	1.02 ± 0.14	1.42 ± 0.17
Skeletal Muscle (pectoral)	0.40 ± 0.04	0.38 ± 0.10	0.18 ± 0.31
Skeletal Muscle (thigh)	0.40 ± 0.04	0.38 ± 0.04	1.20 ± 0.15
Skin, Nonpigmented	0.55 ± 0.02	0.73 ± 0.08	0.48 ± 0.84
Skin, Pigmented	0.58 ± 0.08	1.11 ± 0.41	5.63 ± 5.45
Spleen	0.84 ± 0.07	0.73 ± 0.14	2.63 ± 0.38
Stomach	2.30 ± 1.36	1.14 ± 0.15	1.12 ± 0.18
Testes	0.25 ± 0.02	0.66 ± 0.07	1.54 ± 0.41
Thyroid	1.53 ± 0.20	1.24 ± 0.30	1.23 ± 2.13
Urinary Bladder	5.33 ± 3.29	41.97 ± 53.88	22.78 ± 17.26

**Additional Information:** Tissue:plasma ratios are only reported through the 12 h time point since plasma levels of radioactivity were not measurable after 12 h.

**Pharmacokinetics: Organ Distribution (Mean Percentage of Radioactive Dose in Rat Tissues)**

Study Description or Title: Tissue Distribution of Radioactivity in Male Long-Evans Rats following Oral Administration of [<sup>14</sup>C]Saxagliptin

Test Article: [<sup>14</sup>C]Saxagliptin

Study Type: Non-GLP

Location in Dossier:

Study No./Document Control No.: DDBS038/930007588

Species/Strain: Rat/Long-Evans  
 Gender (M/F) / Number of Animals: M/24 (3 rats per time point)  
 Feeding condition: Fasted overnight and for 4 h after dosing, then fed *ad libitum* for the remainder of the study  
 Vehicle/Formulation: 0.011 M hydrochloric acid in water  
 Method of Administration: Oral  
 Dose (mg/kg): 20 mg /kg (100 µCi/kg)  
 Radionuclide: <sup>14</sup>C  
 Specific Activity: 5.84 µCi/mg  
 Sampling time: 0, 1, 4, 12, 24, 48, 96 and 168 h

Tissues/Organs	Mean Percentage of the Radioactive Dose in Tissues and Gastrointestinal Tract Tissues						
	1 h	4 h	12 h	24 h	48 h	96 h	168 h
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
Adipose (subcutaneous)	0.63 ± 0.22	0.14 ± 0.02	ND	ND	ND	ND	ND
Adrenal Glands	ND	ND	ND	ND	ND	ND	ND
Blood	1.04 ± 0.06	0.30 ± 0.04	0.06 ± 0.00	ND	ND	ND	ND
Bone (femur)	0.16 ± 0.03	0.03 ± 0.03	ND	ND	ND	ND	ND
Bone Marrow (femur)	0.05 ± 0.01	0.01 ± 0.00	ND	ND	ND	ND	ND
Brain	0.00 ± 0.01	0.02 ± 0.03	ND	ND	ND	ND	ND
Cecum	0.04 ± 0.01	0.65 ± 0.50	0.29 ± 0.25	0.00 ± 0.01	ND	ND	ND
Cecum Contents	0.22 ± 0.22	21.07 ± 0.33	7.74 ± 6.23	0.18 ± 0.08	0.06 ± 0.03	ND	ND
Duodenum	0.23 ± 0.01	0.30 ± 0.08	0.01 ± 0.01	0.00 ± 0.01	ND	ND	ND
Eyes	0.01 ± 0.00	ND	ND	ND	ND	ND	ND
Heart	0.05 ± 0.01	0.01 ± 0.00	ND	ND	ND	ND	ND
Ileum	0.06 ± 0.03	8.48 ± 5.38	0.02 ± 0.01	0.01 ± 0.00	ND	ND	ND
Intestinal Contents, Large	0.09 ± 0.06	0.14 ± 0.13	8.16 ± 7.39	0.21 ± 0.15	0.03 ± 0.03	ND	ND
Intestinal Contents, Small	29.71 ± 9.66	9.69 ± 5.61	0.34 ± 0.11	0.07 ± 0.03	0.02 ± 0.02	ND	ND
Intestine, Large	0.13 ± 0.04	0.12 ± 0.07	0.24 ± 0.12	0.01 ± 0.00	ND	ND	ND
Jejunum	10.38 ± 2.05	0.61 ± 0.67	0.06 ± 0.01	0.01 ± 0.01	0.00 ± 0.01	ND	ND
Kidneys	0.67 ± 0.10	0.20 ± 0.02	0.08 ± 0.01	0.06 ± 0.01	0.04 ± 0.01	0.01 ± 0.00	ND
Liver	10.71 ± 0.99	4.97 ± 0.99	1.60 ± 0.20	0.54 ± 0.25	0.14 ± 0.03	0.07 ± 0.01	0.03 ± 0.01
Lungs	0.08 ± 0.01	0.03 ± 0.01	0.01 ± 0.00	ND	ND	ND	ND
Pancreas	0.07 ± 0.02	0.01 ± 0.00	ND	ND	ND	ND	ND
Skeletal Muscle (thigh)	3.70 ± 0.27	0.81 ± 0.16	0.30 ± 0.03	0.04 ± 0.06	ND	ND	ND
Skin, Nonpigmented	1.08 ± 0.09	0.33 ± 0.07	0.03 ± 0.05	ND	ND	ND	ND
Skin, Pigmented	0.57 ± 0.01	0.25 ± 0.11	0.13 ± 0.10	0.01 ± 0.01	0.02 ± 0.03	0.01 ± 0.01	ND
Spleen	0.04 ± 0.00	0.01 ± 0.00	ND	ND	ND	ND	ND
Stomach	0.31 ± 0.16	0.04 ± 0.01	ND	ND	ND	ND	ND
Stomach Contents	2.90 ± 3.40	0.03 ± 0.02	ND	ND	ND	ND	ND
Testes	0.05 ± 0.01	0.03 ± 0.00	0.01 ± 0.00	ND	ND	ND	ND
Thyroid	ND	ND	ND	ND	ND	ND	ND
Urinary Bladder	0.03 ± 0.02	0.07 ± 0.09	0.01 ± 0.01	ND	ND	ND	ND

Abbreviations: SD = Standard Deviation; ND = Not detected. Concentrations of [<sup>14</sup>C]saxagliptin-derived radioactivity were below the limit of quantification.

**Pharmacokinetics: Organ Distribution (Saxagliptin, Sprague-Dawley Rat)**

**Study Description or Title:** Tissue Distribution of Saxagliptin and BMS-510849 in Sprague-Dawley Rats following Single Dose Intraarterial Administration of Saxagliptin

**Test Article:** Saxagliptin

**Study Type:** Non-GLP

**Location in Dossier:**

**Study No./Document Control No.:** 930009229

Tissues/organs	Saxagliptin Dose (mg/kg)	Saxagliptin		BMS-510849 <sup>a</sup>	
		Average Concentration	Tissue:Plasma	Average Concentration	Tissue:Plasma
		(ng/g) ± SD	Ratio ± SD	(ng/g) ± SD	Ratio ± SD
Plasma	0.1	4 ± 2	1	1.3 ± 0.4	1
	2.5	97 ± 23	1	48 ± 18	1
Small Intestine	0.1	159 ± 15	46 ± 18	28 ± 5	24 ± 10
	2.5	942 ± 193	10 ± 1	2396 ± 348	55 ± 21
Large Intestine	0.1	71 ± 13	20 ± 5	BLQ	NA
	2.5	2205 ± 411	24 ± 8	113 ± 20	3 ± 1
Duodenum	0.1	50 ± 22	15 ± 9	15 ± 1	14
	2.5	134 ± 10	1 ± 0.3	329 ± 106	7 ± 1
Kidney	0.1	1243 ± 76	349 ± 102	68 ± 9	53 ± 9
	2.5	1373 ± 282	14 ± 1	417 ± 110	9 ± 2
Spleen	0.1	80 ± 23	21 ± 3	2	1
	2.5	323 ± 50	4 ± 1	13 ± 9	0.3 ± 0.1
Heart	0.1	39 ± 7	11 ± 3	BLQ	NA
	2.5	224 ± 34	2 ± 0.3	27 ± 8	0.6 ± 0.1
Pancreas	0.1	26 ± 5	7 ± 2	12	10
	2.5	186 ± 20	2 ± 0.3	32 ± 5	0.7 ± 0.2
Brain	0.1	1	0.1	BLQ	NA
	2.5	8 ± 1	0.08 ± 0.01	BLQ	NA
Muscle	0.1	11 ± 0.5	3 ± 1	BLQ	NA
	2.5	291 ± 21	3 ± 1	7 ± 4	0.1 ± 0.04

Additional Information: Plasma and tissue homogenates were analyzed for saxagliptin and BMS-510849 using LC-MS/MS. SD = Standard Deviation; BLQ = below the lower limit of quantification; NA = not applicable. BMS-510849 lower limit of quantification: 8.6 ng/g (large intestine), 10.3 ng/g (heart), 2 ng/g (brain), and 1.8 ng/g (muscle)

<sup>a</sup> The bioanalytical assay that was used to measure concentrations of BMS-510849 in this study may not have been completely specific for BMS-510849. Other mono-hydroxylated metabolites with the same MRM transition as BMS-510849 in the LC/MS/MS (332 → 196) may have co-eluted with BMS-510849 under the conditions employed in the assay. Therefore the values for BMS-510849 concentration reported here may include BMS-510849 and other mono-hydroxylated metabolites. The measurement of saxagliptin was not impacted in this method.

**Pharmacokinetics: Organ Distribution (BMS-510849, Sprague-Dawley Rat)**

**Study Description or Title:** Tissue Distribution of BMS-510849 in Sprague-Dawley Rats following Single Dose Intraarterial Administration of BMS-510849

**Test Article:** BMS-510849

**Study Type:** Non-GLP

**Location in Dossier:**

**Study No./Document Control No.:** 930009229

Species/Strain:	Rat/Sprague-Dawley
Gender (M/F) / Number of Animals:	Male/3 per dose
Feeding condition:	Fasted overnight and for duration of study
Vehicle/Formulation:	Propylene glycol:water (50:50)
Method of Administration:	Intraarterial Bolus
Dose (mg/kg):	0.1 or 2.5 mg/kg BMS-510849
Radionuclide:	None
Specific Activity:	NA
Sampling time:	1 h

Tissues/organs	BMS-510849 Dose (mg/kg)	BMS-510849	
		Average Concentration (ng/g) ± SD	Tissue:Plasma Ratio ± SD
Plasma	0.1	5 ± 1	1
	2.5	103 ± 18	1
Small Intestine	0.1	27 ± 9	5 ± 2
	2.5	1953 ± 1029	20 ± 14
Large Intestine	0.1	16 ± 12	3 ± 3
	2.5	319 ± 81	3 ± 0.5
Duodenum	0.1	14	2.7
	2.5	229 ± 24	2 ± 0.5
Kidney	0.1	1411 ± 138	271 ± 39
	2.5	3030 ± 282	30 ± 8
Spleen	0.1	49 ± 14	9 ± 3
	2.5	139 ± 39	1 ± 0.2
Heart	0.1	30 ± 5	6 ± 1
	2.5	146 ± 25	1 ± 0.01
Pancreas	0.1	36 ± 6	7 ± 0.4
	2.5	488 ± 42	5 ± 0.6
Brain	0.1	BLQ	NA
	2.5	8.5 ± 2.5	0.09 ± 0.04
Muscle	0.1	3.3	0.7
	2.5	64.6 ± 9.2	0.6 ± 0.1

Additional Information: Plasma and tissue homogenates were analyzed for BMS-510849 using LC-MS/MS. SD = Standard Deviation; BLQ = below the lower limit of quantification; NA = not applicable. BMS-510849 lower limit of quantification in rat brain homogenate: 2 ng/g.

**Pharmacokinetics: Organ Distribution (Saxagliptin, Zucker Diabetic Fatty Rat)**

**Study Description or Title:** Tissue Distribution of Saxagliptin and BMS-510849 in Zucker Diabetic Fatty Rats following Single Dose Intraarterial Administration of Saxagliptin

**Test Article:** Saxagliptin

**Study Type:** Non-GLP

**Location in Dossier:**

**Study No./Document Control No.:** 930009229

<b>Species/Strain:</b>	Rat/Zucker Diabetic Fatty				
<b>Gender (M/F) / Number of Animals:</b>	Male/3 per dose				
<b>Feeding condition:</b>	Fasted overnight and for duration of study				
<b>Vehicle/Formulation:</b>	Propylene glycol:water (50:50)				
<b>Method of Administration:</b>	Intraarterial Bolus				
<b>Dose (mg/kg):</b>	0.1 or 2.5 mg/kg Saxagliptin				
<b>Radionuclide:</b>	None				
<b>Specific Activity:</b>	NA				
<b>Sampling time:</b>	1 h				
Tissues/organs	Saxagliptin Dose (mg/kg)	Saxagliptin			
		Average Concentration (ng/g) ± SD	Tissue:Plasma Ratio ± SD	Average Concentration (ng/g) ± SD	Tissue:Plasma Ratio ± SD
Plasma	0.1	14 ± 11	1	0.7 ± 0.03	1
	2.5	148 ± 70	1	34 ± 7	1
Small Intestine	0.1	164 ± 21	17 ± 9	29 ± 10	39 ± 15
	2.5	759 ± 392	6 ± 4	943 ± 475	28 ± 15
Large intestine	0.1	81 ± 43	10 ± 8	18	24
	2.5	1547 ± 524	13 ± 8	140 ± 63	4 ± 3
Duodenum	0.1	60 ± 13	6 ± 4	19 ± 8	24 ± 9
	2.5	179 ± 74	1 ± 0.2	254 ± 119	7 ± 2
Kidney	0.1	923 ± 316	87 ± 34	20 ± 0.2	27 ± 1
	2.5	2662 ± 340	21 ± 11	271 ± 41	8 ± 2
Spleen	0.1	139 ± 43	16 ± 12	BLQ	NA
	2.5	1300 ± 656	9 ± 1	22 ± 9	0.7 ± 0.3
Heart	0.1	26 ± 9	8 ± 5	BLQ	NA
	2.5	236 ± 108	3 ± 1	17 ± 5	0.5 ± 0.1
Pancreas	0.1	115 ± 70	36 ± 30	BLQ	NA
	2.5	940 ± 671	10 ± 8	BLQ	NA
Brain	0.1	BLQ	NA	<sup>b</sup>	NA
	2.5	10 ± 1	0.07 ± 0.02	0.8	0.02
Muscle	0.1	23 ± 2	2 ± 1	BLQ	NA
	2.5	500 ± 170	4 ± 0.5	5.6 ± 2	0.2 ± 0.04

Additional Information: Plasma and tissue homogenates were analyzed for saxagliptin and BMS-510849 using LC-MS/MS. SD = Standard Deviation; BLQ = below the lower limit of quantification; NA = not applicable. Saxagliptin lower limit of quantification: 0.77 ng/g (brain); BMS-510849 lower limit of quantification: 22.3 ng/g (pancreas), 2.5 ng/g (heart), 1.8 ng/g (spleen), 0.8 ng/g (muscle), and 0.77 ng/g (brain).

<sup>a</sup> The bioanalytical assay that was used to measure concentrations of BMS-510849 in this study may not have been completely specific for BMS-510849. Other mono-hydroxylated metabolites with the same MRM transition as BMS-510849 in the LC-MS/MS (332 → 196) may have co-eluted with BMS-510849 under the conditions employed in the assay. Therefore the values for BMS-510849 concentration reported here may include BMS-510849 and other mono-hydroxylated metabolites. The measurement of saxagliptin was not impacted in this method.

<sup>b</sup> Represents mean value of 2 rats (Rat 1 = 16.6 ng/g, Rat 2 = 1.2 ng/g, Rat 3 = BLQ)

**Pharmacokinetics: Organ Distribution (BMS-510849, Zucker Diabetic Fatty Rat)**

**Study Description or Title:** Tissue Distribution of BMS-510849 in Zucker Diabetic Fatty Rats following Single Dose Intraarterial Administration of BMS-510849

**Test Article:** BMS-510849

**Study Type:** Non-GLP

**Location In Dossier:**

**Study No./Document Control No.:** 930009229

<b>Species/Strain:</b>	Rat/Zucker Diabetic Fatty
<b>Gender (M/F) / Number of Animals:</b>	Male/3 per dose
<b>Feeding condition:</b>	Fasted overnight and for duration of study
<b>Vehicle/Formulation:</b>	Propylene glycol:water (50:50)
<b>Method of Administration:</b>	Intraarterial Bolus
<b>Dose (mg/kg):</b>	0.1 or 2.5 mg/kg BMS-510849
<b>Radionuclide:</b>	None
<b>Specific Activity:</b>	NA
<b>Sampling time:</b>	1 h

Tissues/organs	BMS-510849	BMS-510849	BMS-510849
	Dose (mg/kg)	Average Concentration (ng/g) ± SD	Tissue:Plasma Ratio ± SD
Plasma	0.1	10 ± 2	1
	2.5	346 ± 52	1
Small Intestine	0.1	25 ± 4	3 ± 0.1
	2.5	920 ± 165	3 ± 0.2
Large Intestine	0.1	17 ± 3	2 ± 0.4
	2.5	389 ± 62	1
Duodenum	0.1	17 ± 9	1.6 ± 0.6
	2.5	407 ± 119	1 ± 0.4
Kidney	0.1	940 ± 106	94 ± 11
	2.5	3019 ± 429	9 ± 2
Spleen	0.1	71 ± 7	7 ± 1
	2.5	317 ± 105	1 ± 0.2
Heart	0.1	12 ± 2	1 ± 0.3
	2.5	218 ± 10	1 ± 0.1
Pancreas	0.1	56 ± 3	5
	2.5	1553 ± 365	5 ± 1
Brain	0.1	0.79	0.06
	2.5	12 ± 2	0.04 ± 0.01
Muscle	0.1	4 ± 2	0.4 ± 0.3
	2.5	155 ± 27	0.5 ± 0.1

Additional Information: SD = Standard Deviation. Plasma and tissue homogenates were analyzed for BMS-510849 using LC-MS/MS.

**Pharmacokinetics: Organ Distribution (Quantitative Whole Body Autoradiography in Male Sprague Dawley Rat)**

**Study Description or Title:** Tissue distribution of radioactivity determined by quantitative whole body autoradiography in male Sprague Dawley rats following oral administration of [<sup>14</sup>C]Saxagliptin

**Test Article:** [<sup>14</sup>C]Saxagliptin

**Study Type:** Non-GLP

**Location in Dossier:**

**Study No./Document Control No.:** 6108-566/930022255

**Species:** Rat/Sprague Dawley  
**Gender (M/F) / Number of Animals:** Male/8  
**Feeding condition:** Non-fasted  
**Vehicle/Formulation:** 50 mM citrate buffer, pH 4.0/Solution (radiolabeled trifluoroacetic acid salt combined with non-labeled free base monohydrate to give 1 mg/mL free base)  
**Method of Administration:** Single dose oral gavage  
**Dose (mg/kg):** 5 mg/kg free base equivalents (radioactive dose: 100 µCi/kg)  
**Radionuclide:** [<sup>14</sup>C]  
**Assay:** Tissue distribution of radioactivity was determined by quantitative whole body autoradiography (QWBA), and blood concentrations of radioactivity were determined by liquid scintillation counting (LSC).  
**Sampling time:** Blood and biological fluids were collected from 1 male rat/time point at pre-dose and 1, 4, 8, 12, 24, 96, and 168 hours post-dose. The animal from which the pre-dose blood sample was collected was dosed with vehicle only. Carcasses were immediately frozen after blood and biological fluid collection for preparation of samples for QWBA.

Pharmacokinetic Parameters				
Tissues/organs	Cmax (ng equivalents/g)	Tmax (h)	AUC(0-infinity) (ng equivalents*h/g)	Terminal T <sub>1/2</sub> (h)
Adrenal gland	972	1	6970	3.73
Blood	438	1	NC	NC
Blood (LSC)	389	1	4320	68.9
Bone	NC	NC	NC	NC
Bone marrow	636	1	2180	1.9
Cecum	20500	4	157000	3.62
Cerebellum	NC	NC	NC	NC
Cerebrospinal fluid	NC	NC	NC	NC
Cerebrum	144	1	NC	NC
Diaphragm	1650	4	NC	NC
Epididymis	479	1	NC	NC
Esophagus	800	1	4080	3.68
Exorbital lacrimal gland	880	1	12500	19.9
Eye	121	1	NC	NC
Eye (lens)	121	1	NC	NC
Fat (abdominal)	117	1	1040	5.77
Fat (brown)	348	1	NC	NC
Fat (reproductive)	107	1	NC	NC
Harderian gland	727	1	2660	2.08
Intraorbital lacrimal gland	765	1	NC	NC
Kidney	4790	1	120000	26.3

**Pharmacokinetics: Organ Distribution (Quantitative Whole Body Autoradiography in Male Sprague Dawley Rat)**

**Study Description or Title:** Tissue distribution of radioactivity determined by quantitative whole body autoradiography in male Sprague Dawley rats following oral administration of [<sup>14</sup>C]Saxagliptin

**Test Article:** [<sup>14</sup>C]Saxagliptin

**Study Type:** Non-GLP

**Location in Dossier:**

**Study No./Document Control No.:** 6108-566/930022255

Tissues/organs	C <sub>max</sub> (ng equivalents/g)	T <sub>max</sub> (h)	AUC(0-infinity) (ng equivalents*h/g)	Terminal T <sub>1/2</sub> (h)
Large intestine	1940	12	22500	4.87
Liver	10400	1	138000	19.8
Lung	704	1	6740	7.33
Lymph nodes (mesenteric)	793	1	5660	3.44
Medulla	NC	NC	NC	NC
Muscle	338	1	NC	NC
Myocardium	464	1	NC	NC
Nasal turbinates	329	1	1130	1.91
Olfactory lobe	224	1	NC	NC
Pancreas	576	1	4090	5.59
Pituitary gland	525	1	NC	NC
Plasma	483	1	3130	22.2
Preputial gland	543	1	NC	NC
Prostate	1020	4	9690	5.57
Renal cortex	4680	1	72300	18.1
Renal medulla	5510	1	141000	33.6
Renal medulla (high)	6910	1	410000	55.2
Salivary gland	742	1	2390	1.74
Seminal vesicle	480	12	NC	NC
Skin	363	1	3230	6.62
Small intestine	5020	1	30400	2.46
Spinal cord	NC	NC	NC	NC
Spleen	770	1	7630	10.5
Stomach	1350	1	6120	2.86
Testis	235	1	1870	5.16
Thymus	516	1	2570	3.05
Thyroid	951	1	NC	NC
Trachea	NC	NC	NC	NC
Urinary bladder	7430	1	108000	3.19

Additional Information: Animal from which pre-dose sample was collected had no detectable radioactivity. NC = not calculable because sample below limit of quantitation (< 75.5 ng equivalents [<sup>14</sup>C]saxagliptin/g) or radioactivity not detectable (sample shape not discernible from background or surrounding tissue). The [<sup>14</sup>C]-saxagliptin-derived radioactivity was extensively distributed in tissues. The highest concentrations were found in GI contents and urine, which is consistent with the route of oral administration and the major elimination pathways of saxagliptin. Besides gastrointestinal tissues and urinary bladder, kidney and liver showed highest radioactivity. A differential distribution of radioactivity was observed in the renal medulla. The radioactivity was quantifiable only in kidney and liver at 96 hours post-dose. The tissues showing lowest radioactivity included cerebellum, CSF, spinal cord, olfactory lobe, trachea, bone, eye and lens of eye. At 168 hours, the final collection time point, the radioactivity was not quantifiable in any tissues. The exposure of total radioactivity in cerebellum, cerebrum and cerebrospinal fluid were much lower than that in plasma, indicating limited distribution to the CNS system due to blood-brain barrier. Radioactivity was detected in testis tissue in male rats.

**Pharmacokinetics: Organ Distribution (Quantitative Whole Body Autoradiography in Female Sprague Dawley Rat)**

**Study Description or Title:** Tissue distribution of radioactivity determined by quantitative whole body autoradiography in female Sprague Dawley rats following oral administration of [<sup>14</sup>C]Saxagliptin

**Test Article:** [<sup>14</sup>C]Saxagliptin

**Study Type:** Non-GLP

**Location in Dossier:**

**Study No./Document Control No.:** 6108-566/93002255

**Species:** Rat/Sprague Dawley  
**Gender (M/F) / Number of Animals:** Non-pregnant female/8  
**Feeding condition:** Non-fasted  
**Vehicle/Formulation:** 50 mM citrate buffer, pH 4.0/Solution (radiolabeled trifluoroacetic acid salt combined with non-labeled free base monohydrate to give 1 mg/mL free base)  
**Method of Administration:** Single dose oral gavage  
**Dose (mg/kg):** 5 mg/kg free base equivalents (radioactive dose: 100 µCi/kg)  
**Radionuclide:** [<sup>14</sup>C]  
**Assay:** Tissue distribution was determined by quantitative whole body autoradiography (WBA). Blood concentrations of radioactivity were determined by liquid scintillation counting (LSC).  
**Sampling time:** Blood and biological fluids were collected from 1 non-pregnant rat/time point at pre-dose and 1, 4, 8, 12, 24, 96, and 168 hours post-dose. The animal from which the pre-dose blood sample was collected was dosed with vehicle only. Carcasses were immediately frozen after blood and biological fluid collection for the preparation of samples for WBA.

**Pharmacokinetic Parameters**

Tissues/organs	C <sub>max</sub> (ng equivalents/g)	T <sub>max</sub> (h)	AUC(0-Infinity) (ng equivalents*h/g)	Terminal T <sub>1/2</sub> (h)
Adrenal gland	887	1	6190	4.34
Blood	331	1	1380	2.46
Blood (LSC)	398	1	2010	7.57
Bone	NC	NC	NC	NC
Bone marrow	833	1	3480	2.47
Cecum	5560	12	70100	3.32
Cerebellum	NC	NC	NC	NC
Cerebrospinal fluid	NC	NC	NC	NC
Cerebrum	77.1	4	NC	NC
Diaphragm	1070	1	3460	1.75
Esophagus	858	1	2760	1.74
Exorbital lacrimal gland	1430	1	11400	5.47
Eye	NC	NC	NC	NC
Eye (lens)	NC	NC	NC	NC
Fat (abdominal)	117	4	NC	NC
Fat (brown)	400	1	NC	NC
Fat (reproductive)	98.7	4	NC	NC
Harderian gland	826	1	5280	4.05
Intraorbital lacrimal gland	878	1	8120	4.80
Kidney	4760	1	126000	32.8
Large intestine	1170	12	21200	14.1

**Pharmacokinetics: Organ Distribution (Quantitative Whole Body Autoradiography in Female Sprague Dawley Rat)**

**Study Description or Title:** Tissue distribution of radioactivity determined by quantitative whole body autoradiography in female Sprague Dawley rats following oral administration of [<sup>14</sup>C]Saxagliptin

**Test Article:** [<sup>14</sup>C]Saxagliptin

**Study Type:** Non-GLP

**Location in Dossier:**

**Study No./Document Control No.:** 6108-566/930022255

Tissues/organs	Cmax (ng equivalents/g)	Tmax (h)	AUC(0-infinity) (ng equivalents*h/g)	Terminal T <sub>1/2</sub> (h)
Liver	6700	1	39800	6.83
Lung	898	1	5690	3.27
Lymph nodes (mesenteric)	780	1	NC	NC
Medulla	75.8	4	NC	NC
Muscle	288	1	2020	4.48
Myocardium	513	1	2260	2.63
Nasal turbinates	195	4	NC	NC
Olfactory lobe	NC	NC	NC	NC
Ovary	312	1	1810	3.63
Pancreas	527	1	3820	4.65
Pituitary gland	698	1	3030	2.58
Plasma	451	1	1790	4.36
Preputial gland	885	1	6490	4.71
Renal cortex	4910	1	80500	23.7
Renal medulla	4300	1	93600	17.1
Renal medulla (high)	7110	1	246000	23.9
Salivary gland	887	1	4950	3.59
Skin	343	1	2730	5.15
Small intestine	4040	8	NC	NC
Spinal cord	NC	NC	NC	NC
Spleen	1020	1	5570	3.79
Stomach	3290	1	10300	1.57
Thymus	613	1	3820	3.78
Thyroid	857	1	NC	NC
Trachea	NC	NC	NC	NC
Urinary bladder	3120	4	14900	1.85
Uterus	624	1	3460	4.07

Additional Information: Animal from which pre-dose sample was collected had no detectable radioactivity. NC = not calculable because sample below limit of quantitation (< 75.5 ng equivalents [<sup>14</sup>C]Saxagliptin/g) or radioactivity not detectable (sample shape not discernible from background or surrounding tissue). The [<sup>14</sup>C]-saxagliptin-derived radioactivity was extensively distributed in tissues. The highest concentrations were found in GI contents and urine, which is consistent with the route of oral administration and the major elimination pathways of saxagliptin. Besides gastrointestinal tissues and urinary bladder, kidney and liver showed highest radioactivity. A differential distribution of radioactivity was observed in the renal medulla. The radioactivity was quantifiable only in kidney and liver at 96 hours post-dose. The tissues showing lowest radioactivity included cerebellum, CSF, spinal cord, olfactory lobe, trachea, bone, eye and lens of eye. At 168 hours, the final collection time point, the radioactivity was not quantifiable in any tissues. The exposure of total radioactivity in cerebellum, cerebrum and cerebrospinal fluid were much lower than that in plasma, indicating limited distribution to the CNS system due to blood-brain barrier.

**Pharmacokinetics: Protein Binding (BMS-477118)**

Study Description or Title: Preliminary Protein Binding of BMS-477118 in Mouse, Rat, Dog, Monkey, and Human Serum  
 Test Article: Saraglitptin  
 Study Type: Non-GLP  
 Location in Dossier:  
 Study No./Document Control No. 930025627

Test system and method: Equilibrium dialysis and LC-MS/MS

Species	Concentration Tested (µg/mL)	% Free (mean ± SD)
Mouse	25	73.3 ± 21.5
Rat	5	82 ± 1.5
Dog	5	109.0 ± 30.2
Monkey	0.1	79.6 ± 25.5
Human	0.1	107.9 ± 34.2

Additional information: SD = Standard Deviation. The experiments were carried out using equilibrium dialysis method in triplicate. The concentrations of saxaglitptin were determined by LC-MS/MS assay. The results indicated that the protein binding of saxaglitptin is very low in mouse, rat, dog, monkey and human serum.

Study Description or Title: Preliminary Protein Binding of BMS-510849 in Mouse, Rat, Dog, Monkey, and Human Serum  
 Test Article: BMS-510849  
 Study Type: Non-GLP  
 Location in Dossier:  
 Study No./Document Control No. 930025627

Test system and method: Equilibrium dialysis and LC-MS/MS

Species	Concentration Tested (µg/mL)	% Free (mean ± SD)
Mouse	25	109.7 ± 16.6
Rat	5	104 ± 8.4
Dog	5	97.8 ± 10.5
Monkey	0.1	89.4 ± 3.0
Human	0.1	103.1 ± 24

Additional information: SD = Standard Deviation. The experiments were carried out using equilibrium dialysis method in triplicate. The concentrations of BMS-510849 were determined by LC-MS/MS assay. The results indicated that the protein binding of BMS-510849 is very low in mouse, rat, dog, monkey and human serum.

**Pharmacokinetics: Study in Pregnant or Nursing Animals**

Study Description or Title: Lactal excretion of radioactivity in lactating female Sprague Dawley rats following oral administration of [<sup>14</sup>C]BMS-477118  
 Test Article: [<sup>14</sup>C]Saxaglitptin  
 Study Type: Non-GLP  
 Location in Dossier:  
 Study No./Document Control No. 6018-566/930022255

**Excretion into milk**

Species: Rat/Sprague Dawley  
 Lactating date/Number of Animals: 7 to 9 days postpartum/21 lactating rats  
 Feeding condition: Non-fasted  
 Vehicle/Formulation: 50 mM citrate buffer, pH 4.0/Solution (radiolabeled trifluoroacetic acid salt combined with non-labeled free base monohydrate to give 1 mg/mL free base)  
 Method of Administration: Single dose oral gavage  
 Dose (mg/kg): 5 mg/kg free base equivalents (radioactive dose: 100 µCi/kg)  
 Analyte: Total [<sup>14</sup>C] radioactivity  
 Assay: Liquid scintillation counting  
 Method: Milk and blood were collected from 3 lactating rats/time point at 1, 4, 8, 12, 24, 48, and 72 hours post-dose to assess the lactal excretion of [<sup>14</sup>C]BMS-477118-derived radioactivity. Pups were removed from their mothers approximately 4 hours before milk collection. Animals received a subcutaneous dose of oxytocin before milking to stimulate lactation.

Matrix	Pharmacokinetic Parameters					
	C <sub>max</sub> (ng equiv/g)	T <sub>max</sub> (h)	AUC (0-infinity) (ng equiv*h/g)	Terminal T <sub>1/2</sub> (h)	Tissue-to-Plasma C <sub>max</sub> Ratio	Tissue-to-Plasma AUC Ratio
Milk	643	1	2610	4.39	1.22	0.808
Blood	582	1	3020	19.7	1.10	0.935
Plasma	528	1	3230	5.56	NA	NA

Additional Information: ND = not determined; NA = not applicable. Blood:plasma and milk:plasma ratios represent mean ± SD of 3 animals. The maximum level of total radioactivity, the time to reach the maximum level of radioactivity and AUC values were comparable between milk and blood or plasma. The t<sub>1/2</sub> value in milk was comparable to that in plasma, but shorter than that in blood. These results indicated that saxaglitptin-derived radioactivity was excreted into milk and showed no accumulation compared to plasma levels.

**Pharmacokinetics: Study in Pregnant or Nursing Animals (Placental Transfer)**

**Study Description or Title:** Fetal and maternal tissue distribution of radioactivity in pregnant female Sprague Dawley rats following oral administration of [<sup>14</sup>C]saxagliptin

**Test Article:** [<sup>14</sup>C]Saxagliptin

**Study Type:** Non-GLP

**Location in Dossier:**

**Study No./Document Control No.** 6108-566/930022255

**Placental Transfer**

**Species:** Rat/Sprague Dawley  
**Gestation day/Number of Animals:** Day 18/21 pregnant females  
**Feeding Condition:** Non-fasted  
**Vehicle/Formulation:** 50 mM citrate buffer, pH 4.0/Solution (radiolabeled trifluoroacetic acid salt combined with non-labeled free base monohydrate to give 1 mg/mL free base)  
**Method of Administration:** Single dose oral gavage  
**Dose (mg/kg):** 5 mg/kg free base equivalents (radioactive dose: 100 µCi/kg)  
**Analyte:** [<sup>14</sup>C]  
**Assay:** Liquid scintillation counting  
**Method:** Blood and selected tissues were collected from 3 pregnant rats/time point and 2 fetuses per dam (6 fetuses/time point) at 1, 4, 8, 12, 24, 48, and 72 hours post-dose to examine [<sup>14</sup>C]BMS-477118-derived radioactivity in maternal and fetal tissues.

**Pharmacokinetic Parameters**

Matrix	Cmax (ng equivalents/g)	Tmax (h)	AUC(0-infinity) (ng equivalents*h/g)	Terminal T <sub>1/2</sub> (h)
Maternal blood	826	1	3640	28.4
Maternal Plasma	949	1	3210	4.89
Placenta	1050	1	11000	23.5
Amniotic fluid	89.8	8	2190	19.0
Uterus	1080	1	6430	6.66
Ovaries	796	1	3280	9.01
Cerebrum (brain)	32.3	1	107	1.82
Heart	931	1	4150	8.58
Kidneys	8240	1	157000	31.5
Liver	8580	1	44600	18.3
Lungs	2180	1	21700	15.5
Fetal blood	236	1	1810	6.63
Fetal brain	173	4	1670	6.50
Fetal kidneys	480	1	9300	21.5
Fetal liver	618	1	5520	8.73
Fetus (residual)	324	1	2470	7.06

**Additional Information:** <sup>14</sup>C-saxagliptin-derived radioactivity was widely distributed in both maternal and fetal tissues. Radioactivity was measurable in all fetal matrices analyzed, indicating transfer of saxagliptin across placenta. The fetal blood exposure (AUC) of total radioactivity was approximately 50% of the maternal blood exposure while the fetal brain exposure was approximately 16-20x the maternal brain exposure. However, the exposures in fetal kidney and liver were much lower than the corresponding maternal tissues.

**Pharmacokinetics: Study in Pregnant or Nursing Animals (Quantitative Whole Body Autoradiography)**

**Study Description or Title:** Fetal and maternal tissue distribution of radioactivity determined by quantitative whole body autoradiography in pregnant female Sprague Dawley rats following oral administration of [<sup>14</sup>C]saxagliptin

**Test Article:** [<sup>14</sup>C]Saxagliptin

**Study Type:** Non-GLP

**Location in Dossier:**

**Study No./Document Control No.** 6018-566/930022255

**Species:** Rat/Sprague Dawley  
**Gestation day/Number of Animals:** Day 18/8 pregnant females  
**Feeding Condition:** Non-fasted  
**Vehicle/Formulation:** 50 mM citrate buffer, pH 4.0/Solution (radiolabeled trifluoroacetic acid salt combined with non-labeled free base monohydrate to give 1 mg/mL free base)  
**Method of Administration:** Single dose oral gavage  
**Dose (mg/kg):** 5 mg/kg free base equivalents (radioactive dose: 100 µCi/kg)  
**Analyte:** Total [<sup>14</sup>C]-saxagliptin radioactivity  
**Assay:** Tissue distribution was determined by quantitative whole body autoradiography (WBA). Blood concentrations of radioactivity were determined by liquid scintillation counting (LSC).  
**Method:** Blood and biological fluids were collected from 1 pregnant rat/time point at pre-dose and 1, 4, 8, 12, 24, 48, and 72 hours post-dose. The animal from which the pre-dose blood sample was collected was dosed with vehicle only. Carcasses were immediately frozen after blood and biological fluid collection for preparation of samples for QWBA

**Pharmacokinetic Parameters**

Matrix	Cmax (ng equivalents/g)	Tmax (h)	AUC(0-infinity) (ng equivalents* <sup>h</sup> /g)	Terminal T <sub>1/2</sub> (h)
Adrenal gland	1550	1	15700	14.7
Amniotic fluid	NC	NC	NC	NC
Blood	472	1	2410	3.13
Blood (LSC)	599	1	3050	25.60
Bone	NC	NC	NC	NC
Bone marrow	1460	1	7350	3.50
Cecum	8830	8	71400	5.30
Cerebellum	NC	NC	NC	NC
Cerebrospinal fluid	NC	NC	NC	NC
Cerebrum	NC	NC	NC	NC
Diaphragm	1160	1	NC	NC
Esophagus	882	1	3280	2.13
Exorbital lacrimal gland	2400	1	20800	4.93
Eye	NC	NC	NC	NC
Eye (lens)	NC	NC	NC	NC
Fat (abdominal)	168.0	12	NC	NC
Fat (brown)	406	1	NC	NC
Fat (reproductive)	482.0	1	7240	14.7
Fetus	261	1	5270	14.2
Harderian gland	1660	1	7170	2.57
Intraorbital lacrimal gland	2360	1	7290	1.64
Kidney	5940	1	NC	NC
Large intestine	4150	8	35800	4.21

**Pharmacokinetics: Study in Pregnant or Nursing Animals (Quantitative Whole Body Autoradiography)**

**Study Description or Title:** Fetal and maternal tissue distribution of radioactivity determined by quantitative whole body autoradiography in pregnant female Sprague Dawley rats following oral administration of [<sup>14</sup>C]saxagliptin

Test Article: [<sup>14</sup>C]Saxagliptin

Study Type: Non-GLP

Location in Dossier:

Study No./Document Control No. 6018-566/930022255

Matrix	C <sub>max</sub> (ng equivalents/g)	T <sub>max</sub> (h)	AUC(0-infinity) (ng equivalents*h/g)	Terminal T <sub>1/2</sub> (h)
Liver	5290	1	38900	14.5
Lung	956	1	10900	11.2
Lymph nodes (mesenteric)	NC	NC	NC	NC
Medulla	NC	NC	NC	NC
Muscle	581	1	2700	3.30
Myocardium	738	1	NC	NC
Nasal turbinates	NC	NC	NC	NC
Olfactory lobe	NC	NC	NC	NC
Ovary	344	1	1590	2.78
Pancreas	846	1	3380	2.69
Pituitary gland	1560	1	NC	NC
Placenta	535	1	2560	3.24
Plasma	661	1	2480	4.45
Preputial gland	1580	1	16200	8.40
Renal cortex	5780	1	125000	29.2
Renal medulla	5410	1	248000	38.7
Renal medulla (high)	6870	8	474000	39.7
Salivary gland	1660	1	11500	11.5
Skin	NC	NC	NC	NC
Small intestine	1440	4	18300	7.38
Spinal cord	NC	NC	NC	NC
Spleen	1880	1	12900	11.8
Stomach	1030	1	6790	4.26
Thymus	1340	1	NC	NC
Thyroid	1630	1	NC	NC
Trachea	NC	NC	NC	NC
Urinary bladder	1080	4	NC	NC
Uterus	985	1	3480	1.99

Additional Information: Animal from which pre-dose sample was collected had no detectable radioactivity. NC = not calculable because sample below limit of quantitation (< 106 ng equivalents [<sup>14</sup>C]BMS-477118/g) or radioactivity not detectable (sample shape not discernible from background or surrounding tissue). [<sup>14</sup>C]-saxagliptin-derived radioactivity was widely distributed in both maternal and fetal tissues. Radioactivity was measurable in all fetus, indicating transfer of saxagliptin across placenta.

**Pharmacokinetics: Other Distribution Study**

**Study Description or Title:** Brain to Plasma Concentration Ratio of Radioactivity in Male and Female Mice and Rats following Oral Administration of [<sup>14</sup>C]saxagliptin

**Test Article:** [<sup>14</sup>C]Saxagliptin  
**Study Type:** Non-GLP

**Location in Dossier:**

**Study No./Document Control No.** MBA00122/930010621  
MBA00113/930010620

<b>Species/Strain</b>	Mouse/CD-1	Rat/Sprague Dawley	
<b>Gender (M/F)/Number of animals</b>	M&F/5 per treatment/sample time or time period	M&F/3 per treatment/sample time or time period	
<b>Feeding condition:</b>	Fasted 4 h/fed 2 h postdose	Fasted overnight/fed 4 h postdose	
<b>Vehicle/Formulation:</b>	Acidified (by HCl) water (pH 5.5)	Acidified (by HCl) water (pH 5.5)	
<b>Method of Administration:</b>	Oral gavage	Oral gavage	
<b>Dose:</b>	600 mg/kg (100 µCi/kg)	300 mg/kg (100 µCi/kg)	
<b>Radionuclide:</b>	<sup>14</sup> C	<sup>14</sup> C	
<b>Specific Activity:</b>	0.16 µCi/mg	0.31 µCi/mg	

  

Time (h)	Brain:Plasma Concentration Ratio of Radioactivity (Mean ± SD, n = 5 from Mouse and n = 3 from Rat)			
	Male Mouse	Female Mouse	Male Rat	Female Rat
1	0.0469 ± 0.0122	0.0494 ± 0.0125	0.0380 ± 0.00206	0.0382 ± 0.00483
4	0.0393 ± 0.0415	0.0344 ± 0.0246	0.0461 ± 0.0154	0.0332 ± 0.0287
8	NC	NC	NC	NC
12	NC	NC	NC	NC
24	NC	NC	NC	NC

  

Time (h)	Concentration (µg equivalents of saxagliptin/g); (Mean ± SD)							
	Male Mouse (n = 5 per time point)		Female Mouse (n = 5 per time point)		Male Rat (n = 3 per time point)		Female Rat (n = 3 per time point)	
	Plasma	Brain	Plasma	Brain	Plasma	Brain	Plasma	Brain
1	108 ± 14.7	5.17 ± 1.84	94.7 ± 23.4	4.55 ± 1.01	61.5 ± 50.6	2.29 ± 1.78	101 ± 3.10	3.85 ± 0.419
4	32.4 ± 13.9	1.37 ± 1.38	29.9 ± 7.92	0.726 ± 0.996	18.6 ± 4.09	0.720 ± 0.624	18.6 ± 4.92	0.710 ± 0.615
8	4.49 ± 4.13	BQL	3.43 ± 4.01	BQL	8.80 ± 3.15	BQL	1.45 ± 0.338	BQL
12	2.76 ± 6.17	BQL	BQL	BQL	9.72 ± 3.71	BQL	BQL	BQL
24	BQL	BQL	BQL	BQL	3.25 ± 2.77	BQL	BQL	BQL

Abbreviations: SD = Standard deviation. BQL = Below quantifiable limit.  
NC = Not Calculated. For both mice and rats, radioactivity in brain samples was below the quantifiable limit (BQL) in all animals after the 4 h time point.

Pharmacokinetics: Metabolism In Vivo (In Plasma)

Study Description or Title: Biotransformation of [<sup>14</sup>C]saxaglipitin in Plasma From Sprague Dawley Rat, Beagle Dog, Cynomolgus Monkey and Human

Test Article: [<sup>14</sup>C]Saxaglipitin  
 Study Type: Non-GLP  
 Location in Dossier:  
 Study No./Document Control No.: NA/930016961

Species/Strain		Rat/Sprague Dawley M24 (n=3 per time point)			Dog/Beagle M3				Monkey/Cynomolgus M3			Human M/6					
Gender (M/F)/Number of animals:																	
Feeding condition:		Fasted overnight/fed 4 h postdose			Fasted overnight/fed 4 h postdose				Fasted overnight/fed 4 h postdose			Fasted for ≥10 h/fed 4 h postdose					
Vehicle/Formulation:		0.01 N HCl			0.01 N HCl				0.01 N HCl			50 mM citrate buffer (pH 4)					
Method of Administration:		Oral gavage			Oral gavage				Oral gavage			Oral					
Dose:		20 mg/kg (100 µCi/kg)			5 mg/kg (10 µCi/kg)				10 mg/kg (26.8 µCi/kg)			50 mg (91.5 µCi)					
Radionuclide:		<sup>14</sup> C			<sup>14</sup> C				<sup>14</sup> C			<sup>14</sup> C					
Specific Activity:		4.61 µCi/mg			1.88 µCi/mg				2.45 µCi/mg			1.8 µCi/mg					
Metabolite ID <sup>a</sup>	[M+H] <sup>+</sup>	% Distribution of Radioactivity in plasma															
		Rat			Dog				Monkey			Human					
		1 h	2 h	4 h	1 h	2 h	4 h	8 h	2 h	4 h	8 h	1 h	2 h	4 h	8 h		
M5	348	0.7	2.3	7.4	- <sup>b</sup>	-	-	-	-	-	-	-	-	-	-	-	
M7	348	-	-	-	-	-	-	-	1.6	-	0.8	-	-	-	-	-	
M10	508	-	-	-	-	-	-	-	1.1	-	-	-	-	-	-	-	
M13	332	-	-	-	-	-	-	-	1.5	2.1 <sup>c</sup>	-	-	-	-	-	-	
M1	332	2.6 <sup>c</sup>	2.5 <sup>c</sup>	4.6 <sup>c</sup>	0.4 <sup>c</sup>	1.7 <sup>c</sup>	1.7 <sup>c</sup>	-	0.5	0.7	3.7 <sup>d</sup>	0.5	0.8	1.7	1.1	-	
M19	508	-	-	-	-	-	-	-	0.9	1.3	1.6	-	-	-	-	-	
M22	508	-	-	-	-	-	-	-	1.1	1.0	1.1	-	-	-	-	-	
M2	332	14.4	11.0	7.0	25.1	37.7	44.8	51.3	52.4	48.7	43.0	57.7	70.7	60.4	64.2	-	
M27	321	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
M3	332	7.5 <sup>f</sup>	12.7 <sup>g</sup>	10.4 <sup>f</sup>	2.8	3.1	5.1	9.0	4.3 <sup>f</sup>	4.9 <sup>f</sup>	5.3 <sup>f</sup>	2.7	1.7	3.1	3.7	-	
M41	319	3.6	-	4.5	-	-	-	-	-	-	-	-	-	-	-	-	
M47	303	2.3	4.7	4.2	1.0 <sup>i</sup>	1.0 <sup>i</sup>	0.8 <sup>i</sup>	3.5 <sup>i</sup>	-	-	-	-	-	-	-	-	
M43	492	-	-	-	-	-	-	-	1.3	1.6	1.4	-	-	-	-	-	
M46	492	-	-	-	1.3	2.7	2.4	1.0	9.1	6.9	6.7	0.3	0.7	1.0	-	-	
Parent	316	25.5	21.3	14.8	53.7	36.5	24.7	16.2	18.3	11.4	11.1	32.4	19.2	24.7	25.5	-	
M45	396	-	-	-	ms <sup>j</sup>	-	-	-	ms <sup>j</sup>	-	-	2.2	1.3	1.7	-	-	
M31	303	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
M4	305	4.8 <sup>k</sup>	4.5 <sup>k</sup>	3.3 <sup>k</sup>	5.7 <sup>k</sup>	5.8 <sup>k</sup>	5.7 <sup>k</sup>	8.0 <sup>k</sup>	-	-	-	-	-	-	-	-	
M24	287	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
Total		63.0	61.2	57.0	91.1	89.1	86.2	52.2	92.3	79.3	76.0	97.3	96.4	95.0	95.4	-	

Additional Information: Samples from all animals (n=3) or humans (n=8) at a particular time point were pooled. The structures of metabolites were determined based on their LC/MS fragmentation pattern. Comparison of HPLC retention times and MS fragmentation patterns of M2, M13, D1 and D2 with synthetic standards BMS-510849, BMS-743894, respectively, provided additional verification on the identities of these metabolites.

<sup>a</sup> Metabolite Structures are presented in Table 2.6.5.11. Metabolites in the 1-h plasma samples from rat, dog and human (2-h for monkey) were identified by mass-spectral analysis. At later time points, metabolite identities were assigned on the basis of their retention times in radioprofiles as compared to the earlier samples. Metabolites that were identified as co-eluting in the 1-h samples (2-h for monkey) were assumed to be present and co-elute at subsequent time points.

<sup>b</sup> A dash (-) means that the metabolite was not detected by radioactivity.

<sup>c</sup> Metabolites M10 and M13 co-eluted on the HPLC in the 4-h monkey plasma sample.

<sup>d</sup> Metabolites M10, M13 and M1 co-eluted on the HPLC in the 8-h monkey plasma sample.

<sup>e</sup> Metabolites M13 and M1 co-eluted on the HPLC in rat and dog plasma samples.

<sup>f</sup> Metabolites M27 and M3 co-eluted on the HPLC in rat (1-h and 4-h) and monkey plasma samples.

<sup>g</sup> Metabolites M27, M3 and M41 co-eluted on the HPLC in the rat 2-h sample.

<sup>h</sup> (-) Metabolite M27 was not detected by mass-spectrometry in the 1-h plasma samples from dog and human. Since M27 may co-elute with another metabolite (M3), it is not known whether it was present in samples from subsequent time points, which were not analyzed by MS.

<sup>i</sup> Metabolites M41 and M47 co-eluted on the HPLC in dog plasma.

<sup>j</sup> ms = Metabolite was detected by mass-spectrometry, but not by radioactivity.

<sup>k</sup> Metabolites M31, M4 and M24 co-eluted on the HPLC in rat and dog plasma.

<sup>l</sup> The total sample radioactivity (sum of radioactive peaks) was less than 100% due to the presence of small unidentified radioactive peaks that were distributed throughout the chromatograms.

b(4)

**Pharmacokinetics: Metabolism In Vivo (in Feces and Urine)**

**Study Description or Title:** Biotransformation of [<sup>14</sup>C]saxagliptin in Feces and Urine From Sprague Dawley Rat, Beagle Dog, Cynomolgus Monkey and Human

**Test Article:** [<sup>14</sup>C]Saxagliptin

**Study Type:** Non-GLP

**Study No./Document Control No.:** NA/930016961

**Location in Dossier:**

Species/Strain Gender (M/F)/Number of animals:	Rat/Sprague Dawley M/3	Dog/Beagle M/3	Monkey/Cynomolgus M/3	Human M/6				
<b>Feeding condition:</b>	Fasted overnight/fed 4 h postdose	Fasted overnight/fed 4 h postdose	Fasted overnight/fed 4 h postdose	Fasted for ≥10 h/fed 4 h postdose				
<b>Vehicle/Formulation:</b>	0.01 N HCl	0.01 N HCl	0.01 N HCl	50 mM citrate buffer (pH 4)				
<b>Method of Administration:</b>	Oral gavage	Oral gavage	Oral gavage	Oral				
<b>Dose:</b>	20 mg/kg (100 µCi/kg)	5 mg/kg (10 µCi/kg)	10 mg/kg (26.8 µCi/kg)	50 mg (91.5 µCi)				
<b>Radionuclide:</b>	<sup>14</sup> C	<sup>14</sup> C	<sup>14</sup> C	<sup>14</sup> C				
<b>Specific Activity:</b>	4.61 µCi/mg	1.88 µCi/mg	2.45 µCi/mg	1.8 µCi/mg				
<b>Metabolite<sup>a</sup></b>	<b>% Distribution of Radioactivity in Urine and Feces Samples -% of Dose<sup>b</sup></b>							
	<b>Feces (0-168 h)</b>				<b>Urine (0-168 h)</b>			
	<b>Rat</b>	<b>Dog</b>	<b>Monkey</b>	<b>Human</b>	<b>Rat</b>	<b>Dog</b>	<b>Monkey</b>	<b>Human</b>
Parent	4.5	0.8	3.2	0.5 <sup>c</sup>	13.8	17.4	11.8	33.6
M5	1.2	- <sup>d</sup>	0.8	-	0.8	0.7	0.7	1.0
M7	-	-	-	-	0.4	-	trace	-
M8	-	-	-	-	trace	-	-	trace
M13	-	-	-	-	-	0.5	-	1.4
M1	1.6 <sup>e</sup>	3.7 <sup>e</sup>	5.6 <sup>e</sup>	2.5 <sup>e</sup>	0.3 <sup>e</sup>	0.9	0.8 <sup>e</sup>	0.7
M16	-	-	-	-	-	-	0.4	-
M17	-	-	-	-	0.9	0.9	-	0.4 <sup>f</sup>
M19	-	-	-	-	-	-	0.4	1.6
M22	-	-	-	-	ndms <sup>g</sup>	-	-	-
M25	-	-	-	-	0.8	-	0.3 <sup>h</sup>	-
M2	5.3	2.7	9.6	8.4	5.0	14.1	15.1	28.2
M28	-	-	-	-	-	trace	-	-
M29	-	-	-	-	-	trace	-	-
M37	-	-	-	-	-	ndms <sup>g</sup>	-	ndms <sup>g</sup>
M27	-	-	-	-	2.7 <sup>i</sup>	-	-	-
M40	-	-	-	-	ndms <sup>g</sup> M40	1.3 <sup>k</sup>	2.1 <sup>j</sup>	0.6 <sup>l</sup>
M3	1.4	2.9	1.9	-	-	ndms <sup>g</sup> M40	-	-
M41	-	-	-	-	-	0.5	-	-
M44	-	-	-	-	-	ndms <sup>g</sup>	ndms <sup>g</sup>	ndms <sup>g</sup>
M47	-	-	-	-	3.4 <sup>m</sup>	4.7	0.4	3.8
M49	-	-	-	-	0.5	-	-	trace
M46	-	-	-	-	-	0.9	1.9	0.5
D2	-	-	-	-	-	-	-	-
M45	-	-	-	-	-	-	0.3	trace
M34	-	-	-	-	0.7	-	-	-
M31	-	-	-	-	0.9	-	-	-
M4	2.0	-	-	-	-	-	-	-
M24	-	-	-	-	1.1 <sup>n</sup>	2.8 <sup>n</sup>	-	-
M30	-	-	-	-	-	0.6	0.5	0.6

**Additional Information:** Samples from all animals (n=3) or humans (n=8) at a particular time point or time interval were pooled. The structures of metabolites were determined based on their LC/MS fragmentation pattern. Comparison of HPLC retention times and MS fragmentation patterns of M2, M13, D1 and D2 with synthetic standards BMS-510849, BMS-743894, respectively, provided additional verification on the identities of these metabolites.

<sup>a</sup> Metabolite structures are presented in Table 2.6.5.11.

<sup>b</sup> The excretion of drug-related radioactivity in urine and bile for these species is reported in Table 2.6.5.13A. Metabolites of saxagliptin in feces and urine were identified by LC/MS analysis of more concentrated samples that were collected over shorter time intervals (ie: 0-12, 0-24, or 0-48 h, DCN930016961). The identities of metabolites in the 0-168-h fecal and urine samples were assigned on the basis of their peak retention times in radioprofiles as compared to the more concentrated samples. Metabolites marked as "trace" were identified in the more concentrated samples, but a corresponding radioactive peak was not observed in the 0-168-h profiles.

<sup>c</sup> In 0-168 h human feces, saxagliptin was detected by radioactivity, but not by mass-spectrometry.

<sup>d</sup> A dash (-) means that the metabolite was not detected in the sample.

<sup>e</sup> Metabolites M13 and M1 co-eluted on the HPLC in fecal samples and in rat and monkey urine samples.

<sup>f</sup> Metabolites M16 and M17 co-eluted on the HPLC in the human urine sample.

<sup>g</sup> ndms = Metabolite was detected by radioactivity, but not by mass-spectrometry.

<sup>k</sup> Metabolites M27 and M3 co-eluted on the HPLC in dog urine.

<sup>h</sup> Metabolites M22 and M25 co-eluted on the HPLC in monkey urine.

<sup>l</sup> Metabolites M27, M40 and M3 co-eluted on the HPLC in human urine.

<sup>i</sup> Metabolites M37, M27 and M3 co-eluted on the HPLC in rat urine.

<sup>m</sup> Metabolites M44 and M47 co-eluted on the HPLC in rat urine.

<sup>j</sup> Metabolites M37, M27, M40 and M3 co-eluted on the HPLC in monkey urine.

<sup>n</sup> Metabolites M4 and M24 co-eluted on the HPLC in rat and dog urine.

b(4)

**Pharmacokinetics: Metabolism In Vivo (Bile-Duct Cannulated Rat)**

**Study Description or Title:** Biotransformation of [<sup>14</sup>C]saxagliptin in Bile-Duct Cannulated Sprague Dawley Rat

**Test Article:** [<sup>14</sup>C]Saxagliptin

**Study Type:** Non-GLP

**Study No./Document Control No.:** NA/930016961

**Location in Dossier:**

<b>Species/Strain</b>	Rat/Sprague Dawley	
<b>Number of animals:</b>	M/3	
<b>Feeding condition:</b>	Fasted overnight and for duration of study	
<b>Vehicle/Formulation:</b>	Water	
<b>Method of Administration:</b>	Oral gavage	
<b>Dose:</b>	20 mg/kg (150 µCi/kg)	
<b>Radionuclide:</b>	<sup>14</sup> C	
<b>Specific Activity:</b>	7.5 µCi/mg	
<b>Metabolite<sup>a</sup></b>	<b>% Distribution of Radioactivity -% of Dose<sup>b</sup></b>	
	<b>Urine (0-24 h)</b>	<b>Bile (0-24 h)</b>
Parent	13.9	5.7
M5	0.8	0.7
M7	0.2	0.1
M8	0.1	0.1
M13	1.0 <sup>c</sup>	1.6 <sup>c</sup>
M1		
M16	- <sup>d</sup>	0.2 <sup>e</sup>
M17	0.5	
M18	-	0.4 <sup>f</sup>
M19	-	
M2	4.1	5.6
M37	2.3 <sup>g</sup>	-
M27		-
M3		0.7
M41		-
M44		0.6
M43	-	0.3
M47	1.9	0.4
D1		
M46	1.1	2.7
M31	0.4	-
M4	0.6 <sup>h</sup>	-
M24		-

**Additional Information:** Samples from BDC rats (n=3) at a particular time point or time interval were pooled. The structures of metabolites were determined based on their LC/MS fragmentation pattern. Comparison of HPLC retention times and MS fragmentation patterns of M2, M13, D1 and D2 with synthetic standards BMS-510849, BMS-743894, respectively, provided additional verification on the identities of these metabolites.

- <sup>a</sup> Structures of metabolites are presented in Table 2.6.5.11. Metabolites in urine and bile samples from BDC rat were identified by LC/MS<sup>2</sup> analysis.
- <sup>b</sup> The excretion of drug-related radioactivity in urine and bile is reported in Table 2.6.5.14A. The relative distribution of saxagliptin and its metabolites in urine and bile is expressed as the % of the dose that each radioactive component represents in a particular matrix.
- <sup>c</sup> Metabolites M13 and M1 co-eluted on the HPLC in urine and bile samples.
- <sup>d</sup> A dash (-) means that the metabolite was not detected in the sample.
- <sup>e</sup> In bile, metabolites M16 and M17 co-eluted on the HPLC.
- <sup>f</sup> In bile, metabolites M18 and M19 co-eluted on the HPLC.
- <sup>g</sup> In urine, metabolites M37, M27, M3, M41 and M44 co-eluted on the HPLC.
- <sup>h</sup> In urine, metabolites M4 and M24 in urine co-eluted on the HPLC.

b(4)

**Pharmacokinetics: Metabolism In Vivo (in Plasma and Brain from Rats and Mice)**

**Study Description or Title:** Biotransformation Profiles of Saxagliptin in Plasma and Brain From Male and Female Harlan Sprague Dawley Rats and CD-1 Mice after Oral Administration of [<sup>14</sup>C]Saxagliptin

**Test Article:** [<sup>14</sup>C]Saxagliptin

**Study Type:** Non-GLP

**Study No./Document Control No.:** NA/930023887

**Location in Dossier:**

Metabolite <sup>a</sup>	% Distribution of Radioactivity in Plasma and Brain (0-24 h) <sup>b</sup>															
	Plasma								Brain							
	Male Rat		Female Rat		Male Mouse		Female Mouse		Male Rat		Female Rat		Male Mouse		Female Mouse	
	1 h	4 h	1 h	4 h	1 h	4 h	1 h	4 h	1 h	4 h	1 h	4 h	1 h	4 h	1 h	4 h
Parent	37.5	27.6	86.2	58.9	31.4	11.1	37.0	17.3	42.2	48.8	90.2	57.1	67.8	40.5	71.2	55.5
M5	0.3	2.6	- <sup>c</sup>	0.6	0.3	3.4	0.5	3.6	-	-	-	-	-	-	-	-
M7	-	- <sup>d</sup>	-	-	0.3 <sup>e</sup>	1.8 <sup>e</sup>	-	1.3 <sup>e</sup>	-	-	-	-	-	-	-	-
M8	0.3	0.6	-	-	-	-	-	-	-	-	-	-	-	-	-	-
M9	0.2	0.5	-	-	-	-	-	-	-	-	-	-	-	-	-	-
M11	-	-	0.3 <sup>h</sup>	1.7 <sup>h</sup>	-	-	-	-	1.5	5.7	-	1.1	-	-	-	-
M13	-	-	-	-	2.0 <sup>i</sup>	3.3 <sup>i</sup>	-	1.7 <sup>j</sup>	-	-	-	-	-	-	-	-
M14	3.6 <sup>f</sup>	8.1 <sup>f</sup>	-	- <sup>g</sup>	-	-	0.8 <sup>j</sup>	1.7 <sup>j</sup>	-	-	-	-	-	-	-	-
M1	nd M14	nd M14	-	-	-	-	nd M14	nd M14	-	-	-	-	-	-	-	-
M16	-	-	0.2 <sup>k</sup>	0.3 <sup>k</sup>	1.0 <sup>l</sup>	1.9 <sup>l</sup>	0.8	-	-	-	-	-	-	-	-	-
M20	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
M23	-	-	-	-	0.8	4.3	3.4	4.9	-	-	-	-	-	-	-	-
M2	15.4	17.8	5.8	22.5	35.1	29.5	37.4	39.9	9.9	17.2	2.9	14.3	8.6	15.5	13.7	15.0
M26	0.5	1.0	-	-	-	-	-	-	-	-	-	-	-	-	-	-
M27	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
M28	4.2 <sup>m</sup>	8.9 <sup>m</sup>	0.9 <sup>m</sup>	3.3 <sup>m</sup>	2.7 <sup>o</sup>	8.8 <sup>o</sup>	2.3 <sup>o</sup>	7.5 <sup>o</sup>	-	-	-	-	-	-	-	-
M3	nd M28	nd M28	nd M28	nd M28	-	-	-	-	2.1	12.1	-	2.6	-	5.2	1.4	4.5
M41	-	-	-	-	4.2 <sup>p</sup>	6.2 <sup>p</sup>	4.2 <sup>p</sup>	6.7 <sup>p</sup>	-	-	-	-	-	-	-	-
M42	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
M44	3.9 <sup>q</sup>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
M43	-	7.2 <sup>q</sup>	-	-	-	-	-	-	-	-	-	-	-	-	-	-
M47	3.5 <sup>q</sup>	-	-	-	1.7 <sup>r</sup>	4.3 <sup>r</sup>	1.6 <sup>r</sup>	2.8 <sup>r</sup>	3.7	3.4	-	-	-	-	-	-
D1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
M51	1.1	0.5	-	-	-	-	-	-	-	-	-	-	-	-	-	-
M52	-	-	-	-	5.0 <sup>s</sup>	5.1 <sup>s</sup>	5.6 <sup>s</sup>	3.6 <sup>s</sup>	-	-	-	-	-	-	-	-
M46	1.5	1.1	0.8	0.4	-	-	-	-	-	-	-	-	-	-	-	-
M31	-	-	-	-	-	-	-	-	1.6	-	-	-	-	-	-	-
M4	6.1 <sup>t</sup>	3.6 <sup>t</sup>	0.7 <sup>t</sup>	1.4 <sup>t</sup>	0.9 <sup>t</sup>	-	0.8 <sup>t</sup>	-	7.1 <sup>u</sup>	8.4 <sup>u</sup>	0.7 <sup>u</sup>	4.2 <sup>u</sup>	-	-	-	-
M24	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
M6	-	-	-	-	10.4	3.0	6.3	3.2	-	-	-	-	4.3 <sup>v</sup>	2.3 <sup>v</sup>	1.7 <sup>v</sup>	-
Total <sup>w</sup>	79.6	79.5	97.1	92.2	97.2	83.3	97.9	92.5	72.1	98.3	96.2	85.3	80.7	66.4	90.3	75.0

Additional Information: Samples from male and female rats (n=3) and mouse (n=5) at a particular time point or time interval were pooled. The structures of metabolites were determined based on their LC/MS fragmentation pattern. Comparison of HPLC retention times and MS fragmentation patterns of M2, M13 and D1 with synthetic standards BMS-510849, BMS-743894 and \_\_\_\_\_ respectively, provided additional verification on the identities of these metabolites.

<sup>a</sup> Metabolite structures are presented in Table 2.6.5.11. Metabolites in the 1-h plasma samples from rats and mice and 1-h brain samples from male rats and male and female mice were identified by LC/MS<sup>n</sup> analysis. For the 1-h samples from female rat brain and the 4-h plasma and brain samples from rats and mice, the identities of metabolites were assigned on the basis of their retention times in the radioprofiles as compared to the 1-h plasma samples, 1-h brain

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- samples from other species, or urine samples. Metabolites that were identified as co-eluting in the 1-h samples were assumed to be present and co-eluting in the 4-h samples.
- <sup>b</sup> The relative distribution in plasma and brain is expressed as the % distribution of radioactivity of each radioactive component in the sample extract.
- <sup>c</sup> A dash (-) means that the metabolite was not detected in the sample.
- <sup>d</sup> (-) Metabolite M7 in 4 h male rat plasma was not detected by mass-spectrometry in the 1-h plasma samples from male rats. Since M7 may co-elute with another metabolite (M8), it is not known whether it was present in samples from the 4 h time point, which were not analyzed by MS.
- <sup>e</sup> Metabolites M7 and M8 were identified by mass-spectrometry in the 1 h plasma samples from male mice, but co-eluted on the HPLC. Both metabolites were assumed to be present in the 4 h sample from female mice.
- <sup>f</sup> Metabolites M11, M13, M1, M16 and M20 in male rat plasma were identified by mass-spectrometry in the 1 h plasma sample from male rats, but co-eluted on the HPLC.
- <sup>g</sup> (-) Metabolite M14 was not detected by mass-spectrometry in the 1 h plasma samples from male rats, female rats, and female mice. Since M14 may co-elute with several other metabolites (M11, M13, M1, M16, or M20), it is not known whether it was present in samples from the 4 h time point, which were not analyzed by MS.
- <sup>h</sup> Metabolites M11 and M13 were identified by mass-spectrometry in the 1 h female rat plasma, but co-eluted on the HPLC.
- <sup>i</sup> Metabolites M11, M13, M14 and M1 were identified by mass-spectrometry in the 1 h male mouse plasma, but co-eluted on the HPLC.
- <sup>j</sup> Metabolites M13 and M1 were identified by mass-spectrometry in the 1 h plasma sample from female mice, but co-eluted on the HPLC.
- <sup>k</sup> Metabolites M1, M16 and M20 were identified by mass-spectrometry in the 1 plasma samples from female rats, but co-eluted on the HPLC.
- <sup>l</sup> Metabolites M16 and M20 were identified by mass-spectrometry in the 1 h plasma samples from male mice, but co-eluted on the HPLC.
- <sup>m</sup> Metabolites M27, M3 and M41 were identified by mass-spectrometry in the 1 h plasma samples from male and female rats, but co-eluted on the HPLC.
- <sup>n</sup> (-) Metabolite M28 was not detected by mass-spectrometry in the 1-h plasma samples from male and female rats. Since M28 may co-elute with several other metabolites (M27, M3, M41 or 42), it is not known whether it was present in samples from the 4 h time point, which were not analyzed by MS.
- <sup>o</sup> Metabolites M27 and M28 were identified by mass-spectrometry in the 1 h plasma samples from male and female mice, but co-eluted on the HPLC.
- <sup>p</sup> Metabolites M3, M41 and M42 were identified by mass-spectrometry in the 1 h plasma samples from male and female mice, but co-eluted on the HPLC.
- <sup>q</sup> Metabolites M44, M43 and M47 were identified by mass-spectrometry in the 1 h plasma samples from male rats, and metabolites M43 and M47 co-eluted on the HPLC. In the 4 h sample, it appears that the three metabolites were co-eluting on the HPLC.
- <sup>r</sup> Metabolites M43 and M47 were identified by mass-spectrometry in 1 h plasma samples from male and female mice, but co-eluted on the HPLC.
- <sup>s</sup> Metabolites M51, M52 and M46 were identified by mass-spectrometry in the 1 h plasma samples from male and female mice, but co-eluted on the HPLC.
- <sup>t</sup> Metabolites M31, M4 and M24 were identified by mass-spectrometry in 1 h plasma samples from male and female rats and mice, but co-eluted on the HPLC.
- <sup>u</sup> Metabolites M4 and M24 were identified by mass-spectrometry in the 1 h brain sample from male rats, but co-eluted on the HPLC. These metabolites were assumed to be present and co-eluting in the 4 h sample from male rats and the 1 and 4 h sample from female rats.
- <sup>v</sup> Metabolite M6 was identified by mass-spectrometry in mouse plasma and urine samples. In brain samples, its identity was assigned on the basis of its retention time relative to the plasma and urine samples.
- <sup>w</sup> The total sample radioactivity (sum of radioactive peaks) was less than 100% due to the presence of small unidentified radioactive peaks that were distributed throughout the chromatograms.

**Pharmacokinetics: Metabolism In Vivo (in Urine from Rat and Mouse Brain Penetration Studies)**

**Study Description or Title:** Biotransformation of [<sup>14</sup>C]saxagliptin in Urine From Male and Female Harlan Sprague Dawley Rats and CD-1 Mice for Brain Penetration Study

**Test Article:** [<sup>14</sup>C]Saxagliptin

**Study Type:** Non-GLP

**Study No./Document Control No.:** NA/930023887

**Location in Dossier:**

Metabolite <sup>a</sup>	% Distribution of Radioactivity in Urine (0-24 h) -% of Dose <sup>b</sup>			
	Rat		Mouse	
	Male	Female	Male	Female
Parent	17.8	45.5	13.2	11.2
M5	0.7	- <sup>c</sup>	0.9	0.6
M7	0.2	-	0.4	0.3 <sup>d</sup>
M8	0.1	-	0.2	-
M11	0.3 <sup>c</sup>	-	1.5 <sup>f</sup>	-
M12	-	-		1.1 <sup>g</sup>
M13	0.7	-		-
M14	-	-		-
M1	0.4	-	-	-
M16	0.3 <sup>h</sup>	-	1.4 <sup>i</sup>	0.7 <sup>h</sup>
M17	-	-		-
M18	-	-	-	-
M20	-	-	1.6 <sup>j</sup>	1.1 <sup>j</sup>
M21	-	-		-
M2	4.3	2.8	14.0 <sup>k</sup>	12.1 <sup>k</sup>
M32	-	-		-
M33	-	-	0.5	0.4
M37	1.8 <sup>l</sup> nd M40	-	2.9 <sup>m</sup>	2.2 <sup>m</sup>
M27		-		-
M40		-	0.6	0.3
M3		0.5	-	-
M42	-	-	4.0 <sup>n</sup> nd M44	3.5 <sup>n</sup> nd M44
M44	3.0 <sup>o</sup>	-		-
M47	-	0.4	-	-
M49	0.6	-	-	-
D1	-----			
M52	-	-	0.8	0.7
M46	0.2	0.2	5.0	3.3
M31	0.7	-	-	-
M4	0.8 <sup>p</sup>	0.1 <sup>p</sup>	0.1 <sup>p</sup>	0.2 <sup>p</sup>
M24				
M6	-	-	3.2	1.7

Additional Information: Urine samples from rats (n=3) and mice (n=5) were pooled by gender over the 0-24 h interval. The structures of metabolites were determined based on their LC/MS fragmentation pattern. Comparison of HPLC retention times and MS fragmentation patterns of M2, M13 and D1 with synthetic standards BMS-510849, BMS-743894 and \_\_\_\_\_ respectively, provided additional verification on the identities of these metabolites. Feces samples were not collected for this study.

<sup>a</sup> Structures of metabolites are presented in Table 2.6.5.11. Metabolites in urine samples from rats and mice were identified by LC/MS<sup>n</sup> analysis.

<sup>b</sup> The excretion of drug-related radioactivity in urine from male and female rats and mice is reported in Table 2.6.5.13B. The relative distribution of saxagliptin and its metabolites is expressed as the % of the dose that each radioactive component represents in urine.

<sup>c</sup> A dash (-) means that the metabolite was not detected in the sample.

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- <sup>d</sup> Metabolites M7 and M8 were identified by mass-spectrometry in female mouse urine, but co-eluted on the HPLC.
- <sup>e</sup> Metabolites M11 and M12 were identified by mass-spectrometry in male rat urine, but co-eluted on the HPLC.
- <sup>f</sup> Metabolites M11, M12, M13, M14 and M1 were identified by mass-spectrometry in male mouse urine, but co-eluted on the HPLC.
- <sup>g</sup> Metabolites M12 and M13 were identified by mass-spectrometry in female mouse urine, but co-eluted on the HPLC.
- <sup>h</sup> Metabolites M16 and M17 were identified by mass-spectrometry in male rat and female mouse urine, but co-eluted on the HPLC.
- <sup>i</sup> Metabolites M16, M17, and M18 were identified by mass-spectrometry in male mouse, but co-eluted on the HPLC.
- <sup>j</sup> Metabolites M20 and M21 were identified by mass-spectrometry in male and female mouse urine, but co-eluted on the HPLC.
- <sup>k</sup> Metabolites M2 and M32 were identified by mass-spectrometry in male and female mouse urine, but co-eluted on the HPLC.
- <sup>l</sup> Metabolites M37, M27 and M3 were identified by mass-spectrometry in male rat urine, but co-eluted on the HPLC. Metabolite M40, which may have co-eluted with these metabolites, was not detected by mass-spectrometry in the male rat urine sample.
- <sup>m</sup> Metabolites M37 and M27 were identified by mass-spectrometry, but co-eluted on the HPLC in urine from male and female mice.
- <sup>n</sup> Metabolites M3, M42 and M47 were identified by mass-spectrometry in male and female mouse urine, but co-eluted on the HPLC. Metabolite M44, which may have co-eluted with these metabolites, was not detected by mass-spectrometry in these samples.
- <sup>o</sup> Metabolites M44 and M47 were identified by mass-spectrometry, but co-eluted on the HPLC in urine from male rats.
- <sup>p</sup> Metabolites M4 and M24 were identified by mass-spectrometry, but co-eluted on the HPLC in urine from male and female rats and mice.

**Pharmacokinetics: Metabolism In Vivo (in Rats after Pre-Treatment with Cimetidine)**

**Study Description or Title:** Biotransformation of [<sup>14</sup>C]Saxagliptin in Male Sprague Dawley Rats after Pre-Treatment with Cimetidine  
**Test Article:** [<sup>14</sup>C]Saxagliptin  
**Study Type:** Non-GLP  
**Study No./Document Control No.:** NA/930016963  
**Location in Dossier:**

<b>Species/Strain</b>	Rat/Sprague Dawley			
<b>Number of animals:</b>	M/18			
<b>Feeding condition:</b>	Fasted overnight and for duration of study			
<b>Vehicle/Formulation:</b>	Groups 1 + 2-Cimetidine/in water, adjusted to pH 7.5 followed 2 h later by [ <sup>14</sup> C]saxagliptin/in acidified water (pH-5.5) Groups 3 + 4-Water, adjusted to pH 7.5 followed 2 h later by [ <sup>14</sup> C]saxagliptin/in acidified water (pH-5.5)			
<b>Method of Administration:</b>	Oral gavage			
<b>Dose:</b>	Cimetidine- 300 mg/kg; [ <sup>14</sup> C] saxagliptin-150 mg/kg (100 µCi/kg)			
<b>Radionuclide:</b>	<sup>14</sup> C			
<b>Specific Activity:</b>	7.5 µCi/mg			
<b>Study System:</b>	Rats in groups 1 (n=3) and 2 (n=6) were administered an oral dose of cimetidine (300 mg/kg) 2 h prior to an oral dose of [ <sup>14</sup> C]saxagliptin (150 mg/kg, 100 µCi/kg). Rats in groups 3 (n=3) and 4 (n=6) were administered a vehicle control (water, adjusted to pH 7.5) 2 h prior to an oral dose of [ <sup>14</sup> C]saxagliptin (150 mg/kg, 100 µCi/kg). Urine was collected from the animals in groups 1 and 3 for the 0-24-h interval after [ <sup>14</sup> C]saxagliptin administration. In addition, terminal blood samples for plasma were collected at 24 h. Terminal blood samples for plasma were collected from the animals in groups 2 and 4 at 2 and 8 h after [ <sup>14</sup> C]Saxagliptin administration (n=3 per treatment per time point). The 0-24 h urine samples and 2 h plasma samples were analyzed by HPLC-radioprofiling. The radioactivity in plasma samples from later time points was too low to produce meaningful profiles.			
<b>Metabolite<sup>a</sup></b>	<b>% Distribution of Radioactivity<sup>b</sup></b>			
	<b>Urine (0-24 h) Group 1 (Cimetidine/Saxagliptin)</b>	<b>Urine (0-24 h) Group 3 (Water/Saxagliptin)</b>	<b>Plasma (2 h) Group 2 (Cimetidine/Saxagliptin)</b>	<b>Plasma (2 h) Group 4 (Water/Saxagliptin)</b>
Parent	20.3	15.7	39.7	33.8
M2	7.2	4.0	32.8	14.8
M4	0.2	1.2	2.2	3.7
<b>Additional Information:</b> Samples from rats (n=3) at a particular time point or time interval were pooled.				

<sup>a</sup> Structures of metabolites are presented in Table 2.6.5.11. Saxagliptin, M2 and M4 were identified in urine and plasma samples on the basis of their retention times relative to radioprofiles in which the identity of these compounds had been confirmed by LC/MS<sup>n</sup> analysis.

<sup>b</sup> The relative distribution in urine and plasma is expressed as the % distribution of radioactivity for each radioactive component in the sample.

**Pharmacokinetics: Metabolism In Vivo (BMS-510849 in BDC Rats)**

Study Description or Title: Biotransformation of [<sup>14</sup>C]BMS-510849 in Male and Female Bile-Duct Cannulated Sprague Dawley Rats

Test Article: [<sup>14</sup>C]BMS-510849

Study Type: Non-GLP

Study No./Document Control No.: NA/930023997

Location in Dossier:

Species/Strain	Rat/Sprague Dawley									
Number of animals:	M/F/4 (2 per gender)									
Feeding condition:	Fasted overnight and for duration of study									
Vehicle/Formulation:	Water									
Method of Administration:	Oral gavage									
Dose:	20 mg/kg (150 µCi/kg)									
Radionuclide:	<sup>14</sup> C									
Specific Activity:	7.5 µCi/mg									
Metabolite <sup>a</sup>	% Distribution of Radioactivity in BDC rats (0-24 h) <sup>b</sup>									
	Urine (% of Dose)		Bile (% of Dose)		Male Plasma (% of Sample)			Female Plasma (% of Sample)		
	Male	Female	Male	Female	1 h	4 h	8 h	1 h	4 h	
BMS-510849 (P)	38.5	23.8	2.4	4.6	69.0	27.5	25.1	76.5	62.0	
M5	0.8	- <sup>c</sup>	0.3	-	-	9.5 <sup>d</sup>	9.0 <sup>d</sup>	-	2.6 <sup>d</sup>	
BMS-743894 (M13)	3.2	1.9	0.5	0.5	11.1	6.1	10.6	9.8	9.3	
M18	-	-	0.1	0.1	-	-	-	-	-	
M37	1.2 <sup>e</sup>	-	-	-	-	-	-	-	-	
M27		-	0.4 <sup>f</sup>	-	2.9	17.3	8.2	1.6	8.0	
M3 <sup>g</sup>	-	-	-	-	-	-	-	-	-	
M47	2.0	0.9	-	-	4.1	5.2	5.5	3.2	5.4	
Total	45.7	26.6	3.7	5.2	87.1	65.6	58.4	91.1	87.3	

Additional Information: Samples from BDC rats (n=3) at a particular time point or time interval were pooled by gender. The structures of metabolites were determined based on their LC/MS fragmentation pattern. For consistency in reporting, drug-related products were assigned metabolite identification numbers according their designations in studies with saxagliptin. BMS-743894 (M13) is a known degradant of BMS-510849 and was identified in studies with saxagliptin. Comparison of HPLC retention times and MS fragmentation patterns of M13 with a synthetic standard, BMS-743894, provided additional verification on the identity of this peak.

<sup>a</sup> Structures of metabolites are presented in Table 2.6.5.11. Metabolites in the 0-24-h urine samples and the 1 h plasma samples from male and female rats were identified by LC/MS<sup>n</sup> analysis. For the 4 h and 8 h plasma samples from male rats and the 4 h sample from female rats, the identities of metabolites were assigned on the basis of their retention times in the radioprofiles as compared to the 1 h plasma samples.

<sup>b</sup> The relative distribution of BMS-510849 and its metabolites in rat urine and bile is expressed as the % of the dose that each radioactive component represents in a particular matrix. The relative distribution in plasma is expressed as the % distribution of radioactivity of each radioactive component in the sample extract.

<sup>c</sup> A dash (-) means that the metabolite was not detected in the sample.

<sup>d</sup> M5 in 4 and 8 h plasma samples was identified based on comparison of the radioactive peaks in the plasma profile with corresponding peaks in the urine and bile samples.

<sup>e</sup> M37 and M27 were identified by mass-spectrometry but co-eluted on the HPLC in male rat urine.

<sup>f</sup> M27 and M3 were identified by mass-spectrometry but co-eluted on the HPLC in male rat bile.

<sup>g</sup> It is unlikely that M3, a hydroxylated metabolite of saxagliptin and an isomer of BMS-510849, was formed by metabolism of BMS-510849. A possible explanation for its presence in the bile of male rats is that M3 was present in the dosing solution and was concentrated upon excretion in the bile.

**Pharmacokinetics: Metabolism *In Vitro* (in Hepatocytes)**

**Study Description or Title:** Comparative Biotransformation of Saxagliptin in Rat, Dog, Monkey and Human Hepatocytes  
**Test Article:** Saxagliptin  
**Study Type:** Non-GLP  
**Location in Dossier:**  
**Study No./Document Control No.** MAP005-477118/930000866

**Study system:** Hepatocytes, 180 min, 5 µM BMS-477118

Metabolite	Rat (RII)	Dog (DE)	Monkey (MKH)	Human (HH)
Parent (BMS-477118)	MS	MS	MS	MS
M2 (BMS-510849)	MS	MS	MS	MS
D1	MS	MS	MS	MS
Mono-hydroxylated metabolite-1	MS	-	MS	-
Mono-hydroxylated metabolite-2	MS	MS	-	-
Mono-hydroxylated metabolite-3	MS	MS	-	-

Additional Information: MS =detected by Mass spectrometry; a dash (-) means that a metabolite was not detected. No conjugated metabolites were detected in the hepatocyte incubations from any of the species evaluated.

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**Pharmacokinetics: Metabolism *In Vitro* (in Liver Microsomes and CYP Enzymes)**

**Study Description or Title:** Biotransformation of [<sup>14</sup>C]Saxagliptin in Mouse, Rat, Dog, and Human Liver Microsomes and in Human cDNA-expressed CYP Enzymes  
**Test Article:** [<sup>14</sup>C]Saxagliptin  
**Study Type:** Non-GLP  
**Location in Dossier:**  
**Study No./Document Control No.** DDBS018-477118/930004422

**Study system:** Mouse, Rat, Dog and Human Liver Microsomes; 1 h, 10 µM [<sup>14</sup>C]Saxagliptin  
 cDNA-Expressed CYP1A2, 2A6, 2B6, 2C8, 2C9, 2C18, 2C19, 2D6, 2E1, 3A4 and 3A5; 30 min, 10 µM [<sup>14</sup>C]Saxagliptin

Metabolite <sup>a</sup>	% Radioactivity					
	Mouse (MLM)	Rat (RLM)	Dog (DLM)	Human (HLM)	Human CYP3A4	Human CYP3A5
Saxagliptin (BMS-477118)	39.62	33.65	79.81	68.11	54.94	86.13
M1	2.75	3.95	0.47	2.57	3.70	1.97
BMS-510849 (M2)	42.68	40.68	12.79	23.00	34.02	7.77
M3	5.92	3.86	0.68	2.55	1.43	1.86
	1.43	4.78	0.82	0.85	1.48	0.65

Additional Information: Radioactive peaks are reported as a percentage of the total radioactivity eluted from the column after background subtraction. Saxagliptin was not metabolized in incubations with cDNA expressed CYP1A2, 2A6, 2B6, 2C8, 2C9, 2C18, 2C19, 2D6, or 2E1.

<sup>a</sup> Structures are presented in Table 2.6.5.11.

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**Pharmacokinetics: Metabolism *In Vitro* (in Monkey Liver Microsomes)**

**Study Description or Title:** Biotransformation of [<sup>14</sup>C]Saxagliptin in Male and Female Monkey Liver Microsomes  
**Test Article:** [<sup>14</sup>C]Saxagliptin  
**Study Type:** Non-GLP  
**Location in Dossier:**  
**Study No./Document Control No.** NA/930022225

**Study system:** Liver microsomes, 30 min, 10 and 100 µM [<sup>14</sup>C]Saxagliptin

Metabolite <sup>a</sup>	% Radioactivity <sup>b</sup>			
	Monkey Liver Microsomes (Male)		Monkey Liver Microsomes (Female)	
	10 µM	100 µM	10 µM	100 µM
Saxagliptin (BMS-477118)	49.4	60.0	54.1	69.1
M	2.0 <sup>c</sup>	2.4	1.4 <sup>c</sup>	1.2
BMS-743894 (M13)				
BMS-510849 (M2)	40.8	23.4	37.4	18.9
M3	2.7	2.0	2.3	1.5
M16	0.6	0.5	0.4	0.3
	2.5	2.6	1.9	2.6
Total <sup>d</sup>	98.0	90.9	97.5	93.6

<sup>a</sup> Structures of Metabolites are presented in Table 2.6.5.11. Metabolites in the 10 µM microsomal samples were identified by mass-spectrometry. Metabolites in the 100 µM microsomal samples were assigned on the basis of their retention times in the radioprofiles as compared to the 10 µM samples.

<sup>b</sup> Radioactive peaks are reported as a percentage of the total radioactivity eluted from the column after background subtraction.

<sup>c</sup> Metabolites M13 and M1 were identified by mass-spectrometry in 10 µM microsomal samples, but co-eluted on the HPLC. These metabolites were assumed to be present and co-eluting in the 100 µM samples.

<sup>d</sup> The total sample radioactivity (sum of radioactive peaks) was less than 100% due to the presence of small unidentified radioactive peaks that were distributed throughout the chromatograms.

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**Pharmacokinetics: Metabolism *In Vitro* (Formation of BMS-510849 in Aroclor-Induced Rat S9)**

**Study Description or Title:** Formation of BMS-510849, a Hydroxylated Metabolite, in Aroclor-Induced Rat S9 Incubation with Saxagliptin

**Test Article:** Saxagliptin

**Study Type:** Non-GLP

**Location in Dossier:**

**Study No./Document Control No.** DDBS042-477118/930008721

**Study system:** Aroclor-Induced rat S9; 0, 1 and 2 h; 0.1, 1.0 and 5.0 mM Saxagliptin

Saxagliptin (mM) in S-9 incubation	Incubation time (h)	% Disappearance of Saxagliptin	Assayed Saxagliptin (mM)	Assayed BMS-510849 (mM) <sup>a</sup>
0.1 mM	0	-	0.104	<LLQ (0.002 mM)
	1	78.8	0.022	0.082
	2	92.3	0.008	0.079
1.0 mM	0	-	0.873	<LLQ (0.018 mM)
	1	42.5	0.502	0.271
	2	68.5	0.275	0.401
5.0 mM	0	-	5.90	<LLQ (0.091 mM)
	1	13.7	5.09	0.294
	2	25.6	4.39	0.570

**Additional Information:** LLQ: Lower Limit of Quantitation. Saxagliptin and BMS-510849 concentrations were determined by LC-MS/MS analysis.

**Results:** These results suggest that BMS-510849, an active, hydroxylated metabolite of saxagliptin, was formed in S9 incubations.

<sup>a</sup> The bioanalytical assay that was used to measure concentrations of BMS-510849 in this study was not completely specific for BMS-510849. Other mono-hydroxylated metabolites with the same MRM transition as BMS-510849 in the LC-MS/MS (332 → 196) co-eluted with BMS-510849 under the conditions employed in the assay. Therefore the values for BMS-510849 concentration reported should be considered as "BMS-510849 and other mono-hydroxylated metabolites." The measurement of saxagliptin was not impacted in this method.

**Pharmacokinetics: Metabolism *In Vitro* (BMS-510849 in Liver Microsomes)**

**Study Description or Title:** Biotransformation of [<sup>14</sup>C]BMS-510849 in Mouse, Rat, Dog, Monkey and Human Liver Microsomes

**Test Article:** [<sup>14</sup>C]BMS-510849

**Study Type:** Non-GLP

**Location in Dossier:**

**Study No./Document Control No.** NA/930023997

**Study system:** Liver microsomes, 30 min, 10 and 100 μM [<sup>14</sup>C]BMS-510849

Metabolite <sup>a</sup>	% Radioactivity <sup>b</sup>									
	Mouse		Rat		Dog	Monkey <sup>c</sup>				Human
	Male	Female	Male	Female	Male	Male		Female		M/F (pooled)
	10 μM	100 μM	100 μM	100 μM	100 μM	10 μM	100 μM <sup>d</sup>	10 μM	100 μM <sup>d</sup>	100 μM
BMS-510849 (Parent)	94.9	88.7	87.1	85.1	87.4	77.0	76.3	74.8	72.5	85.8
M13	4.2	4.8	4.7	4.3	4.4	5.8	7.1	7.9	8.6	4.8
Total <sup>e</sup>	99.1	93.5	91.8	89.4	91.8	82.8	83.4	82.7	81.1	90.6

<sup>a</sup> Structures are presented in Table 2.6.5.11.

<sup>b</sup> Radioactive peaks are reported as a percentage of the total radioactivity eluted from the HPLC column after background subtraction.

<sup>c</sup> The lot of [<sup>14</sup>C]BMS-510849 used for incubations with monkey liver microsomes had a lower radiochemical purity (93%) than the lot used for incubations with liver microsomes from other species (99%).

<sup>d</sup> The 100 μM incubations with monkey liver microsomes were not analyzed by mass-spectrometry. In these samples, BMS-510849 and BMS-743894 were identified by comparison of the radioactive peaks in these profiles to the corresponding peaks in the radioprofiles from the 10 μM monkey liver microsomal incubations.

<sup>e</sup> The total sample radioactivity (sum of radioactive peaks) was less than 100% due to the presence of small radioactive peaks that were distributed throughout the chromatogram that could not be identified by LC-MS/MS analysis. Most of these peaks were also present in the [<sup>14</sup>C]BMS-510849 stock solution and the control incubations without NADPH.

**Pharmacokinetics: Metabolism *In Vitro* ( $K_m$  and  $V_{max}$  in HLM and CYP 3A4/5)**

**Study Description or Title:** Determination of  $K_m$  and  $V_{max}$  for the Formation of BMS-510849 (M2) from Saxagliptin in Human Liver Microsomes, and cDNA-Expressed CYP3A4 and CYP3A5

**Study Type:** Non-GLP

**Test Articles:** Saxagliptin

**Location in Dossier:** Study No./Document Control No. NA/930024372

**Study system:** Pooled human liver microsomes, 0.25 mg/mL, 30 min  
cDNA-expressed CYP3A4 and CYP3A5, 10 pmol/mL, 10 min  
Saxagliptin concentrations 1-800  $\mu$ M

Incubation System	$K_m$ ( $\mu$ M) <sup>a</sup>	$V_{max}$ <sup>a,b</sup>
HLM	94.8 $\pm$ 8.3	496 $\pm$ 11
CYP3A4	81.7 $\pm$ 7.3	31.7 $\pm$ 0.7
CYP3A5	252 $\pm$ 27	24.0 $\pm$ 1.0

Additional Information: BMS-510849 concentrations were determined by LC/MS/MS analysis.

<sup>a</sup> Kinetic parameters were calculated within Sigmaplot (v 10) by fitting data to the Michaelis-Menten equation ( $V = V_{max} * [S] / (K_m + [S])$ ) using non-linear regression.

<sup>b</sup> The units for  $V_{max}$  were pmol BMS-510849/mg protein/min for HLM and pmol BMS-510849/pmol CYP/min for CYP3A4 and CYP3A5.

**Study Description or Title:** Correlation between Experimentally Determined Rate of BMS-510849 (M2) Formation from Saxagliptin and Reported Activities of CYP Enzymes in a Panel of HLM from 16 Individuals

**Test Articles:** Saxagliptin

**Study Type:** Non-GLP

**Location in Dossier:**

**Study No./Document Control No.** NA/930024372

**Study system:** 16 individual lots of human liver microsomes, 0.25 mg/mL, 30 min; saxagliptin at concentrations 1 and 10  $\mu$ M.

BMS-510849 (M2) concentrations in each of the incubations were determined by LC/MS/MS analysis. The rate of BMS-510849 formation in each of the incubations was then calculated and the correlation between the rate of BMS-510849 formation and the reported CYP activities was determined.

		Correlation Coefficient (r)													
	M2 1 $\mu$ M	M2 10 $\mu$ M	CYP3A Test	CYP3A Midaz	1A2 7ER	1A2 Phen	2A6 Coum	2B6 S-Meph	2B6 Bup	2C8 Pacl	2C9 Diclo	2C19 Meph	2D6 Dex	2E1 Chl	4A11 Lau
M2 1 $\mu$ M	1.000	0.984	0.985	0.949	0.013	0.198	0.363	0.825	0.311	0.565	0.217	0.172	0.490	0.094	0.003
M2 10 $\mu$ M		1.000	0.988	0.928	0.027	0.223	0.324	0.587	0.259	0.580	0.303	0.162	0.481	0.120	0.078
CYP3A Test			1.000	0.912	0.033	0.218	0.302	0.576	0.274	0.538	0.225	0.184	0.477	0.091	0.003
CYP3A Midaz				1.000	0.137	0.373	0.329	0.590	0.281	0.656	0.220	0.086	0.415	0.226	-0.028
1A2 7ER					1.000	0.898	-0.035	-0.192	-0.205	0.233	0.186	0.056	-0.234	0.354	0.270
1A2 Phen						1.000	0.043	-0.024	-0.089	0.390	0.204	-0.025	-0.167	0.459	0.089
2A6 Coum							1.000	0.703	0.576	0.409	0.148	0.285	-0.001	-0.161	-0.254
2B6 S-Meph								1.000	0.854	0.566	-0.071	0.414	0.293	-0.066	-0.197
2B6 Bup									1.000	0.253	-0.440	0.642	0.018	-0.159	-0.317
2C8 Pacl										1.000	0.432	-0.043	0.068	0.455	0.263
2C9 Diclo											1.000	-0.208	-0.105	0.461	0.374
2C19 Meph												1.000	-0.308	-0.086	-0.098
2D6 Dex													1.000	-0.438	-0.041
2E1 Chl														1.000	0.176
4A11 Lau															1.000

Additional Information: The activities of the CYP enzymes in each of the individual lots of HLM were determined by the vendor using marker substrates specific for each of the CYP enzymes.

These data suggest that the formation of BMS-510849 from saxagliptin correlated closely with the reported activities of CYP3A4/5 as measured both by testosterone 6 $\beta$ -hydroxylation and midazolam 1'-hydroxylation.

Abbreviations: M2 = BMS-510849

Test = Testosterone 6 $\beta$ -hydroxylation (CYP3A4/5 activity)

Midaz = Midazolam 1'-hydroxylation (CYP3A4/5 activity)

7ER = 7-Ethoxyresorufin O-dealkylation (CYP1A2 activity)

Phen = Phenacetin O-deethylation (CYP1A2 activity)

Coum = Coumarin 7-hydroxylation (CYP2A6 activity)

S-Meph = S-Mephenytoin N-demethylation (CYP2B6 activity)

Bup = Bupropion hydroxylation (CYP2B6 activity)

Pacl = Paclitaxel 6 $\alpha$ -hydroxylation (CYP2C8 activity)

Diclo = Diolofenao 4'-hydroxylation (CYP2C9 activity)

Meph = S-Mephenytoin 4'-hydroxylation (CYP2C19 activity)

Dex = Dextromethorphan O-demethylation (CYP2D6 activity)

Chl = Chlorzoxazone 6-hydroxylation (CYP2E1 activity)

Lau = Lauric acid 12-hydroxylation (CYP4A11 activity)

**Pharmacokinetics: Metabolism *In Vitro* (in HLM and CYP Chemical Inhibitors)**

**Study Description or Title:** Relative Formation of BMS-510849 from Saxagliptin in HLM in the Presence of Chemical Inhibitors of CYP Enzymes

**Test Articles:** Saxagliptin

**Study Type:** Non-GLP

**Location in Dossier:**

**Study No./Document Control No. NA/930024372**

**Study system:** Pooled human liver microsomes (0.25 mg/mL) were incubated with saxagliptin (1 and 10 µM) for 30 min in the presence of chemical inhibitors specific for CYP enzymes

For time dependent inhibitors, namely furafylline (CYP1A2), orphenadrine (CYP2B6), diethyldithiocarbamate (CYP2E1), troleanomyoin (CYP3A4/5), and 1-aminobenzotriazole (all CYPs), microsomes were pre-incubated with the inhibitors and 1 mM NADPH for 15 min at 37°C prior to the addition of saxagliptin. After the pre-incubation period, additional NADPH (1 mM) and saxagliptin (1 or 10 µM) were added and the incubations were continued for 30 min. BMS-510849 (M2) concentrations in each of the incubations were determined by LC-MS/MS analysis. BMS-510849 formation in the presence of chemical inhibitors was compared to control incubations without inhibitor.

Inhibitor (Concentration)	Enzyme(s) Inhibited	% BMS-510849 Formation Relative to Control (± SD)	
		1 µM Saxagliptin	10 µM Saxagliptin
Ketoconazole (1 µM)	CYP3A4/5	0.9 ± 0.55	2.3 ± 0.3
Troleandomycin (20 µM)	CYP3A4/5	0.5 ± 0.60	1.1 ± 0.05
1-Aminobenzotriazole (1000 µM)	All CYPs	0.0	0.3 ± 0.08
Furafylline (10 µM)	CYP1A2	96.8 ± 3.6	92.3 ± 4.2
Tranyloypromine (2 µM)	CYP2A6	77.5 ± 2.5	78.0 ± 1.8
Orphenadrine (50 µM)	CYP2B6	50.2 ± 0.6	57.2 ± 6.2
Montelukast (3 µM)	CYP2C8	47.4 ± 1.1	53.5 ± 3.6
Sulfaphenazole (10 µM)	CYP2C9	73.1 ± 6.5	70.2 ± 3.0
Benzylmivrianol (1 µM)	CYP2C19	58.4 ± 7.6	59.1 ± 5.4
Quinidine (1 µM)	CYP2D6	74.3 ± 3.1	79.5 ± 3.8
Diethyldithiocarbamate (50 µM)	CYP2E1	70.9 ± 0.5	72.3 ± 8.4
Heat killed microsomes	All CYPs	0.0	0.0
No NADPH	All CYPs	0.0	0.0
HLM control	-	100 ± 8.9	100 ± 4.1
HLM control (time-dependent)	-	100 ± 3.8	100 ± 7.9

Abbreviations: SD = Standard Deviation

These data suggest that the formation of BMS-510849 is an NADPH-dependent reaction catalyzed by CYP enzymes and CYP3A4/5 are the primary enzymes involved in the metabolism of saxagliptin to BMS-510849. Although the formation of BMS-510849 was also inhibited in the presence of chemical inhibitors of other CYP enzymes, the other CYP enzymes were not capable of metabolizing saxagliptin in expressed enzyme systems (Table 2.6.5.10B).

**Pharmacokinetics: Metabolism *In Vitro* (in HLM and CYP Antibody Inhibitors)**

**Study Description or Title:** Relative Formation of BMS-510849 from Saxagliptin in HLM in the Presence of Monoclonal Antibody Inhibitors of CYP Enzymes

**Test Articles:** Saxagliptin

**Study Type:** Non-GLP

**Location in Dossier:**

**Study No./Document Control No. NA/930024372**

**Study system:** Pooled human liver microsomes (0.25 mg/mL), antibody inhibitors of CYP enzymes, saxagliptin (1 and 10 µM)

HLMs were pre-incubated with antibodies for 20 min on ice followed by 10 min at 37°C. After the pre-incubation period, saxagliptin (1 or 10 µM) and NADPH (1 mM) were added and incubations were continued for 30 min at 37°C. BMS-510849 (M2) concentrations in each of the incubations were determined by LC-MS/MS analysis. BMS-510849 formation in the presence of antibody inhibitors was compared to control incubations that contained an antibody against egg lysozyme.

Antibody	Amount of Antibody Used <sup>a</sup>	% BMS-510849 Formation Relative to Control (± SD)	
		1 µM Saxagliptin	10 µM Saxagliptin
Antibody to CYP1A2	20 µL/mL	96.1 ± 9.9	93.1 ± 4.7
Antibody to CYP2B6	28 µL/mL	102.3 ± 4.8	105.9 ± 4.9
Antibody to CYP2C8	20 µL/mL	101.8 ± 4.3	98.8 ± 0.8
Antibody to CYP2C19	20 µL/mL	109.8 ± 5.1	82.6 ± 29.9
Antibody to CYP2D6	20 µL/mL	106.8 ± 8.8	103.5 ± 2.2
Antibody to CYP3A4/5	20 µL/mL	9.5 ± 0.5	10.6 ± 0.7
Antibody to egg lysozyme	20 µL/mL	100 ± 2.8	100 ± 4.4
No antibody	-	73.4 ± 18.7	94.0 ± 4.8
No NADPH, no antibody	-	0	0

These data suggest that CYP3A4/5 are the primary enzymes involved in the metabolism of saxagliptin to BMS-510849.

<sup>a</sup> Anti-CYP antibodies were obtained from the Laboratory of Metabolism at the National Institute of Health (NIH, Bethesda, MD) and were used "as is". The antibody concentration was not known. The amount used in each incubation is indicated as the volume of antibody solution per mL of microsomal incubation mixture.

**Pharmacokinetics: Metabolism *In Vitro* (Cyanide Release in Liver Microsomes)**

**Study Description or Title:** Measurement of Cyanide Release After Incubation of Saxagliptin with Liver Microsomes from Mouse, Rat, Dog, Monkey and Human and cDNA Expressed CYP450 Enzymes from Rat, Dog and Human

**Test Articles:** Saxagliptin

**Study Type:** Non-GLP

**Location in Dossier:**

**Study No./Document Control No.** NA/930021240

<b>Study system:</b> Liver Microsomes and cDNA-Expressed CYP450 Enzymes, 30 min, 100 µM Saxagliptin			
<b>Liver Microsomes (Gender M/F)</b>	<b>Final Cyanide Concentration in Incubation (µM)</b>	<b>CYP Enzymes (Species)</b>	<b>Final Cyanide Concentration in Incubation (µM)</b>
Mouse (M)	<LLOQ (0.77)	CYP2C11 (Rat)	42
Rat (M)	9.9	CYP2C12 (Rat)	<LLOQ (0.77)
Rat (F)	<LLOQ (0.77)	CYP2C13 (Rat)	<LLOQ (0.77)
Dog (M)	<LLOQ (0.77)	CYP2C21 (Dog)	8.1
Monkey (M)	3.0	CYP2C8 (Human)	1.7
Monkey (F)	1.5	CYP2C9 (Human)	<LLOQ (0.77)
Human (M/F pool)	<LLOQ (0.77)	CYP2C18 (Human)	<LLOQ (0.77)
		CYP2C19 (Human)	1.4

**Additional Information:** The concentration of cyanide in the incubation mixtures was quantified by HPLC with electrochemical detection. <LLOQ = below the Lower Limit of Quantitation (0.77 µM). Samples were analyzed in duplicate and the average value is reported.

**Pharmacokinetics: Metabolism *In Vitro* (Cyanide Release in RLM and CYP2C11)**

**Study Description or Title:** Measurement of Cyanide Release After Incubation of Saxagliptin with Rat Liver Microsomes and Expressed Rat CYP2C11 in the Presence and Absence of Cimetidine

**Test Articles:** Saxagliptin

**Study Type:** Non-GLP

**Location in Dossier:**

**Study No./Document Control No.** NA/930021240

<b>Study system:</b> Rat Liver Microsomes and Expressed Rat CYP2C11, 30 min, 100 µM Saxagliptin		
<b>Incubation System</b>	<b>Final Cyanide Concentration (µM)</b>	
	<b>Saxagliptin (100 µM)</b>	<b>Saxagliptin (100 µM) + Cimetidine (1 mM)</b>
RLM (M)	5.4	<LLOQ
RLM (F)	<LLOQ	<LLOQ
CYP2C11	37	12

**Additional Information:** The concentration of cyanide in the incubation mixtures was quantified by HPLC with electrochemical detection. <LLOQ = below the Lower Limit of Quantitation (0.77 µM). Samples were analyzed in duplicate and the average value is reported.

**Pharmacokinetics: Metabolism *In Vitro* (Cyanide Release after BMS-510849 in LM and CYP)**

**Study Description or Title:** Measurement of Cyanide Release After Incubation of BMS-510849 with Liver Microsomes from Mouse, Rat, Dog, Monkey and Human and cDNA Expressed CYP450 Enzymes from Rat, Dog and Human

**Test Articles:** BMS-510849

**Study Type:** Non-GLP

**Location in Dossier:**

**Study No./Document Control No.** NA/930021240

**Study system:** Liver Microsomes and cDNA-Expressed CYP450 Enzymes, 30 min, 100 µM BMS-510849

Liver Microsomes (Gender M/F)	Final Cyanide Concentration in Incubation (µM)	CYP Enzymes (Species)	Final Cyanide Concentration in Incubation (µM)
Mouse (M)	<LLOQ (0.77)	CYP2C11 (Rat)	<LLOQ (0.77)
Rat (M)	<LLOQ (0.77)	CYP2C12 (Rat)	<LLOQ (0.77)
Rat (F)	<LLOQ (0.77)	CYP2C13 (Rat)	<LLOQ (0.77)
Dog (M)	<LLOQ (0.77)	CYP2C21 (Dog)	<LLOQ (0.77)
Monkey (M)	4.1	CYP2C8 (Human)	<LLOQ (0.77)
Monkey (F)	3.0	CYP2C9 (Human)	<LLOQ (0.77)
Human (M/F pool)	<LLOQ (0.77)	CYP2C18 (Human)	<LLOQ (0.77)
		CYP2C19 (Human)	<LLOQ (0.77)

Additional Information: The concentration of cyanide in the incubation mixtures was quantified by HPLC with electrochemical detection. <LLOQ = below the Lower Limit of Quantitation (0.77 µM). Samples were analyzed in duplicate and the average value is reported.

**Pharmacokinetics: Metabolism *In Vitro* (in Rat CYP450)**

**Study Description or Title:** Formation of BMS-510849 from Saxagliptin in Incubations with cDNA-expressed CYP450 Enzymes from Rat

**Test Articles:** Saxagliptin

**Study Type:** Non-GLP

**Location in Dossier:**

**Study No./Document Control No.** NA/930016962

**Study system:** cDNA-Expressed Rat CYP450 Enzymes, 30 min, 10 and 100 µM Saxagliptin

CYP Enzyme	Incubation with Saxagliptin (10 µM)		Incubation with Saxagliptin (100 µM)	
	Saxagliptin Conc (µM)	BMS-510849 Conc (µM) <sup>a</sup>	Saxagliptin Conc (µM)	BMS-510849 Conc (µM) <sup>a</sup>
CYP1A1	8.6	<0.03	98.3	0.10
CYP1A2	9.3	<0.03	92.1	0.06
CYP2A2	7.2	<0.03	89.7	0.06
CYP2B1	8.4	<0.03	88.9	0.08
CYP2C6	9.0	<0.03	95.4	<0.03
CYP2C11	0.43	<0.03	28.0	0.20
CYP2C12	8.6	<0.03	96.5	0.03
CYP2C13	9.3	<0.03	90.6	0.11
CYP2D1	10.0	0.09	94.3	0.75
CYP2D2	9.2	<0.03	89.2	0.12

**Pharmacokinetics: Metabolism *In Vitro* (in Rat CYP450)**

**Study Description or Title:** Formation of BMS-510849 from Saxagliptin in Incubations with cDNA-expressed CYP450 Enzymes from Rat

**Test Articles:** Saxagliptin

**Study Type:** Non-GLP

**Location in Dossier:**

**Study No./Document Control No. NA/930016962**

CYP Enzyme	Incubation with Saxagliptin (10 µM)		Incubation with Saxagliptin (100 µM)	
	Saxagliptin Conc (µM)	BMS-510849 Conc (µM) <sup>a</sup>	Saxagliptin Conc (µM)	BMS-510849 Conc (µM) <sup>a</sup>
CYP3A1	0.59	7.7	34.1	63.9
CYP3A2	0.39	8.9	41.1	57.9
Buffer, 0 min	8.7	<0.03	92.4	<0.04
Buffer, 30 min	9.6	<0.03	91.9	<0.03

Additional Information: The concentration of saxagliptin and BMS-510849 in the incubation mixtures was quantified by LC/MS/MS. The lower limit for quantification of saxagliptin was 0.16 µM, the lower limit for quantification of BMS-510849 was 0.030 µM. Values are the average of duplicate analyses. These data suggested that in rats, CYP3A1 and CYP3A2 were the primary enzymes responsible for the metabolism of saxagliptin to BMS-510849. There was appreciable metabolism of saxagliptin in the CYP2C11 incubations, but this metabolism did not result in the formation of BMS-510849. Further LC/MS/MS analysis of the CYP2C11 incubation mixtures indicated that M4, a des-cyano metabolite of saxagliptin was formed in these incubations (DCN930016962). The structure of M4 is shown in Table 2.6.5.11

<sup>a</sup> The bioanalytical assay that was used to measure concentrations of BMS-510849 in this study may not have been completely specific for BMS-510849. Other mono-hydroxylated metabolites with the same MRM transition as BMS-510849 in the LC/MS/MS (332 → 196) may have co-eluted with BMS-510849 under the conditions employed in the assay. Therefore the values reported for BMS-510849 concentration may include BMS-510849 and other mono-hydroxylated metabolites. The measurement of saxagliptin was not impacted in this method.

**Pharmacokinetics: Metabolism *In Vitro* (in LM from Rats Treated with Chemical Inducers)**

**Study Description or Title:** Formation of BMS-510849 from Saxagliptin in Incubations with Liver Microsomes from Rats Treated with Chemical Inducers of CYP Enzymes

**Test Articles:** Saxagliptin

**Study Type:** Non-GLP

**Location in Dossier:**

**Study No./Document Control No. NA/930016962**

**Study system:** Rat liver microsomes, 30 min, 10 and 100 µM Saxagliptin

Chemical Inducer (Primary CYP Enzymes Induced)	Incubation with Saxagliptin (10 µM)		Incubation with Saxagliptin (100 µM)	
	Saxagliptin Conc (µM)	BMS-510849 Conc (µM) <sup>a</sup>	Saxagliptin Conc (µM)	BMS-510849 Conc (µM) <sup>a</sup>
β-Naphthoflavone (CYP1A)	5.4	3.9	71.1	23.9
Phenobarbital (CYP2B)	0.26	8.9	12.9	72.2
β-Naphthoflavone + Phenobarbital (CYP1A+2B)	1.1	7.8	35.7	55.3
Isoniazid (CYP2E)	5.9	2.7	69.7	18.5
Dexamethasone (CYP3A)	0.08	8.7	1.9	83.2
Clofibrin Acid (CYP4A)	1.5	7.0	30.0	51.1
Saline (vehicle control)	1.5	5.7	33.3	40.9
Corn Oil (vehicle control)	2.5	5.0	43.4	37.5
Non-Induced (control microsomes)	2.9	4.3	52.7	28.5

**Pharmacokinetics: Metabolism *In Vitro* (in LM from Rats Treated with Chemical Inducers)**

**Study Description or Title:** Formation of BMS-510849 from Saxagliptin in Incubations with Liver Microsomes from Rats Treated with Chemical Inducers of CYP Enzymes

**Test Articles:** Saxagliptin

**Study Type:** Non-GLP

**Location in Dossier:**

**Study No./Document Control No.** NA/930016962

Chemical Inducer (Primary CYP Enzymes Induced)	Incubation with Saxagliptin (10 µM)		Incubation with Saxagliptin (100 µM)	
	Saxagliptin Conc (µM)	BMS-510849 Conc (µM) <sup>a</sup>	Saxagliptin Conc (µM)	BMS-510849 Conc (µM) <sup>a</sup>
Non-Induced/No NADPH (negative control)	9.5	<0.03	100.8	0.05
Buffer, 0 min	8.7	<0.03	92.4	<0.04
Buffer, 30 min	9.6	<0.03	91.9	<0.03

Additional Information: The concentration of saxagliptin and BMS-510849 in the incubation mixtures was quantified by LC-MS/MS. Values are the average of duplicate analyses. The lower limit for quantification of saxagliptin was 0.16 µM, the lower limit for quantification of BMS-510849 was 0.030 µM.

In conjunction with the data from the metabolism of saxagliptin in cDNA-expressed CYP Enzymes from Rat (Table 2.6.5.10M), these findings suggest that CYP3A enzymes are primarily responsible for the metabolism of saxagliptin to BMS-510849 in rat.

<sup>a</sup> The bioanalytical assay that was used to measure concentrations of BMS-510849 in this study may not have been completely specific for BMS-510849. Other mono-hydroxylated metabolites with the same MRM transition as BMS-510849 in the LC/MS/MS (332 → 196) may have co-eluted with BMS-510849 under the conditions employed in the assay. Therefore the values reported for BMS-510849 concentration may include BMS-510849 and other mono-hydroxylated metabolites. The measurement of saxagliptin was not impacted in this method.

**Pharmacokinetics: Possible In Vivo Metabolic Pathways**

**Test Article:** [<sup>14</sup>C]Saxagliptin

**Study Type:** Non-GLP

**Location in Dossier:**

**Study No./Document Control No.:** NA/930016961  
NA/930023887

References for studies to determine the in vivo metabolic pathways of saxagliptin

Study System	Mouse (Brain)	Intact Rat	BDC Rat	Rat (Brain)	Intact Dog	Intact Monkey	Human
Tabulated Summary in CTD	2.6.5.9D	2.6.5.9A	2.6.5.9C	2.6.5.9D	2.6.5.9A	2.6.5.9A	2.6.5.9A
	2.6.5.9E	2.6.5.9B		2.6.5.9E	2.6.5.9B	2.6.5.9B	2.6.5.9B
BMS DCN	930023887	930016961	930016961	930023887	930016961	930016961	930016961

**Pharmacokinetics: Induction/Inhibition of Drug-Metabolizing Enzymes**

**Study Description or Title:** Evaluation of Saxagliptin as an Inducer of Cytochrome P450 Enzymes in Primary Cultures of Human Hepatocytes

**Test Article:** Saxagliptin

**Study Type:** Non-GLP

**Location in Dossier:**

**Study No./Document Control No:** PD277104c/930015211

**Method:** The potential for saxagliptin to induce CYP1A2, 2B6, 2C9, and 3A4 was investigated using primary cultures of human hepatocytes. Human Hepatocytes were isolated from the livers of 3 female donors. Following exposure to the test article for 3 days, the hepatocytes were harvested and microsomes were prepared. Incubations with known substrates of CYP enzymes were conducted with the microsomes to evaluate enzyme activities. In addition, levels of mRNA encoding the CYP enzymes were measured using TaqMan<sup>®</sup> Real-Time quantitative PCR analysis.

**Study System:** Primary human hepatocytes (3/F)

**Incubation Time:** 3 days

**Concentration:** Saxagliptin (0.2, 1.0, 5.0, 25 µM); Reference: 0.1% di-methylsulfoxide (DMSO); Positive Controls: 2 µM 3-methylcholanthrene; 1000 µM phenobarbital; 10 µM rifampicin.

**Assay:** 1) Enzyme Activity- 1A2 (phenacetin O-deethylation); 2B6 (bupropion hydroxylation); 2C9 (diolofenac 4'-hydroxylation); 3A4 (testosterone 6β-hydroxylation). Products of these enzymatic reactions were quantitated by LC-MS analysis.  
2) mRNA Levels- Levels of mRNA encoding CYP1A2, 2B6, 2C9 and 3A4 enzymes were measured using TaqMan<sup>®</sup> Real-Time quantitative PCR

CYP Enzyme Activity					
Fold-change (Compared to 0.1% DMSO control)					
Test Article	Concentration (µM)	CYP1A2	CYP2B6	CYP2C9	CYP3A4
Saxagliptin	0.2	0.7-1.8	0.6-1.7	0.7-1.8	0.7-2.2
Saxagliptin	1	0.9-1.7	0.8-1.6	0.7-1.6	0.9-1.9
Saxagliptin	5	0.7-2.0	0.7-1.6	0.6-1.8	0.7-2.0
Saxagliptin	25	1.0-2.1	1.0-1.8	0.8-1.6	1.2-2.6
DMSO control	0.1%	1	1	1	1
3-methylcholanthrene	2	15.6-19.7	1.6-1.9	1.0-1.2	0.6-0.9
Phenobarbital	1000	2.6-3.2	7.9-13.1	1.3-3.2	3.4-5.2
rifampicin	10	1.1-2.1	1.8-8.9	1.9-2.2	3.3-6.0
Saxagliptin	0.2	0.85-1.2	0.67-2.66	1.39-3.22	0.18-0.55
Saxagliptin	1	0.75-0.88	0.76-2.03	1.77-3.06	0.36-0.51
Saxagliptin	5	0.8-1.22	0.73-1.25	1.24-2.43	0.23-0.42
Saxagliptin	25	0.7-1.06	0.72-1.27	2.25-3.75	0.53-0.81
DMSO control	0.1%	1	1	1	1
3-methylcholanthrene	2	71.5-385.3	0.90-3.33	1.00-2.68	0.52-1.00
Phenobarbital	1000	ND	5.96-29.34	3.42-6.41	4.81-10.02
rifampicin	10	ND	2.63-14.52	3.15-4.64	7.39-10.85

**Additional Information:** Abbreviations: DMSO = dimethylsulfoxide. Microsomal activity rates and mRNA levels for each of the CYP enzymes were determined separately for each individual donor. Fold-change (compared to control incubations with 0.1% DMSO) for each enzyme/each donor was then calculated. The range of values across the three donors is reported.

These results suggest that exposure of human hepatocytes to saxagliptin at concentrations <25 µM was not associated with significant induction of CYP1A2, 2B6, 2C9, or 3A4 activities or mRNA expression.

**Pharmacokinetics: Induction/Inhibition of Drug-Metabolizing Enzymes (Induction in Primary Human Hepatocytes)**

**Study Description or Title:** Evaluation of BMS-510849 as an Inducer of Cytochrome P450 Enzymes in Primary Cultures of Human Hepatocytes

**Test Article:** BMS-510849

**Study Type:** Non-GLP

**Location in Dossier:**

**Study No./Document Control No:** PD278704c/930015454

**Method:** The potential for BMS-510849 to induce CYP1A2, 2B6, 2C9, and 3A4 was investigated using primary cultures of human hepatocytes. Human Hepatocytes were isolated from the livers of 3 female donors. Following exposure to the test article for 3 days, the hepatocytes were harvested and microsomes were prepared. Incubations with known substrates of CYP enzymes were conducted with the microsomes to evaluate enzyme activities. In addition, levels of mRNA encoding the CYP enzymes were measured using TaqMan<sup>®</sup> Real-Time quantitative PCR analysis.

**Study System:** Primary human hepatocytes (3/F)

**Incubation Time:** 3 days

**Concentration:** BMS-510849 (0.2, 1.0, 10.0, 100 µM); Reference: 0.1% di-methylsulfoxide (DMSO); Positive Controls: 2 µM 3-methylcholanthrene; 1000 µM phenobarbital; 10 µM rifampicin.

**Assay:**  
 1) Enzyme Activity- 1A2 (phenacetin O-deethylation); 2B6 (bupropion hydroxylation); 2C9 (diclofenac 4'-hydroxylation); 3A4 (testosterone 6β-hydroxylation). Products of these enzymatic reactions were quantitated by LC-MS analysis.  
 2) mRNA Levels- Levels of mRNA encoding CYP1A2, 2B6, 2C9 and 3A4 enzymes were measured using TaqMan<sup>®</sup> Real-Time quantitative PCR

CYP Enzyme Activity					
Fold-change (Compared to 0.1% DMSO control)					
Test Article	Concentration (µM)	CYP1A2	CYP2B6	CYP2C9	CYP3A4
BMS-510849	0.2	0.8-1.3	0.7-1.2	0.6-1.2	0.6-1.4
BMS-510849	1	0.9-1.8	0.8-1.3	0.7-1.3	0.7-1.9
BMS-510849	10	0.8-1.5	0.8-1.5	0.7-1.6	0.7-1.9
BMS-510849	100	0.6-1.7	0.7-2.0	0.6-1.6	0.7-2.0
DMSO control	0.1%	1	1	1	1
3-methylcholanthrene	2	15.6-19.7	1.6-1.9	1.0-1.2	0.6-0.9
Phenobarbital	1000	2.6-3.2	7.9-13.1	1.3-3.2	3.4-5.2
rifampicin	10	1.1-2.1	1.8-8.9	1.9-2.2	3.3-6.0
BMS-510849	0.2	0.45-0.99	0.56-0.88	1.21-2.03	0.18-0.39
BMS-510849	1	0.74-1.08	0.58-0.75	1.52-2.36	0.17-0.61
BMS-510849	10	0.31-0.54	0.59-0.74	1.71-2.16	0.17-0.42
BMS-510849	100	0.39-1.00	0.66-1.92	1.65-2.71	0.22-0.52
DMSO control	0.1%	1	1	1	1
3-methylcholanthrene	2	71.5-385.3	0.90-3.33	1.00-2.68	0.52-1.00
Phenobarbital	1000	ND	5.96-29.34	3.42-6.41	4.81-10.02
rifampicin	10	ND	2.63-14.52	3.15-4.64	7.39-10.85

**Additional Information:** Abbreviations: DMSO = dimethylsulfoxide. Microsomal activity rates and mRNA levels for each of the CYP enzymes were determined separately for each individual donor. Fold-change (compared to control incubations with 0.1% DMSO) for each enzyme/each donor was then calculated. The range of values across the three donors is reported.

These results suggest that exposure of human hepatocytes to BMS-510849 at concentrations <100 µM was not associated with significant induction of CYP1A2, 2B6, 2C9, or 3A4 activities or mRNA expression.

**Pharmacokinetics: Induction/Inhibition of Drug-Metabolizing Enzymes (Inhibition of Recombinant CYP450 Enzymes)**

**Study Description or Title:** Limited Evaluation of the Inhibition of CYP Enzymes by Saxagliptin  
**Test Article:** Saxagliptin  
**Study Type:** Non-GLP  
**Location in Dossier:**  
**Study No./Document Control No.:** MAP005-477118/93000866

**Type of Study:** Evaluation of the Potential for BMS-477118 to Inhibit Selected CYP450 Isoforms  
**Methods:**  
 The potential for BMS-477118 to inhibit the major human CYPs was evaluated in vitro using recombinant human c-DNA expressed CYPs.

Compound	IC <sub>50</sub> for CYP Inhibition (µM)					
	CYP1A2	CYP2C9	CYP2C19	CYP2D6	CYP3A4 (BFC)	CYP3A4 (BzRes)
BMS-477118	>100	>100	>100	>100	>100	>100

The substrates used for evaluation of CYP inhibition were:  
 CYP1A2, CYP2C19 - 3-Cyano-7-ethoxycoumarin  
 CYP2C9 - Dibenzylfluorescein  
 CYP2D6 - 3-[2-(N, N-diethyl-N-methylamino)ethyl]-7-methoxy-4-methyloumarin  
 CYP3A4 - 7-benzyloxy-4-trifluoromethyloumarin (BFC), and resorufin benzyl ether (BzRes)

**Additional Information:**  
 These results suggest that BMS-477118 is unlikely to alter the metabolic clearance of drugs metabolized by CYP1A2, CYP2C9, CYP2C19, CYP2D6 and CYP3A4 at concentrations <100 µM.

**Pharmacokinetics: Induction/Inhibition of Drug-Metabolizing Enzymes (Saxagliptin as an Inhibitor)**

**Study Description or Title:** Evaluation of Saxagliptin as an Inhibitor of Cytochrome P450 Enzymes in Human Liver Microsomes  
**Test Article:** Saxagliptin  
**Study Type:** Non-GLP  
**Location in Dossier:**  
**Study No./Document Control No.:** PD277204a/930014645

**Method:** The potential for saxagliptin to cause direct and time-dependent inhibition of CYP1A2, 2A6, 2B6, 2C8, 2C9, 2C19, 2D6, 2E1 and 3A4 was investigated in human liver microsomes (HLM, a pooled lot from 15 individual donors). HLM were incubated in duplicate with probe substrates in the presence of varying concentrations of saxagliptin.<sup>a</sup> Specific products of the CYP-catalyzed enzymatic reactions with the probe substrates were quantitated by LC-MS analysis. For time-dependent inhibition experiments, saxagliptin was pre-incubated with HLM in the presence of NADPH for 15 min prior to the measurement of CYP activity.

<b>Study System:</b>	Human Liver Microsomes (pooled lot from 15 individual donors, male and female)	
<b>Incubation Time:</b>	4 min (3A4-midazolam); 5 min 2A6; 7 min (3A4-testosterone); 10 min 2C8; 15 min 2D6; 20 min 2B6, 2C9, 2E1; 30 min 1A2, 2C19	
<b>Concentration:</b>	Saxagliptin: 0.1, 1, 5, 20 and 50 µM; Probe substrates: Phenacetin 50 µM (1A2), Coumarin 1 µM (2A6), Bupropion 125 µM (2B6), Paclitaxel 5 µM (2C8), Tolbutamide 140 µM (2C9), (S)-Mephenytoin 50 µM (2C19), Bufuralol 40 µM (2D6), Chlorzoxazone 50 µM (2E1), Midazolam 5 µM (3A4), Testosterone 50 µM (3A4).	
<b>Assay:</b>	After incubation of CYP enzymes with probe substrates in the presence of varying concentrations of saxagliptin, samples were analyzed by LC-MS for quantification of specific products of the enzymatic reactions.	
CYP Enzyme (probe substrate reaction monitored)	IC <sub>50</sub> Values (µM)	
	Direct Inhibition	Time-Dependent Inhibition
CYP1A2 (Phenacetin O-deethylation)	> 50	> 50
CYP2A6 (Coumarin 7-hydroxylation)	> 50	> 50
CYP2B6 (Bupropion hydroxylation)	> 50	> 50
CYP2C8 (Paclitaxel 6-hydroxylation)	> 50	> 50
CYP2C9 (Tolbutamide hydroxylation)	> 50	> 50
CYP2C19 ((S)-Mephenytoin 4'-hydroxylation)	> 50	> 50

**Pharmacokinetics: Induction/Inhibition of Drug-Metabolizing Enzymes (Saxagliptin as an Inhibitor)**

**Study Description or Title:** Evaluation of Saxagliptin as an Inhibitor of Cytochrome P450 Enzymes in Human Liver Microsomes  
**Test Article:** Saxagliptin  
**Study Type:** Non-GLP  
**Location in Dossier:**  
**Study No./Document Control No.:** PD277204a/930014645

CYP Enzyme (probe substrate reaction monitored)	IC <sub>50</sub> Values (µM)	
	Direct Inhibition	Time-Dependent Inhibition
CYP2D6 (Bufuralol 1'-hydroxylation)	> 50	> 50
CYP2E1 (Chlorzoxazone 6-hydroxylation)	> 50	> 50
CYP3A4 (Midazolam 1'-hydroxylation)	> 50	> 50
CYP3A4 (Testosterone 6β-hydroxylation)	> 50	> 50

**Additional Information:** These data suggest that saxagliptin, was not associated with inhibition of these 9 CYP enzymes at concentrations < 200 µM.

<sup>a</sup> Positive control incubations were performed using reference inhibitors of individual CYP enzymes: α-Naphthoflavone (1A2), Pilocarpine (2A6), Orphenadrine (2B6), Quercetin (2C8), Sulfaphenazole (2C9), Modafinil (2C19), Quinidine (2D6), 4-Methylpyrazole (2E1), and Ketoconazole (3A4).

**Pharmacokinetics: Induction/Inhibition of Drug-Metabolizing Enzymes (BMS-510849 as an Inhibitor)**

**Study Description or Title:** Evaluation of BMS-510849 as an Inhibitor of Cytochrome P450 Enzymes in Human Liver Microsomes

**Test Article:** BMS-510849

**Study Type:** Non-GLP

**Location in Dossier:**

**Study No./Document Control No:** PD278604/930014647

**Method:** The potential for BMS-510849 to inhibit CYP1A2, 2A6, 2B6, 2C8, 2C9, 2C19, 2D6, 2E1 and 3A4 in a direct and time-dependent manner was investigated in human liver microsomes (HLM, a pooled lot from 15 individual donors). HLM were incubated in duplicate with probe substrates in the presence of varying concentrations of BMS-510849.<sup>a</sup> Specific products of the CYP-catalyzed enzymatic reactions with the probe substrates were quantified by LC-MS analysis. For time-dependent inhibition experiments, BMS-510849 was pre-incubated with HLM in the presence of NADPH for 15 min prior to the measurement of CYP activity.

<b>Study System:</b>	Human Liver Microsomes (pooled lot from 15 individual donors, male and female)
<b>Incubation Time:</b>	4 min (3A4-midazolam); 5 min 2A6; 7 min (3A4-testosterone); 10 min 2C8; 15 min 2D6; 20 min 2B6, 2C9, 2E1; 30 min 1A2, 2C19
<b>Concentration:</b>	BMS-510849: 0.1, 1, 10, 50 and 200 µM; Probe substrates: Phenacetin 50 µM (1A2), Coumarin 1 µM (2A6), Bupropion 125 µM (2B6), Paolitalaxel 5 µM (2C8), Tolbutamide 140 µM (2C9), (S)-Mephenytoin 50 µM (2C19), Bufuralol 40 µM (2D6), Chlorzoxazone 50 µM (2E1), Midazolam 5 µM (3A4), Testosterone 50 µM (3A4).
<b>Assay:</b>	After incubation of CYP enzymes with probe substrates in the presence of varying concentrations of BMS-510849, samples were analyzed by LC-MS for quantification of specific products of the enzymatic reactions.

CYP Enzyme (probe substrate reaction monitored)	IC <sub>50</sub> Values (µM)	
	Direct Inhibition	Time-Dependent Inhibition
CYP1A2 (Phenacetin O-deethylation)	> 200	> 200
CYP2A6 (Coumarin 7-hydroxylation)	> 200	> 200
CYP2B6 (Bupropion hydroxylation)	> 200	> 200
CYP2C8 (Paolitalaxel 6-hydroxylation)	> 200	> 200
CYP2C9 (Tolbutamide hydroxylation)	> 200	> 200
CYP2C19 ((S)-Mephenytoin 4'-hydroxylation)	> 200	> 200
CYP2D6 (Bufuralol 1'-hydroxylation)	> 200	> 200
CYP2E1 (Chlorzoxazone 6-hydroxylation)	> 200	> 200
CYP3A4 (Midazolam 1'-hydroxylation)	> 200	> 200
CYP3A4 (Testosterone 6β-hydroxylation)	> 200	> 200

**Additional Information:** These data suggest that BMS-510849, a major metabolite of saxagliptin, was not associated with inhibition of these 9 CYP enzymes at concentrations < 200 µM.

<sup>a</sup> Positive control incubations were performed using reference inhibitors of individual CYP enzymes: α-Naphthoflavone (1A2), Pilocarpine (2A6), Orphenadrine (2B6), Quercetin (2C8), Sulfaphenazole (2C9), Modafinil (2C19), Quinidine (2D6), 4-Methylpyrazole (2E1), and Ketoconazole (3A4).

**Pharmacokinetics: Excretion (From Rats, Dogs, Monkeys and Humans)**

**Study Description or Title:** Mass Balance of Radioactivity after Oral Administration of [<sup>14</sup>C]Saxagliptin to Male Rats, dogs, Monkeys and Humans

**Test Article:** [<sup>14</sup>C]saxagliptin

**Study Type:** Non-GLP

**Location in Dossier:**

**Study No./Document Control Numbers:** MBA00010/930008675  
MBA00011/930008676  
MBA00169/930013543  
CV181004/930017078

Species/Strain	Rat/Sprague Dawley	Dog/Beagle	Monkey/Cynomolgus	Human	
<b>Gender (M/F)/Number of animals</b>	M/3	M/3	M/3	M/6	
<b>Feeding condition:</b>	Fasted overnight/fed 4 h postdose	Fasted overnight/fed 4 h postdose	Fasted overnight/fed 4 h postdose	Fasted for ≥10 h/fed 4 h postdose	
<b>Vehicle/Formulation:</b>	0.01 N HCl	0.01 N HCl	0.01 N HCl	50 mM citrate buffer (pH 4)	
<b>Method of Administration:</b>	Oral gavage	Oral gavage	Oral gavage	Oral	
<b>Dose:</b>	20 mg/kg (100 µCi/kg)	5 mg/kg (10 µCi/kg)	10 mg/kg (26.8 µCi/kg)	50 mg (91.5 µCi)	
<b>Radionuclide:</b>	<sup>14</sup> C	<sup>14</sup> C	<sup>14</sup> C	<sup>14</sup> C	
<b>Specific Activity:</b>	4.61 µCi/mg	1.88 µCi/mg	2.45 µCi/mg	1.8 µCi/mg	
Species	Time Interval	% of Radioactive Dose Recovered (Mean ± SD)			
		Urine	Feces	Cage Wash/Rinse/Wipe	Total
Rat	0-168 h	46.3 ± 6.7	42.1 ± 8.5	5.0 ± 2.3	93.4 ± 7.9
Dog	0-168 h	57.5 ± 3.6	28.0 ± 2.8	9.2 ± 5.6	94.7 ± 1.1
Monkey	0-168 h	36.4 ± 9.0	47.3 ± 10.9	5.4 ± 3.4	89.1 ± 2.0
Human	0-168 h	74.9 ± 5.1 <sup>a</sup>	22.1 ± 9.5	NA	97.0 ± 9.1

<sup>a</sup> In the calculation of mean urinary excretion for the human study, the data from one subject was excluded because of a suspected missed sample.

**Pharmacokinetics: Excretion**

Study Description or Title: Urinary Excretion of Radioactivity after Oral Administration of [<sup>14</sup>C]Saxagliptin to Male and Female Mice and Rats

Test Article: [<sup>14</sup>C]saxagliptin

Study Type: Non-GLP

Study No./Document Control Numbers: MBA00122/930010621

MBA00113/930010620

Species/Strain	Mouse/CD-1	Rat/Sprague Dawley
Gender (M/F)/Number of animals	M & F/5 per gender	M & F/3 per gender
Feeding condition:	Fasted 4 h/fed 2 h postdose	Fasted overnight/fed 4 h postdose
Vehicle/Formulation:	Acidified (by HCl) water (pH 5.5)	Acidified (by HCl) water (pH 5.5)
Method of Administration:	Oral gavage	Oral gavage
Dose:	600 mg/kg (100 µCi/kg)	300 mg/kg (100 µCi/kg)
Radionuclide:	<sup>14</sup> C	<sup>14</sup> C
Specific Activity:	0.16 µCi/mg	0.31 µCi/mg

  

Matrix	Time Interval	% of Radioactive Dose Recovered (Mean ± SD)			
		Male Mouse <sup>a</sup>	Female Mouse <sup>a</sup>	Male Rat	Female Rat
Urine	0-24 h	52.51	41.14	38.36 ± 2.87	51.75 ± 16.24

<sup>a</sup> The standard deviation for the urinary recovery of the dose in mice was not reported since mouse urine was collected as a pooled sample of total voided urine from all animals (n = 5) housed together in an individual metabolism cage.

**Pharmacokinetics: Excretion into Bile**

Study Description or Title: Excretion of Radioactivity after Oral Administration of [<sup>14</sup>C]Saxagliptin to Bile Duct-Cannulated Sprague-Dawley Rats

Test Article: [<sup>14</sup>C]Saxagliptin

Study Type: Non-GLP

Study No./Document Control Number: NA/930016961

Species/Strain	Rat/Sprague Dawley					
Gender (M/F)/Number of animals	M/3					
Feeding condition:	Fasted overnight and for duration of study					
Vehicle/Formulation:	Water					
Method of Administration:	Oral gavage					
Dose:	20 mg/kg (150 µCi/kg)					
Radionuclide:	<sup>14</sup> C					
Specific Activity:	7.5 µCi/mg					

  

Species	Route	Time Interval	Mean % of Radioactive Dose Recovered <sup>a</sup>					
			Urine	Bile	Feces	Cage Wash	Carcass	Total
Rat	PO	0-24 h	32.9	25.5	3.2	NA	10.2	76.8

<sup>a</sup> Only 2 of the 3 animals were dosed successfully. The mean values from 2 animals were reported.

**Pharmacokinetics: Excretion into Bile (BMS-510849 to BDC Rats)**

Study Description or Title: Excretion of Radioactivity after Oral Administration of [<sup>14</sup>C]BMS-510849 to Bile Duct-Cannulated Sprague-Dawley Rats

Test Article: [<sup>14</sup>C]BMS-510849

Study Type: Non-GLP

Location in Dossier:

Study No./Document Control Number: NA/930023997

Species/Strain	Rat/Sprague Dawley					
Gender (M/F)/Number of animals	M/F/2 per gender					
Feeding condition:	Fasted overnight and for duration of study					
Vehicle/Formulation:	Water					
Method of Administration:	Oral gavage					
Dose:	20 mg/kg (150 µCi/kg)					
Radionuclide:	<sup>14</sup> C					
Specific Activity:	7.5 µCi/mg					

  

Species	Gender	Route	Time Interval	Mean % of Radioactive Dose Recovered <sup>a</sup>			
				Urine	Bile	Feces	Total
Rat	Male	PO	0-24 h	51.0	6.6	1.6	59.2
	Female	PO	0-24 h	28.9	7.4	4.2	40.5

<sup>a</sup> The standard deviation was not reported since only two animals were evaluated per gender.

**Pharmacokinetics: Other (Absorption with PAMPA Model)**

**Study Description or Title:** In Vitro Assessment of Absorption of Saxagliptin using Parallel Artificial Membrane Permeability Assay (PAMPA)

**Test Article:** Saxagliptin

**Study Type:** Non-GLP

**Location in Dossier:**

**Study No./Document Control No.:** 930023241

**Method:** Saxagliptin was incubated in PAMPA assay plate for 4 hours at room temperature and samples from donor and acceptor compartments were analyzed by LC-MS/MS. The study was conducted at an initial saxagliptin donor concentration of 100 µM at donor pH of 5.5 and 7.4 and acceptor pH of 7.4.

**Tabulated Results:**

	Po (nm/sec)	
	Donor pH 5.5	Donor pH 7.4
Saxagliptin	4 ± 1	59 ± 10

**Additional Information:** Reported permeability coefficient (Po) values represent mean ± SD from 3 independent experiments, with sextuplet determinations performed in each experiment. The results indicated that saxagliptin exhibits low intrinsic membrane permeability.

**Pharmacokinetics: Other (Absorption with Caco-2 Model)**

**Study Description or Title:** In Vitro Assessment of Permeability of Saxagliptin using Caco-2 Cells

**Test Article:** Saxagliptin

**Study Type:** Non-GLP

**Location in Dossier:**

**Study No./Document Control No.:** 930018000

**Method:** Saxagliptin was incubated with Caco-2 cell monolayers for 4 hours at 37°C at apical pH of 5.5, 6.5, or 7.4 and basolateral pH of 7.4. The studies were initiated by adding 200 µM saxagliptin to the apical side of the monolayer and permeability was measured in the apical-to-basolateral (A-to-B) direction. Samples collected from the apical and the basolateral compartments were analyzed by HPLC/UV. Quality control compounds were evaluated in the experiments.

**Tabulated Results:**

Compound	Apical pH	A-to-B permeability coefficient (nm/sec, mean ± SD)
Saxagliptin	5.5	10 ± 2
Saxagliptin	6.5	17 ± 11
Saxagliptin	7.4	12 ± 1
Ranitidine	6.5	24
Mannitol	6.5	32
Timolol	6.5	47
Desipramine	6.5	81
Metoprolol	6.5	137
Sulfisoxazole	6.5	245

**Additional Information:** The A-to-B permeability was determined in triplicate. Expected A-to-B Po values were observed for the control compounds. The results indicated that saxagliptin was poorly permeable across Caco-2 cell monolayers.

Pharmacokinetics: Other (P-gp-Mediated Transport of Saxagliptin in LLC-PK<sub>1</sub> Cells)Study Description or Title: Assessment of P-gp-Mediated Transport in LLC-PK<sub>1</sub> Cell Monolayers

Test Article: Saxagliptin

Study Type: Non-GLP

Location in Dossier:

Study No./Document Control No.: 930022259

Type of Study: In vitro transport

Method: LLC-PK<sub>1</sub> cells transfected with empty vector or human P-gp cDNA were designated as CLD and 22L1, respectively. Concentration- and time-dependent transport of saxagliptin was evaluated in the apical-to-basolateral (A-to-B) and basolateral-to-apical (B-to-A) directions using LLC-PK<sub>1</sub> cell monolayers. [<sup>14</sup>C]saxagliptin concentrations of 3, 10, and 100 μM were incubated with cell monolayers for 60, 90, and 120 minutes at 37°C, pH 7.4. Samples collected from the apical and basolateral compartments were analyzed by liquid scintillation counting.

## Tabulated Results:

Compound	Nominal Concentration (μM)	Time (min)	Cell Line	P <sub>app</sub> (A-to-B) (cm/sec, mean ± SD)	P <sub>app</sub> (B-to-A) (cm/sec, mean ± SD)	Efflux Ratio (B-to-A)/(A-to-B) (mean ± SD)
Saxagliptin	3	60	CLD	9.9 × 10 <sup>-07</sup> ± 2.1 × 10 <sup>-07</sup>	7.6 × 10 <sup>-07</sup> ± 1.3 × 10 <sup>-07</sup>	NC <sup>a</sup>
Saxagliptin	3	90	CLD	1.1 × 10 <sup>-06</sup> ± 1.1 × 10 <sup>-07</sup>	1.1 × 10 <sup>-06</sup> ± 1.9 × 10 <sup>-07</sup>	1.0 ± 0.1
Saxagliptin	3	120	CLD	1.4 × 10 <sup>-06</sup> ± 6.4 × 10 <sup>-08</sup>	1.4 × 10 <sup>-06</sup> ± 1.2 × 10 <sup>-07</sup>	1.0 ± 0.1
Saxagliptin	10	60	CLD	1.2 × 10 <sup>-06</sup> ± 7.7 × 10 <sup>-08</sup>	9.4 × 10 <sup>-07</sup> ± 1.6 × 10 <sup>-07</sup>	0.8 ± 0.1
Saxagliptin	10	90	CLD	1.0 × 10 <sup>-06</sup> ± 1.7 × 10 <sup>-07</sup>	1.0 × 10 <sup>-06</sup> ± 1.9 × 10 <sup>-07</sup>	1.0 ± 0.3
Saxagliptin	10	120	CLD	1.1 × 10 <sup>-06</sup> ± 7.7 × 10 <sup>-08</sup>	1.2 × 10 <sup>-06</sup> ± 1.5 × 10 <sup>-07</sup>	1.1 ± 0.1
Saxagliptin	100	60	CLD	1.2 × 10 <sup>-06</sup> ± 3.0 × 10 <sup>-08</sup>	9.2 × 10 <sup>-07</sup> ± 1.5 × 10 <sup>-07</sup>	0.7 ± 0.1
Saxagliptin	100	90	CLD	1.3 × 10 <sup>-06</sup> ± 6.9 × 10 <sup>-08</sup>	1.1 × 10 <sup>-06</sup> ± 1.3 × 10 <sup>-07</sup>	0.8 ± 0.1
Saxagliptin	100	120	CLD	1.1 × 10 <sup>-06</sup> ± 2.3 × 10 <sup>-08</sup>	1.2 × 10 <sup>-06</sup> ± 1.6 × 10 <sup>-07</sup>	1.0 ± 0.1
Digoxin	5	90	CLD	3.3 × 10 <sup>-06</sup>	5.8 × 10 <sup>-06</sup>	1.8
Saxagliptin	3	60	22L1	9.2 × 10 <sup>-07</sup> ± 7.8 × 10 <sup>-07</sup>	4.6 × 10 <sup>-06</sup> ± 9.2 × 10 <sup>-07</sup>	NC <sup>a</sup>
Saxagliptin	3	90	22L1	1.2 × 10 <sup>-06</sup> ± 4.0 × 10 <sup>-07</sup>	4.6 × 10 <sup>-06</sup> ± 6.6 × 10 <sup>-07</sup>	3.7 ± 1.7
Saxagliptin	3	120	22L1	1.9 × 10 <sup>-06</sup> ± 7.6 × 10 <sup>-09</sup>	5.7 × 10 <sup>-06</sup> ± 4.3 × 10 <sup>-07</sup>	3.0 ± 0.2
Saxagliptin	10	60	22L1	1.1 × 10 <sup>-06</sup> ± 2.1 × 10 <sup>-07</sup>	3.5 × 10 <sup>-06</sup> ± 4.8 × 10 <sup>-07</sup>	3.2 ± 0.9
Saxagliptin	10	90	22L1	1.8 × 10 <sup>-06</sup> ± 1.2 × 10 <sup>-06</sup>	3.8 × 10 <sup>-06</sup> ± 4.1 × 10 <sup>-07</sup>	2.1 ± 1.4
Saxagliptin	10	120	22L1	1.8 × 10 <sup>-06</sup> ± 3.5 × 10 <sup>-07</sup>	4.3 × 10 <sup>-06</sup> ± 3.7 × 10 <sup>-07</sup>	2.3 ± 0.3
Saxagliptin	100	60	22L1	1.0 × 10 <sup>-06</sup> ± 4.3 × 10 <sup>-08</sup>	3.6 × 10 <sup>-06</sup> ± 8.5 × 10 <sup>-08</sup>	3.5 ± 0.1
Saxagliptin	100	90	22L1	1.1 × 10 <sup>-06</sup> ± 3.2 × 10 <sup>-08</sup>	3.8 × 10 <sup>-06</sup> ± 2.8 × 10 <sup>-07</sup>	3.3 ± 0.3
Saxagliptin	100	120	22L1	1.5 × 10 <sup>-06</sup> ± 5.6 × 10 <sup>-08</sup>	4.6 × 10 <sup>-06</sup> ± 3.6 × 10 <sup>-07</sup>	3.1 ± 0.4
Digoxin	5	90	22L1	9.7 × 10 <sup>-07</sup>	9.2 × 10 <sup>-06</sup>	9.4

Additional Information: Reported apparent permeability (P<sub>app</sub>) values for saxagliptin represent mean ± SD of triplicate determinations. Mass balance recoveries of saxagliptin from the apical and basolateral compartments at the end of the incubation were > 84%. Saxagliptin was subject to active B-to-A transport with efflux ratios in 22L1 (P-gp expressing) cells approximately 3-fold higher than that in CLD (control) cells. The efflux ratios in 22L1 cells were similar in the concentration range tested in the study. The results indicate that saxagliptin is subject to P-gp mediated transport with an active efflux potential lower than that of the positive control digoxin.

<sup>a</sup> NC= not calculated due to high variability in A-to-B P<sub>app</sub> values (dpm values < 3x blank).

**Pharmacokinetics: Other (P-gp Transport of BMS-510849 in LLC-PK1 Cells)**

**Study Description or Title:** Assessment of P-gp-Mediated Transport in LLC-PK1 Cell Monolayers

**Test Article:** BMS-510849

**Study Type:** Non-GLP

**Location in Dossier:**

**Study No./Document Control No.:** 930023811

**Type of Study:** In vitro transport

**Method:** LLC-PK1 cells transfected with empty vector or human P-gp cDNA were designated as CLD and 22L1, respectively. Concentration- and time-dependent transport of BMS-510849 was evaluated in the apical-to-basolateral (A-to-B) and basolateral-to-apical (B-to-A) directions using LLC-PK1 cell monolayers. [<sup>14</sup>C]BMS-510849 nominal concentrations of 3, 10, and 100 µM were incubated with cell monolayers for 60, 90, and 120 minutes at 37°C, pH 7.4. Samples collected from the apical and basolateral compartments were analyzed by liquid scintillation counting.

**Tabulated Results:**

Compound	Nominal Concentration (µM)	Time (min)	Cell Line	P <sub>app</sub> (A-to-B) (cm/sec, mean ± SD)	P <sub>app</sub> (B-to-A) (cm/sec, mean ± SD)	Efflux (Polarization) Ratio (B-to-A)/(A-to-B) (mean ± SD)
BMS-510849	3	60	CLD	<LLOQ	<LLOQ	NA
BMS-510849	3	90	CLD	<LLOQ	<LLOQ	NA
BMS-510849	3	120	CLD	7.4 x 10 <sup>-07</sup> ± 3.1 x 10 <sup>-07</sup>	5.8 x 10 <sup>-07</sup> ± 8.3 x 10 <sup>-08</sup>	0.8 ± 0.2
BMS-510849	10	60	CLD	4.0 x 10 <sup>-07</sup> ± 3.4 x 10 <sup>-08</sup>	4.3 x 10 <sup>-07</sup> ± 4.6 x 10 <sup>-08</sup>	1.1 ± 0.2
BMS-510849	10	90	CLD	4.4 x 10 <sup>-07</sup> ± 9.0 x 10 <sup>-08</sup>	4.3 x 10 <sup>-07</sup> ± 4.2 x 10 <sup>-08</sup>	1.0 ± 0.3
BMS-510849	10	120	CLD	5.4 x 10 <sup>-07</sup> ± 8.2 x 10 <sup>-08</sup>	5.9 x 10 <sup>-07</sup> ± 7.4 x 10 <sup>-08</sup>	1.1 ± 0.3
BMS-510849	100	60	CLD	4.6 x 10 <sup>-07</sup> ± 5.4 x 10 <sup>-08</sup>	4.2 x 10 <sup>-07</sup> ± 4.7 x 10 <sup>-08</sup>	0.9 ± 0.2
BMS-510849	100	90	CLD	5.2 x 10 <sup>-07</sup> ± 4.7 x 10 <sup>-08</sup>	4.7 x 10 <sup>-07</sup> ± 2.1 x 10 <sup>-08</sup>	0.9 ± 0.1
BMS-510849	100	120	CLD	5.9 x 10 <sup>-07</sup> ± 5.0 x 10 <sup>-08</sup>	6.1 x 10 <sup>-07</sup> ± 5.2 x 10 <sup>-08</sup>	1.0 ± 0.1
Digoxin	5	90	CLD	4.9 x 10 <sup>-06</sup>	6.9 x 10 <sup>-06</sup>	1.4
BMS-510849	3	60	22L1	<LLOQ	<LLOQ	NA
BMS-510849	3	90	22L1	<LLOQ	<LLOQ	NA
BMS-510849	3	120	22L1	<LLOQ	<LLOQ	NA
BMS-510849	10	60	22L1	4.6 x 10 <sup>-07</sup> ± 1.0 x 10 <sup>-07</sup>	4.0 x 10 <sup>-07</sup> ± 1.3 x 10 <sup>-08</sup>	0.9 ± 0.2
BMS-510849	10	90	22L1	4.5 x 10 <sup>-07</sup> ± 5.9 x 10 <sup>-08</sup>	4.6 x 10 <sup>-07</sup> ± 6.0 x 10 <sup>-08</sup>	1.0 ± 0.2
BMS-510849	10	120	22L1	5.0 x 10 <sup>-07</sup> ± 5.1 x 10 <sup>-08</sup>	5.2 x 10 <sup>-07</sup> ± 2.0 x 10 <sup>-08</sup>	1.0 ± 0.1
BMS-510849	100	60	22L1	4.9 x 10 <sup>-07</sup> ± 1.2 x 10 <sup>-07</sup>	4.1 x 10 <sup>-07</sup> ± 1.3 x 10 <sup>-08</sup>	0.8 ± 0.1
BMS-510849	100	90	22L1	5.0 x 10 <sup>-07</sup> ± 7.5 x 10 <sup>-08</sup>	4.6 x 10 <sup>-07</sup> ± 2.5 x 10 <sup>-08</sup>	0.9 ± 0.1
BMS-510849	100	120	22L1	5.5 x 10 <sup>-07</sup> ± 8.1 x 10 <sup>-08</sup>	5.3 x 10 <sup>-07</sup> ± 2.8 x 10 <sup>-08</sup>	1.0 ± 0.2
Digoxin	5	90	22L1	1.7 x 10 <sup>-06</sup>	1.6 x 10 <sup>-05</sup>	9.6

Additional Information: LLOQ = lower limit of quantification; one or more samples obtained at the specified time point was < 50 dpm (background: 22 dpm); NA = not applicable. Reported apparent permeability (P<sub>app</sub>) values for BMS-510849 represent mean ± SD of triplicate determinations. Mass balance recoveries of BMS-510849 from the apical and basolateral compartments at the end of the incubation were > 92%. BMS-510849 was not subject to active B-to-A transport with similar efflux ratios in CLD (control) and 22L1 (P-gp expressing) cells. The results indicate that BMS-510849 is not a P-gp substrate.

**Pharmacokinetics: Other (P-gp interaction)**

**Study Description or Title:** Evaluation of interaction between P-glycoprotein and Saxagliptin

**Test Article:** Saxagliptin

**Study Type:** Non-GLP

**Location in Dossier:**

**Study No./Document Control No.:** MAP005-477118/930000866

**Type of Study:** In vitro radiolabeled competitive binding assay

**Method:** A photoaffinity assay was used to examine whether saxagliptin binds to P-glycoprotein (P-gp). A reduction of <sup>125</sup>I-prazosin binding to P-gp in the presence of test compound suggests the test compound binds to P-gp.

**Tabulated Results:**

Concentration (µM)	% decrease in radioactive band intensity
0.5	-21%
5.0	-5%
50	19%

**Additional Information:** A slight decrease in radioactive band intensity (19%), indicative of prazosin binding, was only observed at the highest concentration of saxagliptin tested in the study (50 µM). The increase in band intensity (negative % value) which was observed at the low concentrations of saxagliptin (0.5 and 5 µM) could be attributed to background noise and is not considered significant. The results suggest that saxagliptin does not strongly bind to P-gp.

**Pharmacokinetics: Other (Uptake Transport of Saxagliptin)**

**Study Description or Title:** Evaluation of Cellular Uptake of Saxagliptin by Drug Transporters

**Test Article:** Saxagliptin

**Study Type:** Non-GLP

**Location in Dossier:**

**Study No./Document Control No.:** 930024169

**Type of Study:** In vitro transport

**Method:** [<sup>14</sup>C]Saxagliptin was incubated at 37°C for 5 min in Hanks' balanced salt solution (pH 7.4) with HEK-293 or MDCK cells transiently or stably transfected with empty vector (mock) or OATP1B1, OATP1B3, OCT1, OCT2, OAT1, OAT3, or PEPT1. [<sup>14</sup>C]Saxagliptin also was incubated at room temperature for 1 hour in sodium uptake buffer (pH 6.5 or 7.5) with *Xenopus laevis* oocytes expressing OCT1, OAT3, or PEPT2. In HEK-293 and MDCK cells, [<sup>14</sup>C]saxagliptin was incubated in the presence or absence of a known inhibitor of each transporter. The uptake of positive control compounds was evaluated in transfected cells or oocytes in parallel with saxagliptin in the presence or absence of a known inhibitor of each transporter. Radioactivity was measured using liquid scintillation counting.

**Tabulated Results:**

Model System	Substrate (µM)	Inhibitor (µM)	Control Uptake Rate <sup>a</sup> (mean ± SD)	Transporter-Mediated Uptake Rate (mean ± SD)
Mock/HEK-293 or OATP1B1/HEK-293 Cells	[ <sup>3</sup> H]estradiol-17β-D glucuronide (1 µM)	None	4.8 ± 0.3	71.0 ± 0.8
	[ <sup>3</sup> H]estradiol-17β-D glucuronide (1 µM)	Bromosulphthalein (50 µM)	3.7 ± 0.3	4.2 ± 0.4
	[ <sup>14</sup> C]Saxagliptin (10 µM)	None	158.5 ± 8.8	150.3 ± 14.4
	[ <sup>14</sup> C]Saxagliptin (10 µM)	Bromosulphthalein (50 µM)	160.6 ± 11.2	152.5 ± 4.9
Mock/HEK-293 or OATP1B3/HEK-293 Cells	[ <sup>3</sup> H]BQ-123 (1 µM)	None	1.4 ± 0.7	5.0 ± 0.2
	[ <sup>3</sup> H]BQ-123 (1 µM)	Bromosulphthalein (50 µM)	1.0 ± 0.1	2.3 ± 0.8
	[ <sup>14</sup> C]Saxagliptin (10 µM)	None	131.9 ± 26.7	137.8 ± 11.6
	[ <sup>14</sup> C]Saxagliptin (10 µM)	Bromosulphthalein (50 µM)	134.1 ± 12.3	137.4 ± 5.2
Mock/HEK-293 or OCT1/HEK-293 Cells	[ <sup>3</sup> H]1-methyl-4-phenylpyridinium (MPP) (1 µM)	None	6.4 ± 0.6	61.9 ± 2.7
	[ <sup>3</sup> H]1-methyl-4-phenylpyridinium (MPP) (1 µM)	Imipramine (200 µM)	3.8 ± 0.6	9.5 ± 0.5

**Pharmacokinetics: Other (Uptake Transport of Saxagliptin)**

Study Description or Title: Evaluation of Cellular Uptake of Saxagliptin by Drug Transporters

Test Article: Saxagliptin

Study Type: Non-GLP

Location in Dossier:

Study No./Document Control No.: 930024169

Model System	Substrate (µM)	Inhibitor (µM)	Control Uptake Rate <sup>a</sup> (mean ± SD)	Transporter-Mediated Uptake Rate (mean ± SD)
	[ <sup>14</sup> C]Saxagliptin (10 µM)	None	95.8 ± 3.7	98.5 ± 13.3
	[ <sup>14</sup> C]Saxagliptin (10 µM)	Imipramine (200 µM)	97.5 ± 1.7	96.9 ± 5.9
Water- or OCT1-injected oocytes	[ <sup>3</sup> H]1-methyl-4-phenylpyridinium (MPP) (1 µM)	None	0.08 ± 0.07	11.62 ± 5.24
	[ <sup>14</sup> C]Saxagliptin (10 µM)	None	1.06 ± 0.14	1.27 ± 0.32
Mock/HEK-293 or OCT2/HEK-293	[ <sup>3</sup> H]1-methyl-4-phenylpyridinium (MPP)	None	9.9 ± 1.7	372.2 ± 12.7
	[ <sup>14</sup> C]Saxagliptin (10 µM)			
	[ <sup>3</sup> H]1-methyl-4-phenylpyridinium (MPP) (1 µM)	Imipramine (200 µM)	6.3 ± 1.0	22.4 ± 4.7
	[ <sup>14</sup> C]Saxagliptin (10 µM)	None	172.1 ± 9.2	164.8 ± 2.6
	[ <sup>14</sup> C]Saxagliptin (10 µM)	Imipramine (200 µM)	170.3 ± 3.2	170.8 ± 6.8
Mock/MDCK or OAT1/MDCK Cells	[ <sup>3</sup> H]para-aminohippurate (PAH) (10 µM)	None	35.5 ± 4.9	784.9 ± 24.4
	[ <sup>3</sup> H]para-aminohippurate (PAH) (10 µM)	Probenecid (0.5 mM)	38.4 ± 6.2	43.6 ± 3.4
	[ <sup>14</sup> C]Saxagliptin (10 µM)	None	92.5 ± 3.2	94.1 ± 10.5
	[ <sup>14</sup> C]Saxagliptin (10 µM)	Probenecid (0.5 mM)	89.5 ± 4.5	91.7 ± 12.2
Mock-HEK-293 or OAT3/HEK-293	[ <sup>3</sup> H]Estrone-3-sulfate (1 µM)	None	4.7 ± 0.5	42.6 ± 6.4
	[ <sup>3</sup> H]Estrone-3-sulfate (1 µM)	Bumetanide (200 µM)	5.9 ± 2.3	6.4 ± 1.3
	[ <sup>14</sup> C]Saxagliptin (10 µM)	None	92.0 ± 9.5	91.6 ± 15.0
Water- or OAT3-injected oocytes	[ <sup>3</sup> H]Estrone-3-sulfate (1 µM)	None	0.11 ± 0.1	0.27 ± 0.09
	[ <sup>14</sup> C]Saxagliptin (10 µM)	None	1.06 ± 0.14	1.27 ± 0.20
Mock/MDCK or PEPT1/MDCK Cells	[ <sup>14</sup> C]Glycylsarcosine (Gly-Sar) (10 µM)	None	34.5 ± 19.7	892.9 ± 3.8
	[ <sup>14</sup> C]Glycylsarcosine (Gly-Sar) (10 µM)	Gly-Sar (10 mM)	44.5 ± 2.2	506.1 ± 29.3
	[ <sup>14</sup> C]Saxagliptin (10 µM)	None	74.1 ± 5.6	81.3 ± 12.7
	[ <sup>14</sup> C]Saxagliptin (10 µM)	Gly-Sar (10 mM)	79.4 ± 18.0	74.7 ± 8.5

**Pharmacokinetics: Other (Uptake Transport of Saxagliptin)**

<b>Study Description or Title:</b> Evaluation of Cellular Uptake of Saxagliptin by Drug Transporters		<b>Test Article:</b> Saxagliptin		
		<b>Study Type:</b> Non-GLP		
		<b>Location in Dossier:</b>		
		<b>Study No./Document Control No.:</b> 930024169		
Model System	Substrate (µM)	Inhibitor (µM)	Control Uptake Rate <sup>a</sup> (mean ± SD)	Transporter-Mediated Uptake Rate (mean ± SD)
Water- or PEPT2-injected oocytes	[ <sup>14</sup> C]Glycylsarcosine (Gly-Sar) (10 µM)	None	0.06 ± 0.06	5.58 ± 1.79
	[ <sup>14</sup> C]Saxagliptin (10 µM)	None	0.47 ± 0.06	0.53 ± 0.16

**Additional Information:** The results from this experiment suggest that saxagliptin is not subject to cellular uptake mediated by the evaluated transporters.

<sup>a</sup> Control uptake rates represent rate of uptake in HEK-293 and MDCK cells-transfected with empty vector or rate of uptake in water-injected oocytes. Units for uptake rates are pmol/mg/5 min for HEK-293 and MDCK cells and pmol/mg/1 hour for oocytes. Uptake rates are reported as mean ± SD of replicate determinations (n=3 for transfected HEK-293 or MDCK cells and n=8-12 for oocytes).

**Pharmacokinetics: Other (Uptake Transport of BMS-510849)**

<b>Study Description or Title:</b> Evaluation of Cellular Uptake of BMS-510849 by Drug Transporters		<b>Test Article:</b> BMS-510849
		<b>Study Type:</b> Non-GLP
		<b>Location in Dossier:</b>
		<b>Study No./Document Control No.:</b> 930024169

**Type of Study:** In vitro transport

**Method:** [<sup>14</sup>C]BMS-510849 was incubated at 37°C for 5 min in Hanks' balanced salt solution (pH 7.4) with HEK-293 or MDCK cells transiently or stably transfected with empty vector (mock) or OATP1B1, OATP1B3, OCT1, OCT2, OAT1, OAT3, or PEPT1. [<sup>14</sup>C]BMS-510849 was also incubated at room temperature for 1 hour in sodium uptake buffer (pH 6.5 or 7.5) with *Xenopus laevis* oocytes expressing OCT1, OAT3, or PEPT2. In HEK-293 or MDCK cells, [<sup>14</sup>C]BMS-477118 was incubated in the presence or absence of a known inhibitor of each transporter. The uptake of positive control compounds was evaluated in transfected cells or oocytes in parallel with BMS-510849 in the presence or absence of a known inhibitor of each transporter. Radioactivity was measured using liquid scintillation counting.

**Tabulated Results:**

Model System	Substrate (µM)	Inhibitor (µM)	Control Uptake Rate <sup>a</sup> (mean ± SD)	Transporter-Mediated Uptake Rate (mean ± SD)
Mock/HEK-293 or OATP1B1/HEK-293 Cells	[ <sup>3</sup> H]estradiol-17β-D glucuronide (1 µM)	None	4.8 ± 0.3	71.0 ± 0.8
	[ <sup>3</sup> H]estradiol-17β-D glucuronide (1 µM)	Bromosulphthalein (50 µM)	3.7 ± 0.3	4.2 ± 0.4
	[ <sup>14</sup> C]BMS-510849 (10 µM)	None	28.2 ± 1.9	29.7 ± 1.4
	[ <sup>14</sup> C]BMS-510849 (10 µM)	Bromosulphthalein (50 µM)	28.5 ± 0.9	28.2 ± 2.2
Mock/HEK-293 or OATP1B3/HEK-293 Cells	[ <sup>3</sup> H]BQ-123 (1 µM)	None	1.4 ± 0.7	5.0 ± 0.2
	[ <sup>3</sup> H]BQ-123 (1 µM)	Bromosulphthalein (50 µM)	1.0 ± 0.1	2.3 ± 0.8
	[ <sup>14</sup> C]BMS-510849 (10 µM)	None	13.8 ± 2.6	15.9 ± 3.7
	[ <sup>14</sup> C]BMS-510849 (10 µM)	Bromosulphthalein (50 µM)	13.2 ± 5.0	13.9 ± 1.2
Mock/HEK-293 or OCT1/HEK-293 Cells	[ <sup>3</sup> H]1-methyl-4-phenylpyridinium (MPP) (1 µM)	None	6.4 ± 0.6	61.9 ± 2.7
	[ <sup>3</sup> H]1-methyl-4-phenylpyridinium (MPP) (1 µM)	Imipramine (200 µM)	3.8 ± 0.6	9.5 ± 0.5
	[ <sup>14</sup> C]BMS-510849 (10 µM)	None	21.9 ± 3.0	21.7 ± 4.9
	[ <sup>14</sup> C]BMS-510849 (10 µM)	Imipramine (200 µM)	22.6 ± 5.2	21.4 ± 4.3

## Pharmacokinetics: Other (Uptake Transport of BMS-510849)

Study Description or Title: Evaluation of Cellular Uptake of BMS-510849 by Drug Transporters

Test Article: BMS-510849

Study Type: Non-GLP

Location in Dossier:

Study No./Document Control No.: 930024169

Model System	Substrate ( $\mu\text{M}$ )	Inhibitor ( $\mu\text{M}$ )	Control Uptake Rate <sup>a</sup> (mean $\pm$ SD)	Transporter-Mediated Uptake Rate (mean $\pm$ SD)
Water- or OCT1-injected oocytes	[ <sup>3</sup> H]1-methyl-4- phenylpyridinium (MPP) (1 $\mu\text{M}$ )	None	0.08 $\pm$ 0.07	11.62 $\pm$ 5.24
	[ <sup>14</sup> C]BMS-510849 (50 $\mu\text{M}$ )	None	0.48 $\pm$ 0.15	0.45 $\pm$ 0.16
Mock/HEK-293 or OCT2/HEK-293	[ <sup>3</sup> H]1-methyl-4- phenylpyridinium (MPP)	None	9.9 $\pm$ 1.7	372.2 $\pm$ 12.7
	[ <sup>3</sup> H]1-methyl-4- phenylpyridinium (MPP) (1 $\mu\text{M}$ )	Imipramine (200 $\mu\text{M}$ )	6.3 $\pm$ 1.0	22.4 $\pm$ 4.7
	[ <sup>14</sup> C]BMS-510849 (10 $\mu\text{M}$ )	None	46.9 $\pm$ 6.6	47.7 $\pm$ 7.8
	[ <sup>14</sup> C]BMS-510849 (10 $\mu\text{M}$ )	Imipramine (200 $\mu\text{M}$ )	45.6 $\pm$ 6.2	49.5 $\pm$ 10.5
Mock/MDCK or OAT1/MDCK Cells	[ <sup>3</sup> H]para-aminohippurate (PAH) (10 $\mu\text{M}$ )	None	35.5 $\pm$ 4.9	784.9 $\pm$ 24.4
	[ <sup>3</sup> H]para-aminohippurate (PAH) (10 $\mu\text{M}$ )	Probenecid (0.5 mM)	38.4 $\pm$ 6.2	43.6 $\pm$ 3.4
	[ <sup>14</sup> C]BMS-510849 (10 $\mu\text{M}$ )	None	44.5 $\pm$ 12.7	39.3 $\pm$ 3.8
	[ <sup>14</sup> C]BMS-510849 (10 $\mu\text{M}$ )	Probenecid (0.5 mM)	47.5 $\pm$ 7.0	43.7 $\pm$ 5.6
Mock-HEK-293 or OAT3/HEK-293	[ <sup>3</sup> H]Estrone-3-sulfate (1 $\mu\text{M}$ )	None	4.7 $\pm$ 0.5	42.6 $\pm$ 6.4
	[ <sup>3</sup> H]Estrone-3-sulfate (1 $\mu\text{M}$ )	Bumetanide (200 $\mu\text{M}$ )	5.9 $\pm$ 2.3	6.4 $\pm$ 1.3
Mock-HEK-293 or OAT3/HEK-293	[ <sup>14</sup> C]BMS-510849 (10 $\mu\text{M}$ )	None	20.4 $\pm$ 2.5	23.8 $\pm$ 6.0
	[ <sup>14</sup> C]BMS-510849 (10 $\mu\text{M}$ )	Bumetanide (200 $\mu\text{M}$ )	22.2 $\pm$ 1.6	20.2 $\pm$ 0.8
Water- or OAT3-injected oocytes	[ <sup>3</sup> H]Estrone-3-sulfate (1 $\mu\text{M}$ )	None	0.11 $\pm$ 0.1	0.27 $\pm$ 0.09
	[ <sup>14</sup> C]BMS-510849 (50 $\mu\text{M}$ )	None	0.48 $\pm$ 0.15	0.40 $\pm$ 0.07
Mock/MDCK or PEPT1/MDCK Cells	[ <sup>14</sup> C]Glycylsarcosine (Gly- Sar) (10 $\mu\text{M}$ )	None	34.5 $\pm$ 19.7	892.9 $\pm$ 3.8
	[ <sup>14</sup> C]Glycylsarcosine (Gly- Sar) (10 $\mu\text{M}$ )	Gly-Sar (10 mM)	44.5 $\pm$ 2.2	506.1 $\pm$ 29.3

**Pharmacokinetics: Other (Uptake Transport of BMS-510849)**

**Study Description or Title:** Evaluation of Cellular Uptake of BMS-510849 by Drug Transporters

**Test Article:** BMS-510849

**Study Type:** Non-GLP

**Location in Dossier:**

**Study No./Document Control No.:** 930024169

Model System	Substrate (µM)	Inhibitor (µM)	Control Uptake Rate <sup>a</sup> (mean ± SD)	Transporter-Mediated Uptake Rate (mean ± SD)
Mock/MDCK or PEPT1/MDCK Cells	[ <sup>14</sup> C]BMS-510849 (10 µM)	None	47.8 ± 7.7	44.9 ± 7.3
	[ <sup>14</sup> C]BMS-510849 (10 µM)	Gly-Sar (10 mM)	50.5 ± 16.2	49.1 ± 14.5
Water- or PEPT2-injected oocytes	[ <sup>14</sup> C]Glycoylsarcosine (Gly-Sar) (10 µM)	None	0.06 ± 0.06	5.58 ± 1.79
	[ <sup>14</sup> C]BMS-510849 (50 µM)	None	0.48 ± 0.15	0.42 ± 0.16

**Additional Information:** The results from this experiment suggest that BMS-510849 is not subject to cellular uptake mediated by the evaluated transporters.

<sup>a</sup> Control uptake rates represent rate of uptake in HEK-293 and MDCK cells-transfected with empty vector or rate of uptake in water-injected oocytes. Units for uptake rates are pmol/mg/5 min for HEK-293 and MDCK cells and pmol/mg/1 hour for oocytes. Uptake rates are reported as mean ± SD of replicate determinations (n=3 for transfected HEK-293 or MDCK cells and n=8-12 for oocytes).

## 2.6.6 TOXICOLOGY

### 2.6.6.1 Overall toxicology summary

#### General toxicology:

##### **Rats**

Saxagliptin prepared in acidified water was administered as a single oral gavage dose to Sprague Dawley (SD) rats at 25, 75, 150, or 300 mg/kg. Although this was a single dose study, it was carried out after chronic studies were completed to bridge the new preparation of saxagliptin in acidified water to the earlier preparation in 1.25% Avicel. There were no drug-related clinical signs at any dose. Systemic exposures to saxagliptin and BMS-510849 (active metabolite) increased approximately linearly with dose in males but greater than linearly in females. Systemic exposure to saxagliptin was higher (2.5 to 5.6X) in females relative to males, whereas exposure to the active metabolite appeared similar in males and females. The ratio of BMS-510849 to saxagliptin AUC was 0.7 to 1.2 for males and 0.2 to 0.5 for females. In humans, the ratio of BMS-510849 to saxagliptin was 4-7x, indicating a much higher ratio of active metabolite to parent in humans compared to rats. However, since rats were given very high doses of saxagliptin, overall exposure to BMS-510849 in rats and dogs were sufficient to cover exposure to both saxagliptin and BMS-510849 in human subjects.

In the 14-day study, SD rats were treated with oral saxagliptin at 2 (3-7x), 20 (20-54x) and 200 (322-1300x MRHD) mg/kg in Avicel (1.25%) suspension. The top dose of 200 mg/kg was 300 to 1300x the clinical dose of 5 mg, based on AUC. The top dose of 200 mg/kg produced a slight decrease (9%) in food consumption. There was no notable change in BW. Among hematological changes were a decrease in platelet count in high dose (HD) males (-19%) and females (-13%), and an increase in lymphocyte counts. Alkaline phosphatase increased in HD animals perhaps indicating cholestasis at high exposure multiples. Other clinical chemistry changes included decrease in serum potassium and total bilirubin in both sexes  $\geq 20$  mg/kg and decrease in cholesterol, globulin and total protein in males at  $\geq 20$  mg/kg. Urine output was increased by nearly 50% in the HD females relative to control. The reason for the increase is not clear. Saxagliptin produced an increase in kidney, liver, and spleen weight in the HD males and increased liver and spleen weight in HD females. Gross examination found inflammation of the esophagus and lung as well as lung hemorrhage in HD males. Whether this was due to gavage error was not clear. Analysis of immunoglobulins in the 2-week study found a 2-fold increase in IgM in females at 20 mg/kg and 2- and 3-fold increase in IgM in HD rats. IgG levels were increased by 3-fold but only in HD females. CD3 and CD45RA cells were increased in HD males. CD3 and CD4 cells were decreased in 20 mg/kg females only. In this early acute rat study there were no notable histopathology findings except an increased incidence of histiocytosis in the lungs of HD rats. Since there were esophageal and lung hemorrhage findings in some rats, it is possible that the incidence of lung histiocytosis was due to gavage error. Since there were increases in kidney and liver weight at 200 mg/kg, the NOAEL of 20 mg/kg was selected for the 2-week rat study. The NOAEL dose was 20-54x the clinical dose of 5 mg, based on AUC. The metabolite exposure in rats was 1-3x the clinical metabolite exposure, based on AUC.

In the 3-month oral toxicity study, saxagliptin was administered to SD rats at 300, 600 and 1200 mg/kg. Exposure multiples for 300mg/kg (M:1307x, F:3127x MRHD), 600mg/kg (M:2941x,

F:6388x MRHD), and 1200mg/kg (M:16172x, F:8797x MRHD) were significantly greater than the clinical AUC at 5 mg (AUC 81 ng.h/ml). Toxicity was noted at every dose level, including an increase in mortality at the low dose (LD, 1/20 rats), mid-dose (MD, 5/20 rats) and high dose (HD, 8/20 rats). Clinical signs at  $\geq 600$ mg/kg included hypoactivity, moribundity, tremor, unkempt appearance, chromorhinorrhea, chromodacryorrhea, labored breathing, respiratory sounds (gasping) and abnormal gait. Body weight gain declined with dose (11%, 27% and 39%) in males at the LD, MD, and HD respectively. Females dosed with 1200 mg/kg had a 13% decrease in body weight gain. Increased white blood cell count occurred in treated males with significance reached at doses  $\geq 600$  mg/kg. A marked increase in reticulocytes occurred at 1200 mg/kg. A marked decrease in eosinophils occurred at doses  $\geq 300$  mg/kg. Dose-related decrease in platelets occurred in females at doses  $\geq 300$  mg/kg. A slight decrease in lymphocytes occurred in males at 1200 mg/kg. Slight decreases in MCH (HD females), MCV (LD females) and hemoglobin (MD and HD females) were observed. Slight but non-dose related increases in AST and ALT were observed in treated females with no correlative histopathology. Increase in kidney (11-25%) weight occurred in MD and HD corresponding to progressive nephropathy. Slight to moderate decrease in weight of the thymus (lymphoid depletion) and marked increase in weight of the spleen (lymphoid hyperplasia) occurred at all doses in both male and females. The target organs of toxicity included the bone marrow (myeloid depletion/hypoplasia), heart (inflammation/mononuclear cell infiltration), kidney (nephropathy), liver (inflammation & necrosis), lung (histiocytosis), ocular accessory gland (mononuclear cell infiltration), spleen (lymphoid hyperplasia), thymus (lymphoid depletion), skeletal muscle (degeneration) and brain (degeneration). Due to toxicity at all doses, a NOAEL was not established. This is not surprising since even the lowest dose (300 mg/kg) in male and female rats was 1309x to 3127x the clinical dose of 5 mg, based on AUC.

In the 6-month rat study (20/sex/dose, PO) with a 1-month recovery period, SD rats were treated with 2 (3-8x MRHD), 20 (35-75x MRHD) and 100 (270-596x MRHD) mg/kg of saxagliptin with 1.25% Avicel serving as vehicle. The exposure to BMS-510849 metabolite at 2, 20 and 100 mg/kg in rats was  $<1x$ , 3-10x and 22-59x the clinical BMS-510849 exposure at clinical saxagliptin dose of 5 mg, based on AUC. Although there were no drug-related deaths, 2/35 males (control), 1/35 females (2 mg/kg), 2/35 males and 1/35 females both in the 20 mg/kg dose group were sacrificed in poor condition due to gavage error. This was supported by necropsy evaluation (esophageal hemorrhage and inflammation, inflammation of heart and lungs). Mammary adenocarcinoma was observed in 1/35 females (2 mg/kg) that was sacrificed. Since there were no drug-related neoplastic findings in the 2-year rat carcinogenicity study, the incidence of mammary adenocarcinoma in a LD female was considered incidental to treatment. Body weight was lower (-18%) in males at 20 mg/kg at the end of the treatment phase and recovery. Blood pressure (BP) measured by tail cuff was lower in MD and HD males (121-125 mmHg) during first week but not at later time points. Since there were no notable changes in blood pressure values during week 25 in either sex, lower BP during the first week appeared to be transient and gender specific and may have been in part due to higher BP values in the controls (150 mmHg) and variability in the method. Female control and treated had BP values ranging 119 to 130 mmHg. There was no change in heart rate in the treated rats vs. controls. Hematology findings included decreases in basophils in MD (-52%) and HD (-54%) males. Reticulocytes were decreased by 13% in females dosed with 100 mg/kg. APTT was increased by 11% and 9% in males dosed 20 and 100 mg/kg respectively and decreased (-6%) in females dosed with 100 mg/kg. Fibrinogen decreased by 17% in MD females. At the end of the recovery period, eosinophils were decreased by 27% in HD females while fibrinogen was decreased in LD males.

Alkaline phosphatase was increased in males and females dosed 20 and 100 mg/kg by 22 to 28% relative to control. Cholesterol was decreased by 26% (males) and 19% (females) at the 100 mg/kg dose. Weight of the spleen increased by 16% to 25% (at doses  $\geq$  20 mg/kg) which correlates with lymphoid hyperplasia observed. There was also a decrease in pituitary weight in females at 20 mg/kg (-19%) and 100 mg/kg (-14%) and thyroid weight (-17%) at 100 mg/kg with no correlative histopathology.

Most histopathology findings were limited to HD males and females except for lymphoid hyperplasia in spleen, which was seen in MD as well as HD rats. Histopathology findings in the HD rats were marked by mononuclear cell infiltration in adrenal gland, epididymides, prostate (control, HD), thyroid, and urinary bladder. Progressive renal nephropathy was seen in control and HD males and females. At the end of the recovery period, liver and kidney weights decreased in the 20 mg/kg males possibly due to the decreased body weight observed. Recovery adrenal weight was increased by 32% (no correlative histopathology) in the 20 mg/kg males. The target organs of toxicity include the prostate, urinary bladder (mononuclear cell infiltration) and spleen (lymphoid hyperplasia). Interestingly, there were no brain lesions in males at saxagliptin doses up to 100 mg/kg, 270x the clinical dose AUC. Since most of the histopathology observations were not present after the recovery period, the multi-organ mononuclear cell infiltration observed after the dosing period was judged to be reversible and of minimal clinical consequence. The reviewer selected 2 mg/kg as the NOAEL due to minor changes in hematological parameters at 20 mg/kg in rats. Exposure at the NOAEL was 3-8x the clinical dose of 5 mg based on AUC. At 20 mg/kg, exposure to saxagliptin in rats was 35-75x the clinical saxagliptin exposure based on AUC (5 mg dose). The safety margins for BMS-510849 metabolite at 2 and 20 mg/kg were less than 1x and 3-10x the clinical metabolite exposure, respectively.

### **Dogs**

In the 2-week dog study, beagle dogs were treated with 1 (LD), 5 (MD) and 25 (HD) mg/kg of saxagliptin in Avicel 1.25% vehicle. Saxagliptin AUC values in male and female dogs at 1, 5 and 25 mg/kg were 18-12x, 106-82x, 627-562x the clinical AUC at 5 mg dose, respectively. Metabolite exposure in male and female dogs at the same saxagliptin doses were 3-4x, 33-15x, 67-84x the metabolite exposure in humans. Oral administration of 25 mg/kg resulted in moribundity in one male resulting in sacrifice on Day 9 of the study. At the time of sacrifice, this HD male dog had lost 20% of its body weight. Histopathology revealed moderate, multifocal congestion, moderate, diffuse enteropathy with epithelial cell necrosis, inflammation, thrombus, mucus cell depletion, hemorrhage, and moderate lymphoid depletion of both large and small intestine. Minimal multifocal congestion, multifocal infiltration of mononuclear cells and minimal, multifocal, subacute inflammation of centrilobular region and central vein of the liver was observed. Moderate, diffuse lymphoid depletion was observed in the thymus. The clinical signs of toxicity at 25 mg/kg (627x the MRHD) suggested that this exposure exceeded tolerability in dogs.

Clinical signs in dogs included blood in feces at MD, emesis (MD, HD), loose feces and bloody discharge from anus in HD dogs with hypoactivity in HD males. There was no apparent hematological change in this acute dog study. Clinical chemistry changes in the HD group included slight decrease in serum albumin, Na, Ca, sodium and calcium and increase in globulin relative to control. Serum IgG and IgA were increased by 3- and 4-fold respectively in HD males.

The clinical significance of these findings at 25 mg/kg, approximately 600x the clinical dose, is not clear since this dose has clearly reached superapharmacological levels of saxagliptin. The target organs of toxicity in dogs included the GI tract - small and large intestines (enteropathy with epithelial cell necrosis, inflammation, thrombus, mucus cell depletion, hemorrhage, and lymphoid depletion), stomach (mineralization), liver (inflammation, thrombus, congestion), pancreas (congestion, thrombus), mesenteric lymph node (lymphoid necrosis, congestion, erythrophagocytosis), thymus (lymphoid depletion), thyroid (lymphocytic thyroiditis) and the urinary bladder (arteritis and inflammation). The NOAEL of 1 mg/kg was selected due to histopathology findings at 5 mg/kg in dogs. Exposure at the NOAEL of 1 mg/kg saxagliptin was 12-18x the clinical AUC exposure at 5 mg.

In a 3-month study with 1 month recovery, dogs were given oral saxagliptin capsules (LD: 0.2, MD: 1, and HD: 5 mg/kg, PO). Saxagliptin exposure at 0.2, 1 and 5 mg/kg in dogs were 2x, 11x, and 64x the clinical saxagliptin AUC at 5 mg dose, respectively. Metabolite exposure was 0.6x, 4x, and 30x the clinical metabolite AUC at 5 mg, respectively. Since earlier studies found blood in feces, a fecal occult test was done in the 3 month study. Two out of 5 HD males tested positive for fecal occult blood. Hematology changes included increased eosinophils (167%) in HD males, and increased platelets in LD (32%) and HD (34%) males. At the end of the recovery period, reticulocytes were decreased by 66% in LD and MD females and 58% in HD females. Eosinophils were increased by 300% in females dosed 0.2 mg/kg. Reversible increases in creatinine,  $Ca^{2+}$  and lactate dehydrogenase were observed in males dosed 5 mg/kg and females dosed 1 and 0.2 mg/kg respectively. Reversible decrease in albumin was observed in males dosed 5 mg/kg. Reversible decrease in phosphorus was observed in MD and HD males. The target organs of toxicity include the heart, brain, epididymides, skeletal muscle, thyroid (mononuclear cell infiltration), liver (inflammation), and stomach (granuloma). At the end of the recovery period, mononuclear cell infiltration was observed in livers of both control and HD groups. The NOAEL again in the 3-month dog study was 1 mg/kg based on clinical signs. At NOAEL dose, the exposure in dogs to saxagliptin and BMS-510849 metabolite were approximately 11x and 4x the clinical exposures at 5 mg/kg respectively.

In a 12-month oral toxicity study, dogs (7/sex/group) were dosed with 1, 5 and 10 mg/kg of saxagliptin in 1.5% Avicel in water. Data was collected at interim (6 months) and at study termination (12-months). These doses represent 4-5X (M-F), 18-19x (M-F), and 53-34X (M-F) the clinical dose of 5 mg, based on AUC. The exposure to BMS-510849 at these doses in dogs was 1x (M-F), 4-5x (M-F) and 11-12x (M-F) the clinical BMS-510849 AUC exposure. Study findings at interim 6-months and study termination at 12-months will be discussed. After 6 months of treatment, 3 dogs/sex/group were scheduled to be euthanized and necropsied. The remaining 4 dogs/sex/group were continued on treatment for an additional 6 months. As observed in the 3-month study, there was an increase in incidence of unformed/mucoid stools and abnormally colored stool in both sexes at 5 and 10 mg/kg (MD and HD). In some cases the stool appeared red, green, yellow or white. The incidence of dogs that tested positive for fecal occult blood during Week 2 was higher in males at 10 mg/kg (3/7) relative to controls (1/7) but similar among groups at other time points including control.

Although there was an initial decrease in BW (LD, HD males, 11-14%) at 6 months and at 12 months in all treated males (LD:16%, MD:11% and HD:20%), changes were not statistically different from control and were not related to a change in food intake. Close observation of dogs found cracking of the food pads in 3 of 4 HD males (WK 41) and 4 of 4 HD females (WK33).

There was also one female at MD with cracking of food pads (19x MRHD). Cracking of the foot pads was supported by histopath findings that were marked by erosion of the epidermis/keratin surface of the food pad. It is reasonable to conclude that erosions of the foot pads in dogs resulted from the same mechanism responsible for ulcerative and erosive skin lesions in monkeys administered saxagliptin.

The most consistent hematological finding was an increase in eosinophil count in HD females at 3 (142%), 6 (173%), 9 (209%) months and but not at 12-months. The decrease in lymphocyte count (22%) in HD males at 6-months was not seen at later time points.

Cardiovascular evaluation found occasional changes in blood pressure (BP) values such as an increase in diastolic and arterial BP in HD females at 9-months but not at earlier or later time points. There was no change in heart rate. ECG evaluation found no meaningful drug-related changes even though there were occasional inconsistent, gender specific changes at some points (increase in QTc in HD female at 3 months). Blood oxygen saturation was increased in all treated males only at 3-months. Variation of values in the control and treated groups had often rendered interpretation of 'true' drug-related effects difficult.

There were no notable changes in ophthalmic values or liver enzymes or urinalysis. Clinical chemistry concerns were restricted to decreased serum total protein, Ca, K, and albumin in HD males.

Gross necropsy evaluation of the dogs at 6-months yielded unremarkable results. At 12-months, however, there were few incidences of ileocolic junction discoloration in MD and HD dogs and sore extremities (foot pads) primarily in HD male and female dogs. Of all the tissues weighed, only the prostate weight was affected (LD: -46%, HD: -42%), but since it was not dose-dependent, the decrease in prostate weight may have been coincidental.

Target organs identified after microscopic evaluation included adrenal cortex (capsular nodules), cracking of the foot pads (skin) of the fore and rear paws (inflammation, hemorrhage, and erosion characterized by vacuolation of epithelium/keratin, parakeratosis, sloughing of keratin and hemorrhage), ileum and ileocolic junction (congestion), kidney (lymphoid cell aggregates, mineral deposits, pyelonephritis), lacrimal gland (lymphoid cell aggregates), mediastinal lymph node (erythrophagia, lymphoid cell hyperplasia) mesenteric lymph node (lymphoid cell depletion/atrophy), salivary gland (lymphoid cell aggregates), thymus (involution/atrophy) and urinary bladder (inflammation, urothelial hyperplasia) at MD and HD dogs. Based on the histopathology findings at MD and HD dogs, 1 mg/kg was selected as the NOAEL. Exposure at the NOAEL dose was 11x and 4x higher than the clinical AUC for saxagliptin and BMS-510849 at 5 mg dose, respectively.

Evaluation of plasma DPP4 activity found maximum DPP4 inhibition at all doses by 97 to 99% suggesting that even the NOAEL dose was very effective in inhibiting DPP4 activity in dogs. It is highly possible that at doses  $\geq$  5 mg/kg, saxagliptin potentially interacted/inhibited other enzymes (i.e. DPP8 and DPP9), leading to potential off target activity.

In summary, oral administration of saxagliptin to dogs for 12-months found no drug-related changes at 1 mg/kg. At 5 (18-19x, MRHD) and 10 mg/kg (53-34x, MRHD), unfurrowed/mucoid feces and erosions of the epidermis/keratin surface of the foot pads (beginning at 7 months) were observed. Additional findings at 10 mg/kg were increased eosinophils in females, increased serum cholesterol and triglycerides in females, and decreased serum protein and albumin in males. Since foot pad surface erosions and adverse clinical signs were seen at  $\geq$  5 mg/kg, the NOAEL dose was limited to 1 mg/kg (11x saxagliptin, 4x BMS-510849 MRHD).

### Monkeys

At the request of the agency, the sponsor carried out several toxicology studies in cynomolgus monkeys. Monkey studies were requested to evaluate the potential for saxagliptin to induce necrotizing cutaneous lesions as observed with multiple other DPP4 inhibitors.

In the initial 1-3-month study in cynomolgus monkeys (3-5/sex/group, 2005), animals were treated with 2, 10, 30/20 mg/kg of saxagliptin in acidified water. The LD was given for 3-months while MD and HD were given for only 4-6 weeks. At these doses, saxagliptin (M-F) exposure was 7-17x, 35-21x, 74-60x the clinical saxagliptin exposure at 5 mg dose, based on AUC, respectively. The metabolite exposure (M-F) was 6-9x, 36-28x, 64-60x the clinical exposure to the metabolite at 5 mg dose of saxagliptin, respectively.

Due to significant toxicity by Day 3 that resulted in termination of 2 HD females (Day3/4), the top dose was reduced to 20 mg/kg. In one HD female, severe thrombocytopenia resulted in dose termination on Day 29 and re-initiated 21 days later when platelet levels reached normal levels. Saxagliptin produced skin lesions at all dose levels (2, 10 and 30/20 mg/day). The incidence and severity of skin lesions increased with dose and treatment duration. These lesions appeared as early as Day 6 in the HD and Day 13 in MD and LD (2 mg/kg). Skin lesions at the low dose of 2mg/kg appeared to be non-necrotizing. Conversely, skin lesions at MD and HD were ulcerative and necrotizing. Lesions were seen on the tail, nose, face and scrotum. The lesions in 2 HD monkeys were severe enough to necessitate surgical amputation of the distal end of the tail. One HD female had generalized edema which was attributed to nephropathy by the sponsor. Since rat studies had found significant brain lesions in male rats, blood cyanide levels were measured to see if the skin lesions were related to cyanide release. There was no detectable cyanide in the whole blood. Serum thiocyanates detected in the HD monkeys (1.1 to 2.7 µg/ml) were within the background levels seen in humans (nonsmoker: 2.9 µg/ml, smoker: 7.1 µg/ml). Cardiovascular evaluation found no notable change in cardiovascular or ECG parameters in monkeys. Hematological changes of significance were the decreases in RBC, Hct, hemoglobin, platelets and increases in reticulocytes and neutrophils and lymphocytes. Similar decreases in platelet counts and albumin levels have been reported in clinical studies. The mechanism and clinical consequence of lower platelet count is not clear at this point. These changes became more pronounced and occurred earlier at 10 (MD) and 30/20 mg/kg (HD) treated monkeys. Earliest clinical chemistry changes (Day 35) were decrease in albumin and globulin and increase in Serum IgM.

In an attempt to assess skin-related lesions, the sponsor had assessed immunological responses and attempted to phenotype peripheral blood lymphocytes. There was no drug-related change in lymphocyte subsets. The total IgG and IgM levels were however significantly increased relative to prestudy values and controls. There were no detectable antinuclear antibodies suggesting that there was no significant immune mediated mechanism for skin lesions in monkeys. Furthermore, immunohistochemistry analysis found IgG and IgM in the glomerulus or tubular epithelium in the two monkeys with (1MD, 1HD) with glomerulopathy, but no such immunoglobulin deposition occurred in the skin lesions to suggest an immune mediated mechanism.

The most significant change in organ weight was the increase in spleen weight which correlated with lymphoid hyperplasia at  $\geq 2$  mg/kg and bone marrow at  $\geq 10$  mg/kg. Histopathology findings included minimal to mild (LD) and minimal to marked (MD, HD) inflammation and

monocellular-cell infiltration in pituitary gland, parathyroid gland, salivary gland, mammary gland, testes, epididymides, seminal vesicles, prostate, choroid plexus, peripheral nerve, liver, kidney and urinary bladder. Additional tissues that displayed minimal to marked active inflammation and cell-infiltration at  $\geq 10$  mg/kg included pancreas, vagina, uterus, esophagus, tongue. At doses  $\geq 10$  mg/kg, there was an increased incidence of nephropathy (1 MD female, 1 HD males) with associated edema and minimal to moderate increases in the incidence of subacute vasculitis in the skeletal muscle, diaphragm, skin, nasal submucosa, vagina, cervix, urinary bladder, lung, thyroid gland and intestine in 5/6 MD monkeys (M+F). Since there were significant skin lesions and changes in hematology and clinical chemistry parameters, no NOAEL was established. Saxagliptin exposure at the lowest dose of 2 mg/kg was 4 to 6 x the clinical saxagliptin exposure at 5 mg, based on AUC.

In a follow up 6-week study, the sponsor compared saxagliptin to two other DPP4 inhibitors, vildagliptin (BMS-471211), and sitagliptin (BMS-730173) in cynomolgus monkeys (3/sex/dose) monkey. Doses were picked to produce the same pharmacodynamic and or pharmacokinetic parameters produced by saxagliptin at 10 mg/kg which was known to produce skin lesions and multiorgan mononuclear cell infiltrates after 6 weeks of dosing and *in-vitro* potency for DPP4 inhibition. Based on these predictions, monkeys were treated with 10 mg/kg of saxagliptin, 40 mg/kg of vildagliptin and 40 mg/kg of sitagliptin. Due to toxicity on Days 12 (F) and Day 13(M), the vildagliptin dose was reduced to 20 mg/kg. In this study, high dose of saxagliptin and vildagliptin appeared to have similar adverse skin effect. Saxagliptin related skin lesions were seen in 5/6 monkeys with onset as early as Day 2-11 for 3 monkeys and post Day 28 in 2 monkeys. The earlier the skin lesions, the more severe they became. In one female the distal portion of the tail had to be amputated. Skin abrasions/ ulcerations were seen in scrotum, skin digits, nose/nasal cavity and tail (necrosis). There were also minimal to mild lymphoid hyperplasia in the spleen and bone marrow and mild lymphoid depletion in thymus. Furthermore, two animals (M, F) had multifocal glomerulopathy. Nephropathy has been seen in saxagliptin treated monkeys suggesting that it is a drug-related effect. Since DPP4 is known to improve GFR (published literature), then a potent DPP4 inhibitor such as saxagliptin was likely the responsible agent for the two incidences of glomerulopathy in monkeys. Glomerulopathy was also seen in rats. Since saxagliptin is cleared by renal filtration/secretion and autoradiography in rats found kidneys to be the second most exposed tissue after liver and GI, glomerulopathy in monkeys was likely saxagliptin related. Vildagliptin basically had similar profile to saxagliptin regarding skin lesions, hematological and lymphoid hyperplasia in spleen and bone marrow. However there was no evidence of glomerulopathy with vildagliptin. In contrast to saxagliptin and vildagliptin, sitagliptin treated monkeys did not display any sign of skin lesion or any other notable histopathology. However, there was presence of reactive lymphocytes and minimal to mild lymphoid hyperplasia in the spleen and bone marrow in sitagliptin treated monkeys suggesting that all DPP4 inhibitors may produce some changes in lymphoid function. It is highly likely that skin related findings are limited to less selective DPP4 inhibitors such as saxagliptin and vildagliptin. Although the exact mechanism is unknown, the available data appear to point to the DPP4 selectively of this class of drug since skin lesions in cynomolgus monkeys were seen only with the two less selective DPP4 inhibitors. Recent publications have highlighted the potential role of DPP8/DPP9 inhibitors in suppression of T-cell proliferation and inflammatory response. In fact the author of one publication regards this as a potential new therapeutic target in patients with autoimmune disease (e.g. Reinhold D. etal, Clin Chem Lab Med 2009).

Interestingly, the AUC exposure for vildagliptin (9-1.5x) and sitagliptin (17-9x) were higher than saxagliptin exposure on Day 1 and Day 40. The AUC on day 40 for saxagliptin, vildagliptin and sitagliptin were 4,851, 8,495 and 43,449 ng.h/ml in monkeys. Saxagliptin exposure at 10 mg/kg was 60x the clinical saxagliptin dose of 5 mg. Since saxagliptin is much more potent than either drug, it is possible that saxagliptin with no protein binding in monkeys reaches tissues that other compounds don't at similar concentrations to produce toxicological effects. It should be noted that although there were differences in AUC exposures, DPP4 inhibition was similar among the three DPP4 inhibitors. It is highly possible that at higher doses, saxagliptin effect was spilling over to other enzymatic/receptor systems since saxagliptin has been shown to produce maximal plasma DPP4 inhibition at 0.3 and 3 mg/kg. The fact that 2.5 mg is similarly efficacious as 5 mg supports the hypothesis that even lower clinical doses of saxagliptin would have been sufficient to produce near maximal inhibition of DPP4.

In an attempt to identify a NOAEL for skin lesions, the sponsor carried out another 3-month study in cynomolgus monkeys with a 1-month recovery phase. Cynomolgus monkeys (4/sex/group + 3/sex/dose for recovery) were treated with 0.03, 0.3 and 3 mg/kg saxagliptin for 3-months. At these doses, saxagliptin exposure in monkeys were <1x, 1-2.5x, 20-27x MRHD, respectively. The metabolite exposure was <1x, 1x, 10-11x the clinical BMS-510849 exposure, respectively. There were no drug-related deaths in the study and no gross or microscopic findings at 0.03 and 0.3 mg/kg. However, oral administration of 3 mg/kg resulted in macroscopic and microscopic findings in 1/4 males and 4/4 females during the treatment phase. Findings included multi-focal lesions of the skin on the feet and tail which correlated with hypertrophy (smooth muscle and endothelial cells) of the microvasculature and small arteries and also associated with inflammatory-cell infiltration (intramural and perivascular mononuclear cells) with minimal to mild epithelial hyperplasia (secondary reparative change). The lesions at 3mg/kg were described as roughened skin with scabs, including multifocal ulcers on the tail tip of one animal. Two animals affected with skin lesions were carried into a non-dosing recovery period, with complete resolution of the lesions by post-dose day 68.

At the high dose of 3mg/kg, there were also mononuclear-cell infiltrates of slight severity (perivascular and/or periglandular) in the mammary gland (3/4 F) and non-necrotizing vascular inflammation in skeletal muscles (minimal, 3/4 F). Minimal lymphoid hyperplasia (1/4 M, 2/4 F) was noted in the spleen (1/4 M, 1/4 F), thymus (1 of 4 F), and/or in the bone marrow (2 of 4 F). There were no drug-related histopathology findings at the end of the recovery. Based on notable skin findings at 3 mg/kg, the NOAEL was 0.3 mg/kg saxagliptin. Saxagliptin doses of 0.3 and 3 mg/kg produced near maximal inhibition of DPP4 activity (Emax 57 to 70%). DPP4 activity was also inhibited by 0.03 mg/kg but to slightly lower extent (Emax 49%). DPP4 inhibition at the end of the 24-hr (Emin) ranged from 9 to 28%, 17 to 52% and 30 to 48% at 0.03, 0.3 and 3 mg/kg, respectively. The exposure multiples at NOAEL dose of 0.3mg/kg was 1-2.5x the clinical saxagliptin exposure. The metabolite exposure was also near unity relative to the clinical dose of 5 mg, based on AUC.

Since relatively mild and reversible skin lesions occur at a 20 to 27x multiple of clinical exposure to saxagliptin, the risk to humans appears relatively limited. By comparison, vildagliptin produced necrotizing, irreversible skin lesions at multiple sites in cynomolgus monkeys at ~3-fold multiple of clinical exposure. A 20-fold multiple of vildagliptin resulted in lesions of such severity in monkeys that humane sacrifice was required. It should be noted that more than 2500

patients have received saxagliptin doses up to 10 mg with no notable clinical signs to suggest saxagliptin will produce skin lesions in humans similar to those in monkeys.

Genetic toxicology:

The genotoxicity of saxagliptin (BMS-477118) drug substance with impurities ( < > < > ) used in toxicology and clinical studies was tested in a standard battery of genotoxicity tests. In addition to saxagliptin, the sponsor also carried out independent tests for the major active metabolite, BMS-510849. Saxagliptin with impurities and its metabolite were negative in the Ames test using standard *Salmonella typhimurium* and *Escherichia coli* strains. In a cytogenetics study using human lymphocytes, the initial saxagliptin product with impurities was clastogenic in vitro at the highest concentration tested (1000 µg/mL), in the absence of S9. However, no clastogenicity or evidence of DNA damage was observed in rats at doses up to 2000 mg/kg for 3 days in a micronucleus assay, 1500 mg/kg in a DNA repair study, or 500 mg/kg for 1 month in an in vivo/in vitro rat cytogenetics study. The positive human lymphocyte assay appears to be due to several degradants in the old manufacturing process. These degradants were identified and removed or reduced in the final product manufactured by Process D for marketing. Overall, the to-be-marketed saxagliptin manufacture by process D and its' major active metabolite (BMS-510849) were not genotoxic under standard assay conditions. b(4)

Carcinogenicity:

The sponsor had carried out standard 2-year carcinogenicity studies in mice and rats. The carcinogenicity protocols submitted by the sponsor were concurred by the Executive Carcinogenicity Assessment Committee (ECAC). The full carcinogenicity study reports were reviewed by Dr. Alavi and the statistical analysis was performed by Dr. Atria Rahman. Results of the carcinogenicity studies were deemed acceptable by ECAC. The full carcinogenicity study reviews are attached as Appendix B (page 341).

104-Week Oral Gavage Carcinogenicity Study in Mice

BMS-477118 was not carcinogenic when tested up to 600 mg/kg for 2 years in mice. Dose-related mortality was observed male mice at 250 and 600mg/kg but a cause of death or other target organ toxicity was not identified. Sufficient numbers of animals survived to week 90 to allow adequate statistical analysis of tumor incidence. The exposure multiples at 600 mg/kg in male and female mice were 869 and 1165 times the clinical saxagliptin exposure at 5 mg, based on AUC. The exposure to metabolite BMS-510849 at 600 mg/kg in male and female mice was 337 (M) and 300 (F) fold the metabolite exposure in humans. If the exposure to the metabolite is reduced by 39%, the exposure at the top dose in mice is still in excess of 150 fold.

Statistical analysis of the tumor incidences by the sponsor found no difference between saxagliptin and control mice. Tumor incidence in mice was also analyzed by the agency's preclinical statistician, Dr. Atiar Rahman which was in agreement with the sponsor's analysis. There was no statistically significant difference in the incidences of tumors between control and saxagliptin treated mice.

104-Week Oral Gavage Carcinogenicity Study in Rats

BMS-477118 was not considered carcinogenic in SD rats. The incidence of tumors between saxagliptin treated rats (up to 150 mg/kg M and 300 mg/kg F) and controls were similar. There was a significant increase in mortality in HD males but not in females. There were minimal

histopathology findings in females at high drug exposure levels. The brain lesions noted in males at  $\geq 75$  mg/kg appeared to be due to cyanide liberation. Since cyanide liberation was demonstrably related to high expression and activity of CYP2C11 enzyme in male rats, this effect has minimal clinical relevance.

The AUC exposure for BMS-477118 and its metabolite were 28742 and 9204 ng.h/ml in males (150mg/kg) and 179606 and 29730 ng.h/ml in females (300mg/kg), respectively. The exposure at 150 mg/kg in males and 300 mg/kg in females were 355 and 2217 fold greater than clinical dose of 5 mg, based on AUC (81 ng.h/ml).

There was no statistically significant difference in tumor incidence in any of the treated groups compared to controls. Non-neoplastic microscopic findings in the treated groups included adverse effects in the brain, harderian gland, epididymis, urinary bladder and liver. Brain lesions were specific to male rats. Tumor incidence data in rats was also analyzed by the FDA's preclinical statistician, Dr. Atiar Rahman, whose analysis was consistent with the sponsor's.

#### **Reproductive toxicology:**

Standard reproductive toxicology studies with saxagliptin included a fertility study in male and female rats, rat and rabbit embryofetal development studies, and a rat pre-and postnatal development study. Saxagliptin did not adversely alter fertility or embryofetal and post-natal development even at very high multiples of clinical exposure. The FDA had also recommended a rat embryofetal development study with saxagliptin in combination with metformin.

Late in the review cycle for the saxagliptin monotherapy NDA (March 2009), BMS submitted a 15 day safety report describing neural tube malformations in 2 fetuses from a single litter treated with 25mg/kg + 200mg/kg saxagliptin/metformin combination. The full study report was submitted in mid-April 2009, and is preliminarily described in this review.

b(4)

#### Fertility Study in male and female rats

The potential effect of saxagliptin on fertility (25/sex/group) was tested in male (100, 200 and 400 mg/kg, LD, MD and HD, respectively) and female rats (125, 300 and 750 mg/kg). Saxagliptin had no notable effect on male fertility at to 200 mg/kg which was 604x the clinical dose of 5 mg, based on AUC. Saxagliptin doses up to 125 mg/kg (775x the clinical dose, 5 mg, based on AUC) had no notable effect on fertility parameters in female rats.

NOAEL for reproductive effects was 200 mg/kg (603x MRHD-AUC) for males (mortality, decreased fertility at HD) and 125 mg/kg (776x MRHD-AUC) for females (increased embryoletality, prolonged estrous, abbreviated proestrus, decreased fertility, reductions in corpora lutea and implantations). Since NOAEL exposure multiples in male and female rats were  $\geq 600$ x the clinical dose of 5 mg based on AUC, saxagliptin appears to present little or no risk of adverse effects on fertility in human subjects.

#### Rat embryofetal development study

The potential embryofetal development effect of saxagliptin was investigated in pregnant rats. For the study, saxagliptin was prepared in 1.25% Avicel® and was administered by oral gavage to presumed pregnant rats once daily from gestation day (GD6) through GD15 at doses of 64, 240,

and 900 mg/kg. These doses represent 291x, 1503x, 7986x the MRHD of 5 mg based on AUC. The exposure to BMS-510849 metabolite was 15x, 66x and 328x the clinical metabolite AUC.

There was a dose-dependent increase in the incidence of fetuses with reduced pelvic ossification at 240 and 900 mg/kg that reached statistical significance at maternally toxic dose of 900 mg/kg but not at 240 mg/kg. The incidence of reduced ossification of pelvis was drug-related but occurred in the absence of any other fetal effects suggesting that this is likely the most sensitive end point for the embryofetal development studies. Since reduced fetal pelvic ossification was statistically significant at a maternally toxic dose of 900 mg/kg (7986x MRHD of 5 mg) and only marginally at 240 mg/kg (1504x MRHD), and the delay may resolve with age, risk to humans is minimal. It should be noted that clinical risk may change if saxagliptin were to be administered with another drug with its own embryofetal development problems. It is concluded that Saxagliptin was not teratogenic in embryofetal development study in rats.

#### Oral Embryofetal development study in rabbits

The potential embryofetal development effect of saxagliptin was also addressed in pregnant rabbits. Saxagliptin was prepared in 1.25% Avicel and given daily by oral gavage to pregnant rabbits from GD 7 through GD19 at 8, 40 and 200 mg/kg. The doses represent 31x, 152x, 1432x the clinical dose of 5 mg, based on AUC. BMS-510849 exposure in pregnant rabbits was 17x, 109 and 992x the metabolite exposure at clinical saxagliptin dose of 5 mg.

Food consumption decreased by 10% to 13% (NS) in all treated does, with no dose-related pattern, during the dosing period (Gestation Days 7-20). Food consumption in does given 8 mg/kg decreased by 11% (NS) but increased by 4% and 3% in does dosed 40 mg/kg and 200 mg/kg respectively on GD 20-29 (postdose period). Slight but significant increase (2%) in ossification sites per fetus per litter (ribs) was observed in the 200 mg/kg group. NOAEL for maternal toxicity could not be established due to decreased food consumption (11%) at the LD of 8 mg/kg (31x). NOAEL for fetal toxicity was 40 mg/kg (152x MRHD of 5 mg) due to the skeletal effects observed at 200 mg/kg (1432x MRHD of 5 mg). Fetal skeletal ossification finding (slight increase) in rabbits suggests that ossification process is a sensitive marker in embryofetal development studies in both rabbits and rats. It is concluded that saxagliptin was not teratogenic in the rabbit embryofetal development study.

#### Oral Study of Pre- and Postnatal Development in Rats:

Oral administration of saxagliptin (40, 250 and 500 mg/kg) to pregnant/lactating rats from gestation day 6 through lactation day 20 resulted in lower body weight and body weight gain in F1-generation offspring. These changes resolved by early adulthood and occurred only at doses of 250 and 500 mg/kg that also caused maternal toxicity, defined as a decrease in body weight and food intake during lactation days 1 to 7. The NOAEL for both dams and offspring in this study was 100 mg/kg (AUC of 38061 and 9573 ng•h/mL for saxagliptin and BMS-510849, respectively). The maternal NOAEL dose of 100 mg/kg was approximately 470x and 22x the clinical dose of 5 mg based on AUC (saxagliptin and BMS-510849, respectively).

#### Rat embryofetal development study with saxagliptin in combination with metformin

The sponsor notified the agency in a 15-day safety report in March 2009 that when saxagliptin (5 and 25 mg/kg, 21x and 114x MRHD) was given in combination with metformin (200 mg/kg, 4x MRHD), notable malformations were observed in the rat embryofetal development study with 25mg/kg saxagliptin+metformin. Malformations were characterized as craniochischisis

(incomplete closure of the skull and spinal column = neural tube defect) with forelimb flexure and absence of renal papillae in 2 fetuses from 1 litter at 25/200 mg/kg of saxagliptin/metformin. The full study was submitted and reviewed (April 09) for this NDA.

) Neural tube defect is very rare teratogenic finding. Since both saxagliptin and metformin alone are not associated with teratogenic findings (metformin is pregnancy category B), the Division was concerned that the teratogenic finding was related to an unexpected drug interaction with the combination. Therefore, the Division has requested that the sponsor repeat the study in rats and perform an additional study in rabbits with saxagliptin in combination with metformin that will include separate arms for saxagliptin and metformin alone. The sponsor had proposed that the neural tube defect was related to metformin since metformin has been shown to reduce plasma folate and Vitamin B12, two cofactors essential for methionine synthesis needed for proper fetal development. Based on this, the Division has requested analysis of plasma glucose, folate and Vit B12 in the two studies. Based on the available data, the reviewer believes that pregnancy category for saxagliptin alone should stay as 'B' for the following reasons: a) the teratogenic finding with the combination may have been incidental, b) saxagliptin alone was clean at very high exposure multiples, c) the exposure multiples for malformation in the combination study was at least 110x MRHD, d) the combination dose study design was inadequate due to lack of separate arms for saxagliptin and metformin, e) it is questionable whether a potential malformation in a combination study should result in pregnancy category change since there are number of well known teratogenic drugs i.e. cholesterol lowering drugs (category X) that will cause malformations in the combination studies with saxagliptin. The reviewer recommends the saxagliptin label to reflect the malformation (neural tube defect) with saxagliptin+metformin combination but the label for the pregnancy category for saxagliptin monotherapy to stay as B until the recommended studies are repeated, at which time the risk will be re-assessed.

b(4)

**Local Tolerance:**

Skin sensitivity to saxagliptin (99.4% purity) was tested using local lymph node assay in mouse since repeated exposure of the skin to certain chemicals have been shown to cause immunologically-mediated, delayed-onset hypersensitivity. Saxagliptin caused skin sensitization and proliferation in the draining lymph node in this assay. The relationship of this finding to ulcerative skin lesions in cynomolgus monkeys and foot pad cracking in chronic dog studies is unclear. Whether the skin effect is immune-mediated or saxagliptin activity via DPP8 and DPP9 has not been definitively sorted out. This study suggests that saxagliptin may produce hypersensitivity in humans.

When the potential skin irritation of saxagliptin was tested in rabbits after topical application of 500 mg of saxagliptin, there was no reaction following single semi-occluded, topical application of saxagliptin to the intact rabbit skin for 4 hours. Based on this study, saxagliptin did not appear to be a direct irritant to rabbit skin. Saxagliptin was also not irritative in studies with corneal tissue.

**Special Toxicology:****Saxagliptin-induced brain lesions in male rats:**

In a series of mechanistic studies in rats, the sponsor tried to sort out what was causing brain lesions in male rats at high doses of saxagliptin. In vitro and in vivo biotransformation studies have suggested that the cytochrome P450 isozyme CYP2C 11, which is highly expressed in male

rats, may be responsible for the de-cyanation of saxagliptin with the concurrent release of cyanide. This hypothesis was tested by administering saxagliptin (1200 mg/kg) to SD rats with and without pretreatment with cimetidine (a CYP2C11 inhibitor). In rats given cimetidine alone or cimetidine followed by saxagliptin no adverse clinical signs were observed and blood cyanide concentrations were below the limit of quantitation. There were 3 deaths (3/6) in saxagliptin group designed to be bled at 2 hours. Consistent with acute cyanide toxicity, clinical signs included decreased activity, ataxia, labored respiration, inactivity, tremor, and cage biting. These findings are consistent with acute cyanide intoxication (Mugford, Kedderis. Sex-dependent metabolism of xenobiotics, Drug Metab. Rev. 1998, 30(3): 441-498.) Clinical signs occurred within 20 minutes. Blood cyanide levels were increased in saxagliptin group at 0.5 hr (2.2 µg/ml) and 2 hours (1.5 µg/ml) post dose. Serum thiocyanate concentrations were similarly increased at 0.5 hr (2.5X control) and 2 hours (7.4X control). Increased serum thiocyanate concentrations (1.7X and 5.2X control at 0.5 and 2 hours after dosing, respectively), but not blood cyanide, were also noted in the rats given both cimetidine and saxagliptin. In summary, the study found an increase in blood cyanide levels after single dose of saxagliptin (1200 mg/kg). Pretreatment with cimetidine eliminated the overt toxicity and reduced blood cyanide levels to below the limit of quantitation. The levels of serum thiocyanate in rats given saxagliptin plus cimetidine were lower than those noted in rats given BMS-477118 alone, thus suggesting that cyanide production was not completely blocked through the inhibition of CYP2C11 by cimetidine.

#### Chronic investigation of CNS toxicity in rats

This study appeared to be a carcinogenicity study that was changed into a chronic CNS toxicity study further exploring the role of cyanide release from saxagliptin and brain lesions in male rats. In an attempt to analyze brain lesions produced by saxagliptin in male rats, a detailed examination of the brain tissues and plasma cyanide levels were made in a chronic rat study. In this study, SD rats (60/sex/group) were treated with 0, 0, 25, 75, 150 or 300 mg/kg of saxagliptin for 81/82 weeks. There was a significant incidence of mortality in males at 300 mg/kg; thus, the study was revised to carry out necropsy at interim week 54 in all surviving high dose males. The controls and 25, 75 and 150 mg/kg groups were necropsied at week 81/82. Survival of males given ≤ 150 mg/kg and females given ≤ 300 mg/kg was similar to that of controls through Week 54. High dose males (300 mg/kg) had a survival rate of 73% relative to control (93%) at Week 54. The survival rate at necropsy in males at 150 mg/kg was 42% vs. 62% in controls. The survival rates in males at ≤ 75 mg/kg were similar to females at ≤ 150 mg/kg and controls.

No drug-related macroscopic findings were observed in any of the unscheduled sacrifice rats, or at the interim and terminal sacrifices. Drug-related microscopic findings were observed only in the brain of male rats at doses ≥ 150 mg/kg (355x MRHD). At 150 mg/kg, microscopic findings in the brain of 9 of 60 males were most commonly in caudate-putamen, but were also present in the corpus callosum, frontal cortex, and/or cerebellum at terminal sacrifice (Weeks 81/82). Drug-related brain lesions were observed at the interim sacrifice (Week 54) in 33 of 60 male rats given 300 mg/kg dose. Findings were most common in the corpus callosum but were also present in the caudate-putamen, thalamus, and/or piriform/temporal cortex and included attenuation and degeneration/rarefaction in the corpus callosum; focal or multifocal gliosis and increased vascularization in the caudate-putamen; focal/multifocal necrosis in the caudate-putamen, piriform/temporal cortex, and thalamus; intracytoplasmic PAS-positive material in glial cells in the corpus callosum, caudate-putamen, piriform/temporal cortex, and thalamus; and increased glial fibrillary acidic protein immunoreactivity in the corpus callosum, caudate-putamen, and thalamus.

Brain penetration of saxagliptin and BMS-510849 was not extensive (brain:plasma concentration ratio  $\leq 0.16$ ), appeared similar (brain:plasma concentration ratio range of 0.04 to 0.16) at each dose and time point, and no gender-related differences were apparent. Cyanide was not measurable in any of the controls and only measurable in 3 males at 150 mg/kg (0.33, 0.48, or 1.2  $\mu\text{g/ml}$ ). Thiocyanate concentrations ranged from 1.8 to 3.4  $\mu\text{g/ml}$  in control rats and 3.8 to 12  $\mu\text{g/ml}$  in rats given 150 mg/kg. Overall, male rats dosed with 150 mg/kg were the only rats with measurable whole blood cyanide concentrations and, when compared with control rats, had increased serum thiocyanate concentrations. The study found a close relationship between cyanide and brain lesions in male rats. These lesions in male rats were similar to known published cyanide-induced brain lesions.

#### Immunotoxicology studies

The T-Cell-Dependent Antibody Response was investigated in rats. The immune response was evaluated following challenge with keyhole limpet hemocyanin (KLH) after 28 days dosing of 10, 50, or 200 mg/kg saxagliptin. The antibody response in males given 10 mg/kg (50x) decreased by 20% relative to control. Diffuse lymphoid hyperplasia of the spleen occurred at doses  $\geq 10$  mg/kg (10-50x for males and females respectively). This was associated with dose dependent increases (NS) in splenic-lymphocytes expressing CD3, CD4<sup>+</sup>CD8<sup>-</sup> and CD8<sup>+</sup>CD4<sup>-</sup> in treated males. In treated females, CD4<sup>+</sup>CD8<sup>-</sup> and CD45RA increased dose-dependently (NS). Biologically significant increases in CD3 (51%), CD4<sup>+</sup>CD8<sup>-</sup> (43%) and CD8<sup>+</sup>CD4<sup>-</sup> (47%) were observed in males dosed 200 mg/kg (19X). In females dosed 200 mg/kg (1281X) increases in CD3 (31%), CD4<sup>+</sup>CD8<sup>-</sup> (20%) and CD8<sup>+</sup>CD4<sup>-</sup> (27%) were observed. Even though there were no significant differences between control and treated rats with regards to antibody response to KLH, biologically significant immune response suppression (51-56% decrease in antibody titer relative to control) was observed in males at doses  $\geq 50$  mg/kg ( $\geq 27x$ ). Only minimal immune response suppression (8-13%) occurred in females at doses  $\geq 50$  mg/kg ( $\geq 135X$ ).

An additional immunotoxicology study was conducted in monkeys to evaluate potential acute clinical and hematologic (lymphocyte) changes following once weekly administration of saxagliptin to monkeys. This study was done to address clinical findings of flu-like symptoms (myalgia, body aches and fever) within 14 hours of a second dose of 100 mg in 5 of 15 subjects enrolled in a DDI study. All 15 subjects had transiently decreased absolute lymphocyte counts (approximately 66%). Similar decreases in lymphocyte counts had not been consistently observed in previous repeat daily dose toxicity studies in mice or rats, and only a transient decrease (25%) was observed at 10 mg/kg (at 6 months) in a 1-year dog study. Cynomolgus monkeys were dosed orally (gavage) with an initial saxagliptin dose of 3 mg/kg given on Day 1 followed by challenge doses on Days 8 (3 mg/kg), 15 (10 mg/kg), and 22 (10 mg/kg). Lymphocyte count decreased 30-60% in males and females (the latter were more affected) starting primarily after the second dose of 3mg/kg and continuing through the subsequent two doses of 10mg/kg. The decrease was observed at both 5hr and 24hrs post-dose, which effectively excludes a 'stress-response' as a possible explanation. T-cell subsets decreased by saxagliptin in females included CD8<sup>+</sup>, CD4<sup>+</sup>, (CD2<sup>+</sup>CD20<sup>-</sup>), and (CD20<sup>+</sup>CD2<sup>-</sup>) populations. Males were less affected, as only the (CD20<sup>+</sup>CD2<sup>-</sup>) population declined. This study along with other data appears to show that saxagliptin has some impact on peripheral lymphocytes but the effect is not always consistent and occasionally gender-specific, thus making it difficult conclude that saxagliptin was substantially immunotoxic or that ulcerative skin lesions had immunity-related etiology.

### 2.6.6.2 Single-dose toxicity

#### Rats

Single dose toxicity studies were carried out to explore the mechanism of saxagliptin-related CNS lesions and role of cyanide release in male rats. Single dose of saxagliptin (300, 600, 1000, 1500 and 2000 mg/kg, PO) were given to mice (12/sex/dose) by oral gavage and blood levels of cyanide was determined at 0.5 or 2 hrs post dose. There were no deaths in mice at any dose level. Decrease in activity and increase in respiration was noted at  $\geq 1000$  mg/kg. There was no detectable cyanide in blood with assay detection limit of 0.05  $\mu\text{g/ml}$ . When a similar study was repeated in rats (75, 150, 300, 600, 1000 and 1200 mg/kg, PO), a measurable increase in blood cyanide levels were seen in rats at single saxagliptin doses  $\geq 150$  mg/kg. The increase in cyanide levels appear to increase with dose.

Mean and Individual Blood Cyanide Concentrations

Dose (mg/kg)	Sample Time (h)	No. samples w/ detectable CN <sup>a</sup>	Cyanide ( $\mu\text{g/ml}$ )	
			Mean	Range
150	0.5	1	0.23	0.23
	2	0	<sup>c</sup>	<sup>c</sup>
300	0.5	4	0.36	0.20 - 0.76
	2	1	1.20	1.20
600	0.5	5	1.43	0.37 - 2.50
	2	4	1.01	0.65 - 1.50
1000	0.5	5	1.15	0.22 - 2.20
	2	3 <sup>b</sup>	1.80	1.50 - 2.30
1200	0.5	5	2.10	1.50 - 3.60
	2	3 <sup>b</sup>	3.15	0.36 - 5.30

<sup>a</sup>n = 5; <sup>b</sup>n = 3; <sup>c</sup>below limit of detection (0.05  $\mu\text{g/ml}$ )

There was decreased activity, increased incidence of ataxia and whole body tremor in rats at  $\geq 600$  mg/kg. Deaths were noted at  $\geq 1000$  mg/kg.

#### Monkeys

Single intravenous dose of saxagliptin (5 mg/kg) was given to a single male cynomolgus monkey. Another male monkey was also treated with single oral 5mg/kg dose of BMS-428425 (Novartis: NVP-728, a non-selective DPP inhibitor). There was a 50 mmHg decrease in blood pressure at 1.5 hr post dose. Blood pressure was effectively normalized with aggressive shock therapy. At 24-hr post dose, there was an elevation in serum AST, LDH and CPL. Punch biopsy found moderate multifocal skeletal muscle myofiber degeneration. Similar to saxagliptin, Novartis drug also decreased BP (<50 mmHg) that also had to be treated with aggressive shock therapy. The non-selective DPP4, 8, 9 inhibitor NVP-728 also produced a marked elevation in serum AST, LDH and CPK.

**Single-Dose Oral Investigative Study in Rats with BMS-477118**

(Colerangle review revised by Alavi).

The objective of this study was to determine BMS-477118 and BMS-510849 (active metabolite) levels in plasma and brain following a single dose thereby providing Day 1 TK data to aid in the interpretation of toxicokinetic data from a chronic central nervous system (CNS) investigative study in rats.

In a chronic (12 month) CNS investigational study, brain lesions were detected as early as 10 weeks following oral administration of BMS-477118 at a dose of 300 mg/kg in male rats. In that study, rats were sacrificed at 12 months to assess brain toxicity and parent levels of drug and active metabolite in plasma and brain tissue. The sponsor stated that in order to interpret these data, it was necessary to determine plasma and brain levels following a single dose at the same doses used in the chronic study. Therefore, doses of 25, 75, 150, and 300 mg/kg were selected for this study.

**Key study findings:**

- No drug-related clinical signs were noted at BMS-477118 doses up to 300 mg/kg.
- Systemic exposures to BMS-477118 and its active metabolite, BMS-510849, were dose related.
- Brain concentrations of BMS-477118 and BMS-510849 were relatively low and showed no gender-related differences.

**Study no:** DN05002.

**Volume # and page #:** Vol. 1, pg. 1.

**Conducting laboratory and location:** Bristol-Myers Squibb, Pharmaceutical Research Institute, New Brunswick, NJ.

**Date of study initiation:** January 3, 2005.

**GLP compliance:** No.

**QA report:** yes (X) no ( )

**Drug, lot #, radiolabel, and % purity:** Batch # 4E84589-02; 94.5% pure.

**Formulation/vehicle:** BMS-477118 in acidified water.

**Methods:**

**Dosing:**

Species/strain: Rat/SD.

#/sex/group or time point (main study): 10/sex/group.

Satellite groups used for toxicokinetics or recovery: 3/sex/group at 0.5 and 4 hr postdose; 3/sex/group at 1 and 8 hr postdose; 4/sex/group at 2 and 24 hr postdose.

Age: 5 weeks old.

Weight: 110 to 210 on day of dosing.

Doses in administered units: 25, 75, 150 and 300 mg/kg.

Route, form, volume, and infusion rate: Oral, 5 ml/kg.

**Observations and times:** (Data only from the following sections parameters were collected)

Clinical signs: Twice after dosing.

Gross pathology: 3 rats/sex/group were euthanized at 4, 8 and 24 hours postdose. The brains were removed for determination of BMS-477118 and BMS-510849.

Toxicokinetics: Blood samples for TK were collected at 0.5, 1, 2, 4, 8 and 24 hours postdose.

**Results:**

Toxicokinetics: Systemic exposures to BMS-477118 and BMS-510849 (active metabolite) were dose related. Between the 25- to 300-mg/kg doses, the AUC values for BMS-477118 and BMS-510849 appeared to increase approximately equal to the dose increment, except in females where AUC values for BMS-477118 appeared to increase greater than the dose increment. Systemic exposure to saxagliptin (BMS-477118) appeared to be higher in females compared to males whereas exposures to BMS-510849 appeared to be similar in males and females. The ratio of AUC values of BMS-510849 to BMS-477118 were 0.7 to 1.2 for males and 0.2 to 0.5 for females.

**TK SUMMARY**

Dose (mg/kg)	C <sub>max</sub> (ng/mL)				AUC(0-T) (ng.h/mL) <sup>a</sup>			
	Males	Females	Males	Females	Males	Females	Males	Females
	<b>BMS-477118</b>		<b>BMS-510849</b>		<b>BMS-477118</b>		<b>BMS-510849</b>	
25	648	2420	468	888	2278	5644	1609	2850
75	2593	11100	1617	1747	6588	19798	6832	8244
150	4063	32767	2767	3053	13820	55698	16220	13890
300	5043	49433	3673	4513	22836	127928	22421	24525
<b>Nominal Dose Ratio</b>	<b>C<sub>max</sub> Ratio</b>				<b>AUC(0-T) ratio</b>			
1:3:6:12	1:4:6:8	1:5:14:20	1:3:6:8	1:2:3:5	1:3:6:10	1:4:10:23	1:4:10:14	1:3:5:9

<sup>a</sup> Calculated from time zero to the time of last measurable concentration, ranging between 8 and 24 h.

**Brain Concentrations**

Brain penetration of BMS-477118 was relatively low and with the exception of the 25 mg/kg dose in males at the 4-hour time point, mean brain BMS-477118 concentrations were less than those observed in plasma at the same time point. Mean brain concentrations of BMS-510849 were less than 10% of those observed in plasma at each given time point. With the exception of BMS-477118 concentrations in the 300-mg/kg group at the 24-hour time point, the plasma/brain concentration ratios for BMS-477118 and BMS-510849 appeared to be similar in males and females. LLQ was not provided.

Dose (mg/kg)	Time post dose (h)	Brain Concentrations (ng/mL)			
		Males	Females	Males	Females
		<b>BMS-477118</b>		<b>BMS-510849</b>	
25	4	63.75	47.90	63.20	a
	8	a	44.85	a	a
	24	a	a	a	a
75	4	38.35	93.80	a	a
	8	a	55.33	a	a
	24	a	a	a	a
150	4	75.80	57.07	106.17	119.70
	8	73.20	149.00	a	83.90
	24	a	a	a	a
300	4	94.20	87.87	a	a
	8	213.00	498.00	a	a
	24	52.30	a	a	a

<sup>a</sup> All brain concentrations were <LLQ.

Note: Data are mean of individual brain concentrations (n=3/time point), data <LLQ were treated as missing for the calculation of mean values.

Dose (mg/kg)	Time postdose (h)	Brain/Plasma Concentration Ratio			
		Males	Females	Males	Females
		BMS-477118		BMS-510849	
25	4	1.33	0.71	0.04	a
	8	a	0.10	a	a
	24	a	a	b	b
75	4	0.51	0.30	a	a
	8	a	0.04	a	a
	24	a	a	b	b
150	4	0.14	0.13	0.03	0.09
	8	0.04	0.05	a	0.03
	24	a	a	b	b
300	4	0.05	0.07	a	a
	8	0.16	0.04	a	a
	24	0.98	a	a	b

<sup>a</sup> No brain/plasma concentration ratio was calculated as all brain concentrations were <LLQ.

<sup>b</sup> No brain/plasma concentration ratio was calculated as all brain and plasma concentrations were <LLQ.

Note: Data are mean of individual brain/plasma ratios (n=3/time point), data <LLQ were treated as missing for the calculation of mean values.

#### Study Summary:

Solutions of saxagliptin in acidified water were administered orally by gavage to SD rats (10/sex/group) as single doses of 25, 75, 150, or 300 mg/kg. No drug-related clinical signs were observed at any dose. Systemic exposures to BMS-477118 and BMS-510849 (active metabolite) increased approximately equal to the dose increment in males and greater than the dose increment in females. Systemic exposures to BMS-477118 appeared to be higher (2.5 to 5.6X) in females relative to males whereas exposures to the active metabolite appeared to be similar in males and females. The AUC ratios of BMS-510849 to BMS-477118 were 0.7 to 1.2 for males and 0.2 to 0.5 for females.

#### Saxagliptin (BMS-477118) and Cimetidine: Single-Dose Oral Investigative Study in Rats (Colerangle review revised by Alavi).

In vitro and in vivo biotransformation studies have suggested that the cytochrome P450 isozyme CYP2C 11, which is highly expressed in male rats, may be responsible for the de-cyanation of BMS-477118 (saxagliptin) with the concurrent release of cyanide. This hypothesis was evaluated by oral administration of saxagliptin to Sprague-Dawley rats (12 males/group) in the presence or absence of cimetidine (a CYP2C11 inhibitor). The control group received a single dose of cimetidine followed by a dose of acidified water (10 ml/kg). Two (2) other groups of rats were dosed with either 1200 mg/kg (in a solution of acidified water) of BMS-477118 alone or following pretreatment with 300 mg/kg of cimetidine (2 hours prior to the dosing of BMS-477118). The rats were monitored after dosing for adverse clinical signs. Blood cyanide (6 rats/group) and serum thiocyanate (6 rats/group) concentrations were determined at 2.5 and 4 hours (cimetidine control group) after dosing with cimetidine and 0.5 and 2 hours after dosing with BMS-477118, which corresponded to 2.5 and 4 hours after cimetidine pretreatment for the BMS-477118 plus cimetidine group.

**Key study findings:**

**Saxagliptin alone (1200 mg/kg)**

- Three (3) of 6 rats died prior to the 2-hour blood collection.
- In all rats decreased activity, ataxia, labored respiration, inactivity, tremor (6/12), and cage biting (2/12). In most rats, clinical signs were observed within approximately 20 minutes of dosing.
- Increases in blood cyanide concentrations at 0.5 (2.2 µg/ml) and 2 hours (1.5 µg/ml) after dosing compared to cimetidine-treated controls (no blood cyanide detected at either time point) and serum thiocyanate concentrations at 0.5 (2.5X control) and 2 hours (7.4X control) after dosing.

**Saxagliptin (1200 mg/kg) plus cimetidine (300 mg/kg)**

- Increased serum thiocyanate concentrations at 0.5 (1.7X control) and 2 hours (5.2X control) after dosing.

**Study no:** DN05038

**Volume # and page #:** electronic

**Conducting laboratory and location:** Bristol-Myers Squibb Pharmaceutical Research Institute

**Date of study initiation:** April 18, 2005.

**GLP compliance:** No.

**Drug, lot #, radiolabel, and % purity:** Batch # 4E84589; purity not provided.

**Formulation/vehicle:** Solutions of BMS-477118 in acidified water; cimetidine in water.

**Methods:**

**Dosing:**

12 Male SD rats/group (~ 9 weeks old) weighting between 272 to 294 g were used in the study. Saxagliptin alone (1200 mg/kg) or in combination with cimetidine (300 mg/kg) were given to rats orally. Blood cyanide (6 rats/group) and serum thiocyanate (6 rats/group) concentrations were determined at 2.5 and 4 hours (cimetidine control group) after dosing with cimetidine and 0.5 and 2 hours after dosing with BMS-477118, which corresponded to 2.5 and 4 hours after cimetidine pretreatment for the BMS-477118 plus cimetidine group.

Group Number	Dose <sup>a</sup>		Sex	Number of Animals	
	mg/kg	mL/kg		At Start	Died
1 <sup>b</sup>	300	26.3	M	12	0
	0	10.0			
2 <sup>c</sup>	1200	10.0	M	12	3
3 <sup>d</sup>	300	26.3	M	12	0
	1200	10.0			

<sup>a</sup> Date of dosing: 18-April-2005.

<sup>b</sup> Cimetidine (300 mg/kg) followed by acidified water (10mL/kg) within 1 hour of the cimetidine dose.

<sup>c</sup> BMS-477118.

<sup>d</sup> Cimetidine (300 mg/kg) followed by BMS-477118 (1200 mg/kg) approximately 2 hours after the cimetidine dose.

**Results:**

**Mortality:** Three (3) of 6 rats given BMS-4771 18 alone (Group 2) died before their intended 2-hour blood collection.

**Clinical signs:** Drug-related clinical signs observed in all rats given BMS-477118 alone included decreased activity, ataxia, labored respiration, inactivity, tremor (6/12), and cage biting (2/12). In most rats, these clinical signs occurred within approximately 20 minutes of dosing.

**Toxicokinetics:** Blood cyanide concentrations were below the limit of Quantitation (50 ng/ml) in rats given cimetidine alone or cimetidine followed by BMS-477118. Blood cyanide was noted in the rats given BMS-477118 alone at 0.5 (2.2 µg/ml) and 2 hours (1.5 µg/ml) after dosing. Serum thiocyanate concentrations were increased at 0.5 (2.5X control) and 2 hours (7.4X control). Increased serum thiocyanate concentrations (1.7X and 5.2X control at 0.5 and 2 hours after dosing, respectively) were also noted in the rats given both cimetidine and BMS-477118.

Blood Cyanide Concentrations

Dose Groups	Cimetidine 300 mg/kg	BMS477118 1200 mg/kg	Cimetidine 300 mg/kg followed by BMS-477118 1200 mg/kg ~2hr after the cimetidine dose
0.5 hr postdose	BLQ (50 ng/ml)	2.18 µg/ml	BLQ (50 ng/ml)
2 hr postdose	BLQ (50 ng/ml)	1.45 µg/ml	BLQ (50 ng/ml)

Blood Thiocyanate Concentrations

Dose Groups	Cimetidine 300 mg/kg	BMS477118 1200 mg/kg	Cimetidine 300 mg/kg followed by BMS-477118 1200 mg/kg ~2hr after the cimetidine dose
0.5 hr postdose	1.72 µg/ml	4.30 µg/ml**	2.87 µg/ml*
2 hr postdose	1.23 µg/ml	9.07 µg/ml**	6.45 µg/ml**

\* p<0.05; \*\* p<0.01

### 2.6.6.3 Repeat-dose toxicity

#### Six-Month Oral Toxicity Study in Rats with 1-Month Postdose recovery

##### Key study findings:

- There were no drug-related deaths.
- Irreversible body weight decrease of 18% was observed in males dosed 20 mg/kg at the end of the treatment and recovery periods. 16% decrease in food consumption was observed in these males at the end of the recovery period.
- There was no notable change in blood pressure or heart rate in either sex at the end of the study.
- Dose-dependent decreases in basophils were observed in treated males. Reticulocytes were decreased in HD females (100 mg/kg). APTT was increased in MD males (20 mg/kg) and MD and HD in both male and female. Fibrinogen decreased in MD females. At the end of the recovery, eosinophils were still lower in HD females.
- Alkaline phosphatase was increased in males and females dosed 20 and 100 mg/kg by 22 to 28% relative to control. Cholesterol was decreased by 26% (males) and 19% (females) at the 100 mg/kg dose.
- Weight of the spleen increased by 16% to 25% (at doses  $\geq$  20 mg/kg) which correlates with lymphoid hyperplasia observed. 19% and 14% pituitary weight decreases were observed in females dosed 20 and 100 mg/kg respectively with no correlative histopathology. Thyroid weight decreased by 17% in the 100 mg/kg females (no correlative histopathology). At the end of the recovery period, liver and kidney weights decreased in the 20 mg/kg males possibly due to the decreased body weight observed. Adrenal weight increased by 32% (no correlative histopathology) in the 20 mg/kg males at the end of recovery.
- The target organs of toxicity include urinary bladder (mononuclear cell infiltration in both) and spleen (lymphoid hyperplasia), all of which were observed at 100mg/kg.
- NOAEL = 2 mg/kg based on the lymphoid hyperplasia in the spleen at 20 mg/kg.

**Study no:** DN02021

**Volume #, and page #:** electronic

**Conducting laboratory and location:** Bristol-Myers Squibb Co., One Squibb Drive, New Brunswick, New Jersey, 08903.

**Date of study initiation:** March 28, 2002.

**GLP compliance:** Yes (USA)

**QA report:** Yes (X) no ( )

**Drug, lot #, radiolabel, and % purity:**

**Formulation/vehicle:** BMS-477118 in 1.25% Avicel [w/v] in water.

**Methods (unique aspects):** N = 35/sex/group.

The first 20 males and 20 females in each group were designated to be euthanatized at the end of a 6-month dosing period. The last five males and five females in each group were retained for a 1-month postdose evaluation. The remaining 10 males and 10 females in each group were designated for interim necropsy following a 3-month dosing period.

**Dosing:** Animals were dosed orally by gavage at 2, 20 and 100 mg/kg QD for 6 months.

**Species/strain:** Rat/Sprague Dawley.

**#/sex/group or time point (main study):** 35/sex/group (main study).

**Satellite groups used for toxicokinetics or recovery:** 5/sex/group for recovery.

**Age:** 6 weeks at study initiation.

**Weight:** 97-160 g (M); 84-130 g (F)

**Doses in administered units:** 2, 20 and 100 mg/kg QD.

**Route, form, volume, and infusion rate:** Oral (gavage), 5 ml/kg.

**Observations and times:**

Clinical signs: Daily.

Body weights: Weekly.

Food consumption: Weekly.

Ophthalmoscopy: pretest, during the third and sixth months, and near the end

EKG: Not conducted.

Blood pressure: Tail cuff for BP, HR in five males and five females in each group after a daily dose during week 1, 3-months and 6-months and end of the recovery period.

Hematology: Blood collected (10/sex/dose) before and month 1, 3 and 6 and recovery

Clinical chemistry: Standard list at times noted above. In addition plasma GLP-1 and coagulation tests were obtained by cardiac puncture prior to necropsy.

Urinalysis: 24-hr urinalysis before and at 1, 3 and 6 month post dose plus recover. Additional analysis included presence of blood, and urobilinogen.

Gross pathology: At 3 and 6 and at the end of recovery

Organs weighed: standard list

Histopathology: All tissues collected from high-dose, control, and dead or moribund-euthanatized animals were examined by light microscopy for drug-related or spontaneous lesions. Only target organs, defined microscopically at the high dose, and gross lesions were examined at lower doses. In recovery animals, only target organs identified in end-of-dose animals and gross lesions were examined microscopically.

Toxicokinetics: Plasma concentration of saxagliptin and its active metabolite BMS-510849 was determined on Day 1, 3-months and 6-months

**Results:**

**Mortality:**

Dose (mg/kg)	0		2		20		100	
Sex	M	F	M	F	M	F	M	F
Unscheduled sacrifice	2/35 D 47-49			1/35 D 127	2/35 D 95-103	1/35 D 63		

D = Day of sacrifice

**Clinical signs:**

Dose (mg/kg)	0		2		20		100	
Sex	M	F	M	F	M	F	M	F
Chromorhinorrhea	1/35		2/35		3/35		1/35	
Blepharospasm					1/35		1/35	
Conjunctivitis					2/35		2/35	

**Blood Pressure: (mm Hg); N = 5/sex/group**

Dose (mg/kg)	0		2		20		100	
Sex	M	F	M	F	M	F	M	F
Week 1	150	125	137	121	121*	125	123*	130
Week 25	131	119	131	120	137	126	143	115
Week 30 (Recovery)	148	129	132	125	140	130	140	123

\*p<0.05; \*\* p<0.01

Heart rate: (beats/min); N = 5/sex/group

Dose (mg/kg)	0		2		20		100	
Sex	M	F	M	F	M	F	M	F
Week 1	432	450	453	480	443	486	477	447
Week 25	381	453	414	459	412	444	399	411
Week 30 (Recovery)	420	435	414	438	416	432	429	426

\* p<0.05; \*\* p<0.01

Body weights: (g)

Dose (mg/kg)	0		2		20		100	
Sex	M	F	M	F	M	F	M	F
Day -3	117	104	116	102	115	102	117	103
Day 184	499	247	489	243	409**	255	447	234
Day 212 (Recovery)	514	254	506	255	423**	269	471	246

\* p<0.05; \*\* p<0.01

Food consumption: (g)

Dose (mg/kg)	0		2		20		100	
Sex	M	F	M	F	M	F	M	F
Week 1	18	16	18	17	19	16	18	17
Week 25	24	17	29	16	22	15	23	16
Week 30 (Recovery)	23	18	25	16	16*	18	25	17

\* p<0.05; \*\* p<0.01

There was no ophthalmoscopy data.

Hematology: Week 26 Data

Dose (mg/kg)	0		2		20		100	
Sex	M	F	M	F	M	F	M	F
Basophils (%)	1.15		0.90		0.55** (52%↓)		0.53** (54%↓)	
Reticulocytes (%)		2.24		2.16		2.02		1.94* (13%↓)
APTT (sec)	17.97	17.56	17.29	18.68	17.96	19.86** (11%↑)	16.43* (6%↓)	19.53** (9%↑)
Fibrinogen (mg/dl)		156		142		129* (17%↓)		145
WEEK 30 (RECOVERY)								
Eosinophils (%)		3.06		2.62		2.52		2.22* (27%↓)
Fibrinogen (mg/dl)	305		237* (22%↓)		258		287	

\* p<0.05; \*\* p<0.01

Clinical chemistry: Week 26 Data

Dose (mg/kg)	0		2		20		100	
Sex	M	F	M	F	M	F	M	F
Glucose (mg/dl)	133		143* (8%↑)		142		143* (8%↑)	
Cholesterol (mg/dl)	147	141	134	133	131	138	109** (26%↓)	114** (19%↓)
Albumin (g/dl)	3.26		3.30		3.32		3.45** (6%↑)	
Globulin (g/dl)		3.35		3.28		3.28		3.19** (5%↓)
A/G Ratio	0.92	1.07	0.93	1.12* (5%↑)	0.96	1.13* (6%↑)	1.01** (10%↑)	1.13* (6%↑)
ALKP (U/L)	168	154	170	173	208** (24%↑)	189* (23%↑)	205** (22%↑)	197** (28%↑)
Potassium (mEq/l)	5.93	5.68	5.74	5.55	5.61* (5%↓)	5.41	5.63* (5%↓)	5.24* (8%↓)

CL (mEq/l)	99.1	100.7	100.4* (1%↑)	101.4	100.6** (2%↑)	101.5	100.7** (2%↑)	102.1** (1%↑)
Phosphate (mg/dl)	5.79		5.53		5.40		5.23** (10%↓)	
Ca (mg/dl)		10.52		10.48		10.44		10.28* (2%↓)
Dose (mg/kg)	0		2		20		100	
Sex	M	F	M	F	M	F	M	F
WEEK 30 (RECOVERY)								
CL (mEq/l)	98.8	101.0	102.2** (3%↑)	101.5	102.3** (3%↑)	103.0	102.6** (4%↑)	103.2* (2%↑)

\* p<0.05; \*\* p<0.01

Plasma glucagon-like peptide-1: Unremarkable.

Urinalysis: Unremarkable at both weeks 26 and 30.

Organ weights: Absolute wt. (g); relative wt. (to body wt.) (%)

INTERIM SACRIFICE (MONTH 3)								
Dose (mg/kg)	0		2		20		100	
Sex	M	F	M	F	M	F	M	F
Brain (%)	0.46		0.49		0.50* (9%↑)		0.49	
Spleen (g)	0.95		0.79* (17%↓)		0.81* (15%↓)		0.94	
Spleen (%)		0.27		0.25		0.27		0.32* (19%↑)
Thymus (g)	0.37		0.30*		0.32		0.31* (16%↓)	
TERMINAL SACRIFICE (MONTH 6)								
Liver (g)	10.71	5.76	11.02	5.59	10.71	5.34* (7%↓)	11.62* (9%↑)	5.36* (7%↓)
Liver (%)	2.41		2.46		2.46		2.68** (11%↑)	
Spleen (g)	0.85	0.61	0.94	0.59	0.93	0.60	1.02** (20%↑)	0.70* (15%↑)
Spleen (%)	0.19	0.24	0.21	0.25	0.22* (16%↑)	0.26	0.23** (21%↑)	0.30** (25%↑)
Brain (%)		0.71		0.74		0.76* (7%↑)		0.76* (7%↑)
Heart (g)		0.97		0.92		0.89* (8%↓)		0.89** (8%↓)
Kidney (g)		1.48		1.43		1.37* (7%↓)		1.37* (7%↓)
Pituitary (g)		0.0155		0.0142		0.0126* (19%↓)		0.0133
Thyroid (g)		0.024		0.022		0.022		0.020* (17%↓)
WEEK 30 (RECOVERY)								
Adrenal (%)	0.0134		0.0131		0.0173* (32%↑)		0.0161	
Kidney (g)	2.68		2.52		2.11** (21%↓)		2.49	
Liver (g)	12.63		11.44		8.88** (30%↓)		10.96	
Liver (%)	2.55		2.36		2.22** (13%↓)		2.43	

\* p<0.05; \*\* p<0.01

Gross pathology Findings:

UNSCHEDULED SACRIFICE								
Dose (mg/kg)	0		2		20		100	
Sex	M	F	M	F	M	F	M	F
Esophagus Rupture						1/1		
Mandibular L. node Red discoloration	1/2				1/2			
Skin Swelling					1/2	1/1		
INTERIM SACRIFICE (MONTH 3)								
Dose (mg/kg)	0		2		20		100	
Sex	M	F	M	F	M	F	M	F
Unremarkable								
TERMINAL SACRIFICE (MONTH 6)								
Uterus Dilatation								2/20
RECOVERY SACRIFICE: Unremarkable								

Histopathology:

UNSCHEDULED SACRIFICE								
Dose (mg/kg)	0		2		20		100	
Sex	M	F	M	F	M	F	M	F
Esophagus Hemorrhage					1/2(2)			
Inflammation	1/2(4)				1/2(2)	1/1(3)		
Heart, infiltration Mononuclear cell					1/2(1)			
Kidneys, Progressive murine nephropathy	2/2(1)			1/1(1)	2/2(2)	1/1(1)		
Lungs Inflammation					1/2(1)			
Mandibular L. node Erythrophagocytosis	1/2(1)				1/2(2)			
Mammary gland Adenocarcinoma				1/1(X)				
Salivary gland Necrosis					1/2(3)			
Skeletal muscle, Mononuclear cell infiltration				1/1(1)				
Skin Inflammation						1/1(3)		
Spleen Necrosis	1/2(1)				1/2(1)			
Thymus Inflammation					1/2(2)			
Lymphoid depletion	1/2(1)				2/2 1/2(3) 1/2(4)	1/1(2)		
Lymphoid necrosis	2/2(1)				2/2 1/2(1) 1/2(4)	1/1(1)		
Thyroid Inflammation					1/2(2)			
Trachea Inflammation						1/1(1)		

1 = minimal; 2 = mild; 3 = moderate; 4 = marked; x = present

Tissues from control and HD groups were evaluated. However, spleen tissue from all dose groups was examined.

INTERIM SACRIFICE (MONTH 3)								
Dose (mg/kg)	0		2		20		100	
Sex	M	F	M	F	M	F	M	F
Adrenal Hypertrophy							1/10(1)	
Esophagus Inflammation		2/10(1)					2/10(1)	1/10(1)
Heart, Infiltration Mononuclear cell	3/10(1)	3/10(1)					4/10(1)	
Lung Deposition								2/10(2)
Histiocytosis							1/10(1)	
Inflammation	1/10(1)						2/10(1)	2/10(1)
Pancreas Inflammation								1/10(2)
Spleen Lymphoid hyperplasia					1/10(1)	7/10(1)	7/10(1)	9/10 6/10(1) 3/10(2)
Thymus Hemorrhage	2/10(1)						2/10(1)	1/10(1)
Urinary bladder Mononuclear cell infiltration								1/10(1)

1 = minimal; 2 = mild; 3 = moderate; 4 = marked

Tissues from control and HD groups were evaluated.

Spleen tissue from all dose groups were examined

TERMINAL SACRIFICE (MONTH 6)								
Dose (mg/kg)	0		2		20		100	
Sex	M	F	M	F	M	F	M	F
Adrenal gland Mononuclear cell infiltration								1/20(1)
Epididymides Mononuclear cell infiltration							1/20(1)	
Kidney, Progressive Murine Nephropathy	19/20 16/20(1) 3/20(2)	17/20 15/20(1) 2/20(2)					19/20 15/20(1) 2/20(2) 2/20(3)	16/20(2)
Prostate, infiltration Mononuclear cell	5/20(1)						12/20 11/20(1) 1/20(2)	
Spleen Lymphoid hyperplasia					7/20(1)	13/20(1)	18/20(1)	12/20(1)
Thyroid, infiltration Mononuclear cell								1/20(1)
Urinary bladder Mononuclear cell infiltration							1/20(1)	3/20(1)
RECOVERY SACRIFICE								
Unremarkable: only spleen and gross lesions were examined.								

1 = minimal; 2 = mild; 3 = moderate; 4 = marked

**Toxicokinetics in 6-month rat study:**

Dose [mg/kg/day]	Study Day	BMS-477118		BMS-510849		BMS-477118		BMS-510849	
		Male	Female	Male	Female	Male	Female	Male	Female
		C <sub>max</sub> [ng/mL]				AUC [ng.h/mL] <sup>a</sup>			
2	1	62	127	54	91	181	326	131	211
	92	77	336	40	184	183	671	50	307
	181	82	223	40	178	217	668	54	333
20	1	917	1579	738	1219	2030	3844	1871	3251
	92	976	2717	431	1706	2468	6127	1300	4738
	181	1118	2363	563	1338	2796	6111	1345	4259
100	1	4946	11444	3552	4332	16758	32804	15317	20130
	92	4475	14182	1884	5219	18060	37795	9148	22995
	181	4607	18741	1993	5948	21869	48261	9464	25992
		C <sub>max</sub> Ratio				AUC Ratio			
1:10:50 Dose Ratio	1	1:15:79	1:12:90	1:14:66	1:13:48	1:11:93	1:12:101	1:14:117	1:15:95
	92	1:13:58	1:8:42	1:11:47	1:9:28	1:13:99	1:9:56	1:26:184	1:15:75
	181	1:14:56	1:11:84	1:14:50	1:8:33	1:13:101	1:9:72	1:25:175	1:13:78

<sup>a</sup> Calculated from time zero to the time of last measurable concentration, ranging between 4 and 24 h.

**Safety margins:**

Species	Dose, mg/kg	Saxagliptin AUC, ng.h/ml	BMS-510849 AUC, ng.h/ml	Safety margins based on AUC (Animal/Human)	
				Saxagliptin	BMS-510849
6-Month rat study, NOAEL= 2 mg/kg	2	M:217, F:668	M:54, F:333	M:2.7, F:8.2	M:0.1, F:0.8
	20	M:2796, F:6111	M:1345, F:4259	M:35, F:75	M:3, F:10
	100	M:21869, F:48261	M:9464, F:25992	M:270, F:596	M:22, F:59
Clinical Dose: 5 mg (BMS-510849)*		81			
		438			

\*Saxagliptin is metabolized in all species primarily to an active metabolite, BMS-510849. This metabolite is half as potent as parent but more selective to DPP4. The initial HPLC analysis used for AUC calculations was apparently overestimated due to inadequate peak resolution from two small metabolites, thus all the submitted AUC values for BMS-510849 in mice, rats, pregnant rabbits, dogs, Cynomolgus monkeys and humans were overestimated by 20%, 42.7%, 11.1%, 36.2%, 15.1% and 6.8%, respectively. Therefore the safety margins for BMS-510849 are lower than safety margin shown in the table above. The lower metabolite exposure was less than 2 fold therefore is unlikely to alter safety profile of saxagliptin and its metabolite.

**Evaluation of Plasma DPP-4 Inhibition:**

Saxagliptin significantly inhibited DPP4 activity at all doses despite no substantial increase in circulating GLP.

Dose (mg/kg/day)	Study Day	E <sub>max</sub> <sup>a</sup> (% inhibition)		AUEC <sup>b</sup> (% inhibition-h)	
		Males	Females	Males	Females
2	1	73	72	843	1217
	92	81	78	1475	1540
	181	74	70	728	1193
20	1	73	75	1533	1394
	92	86	82	1714	1713
	181	84	81	1628	1396
100	1	72	79	1440	1652
	92	91	86	1824	1836
	181	84	77	1621	1056
		E <sub>max</sub> ratios		AUEC ratios	
Nominal Dose ratio 1:10:50	1	1:1.0:1.0	1:1.0:1.1	1:1.8:1.7	1:1.1:1.4
	92	1:1.1:1.1	1:1.0:1.1	1:1.2:1.2	1:1.1:1.2
	181	1:1.1:1.1	1:1.2:1.1	1:2.2:2.2	1:1.2:0.9

<sup>a</sup> Maximum plasma % DPP-4 inhibition (maximum effect). Data are % decrease in DPP-4 activity from the mean drug-free control group value for each sex.

**Twelve-Month Oral Toxicity Study in Dogs - Final Study Report**

The sponsor had submitted the results of this study at interim 6-month and at the end of 12-months.

**Key study findings:**Findings at 12-months:

- All animals survived to scheduled termination.
- Unformed/mucoid/abnormally colored stools (red, green, yellow, and white) were observed in both sexes at 5 and 10 mg/kg over the duration of the study. However, the incidence decreased with the duration of the study.
- Cracking of the foot pads was first noted in males at Week 41 and females at Week 33 and continued through termination of the study, despite topical treatment of the pads. Animals affected included 3/4 males and 4/4 females at 10 mg/kg and 1/4 females at 5 mg/kg. These observations correlated with microscopic findings of erosion of the epidermis/keratin surface of the foot pads.
- Body weight decreased without a dose-dependence in males at 1, 5 and 10 mg/kg, but females were unaffected.
- Mean diastolic and arterial pressure in females given 10 mg/kg increased during the first 9 months of treatment, but was comparable to control by month 12. Mean heart rate in males given 10 mg/kg increased at 9 months of treatment, but was also comparable to controls by month 12. Mean QT interval was not significantly altered by treatment.
- Blood oxygen saturation was slightly increased in all treated males by month 3 relative to controls. By month 12, blood oxygen saturation values in treated males were comparable to the control value.
- Increase in eosinophils was observed in males at 10 mg/kg at months 3, 6, 9 and 12 being significant at all these timepoints except for the 12 month time point.
- Weight of the prostate gland was moderately decreased in males at 1 and 10 mg/kg with no correlative histopathology.
- Histopathologic target organs included the adrenal cortex (capsular nodules), cracking of the foot pads of the fore and rear paws (inflammation, hemorrhage, and erosion characterized by vacuolation of epithelium/keratin, parakeratosis, sloughing of keratin), ileum and ileocolic junction (congestion), kidney (lymphoid cell aggregates, mineral deposits, pyelonephritis), lacrimal gland (lymphoid cell aggregates), mediastinal lymph node (erythrophagia, lymphoid cell hyperplasia) mesenteric lymph node (lymphoid cell depletion/atrophy), salivary gland (lymphoid cell aggregates), thymus (involution/atrophy) and urinary bladder (inflammation, urothelial hyperplasia).
- A NOAEL of 1 mg/kg is based on findings of epidermal footpad erosion/inflammation and ileocolic congestion at 5mg/kg.
- Exposure to parent at 1 mg/kg was 4x (M) and 5x (F) clinical exposure, and for metabolite was 0.8x (M) and 1x (F) clinical exposure based on AUC.
- Actual exposure multiples are likely to be ~30% lower due to changes in toxicokinetic methods, but the overall impact on safety is unlikely to be altered by the change.

**Study no:** DN02057

**Volume # and page #:** Vol. 2, pg. 001.

**Conducting laboratory and location:** \_\_\_\_\_

b(4)

**Date of study initiation:** September 12, 2002.

**GLP compliance:** Yes (USA).

**QA report:** yes (X) no ( )

**Drug, lot #, radiolabel, and % purity:** Lot # 2F59952, 68.3% pure; Lot # 2E59572, 68.6% pure.

**Formulation/vehicle:** BMS-477118 (benzoate salt) dissolved in vehicle (1.25% Avicel in water).

**Methods:**

**Dosing:**

Species/strain: Dog/Beagle.

#/sex/group or time point (main study): 7/sex/group; 3/sex/group for 6-month interim sacrifice.

Satellite groups used for toxicokinetics or recovery: 7/sex/group for TK.

Age: 5-6 months at study initiation.

Weight: 5.8-7.7 kg (M); 5.3-6.9 kg (F).

Doses in administered units: 1, 5, 10 mg/kg.

Route, form, volume, and infusion rate: Oral (gavage), 5 ml/kg.

**Observations and times:**

Clinical signs: Daily.

Body weights: Weekly.

Food consumption: Weekly.

Ophthalmoscopy: Conducted pretest and at months 3, 6, 9 and 12.

EKG: Tracings were taken on unanesthetized dogs pretest and at months 3, 6, 9 and 12.

Indirect BP and heart rate measurements were also recorded pretest and at months 3, 6, 9 and 12.

Hematology: Blood samples for hematology evaluation were collected pretest and at months 3, 6, 9 and 12.

Clinical chemistry: the same as above time points.

Urinalysis: Urine samples were obtained via a 16-hour overnight collection, pretest and at months 3, 6, 9 and 12. Animals were fasted and deprived water during the collection period.

Gross pathology: Tissues/organs collected for gross examination are indicated in the histopathology table.

Organs weighed: Organs weighed are indicated in the histopathology table.

Histopathology: Tissues from all dose groups were processed for evaluation.

Toxicokinetics: Blood samples for TK were obtained from all animals on Day 0 and at months 6 and 12 at 1, 2, 3, 4, 8 and 24 hours post-dose.

Fecal Evaluation: Fecal samples were collected from all animals at the end of the 2<sup>nd</sup> week of dosing for the determination of occult blood.

**Results:**

**Mortality:** There were no deaths for the duration of the study.

Clinical signs: Increased incidences of unformed/mucoid stool and abnormally colored stool (red, green, yellow, and white) were noted at 5 and 10 mg/kg, relative to the controls. Based on individual daily observations, the incidence of unformed/mucoid stool and/or abnormally colored stools was greater than control values in 6/7 males each at 5 and 10 mg/kg and 5/7 and 6/7 females at 5 and 10 mg/kg, respectively. These observations were noted during the first week of the study and continued through 6 months. For males there appeared to be a slight decrease in incidence after approximately 3 months of dosing.

**Cumulative Incidence of Unformed/mucoid stool**

Text Table 1								
Group (mg/kg/day)	Sex	Cumulative Daily Observations of Unformed/Mucoid Stool (Days 0-187)						
		Weeks						
		0-3	4-7	8-11	12-15	16-19	20-23	24-26 <sup>a</sup>
1 (0)	M	11	7	7	10	17	11	3
	F	7	8	2	9	27	7	7
2 (1)	M	9	10	5	8	10	4	16
	F	17	11	9	4	10	12	7
3 (5)	M	45	54	38	34	44	36	16
	F	31	36	30	17	36	27	10
4 (10)	M	111	102	88	67	61	33	29
	F	86	98	99	59	70	74	48

<sup>a</sup>Three-week interval.

Text Table 2								
Group (mg/kg/day)	Sex	Cumulative Daily Observations of Unformed/Mucoid Stool (Days 189-370)						
		Weeks						
		27-30	31-34	35-38	39-42	43-46	47-50	51-52 <sup>a</sup>
1 (0)	M	6	2	3	1	3	1	0
	F	4	9	8	6	9	7	5
2 (1)	M	6	6	5	6	11	4	3
	F	1	8	3	5	4	12	0
3 (5)	M	25	23	19	16	21	27	9
	F	5	11	9	9	12	7	0
4 (10)	M	34	49	45	43	40	32	16
	F	43	41	40	32	44	39	17

<sup>a</sup>Two-week interval.

Increased incidences of unformed/mucoid stools and abnormally colored stool (red, green, yellow, and white) continued to be observed during months 7 to 12 in males at 5 and 10 mg/kg and females at 10 mg/kg, relative to the controls. The number of animals affected were 3/4 males and 4/4 males at 5 and 10 mg/kg, respectively, and 3/4 females at 10 mg/kg. In contrast to the findings at months 1 to 6, the incidences of unformed/mucoid stools and abnormally colored stool in females at 5 mg/kg were generally comparable to those of the controls. In addition, there were fewer observations of red stool at 7 to 12 months.

Other findings included cracking of the foot pads and thinness. Cracking of the foot pads was first noted in males at Week 41 and females at Week 33 and continued through termination of the study, despite topical treatment of the pads. Animals affected included 3/4 males and 4/4 females at 10 mg/kg and 1/4 females at 5 mg/kg. These observations correlated with microscopic findings of erosion of the epidermis/keratin surface of the foot pads.

Thinness was noted at 10 mg/kg in 3/4 males and 3/4 males at various intervals throughout 7 to 12 months, which generally correlated with the finding of decreased body weights in these animals.

Vomiting was generally sporadic and did not appear to be drug related. One (1) female at 10 mg/kg (Animal No. 4575), however, had frequent episodes of vomiting immediately after dosing, starting in Week 38. Based on the possibility that vomiting was caused by irritation in placing the oro-gastric dosing tube, the dose suspension was put into gelatin capsules for dosing starting in Week 41. After the change in the dosing procedure, the incidence of vomiting gradually decreased over the following month, indicating that this effect was most likely not drug related.

**Body weights at 6-months: (kg)**

Dose (mg/kg)	0		1		5		10	
	M	F	M	F	M	F	M	F
Week 0	6.9	6.0	6.8	6.0	6.9	6.0	6.6	6.0
Week 26	9.9	7.4	8.8	7.6	9.5	7.4	8.5	7.7
% ↓ in body wt.	-	-	11	-	4	-	14	-

**Body weights (kg) at 12 Months Data**

Dose (mg/kg)	0		1		5		10	
	M	F	M	F	M	F	M	F
Week 0	6.9	6.0	6.8	6.0	6.9	6.0	6.6	6.0
Week 52	11.7	8.7	9.8	9.5	10.4	8.7	9.4	8.4
% ↓ in body wt.	-	-	16	-	11	-	20	3

Food consumption: No treatment-related changes in food consumption.

Ophthalmoscopy: No treatment-related ocular findings at 3 or 6 months.

Electrocardiography:

- No treatment-related changes in ECG at 3 or 6 months.
- There was a statistically significant decrease in systolic blood pressure for males at 1 mg/kg in Month 3. Since no effects were observed at higher doses, this decrease was not considered drug related. There were no additional changes in systolic, diastolic, or mean arterial pressure at 3 or 6 months in the drug-treated groups.

Mean Systolic Blood Pressure Values (mmHg) - Males

	Pretest	Month 3	Month 6	Month 9	Month 12
<b>Group 1 – 0 mg/kg/day</b>					
Mean	132.7	131.6	138.9	141.0	140.5
S.D.	10.9	19.0	13.2	7.5	9.1
N	7	7	7	4	4
<b>Group 2 – 1 mg/kg/day</b>					
Mean	126.9	**	145.6	132.3	136.5
S.D.	11.6	20.7	13.4	12.3	8.7
N	7	7	7	4	4
<b>Group 3 – 5 mg/kg/day</b>					
Mean	119.6	129.0	131.6	142.5	148.3
S.D.	17.1	14.9	16.9	10.5	16.0
N	7	7	7	4	4
<b>Group 4 – 10 mg/kg/day</b>					
Mean	134.6	122.0	136.9	141.8	118.0
S.D.	19.2	17.3	21.3	9.7	47.5
N	7	7	7	4	4

\* p<0.05; \*\* p<0.01

Mean Systolic Blood Pressure Values (mmHg) - Females

	Pretest	Month 3	Month 6	Month 9	Month 12
<b>Group 1 – 0 mg/kg/day</b>					
Mean	129.3	116.7	140.6	141.0	131.8
S.D.	11.5	14.9	8.8	18.5	11.0
N	7	7	7	4	4
<b>Group 2 – 1 mg/kg/day</b>					
Mean	116.4	127.6	142.7	153.5	152.3
S.D.	11.6	16.9	23.0	15.2	20.6
N	7	7	7	4	4
<b>Group 3 – 5 mg/kg/day</b>					
Mean	137.9	128.6	140.6	143.3	141.8
S.D.	17.9	16.3	13.4	18.7	12.8
N	7	7	7	4	4
<b>Group 4 – 10 mg/kg/day</b>					
Mean	123.6	121.6	134.6	147.0	147.8
S.D.	13.6	9.6	18.3	11.0	16.1
N	7	7	7	4	4

Mean Diastolic Blood Pressure Values (mmHg)

Dose (mg/kg)	Pretest		Month 3		Month 6		Month 9		Month 12	
	M	F	M	F	M	F	M	F	M	F
0	85.7	83.9	87.0	75.3	88.0	89.0	78.8	69.5	87.3	80.5
1	72.0	74.1	67.9	84.4	95.0	92.6	69.5	90.0	80.0	95.0
5	75.5	98.7	79.7	86.3	83.0	91.9	86.8	78.0	89.8	81.0
10	91.0	87.0	78.0	75.7	88.7	84.9	80.5	91.8*	70.0	84.8

\* p<0.05

Mean Arterial Pressure Values (mmHg)

Dose (mg/kg)	Pretest		Month 3		Month 6		Month 9		Month 12	
	M	F	M	F	M	F	M	F	M	F
0	102.0	99.4	103.4	88.7	105.9	109.0	101.8	93.3	105.5	99.0
1	88.4	88.0	79.0	100.3	109.0	109.3	93.3	117.8	101.8	115.0
5	92.0	112.7	96.1	100.9	97.6	109.7	108.3	101.5	108.5	107.0
10	107.0	100.3	91.9	90.3	105.1	99.1	98.8	113.5*	88.5	103.0

\* p<0.05

Mean Heart Rate Values (bpm)

Dose (mg/kg)	Pretest		Month 3		Month 6		Month 9		Month 12	
	M	F	M	F	M	F	M	F	M	F
0	136	135	107	102	94	98	82	87	92	81
1	120	131	114	122	107	107	89	98	89	101
5	129	132	93	108	83	127	83	93	89	81
10	129	134	105	108	100	114	101	95	117	95

Mean Cardiologist-Derived Heart Rate Values (bpm)

Dose (mg/kg)	Pretest		Month 3		Month 6		Month 9		Month 12	
	M	F	M	F	M	F	M	F	M	F
0	119	125	106	101	91	106	76	82	84	75
1	119	128	104	123	97	117	79	88	77	97
5	103	126	84	101	81	115	84	97	84	92
10	121	134	116	121	95	111	104*	92	103	99

\* p<0.05

Mean QT Values (sec)

Dose (mg/kg)	Pretest		Month 3		Month 6		Month 9		Month 12	
	M	F	M	F	M	F	M	F	M	F
0	0.19	0.19	0.21	0.20	0.21	0.20	0.20	0.21	0.23	0.22
1	0.20*	0.19	0.21	0.20	0.22	0.20	0.22	0.20	0.22	0.21
5	0.21*	0.19	0.22	0.20	0.22	0.20	0.22	0.20	0.22	0.21
10	0.20*	0.19	0.21	0.21	0.21	0.21	0.20	0.21	0.21*	0.22

\* p<0.05

Mean QTc Values (sec)

Dose (mg/kg)	Pretest		Month 3		Month 6		Month 9		Month 12	
	M	F	M	F	M	F	M	F	M	F
0	0.23	0.23	0.24	0.23	0.24	0.24	0.23	0.23	0.25	0.23
1	0.24*	0.23	0.24	0.24	0.25	0.24	0.23	0.22	0.24	0.24
5	0.24*	0.23	0.24	0.24	0.24	0.24	0.24	0.24	0.25	0.24
10	0.24*	0.24	0.25	0.25*	0.24	0.25	0.24	0.24	0.24	0.25

\* p<0.05

Percent Blood Oxygen Saturation Values: At 3 months, there were statistically significant increases in percent blood oxygen saturation for all drug-treated male groups, as compared to the controls. The sponsor stated that this was due to low percent oxygen saturation values in the control group and was not drug related.

Mean Percent Blood Oxygen Saturation Values

Dose (mg/kg)	Pretest		Month 3		Month 6		Month 9		Month 12	
	M	F	M	F	M	F	M	F	M	F
0	87.0	87.3	75.4	82.6	92.1	89.4	90.3	82.5	92.0	93.0
1	77.6	73.1	84.7*	78.0	82.1	90.0	89.8	78.0	92.0	94.3
5	72.1	78.9	85.7*	84.1	86.7	80.4	86.3	89.0	95.3	98.0
10	84.9	75.7	85.4*	78.1	89.3	83.1	86.3	84.0	92.8	96.5

\* p<0.05

Hematology: 12 Month Data

Significantly (\*  $p \leq 0.05$ ) increase eosinophils was observed at Months 3 (142%), 6 (173%) and 9 (209%) in females at 10 mg/kg. At month 12, eosinophil count was still increased (123%; not SS) in females at 10 mg/kg.

$a = p \leq 0.05;$

Group (mg/kg/day)	Mean Eosinophil Counts ( $\times 10^3/\mu\text{L}$ ) in Females				
	Pretest to Month 12				
	Pretest	Month 3	Month 6	Month 9	Month 12
1 (0)	0.21	0.24	0.26	0.23	0.35
2 (1)	0.27	0.25	0.38	0.49	0.47
3 (5)	0.27	0.23	0.45	0.49	0.53
4 (10)	0.26	0.58 <sup>a</sup>	0.71 <sup>a</sup>	0.71 <sup>a</sup>	0.78

<sup>a</sup>Statistically significant, as compared to control value.

Clinical chemistry: 6 month data

Dose (mg/kg)	0		1		5		10	
Sex	M	F	M	F	M	F	M	F
Triglycerides (mg/dl)		30		35		34		39*(30%↑)

\*  $p \leq 0.05$ ; empty cells = no significant difference relative to control

Clinical chemistry: 12 Month Data

Dose (mg/kg)	0		1		5		10	
Sex	M	F	M	F	M	F	M	F
T. Protein (m/dl)	6.0		5.9		5.7		5.3*(12%↓)	
Ca (mg/dl)	9.8		10.0		9.7		9.3*(5%↓)	
K (mg/dl)	4.6		4.4		4.5		4.2*(9%↓)	
Albumin (g/dl)	3.5		3.3		3.4		3.0*(14%↓)	
Cholesterol (mg/dl)		178		177		169		227*(28%↑)
Triglycerides (mg/dl)		36		30		42		60*(67%↑)

Urinalysis: There were no treatment-related effects on urinalysis during the study.

Fecal Evaluation: Stool samples were analyzed for fecal occult blood at the end of the second week of dosing because of observation of abnormal stools. The incidence of dogs with positive findings was only slightly higher in males at 10 mg/kg (3/7) than in the controls (1/7). Two males at 10 mg/kg (# 4077 and 4078) had slightly low red blood cell values at 3 months, which is consistent with loss of blood in the feces. The incidence of positive findings in all other drug-treated groups was generally comparable to controls.

Organ weights:

- There were no treatment-related effects on organ weights at the 6-month interim necropsy. However, at 12-month, prostate weight at 1 and 10 mg/kg were significantly lower.

Organ weight at 12 months

Dose (mg/kg)	0	1	5	10
Sex	M	M	M	M
Prostate (g)	13.2	7.1*(46%↓)	9.8	7.7*(42%↓)
Prostate (% of B.wt.)	0.11	0.07*(36%↓)	0.10	0.08

\*  $p \leq 0.05$

Gross pathology: There were no treatment-related gross pathology effects at the 6-month interim necropsy.

12 Month Data

Dose (mg/kg)	0		1		5		10	
N	4	4	4	4	4	4	4	4
Sex	M	F	M	F	M	F	M	F
Ileum: Discolored	0	0	0	0	0	0	0	1
Kidney: Irregular shape	0	0	0	0	0	0	0	1
Kidney: Small	0	0	0	0	0	0	0	1
Lung: Discolored	1	0	1	0	1	0	0	2
Ileocolic junction: Discolored	0	0	1	1	3	1	2	3
Extremity: Sore	0	0	0	0	0	1	3	4

Histopathology:

- At 6-months interim analyses, notable findings were limited to epididymis, testes and salivary gland in dogs.
- At 12-months notable findings were seen in adrenal gland (cortex), dermis, ileocolic junction, ileum, renal cortex, mediastinal and mesenteric lymph node, LN, urinary bladder and thymus (in all groups)

Histopath findings at 6-month interim (tissue from 3 dogs /sex/group were evaluated)

Dose (mg/kg)	0		1		5		10	
N	3	3	3	3	3	3	3	3
Sex	M	F	M	F	M	F	M	F
Epididymis: Tubular Lumen Degenerated germ cells/cell debris	3 3(1)		3 2(1) 1(2)		3 3(1)		3/3 1(1) 1(2) 1(3)	
Testes: Germinal epithelium Segmental hypospermatogenesis /vacuolated	2 1(1) 1(2)		3 1(1) 2(2)		3 2(1) 1(2)		3 1(1) 2(2)	
Salivary gland: Lymphoid cell aggregate(s)	0	0	0	0	2 2(2)	0	3 2(1) 1(2)	1 1(2)

1 = minimal; 2 = slight; 3 = moderate

Histopath findings at 12 Month

Dose (mg/kg)	0		1		5		10	
N	4	4	4	4	4	4	4	4
Sex	M	F	M	F	M	F	M	F
Adrenal gland: Cortex Capsular/Extra capsular nodule	1 1(1)	1 1(1)	2 1(1) 1(2)	1 1(1)	1 1(1)	2 1(1) 1(2)	2 1(1) 1(2)	2 1(1) 1(2)
Extremity: Surface Free erythrocytes	0	0	0	0	0	1 1(1)	3 1(1) 2(2)	1 1(2)
Extremity: Dermis Inflammation	0	0	0	0	0	1 2(2)	2 2(1)	3 1(1) 2(2)
Extremity: Dermis Hemorrhage	0	0	0	0	0	0	0	1 1(2)
Epidermis/keratin surface: Erosion characterized by vacuolation of epithelium/keratin, parakeratosis, sloughing of keratin and/or hemorrhage	0	0	0	0	0	1 1(2)	3 3(2)	4 2(1) 2(2)
Ileocolic junction: Mucosa Congestion	0	0	1 1(2)	0	3 3(2)	1 1(2)	2 1(1) 1(2)	2 1(2)

<b>Ileum: Mucosa</b> Congestion	0	0	0	0	0	0	0	1 1(2)
<b>Kidney: Cortex</b> Lymphoid cell aggregates	0	0	0	1 1(1)	1 1(1)	1 1(1)	0	1 1(1)
<b>Cortico-medullary junction</b> Mineral deposits	0	0	0	1 1(1)	0	0	0	1 1(1)
Pyelonephritis	0	0	0	0	0	0	0	1 1(1)
<b>Lacrimal gland</b> Lymphoid cell aggregates	0	1 1(1)	1 1(1)	1 1(2)	0	1 1(2)	0	3 3(1)
<b>Mediastinal LN: Sinuses</b> Erythrocytes/Erythrophagia	1 1(2)	0	1 1(1)	2 1(1) 1(2)	2 1(1) 1(2)	0	3 2(1) 1(3)	1 1(1)
Lymphoid cell hyperplasia	0	1 1(1)	0	0	0	1 1(2)	0	1 1(1)
<b>Mesenteric LN</b> Lymphoid cell depletion/atrophy	0	0	0	0	0	0	1 1(3)	0
<b>Salivary gland</b> Lymphoid cell aggregate	2 1(1) 1(2)	3 3(1)	2	1 1(2)	1 1(2)	3 1(1) 2(2)	3 3(1)	4 3(1) 1(2)
<b>Thymus</b> Involution/atrophy	2 2(2)	0	2 1(2) 1(3)	1 1(2)	4 3(2) 1(3)	2 2(2)	2 2(2)	2 2(2)
Interstitial: free erythrocytes	1 1(3)	0	0	0	1 1(2)	1 1(3)	1 1(3)	1 1(2)
<b>Urinary bladder</b> Inflammation	0	0	0	0	0	0	0	1 1(2)
Urothelial hyperplasia	0	0	0	0	0	0	0	1 1(2)

1 = minimal; 2 = slight; 3 = moderate

**Toxicokinetics:**

- Systemic exposure to saxagliptin and its metabolite, BMS-510849 in dogs were dose-related and increase in both AUC and Cmax appear to be dose-proportional
- Systemic exposures to saxagliptin and BMS-510849 appeared to be similar in females compared to males. The mean BMS-510849 to saxagliptin molar AUC<sub>(0-T)</sub> ratio ranged from 0.8 to 1.7 and appeared to be independent of gender, dose of saxagliptin or study day.
- After once-daily 1- to 10-mg/kg doses BMS-477118, the AUC ratios of BMS-477118 were 0.5-0.7 (males) and 0.6-1.0 (females) on day 179, and 0.4-0.5 (males) and 0.4-0.6 (females) on day 361, each compared to day 0. After once-daily 1- to 10-mg/kg doses of BMS-477118, the AUC ratios of BMS-510849 were 0.2-0.5 (males) and 0.6-1.0 (females) on day 179, and 0.3-0.4 (males) and 0.4-0.5 (females) on day 361, each compared to the first day of dosing.
- A modest reduction in systemic exposure to saxagliptin and BMS-510849 was observed between day 0 and 6 months and a further reduction was observed between 6 and 12 months.

Dose (mg/kg/day)	Study Day	BMS-477118		BMS-510849		BMS-477118		BMS-510849	
		Male	Female	Male	Female	Male	Female	Male	Female
		Cmax (ng/mL)				AUC (ng.h/mL) <sup>a</sup>			
1	0	297	340	265	259	722	754	1072	911
	179	210	257	156	188	433	461	428	542
	361	167	238	127	172	286	415	359	454
5	0	1728	1785	1439	1585	3688	3582	5635	5206
	179	1222	1912	952	1216	2406	3583	3016	4238
	361	749	830	666	619	1470	1544	1872	1964
10	0	3913	2997	2998	2497	8979	7723	13539	10493
	179	1960	3613	916	2926	4141	6944	3296	9985
	361	2080	1479	1337	1363	4278	2782	4767	5088
		Cmax Ratio				AUC Ratio			
1:5:10 Dose Ratio	0	1:6:13	1:5:9	1:5:11	1:6:10	1:5:12	1:5:10	1:5:13	1:6:12
	179	1:6:9	1:7:14	1:6:6	1:6:16	1:6:10	1:8:15	1:7:8	1:8:18
	361	1:4:12	1:3:6	1:5:11	1:4:8	1:5:15	1:4:7	1:5:13	1:4:11

<sup>a</sup>Calculated from time zero to the time of last measurable concentration, ranging between 4 and 24 h.

**DPP4 inhibition:**

For nominal doses increasing in a 1:5:10 proportion, the E<sub>max</sub> and AUEC values increased in the proportions listed in the Table above. Between 1- and 10-mg/kg, the E<sub>max</sub> and AUEC values appeared to increase less than proportional to the dose; however, the values appeared to increase approximately equal to the logarithm of the daily dose of BMS-477118. Plasma DPP4 inhibition following dosing with BMS-477118 appeared to be similar in females and males.

**DPP-IV Activity: Plasma DPP-4 inhibition in the dogs following administration of 1- to 10-mg/kg doses of BMS-477118 for 12 months was dose-related**

Dose (mg/kg/day)	Study Day	Emax <sup>a</sup> (% inhibition)		AUEC <sup>b</sup> (% inhibition-h)	
		Males	Females	Males	Females
1	0	91.9	92.6	1857	1898
	179	94.3	94.3	1988	2015
	361	97.1	96.6	2151	2126
5	0	95.7	95.4	2114	2121
	179	96.9	98.0	2192	2225
	361	97.8	98.2	2262	2292
10	0	96.3	96.2	2119	2131
	179	97.7	98.1	2208	2242
	361	99.1	98.5	2309	2304
		Emax ratios		AUEC ratios	
Nominal	0	1:1.04:1.05	1:1.03:1.04	1:1.14:1.14	1:1.12:1.12
Dose ratio	179	1:1.03:1.04	1:1.04:1.04	1:1.10:1.11	1:1.10:1.11
1:5:10	361	1:1.01:1.02	1:1.02:1.02	1:1.05:1.07	1:1.08:1.08

<sup>a</sup>Maximum plasma % DPP4 inhibition (maximum effect). Data are % decrease in DPP4 activity from predose values on day 0.

<sup>b</sup>Trapezoidal area under the effect (plasma % DPP4 inhibition) versus time curve, calculated from predose to the time of the last measurement at 24 h postdose.