

One- to Three-Month Oral Toxicity Study in Monkeys**Key study findings:**

- Skin lesions were observed at all dose levels, ranging from relatively minimal and self-resolving at the low dose to severe and ulcerative requiring surgical amputation of the distal tail and moribund sacrifice at the mid and high doses.
- Lesion severity and time to lesion emergence was dose and time-dependent. For example, lesions erupted earlier with higher doses.
- There was no evidence of increased cyanide levels or immunoglobulin deposition in tissues at any dose, which appears to discount these mechanisms as causative of the lesions.
- Two females given the high dose became acutely moribund after 1 or 2 doses, and were sacrificed.
- One high dose female became thrombocytopenic. This animal was re-challenged with drug after a dosing holiday and thrombocytopenia did not recur.
- Two animals showed histologically confirmed renal glomerulopathy with associated clinical chemistry abnormalities in protein excretion and albumin.
- Exposure at lowest dose, 2 mg/kg was 4 to 6 x the clinical exposure at 5 mg, based on AUC.

Study no.: DN05063

Volume #, and page #: eNDA

Conducting laboratory and location: BMS, One Squibb Drive, New Brunswick, NJ

Date of study initiation: August 30, 2005

GLP compliance: No

QA report: yes () no (x)

Drug, lot #, and % purity: Batch 4K85994 with free base purity of 93.5%

Methods

Intended as a one month dose-ranging study, observations of severe skin lesions altered the study design to investigate the nature of the drug-induced toxicities in monkeys. Doses and treatment duration are listed in the following table:

Group Number	Daily Dose		Concentration	Number of Animals	Dosing Duration
	Saxagliptin (BMS-477118) (mg/kg)	Volume (mL/kg)	Saxagliptin (BMS-477118) (mg/mL)		Weeks
1	0	1	0	5M, 5F	4, 13 ^a
2	2	1	2	3M, 3F	13
3	10	1	10	3M, 3F	6
4	30/20 ^b	1	30/20	5M, 5F	4, 6, 13 ^c

^a A subset of control monkeys (2M, 2F) were necropsied after 4 weeks of dosing. The remaining 3M, 3F were necropsied after 13 weeks of dosing.

^b The high-dose was reduced from 30 mg/kg/day to 20 mg/kg/day after 3 days of dosing in the males and 2 days of dosing in the females.

^c A subset of monkeys were necropsied at 4 and 6 weeks. Animal 4102 was necropsied at the end of 13 weeks (see text for details).

Doses: 2, 10, 30/20 mg/kg (free base used in the study)

Species/strain: cynomolgus monkeys from _____

Route, formulation, volume, and infusion rate: oral gavage

b(4)

Age: 23 to 38 months old

Weight: 2.4 to 4.7 kg

Sampling times: PK data for saxagliptin and active metabolite were collected on Day 1, 28, 84/85 at 0.5, 1, 2, 4, 8 and 24 hrs post dose. Prior to freezing, an aliquot of plasma was taken for DPP4 inhibition evaluation. Plasma samples were analyzed at _____

b(4)

Unique study design or methodology: Animals were acclimated for at least 21 days and had ad lib access to water. They received daily ration of approximately 12 biscuits each. Cyanide and thiocyanate levels were also measured during the first 2 TK time points on Day 1 and Day 28. Peripheral blood lymphocyte phenotyping was also made in samples collected before and on week 2, 3, 4 and 8. The sponsor also investigated serum immunoglobulin (Ig) levels, antinuclear antibody (ANA) levels and Ig reactivity to red blood cells and platelets. Attempts were made to look at skin and kidney samples using electron microscopy. Based on histopath findings, paraffin-embedded tail, nose, scrotum, foot, skin (face and torso), urinary bladder, kidney, tongue, lung, vagina, testes, skeletal muscle intestine (jejunum) were stained for IgG, IgM and IgA deposition by immunohistochemistry staining.

Observations and times:

Mortality: daily

Clinical signs: twice daily

Body weights: weekly

Food consumption: daily

Ophthalmoscopy: before and at weeks 4 and 8

ECG: before and at weeks 4 and 8

Hematology: Blood samples were collected from fasted monkeys twice prior to first dose and at week 2, 3, 4, 5, 6, 8, 10 and 13

Clinical chemistry: The same as above

Urinalysis: before and at week 4, 6 and 13

Gross pathology: at the end of each phase (treatment and recovery).

Organ weights: standard list with greater emphasis on the skin lesions to determine NOAEL.

Histopathology: Adequate Battery: yes (x)

Peer review: yes (x)

Results

Mortality and clinical signs:

2mg/kg (3 month dosing duration)

Five of the six monkeys developed skin 'abrasions/ulcerations' on the tail and digits during the dosing period. Lesions developed within 13 days of dosing. The lesions largely resolved despite continued dosing, with only one female harboring minimal lesions at the end of 3 months of treatment.

10mg/kg (6 weeks dosing duration)

Four of six monkeys developed abrasions/ulcerations on the tail, digits, and scrotum within 13 days of dosing. While some lesions resolved, there remained ulcerative, inflamed lesions of the tail and multifocal scabs of the skin/subcutis in some animals at the end of 6 weeks treatment.

30/20mg/kg (Dose reduction at day 2/3, then 4 & 6 weeks at 20mg/kg)

Two females (#s 4202 & 4205) were sacrificed after the first or second dose after becoming acutely moribund (ataxia, collapse). Skin lesions were not observed in these two acutely ill females. The dose was reduced in remaining animals to 20mg/kg for an additional 4 or 6 weeks duration.

Severe ulcerative lesions were identified in 7 of 8 monkeys as early as day 6. Surgical amputation of the tail was required in one male and female; the female was found dead the following morning. An additional male was sacrificed moribund on day 39. Edema was feature of the skin lesions.

Body weights: No drug-related change in BW

Food consumption: No drug-related change in food intake

Ophthalmoscopy: No drug-related change

EKG and arterial oxygenation:

There was no drug-related change in cardiovascular parameters. One monkey had inverted QRS before treatment and at week 4. This appeared to be a pre-existing condition and not a drug-related finding.

Hematology: The decrease in RBC parameters may have been due to some form of hemorrhage from vascular damage and skin lesions due to the fact that reticulocyte levels were increased and albumin levels were increased. Increase in WBC parameters and albumin to globulin ratio is consistent with inflammation and inflammatory cell infiltration in the damaged tissue identified microscopically.

Hematological changes relative to pre-dose levels at 2 mg/kg

- Decrease in RBC, Hgb, Hct, decrease in platelets
- Compensatory 2.4x increase in reticulocyte on Days 35 to 56, increase in neutrophils
- Presence of reactive lymphocytes (typical of lymphoid activation) on Day 35; and,

Hematological changes at 10 mg/kg

- Decreased in RBC, Hgb, Hct and decrease in platelet count
- Increase in reticulocytes and WBC (predominantly due to increased neutrophil count) and monocytes (1.8 to 2.9x) and increase in lymphocytes (1.4x),

Hematological changes at 30/20 mg/kg,

- Decrease in RBC, hemoglobin, Hct and decreased in platelet
- Increase in reticulocyte, Neutrophils, monocytes
- Presence of reactive lymphocytes
- Additionally 1 (4102) developed severe thrombocytopenia (-77 to -94%) at 2weeks leading to temporary halt in treatment until platelet counts recovered. This animal was re-challenged with drug without recurrence of the thrombocytopenia.

Hematological change in cynomolgus monkeys relative to pretest values

Dose	2 mg/kg	10 mg/kg	30/20 mg/kg
RBC count	-12 to -18%	-11 to -27%	-8 to -36%
Hgb	-15 to -24%	-11 to -26%	-10 to -41%
Hct	-15 to 27%	-14 to -28%	-10 to -40%
Reticulocytes	2.40 x	1.70 to 7.5x	1.9 to 8.1x
Neutrophil	2.4x	1.5 to 2.5x	1.8 to 3.3x
Platelet	-38%	-31 to -54%	-36 to -69%

Clinical chemistry:

- There were decreases in albumin and albumin/globulin ratio and increases in globulin, total protein and fibrinogen at saxagliptin doses \geq 2 mg/kg
- In contrast to other MD and HD animals, a MD female monkeys (#3203) and a HD male monkey (#4103) with kidney lesions (glomerulopathy) had decreased levels of total protein and albumin

Dose	2 mg/kg	10 mg/kg	30/20 mg/kg
globulin	1.2 to 1.5x	1.2 to 1.9x	1.2 to 1.9x
albumin,	-4 to -16%	-10 to -31%	-12 to -30%
albumin/globulin ratio	-12 to -44%	-29 to -67%	-25 to -63%
total protein, Day 42		1.1 to 1.2x	1.1 to 1.2x
fibrinogen		1.5 to 2.2x	1.3 to 5.5x

Cyanide levels:

There were no detectable levels of cyanide in the whole blood of any monkey. Serum thiocyanate were detected only in the HD monkeys (1.1 to 2.7 $\mu\text{g/mL}$) but were within the background levels noted in humans (nonsmokers: 2.9 and smokers 7.1 $\mu\text{g/mL}$). The skin lesions were therefore not related to liberation of cyanide from saxagliptin.

Peripheral-Blood Lymphocyte Phenotyping and Immunology Assessment:

- There were no drug-related changes in lymphocyte subsets.
- Total IgG and IgM levels in drug-treated monkeys were significantly increased compared to individual pre-study levels and controls
- There were no detectable antinuclear antibodies (ANAs).
- Overall findings do not support a significant immune-mediated mechanism for skin lesions in monkeys.

Saxagliptin: One-Month Oral Toxicity Study in Monkeys
Summary of Absolute Number of Lymphocytes (10e³ /μL)
for Male Monkeys at Prestudy

Saxagliptin: One-Month Oral Toxicity Study in Monkeys
Summary of Absolute Number of Lymphocytes (10e³ /μL)
for Male Monkeys at Day 22

Parameter	Group	N	Mean (SD)	Parameter	Group	N	Mean (SD)
CD2+CD20-	Control	5	3.66 (1.45)	CD2+CD20-	Control	5	3.47 (0.87)
	BMS-477118 2 mg/kg	3	3.52 (0.73)		BMS-477118 2 mg/kg	3	2.83 (0.63)
	BMS-477118 10 mg/kg	3	2.38 (0.72)		BMS-477118 10 mg/kg	3	2.80 (1.04)
	BMS-477118 30/20 mg/kg	5	3.78 (1.38)		BMS-477118 30/20 mg/kg	5	2.89 (1.54)
CD20+CD2-	Control	5	0.63 (0.45)	CD20+CD2-	Control	5	0.70 (0.23)
	BMS-477118 2 mg/kg	3	0.61 (0.17)		BMS-477118 2 mg/kg	3	0.54 (0.24)
	BMS-477118 10 mg/kg	3	0.68 (0.25)		BMS-477118 10 mg/kg	3	0.91 (0.13)
	BMS-477118 30/20 mg/kg	5	0.67 (0.26)		BMS-477118 30/20 mg/kg	5	0.74 (0.20)
CD4+CD8-	Control	5	1.80 (0.55)	CD4+CD8-	Control	5	1.74 (0.39)
	BMS-477118 2 mg/kg	3	1.43 (0.27)		BMS-477118 2 mg/kg	3	1.14 (0.11)
	BMS-477118 10 mg/kg	3	1.08 (0.33)		BMS-477118 10 mg/kg	3	1.19 (0.40)
	BMS-477118 30/20 mg/kg	5	1.67 (0.67)		BMS-477118 30/20 mg/kg	5	1.41 (0.73)
CD8+CD4-	Control	5	2.43 (1.19)	CD8+CD4-	Control	5	2.22 (0.56)
	BMS-477118 2 mg/kg	3	2.58 (0.39)		BMS-477118 2 mg/kg	3	1.97 (0.70)
	BMS-477118 10 mg/kg	3	1.65 (0.30)		BMS-477118 10 mg/kg	3	1.86 (0.56)
	BMS-477118 30/20 mg/kg	5	2.67 (0.66)		BMS-477118 30/20 mg/kg	5	1.83 (0.80)
Female Monkeys at Prestudy				Female Monkeys at Day 21			
Parameter	Group	N	Mean (SD)	Parameter	Group	N	Mean (SD)
CD2+CD20-	Control	5	2.89 (0.88)	CD2+CD20-	Control	5	3.89 (0.93)
	BMS-477118 2 mg/kg	3	3.49 (0.56)		BMS-477118 2 mg/kg	3	3.40 (0.99)
	BMS-477118 10 mg/kg	3	2.45 (0.75)		BMS-477118 10 mg/kg	3	3.06 (0.66)
	BMS-477118 30/20 mg/kg	5	1.92 (0.57)				
CD20+CD2-	Control	5	0.65 (0.25)	CD20+CD2-	Control	5	0.76 (0.27)
	BMS-477118 2 mg/kg	3	0.62 (0.14)		BMS-477118 2 mg/kg	3	0.60 (0.23)
	BMS-477118 10 mg/kg	3	0.57 (0.28)		BMS-477118 10 mg/kg	3	0.51 (0.40)
	BMS-477118 30/20 mg/kg	5	0.53 (0.14)				
CD4+CD8-	Control	5	1.52 (0.63)	CD4+CD8-	Control	5	2.01 (0.74)
	BMS-477118 2 mg/kg	3	1.65 (0.31)		BMS-477118 2 mg/kg	3	1.51 (0.17)
	BMS-477118 10 mg/kg	3	1.27 (0.58)		BMS-477118 10 mg/kg	3	1.79 (0.48)
	BMS-477118 30/20 mg/kg	5	0.89 (0.32)				
CD8+CD4-	Control	5	1.59 (0.28)	CD8+CD4-	Control	5	2.01 (0.25)
	BMS-477118 2 mg/kg	3	2.19 (0.77)		BMS-477118 2 mg/kg	3	2.17 (0.85)
	BMS-477118 10 mg/kg	3	1.67 (0.50)		BMS-477118 10 mg/kg	3	1.52 (0.05)
	BMS-477118 30/20 mg/kg	5	1.36 (0.26)				

No significant differences from the vehicle control group were observed, based on the Dunnett multiple-comparison t-test procedure (p≥0.05).

**One- to Three-Month Oral Toxicity Study in Monkeys
Attachment 3: Individual Anti-Nuclear Antibody (ANA) Reactivity**

Group	Animal No.	Sex	Study Day				Animal No.	Sex	Study Day											
			Prestudy	Day 14	Day 35	Day 43			Prestudy	Day 13	Day 34	Day 42								
			Ratio ^a	ANA ^b																
1 BMS-477118 (0 mg/kg)	1101	M	0.24	-	0.24	-	0.22	-	0.23	-	1201	F	0.24	-	0.25	-	0.24	-	0.26	-
	1102	M	0.26	-	0.24	-	0.25	-	0.24	-	1202	F	0.28	-	0.29	-	0.32	-	0.29	-
	1103	M	0.29	-	0.39	-	0.32	-	0.35	-	1203	F	0.24	-	0.24	-	0.27	-	0.25	-
	1104	M	0.28	-	0.22	-	NA	NA	NA	NA	1204	F	0.28	-	0.26	-	NA	NA	NA	NA
	1105	M	0.25	-	0.28	-	NA	NA	NA	NA	1205	F	0.30	-	0.27	-	NA	NA	NA	NA
2 BMS-477118 (2 mg/kg)	2101	M	0.25	-	0.28	-	0.35	-	0.35	-	2201	F	0.28	-	0.32	-	0.38	-	0.38	-
	2102	M	0.38	-	0.44	-	0.97	+/-	0.50	-	2202	F	0.32	-	0.29	-	0.51	-	0.48	-
	2103	M	0.25	-	0.31	-	0.25	-	0.38	-	2203	F	0.27	-	0.32	-	0.39	-	0.37	-
3 BMS-477118 (10 mg/kg)	3101	M	0.25	-	0.39	-	0.71	+/-	0.61	-	3201	F	0.20	-	0.24	-	0.50	-	0.49	-
	3102	M	0.24	-	0.27	-	0.36	-	0.28	-	3202	F	0.28	-	0.39	-	0.48	-	0.64	-
	3103	M	0.25	-	0.34	-	0.57	-	0.57	-	3203	F	0.24	-	0.30	-	NA	NA	NA	NA
4 BMS-477118 (30/20 mg/kg)	4101	M	0.30	-	0.35	-	0.41	-	0.39	-	4201	F	0.29	-	0.34	-	NA	NA	NA	NA
	4102*	M	0.22	-	0.28	-	0.42	-	0.28	-	4202	F	0.26	-	NA	-	NA	NA	NA	NA
	4103	M	0.30	-	0.38	-	0.75	+/-	NA	NA	4203	F	0.25	-	0.29	-	NA	NA	NA	NA
	4104	M	0.28	-	0.30	-	NA	NA	NA	NA	4204	F	0.25	-	0.25	-	NA	NA	NA	NA
	4105	M	0.26	-	0.27	-	NA	NA	NA	NA	4205	F	0.29	-	NA	-	NA	NA	NA	NA

^a Calculated as the mean sample OD divided by the reference control OD

^b Evaluated serum sample collected on Day 42.

^b Reactivity considered:

NA - Not available.

negative (-) if ratio is less than 0.7

positive (+) if ratio is greater than or equal to 1.0

borderline (+/-) if ratio is greater than or equal to 0.7 and less than 1.0

Immunohistochemistry:

- Except for presence of IgG and IgM, in the glomerulus or tubular epithelium of 2 monkeys (1 MD, 1HD) with glomerulopathy, there was no immunoglobulin deposition in any of the other tissues evaluated.
- Similar to immunoassessment data, the absence of immunoglobulin deposition suggests that skin lesions were unlikely to be an autoimmune or complement-mediated mechanism.

Individual immunoglobulin levels in male and female monkeys:

One- to Three-Month Oral Toxicity Study in Monkeys
Attachment 4a: Individual Serum Immunoglobulin Levels in Males (mg/dL)

Group	Animal No.	Study Day											
		Prestudy			Day 14			Day 35			Day 43		
		IgG	IgA	IgM	IgG	IgA	IgM	IgG	IgA	IgM	IgG	IgA	IgM
1 BMS-477118 (0 mg/kg)	1101												
	1102												
	1103												
	1104												
	1105												
	Mean	1232	230	68	1209	239	68	1148	225	65	1082	220	62
	StdDev	103	41	15	44	47	17	41	41	4	59	36	2
2 BMS-477118 (2 mg/kg)	2101												
	2102												
	2103												
	Mean	1229	242	72	1359	258	83	1554	268	87	1461	262	90*
	StdDev	111	39	41	115	48	51	302	70	64	210	68	76
3 BMS-477118 (10 mg/kg)	3101												
	3102												
	3103												
	Mean	1256	226	67	1522	248	97*	1888*	253	71	1854*	244	72
	StdDev	167	11	7	130	42	16	500	70	4	425	55	6
4 BMS-477118 (30/20 mg/kg)	4101												
	4102*												
	4103												
	4104												
	4105												
	Mean	1219	249	71	1513	303	92*	1968*	286	64	1789*	271	54
	StdDev	138	61	26	83	83	29	542	73	21	718	75	21

Bold values indicates that the Day mean was significantly different from the Prestudy mean (p<0.05). Arithmetic means are presented.
 * Indicates significant difference from vehicle control (p<0.05). Comparisons were based on least squares adjusted mean from ANOCOV model.
 * Evaluated using serum collected on Day 42.
 NA = Not applicable.

One- to Three-Month Oral Toxicity Study in Monkeys
Attachment 4b: Individual Serum Immunoglobulin Levels in Females (mg/dL)

Group	Animal No.	Study Day											
		Prestudy			Day 13			Day 34			Day 42		
		IgG	IgA	IgM	IgG	IgA	IgM	IgG	IgA	IgM	IgG	IgA	IgM
1 BMS-477118 (0 mg/kg)	1201												
	1202												
	1203												
	1204												
	1205												
	Mean	1498	240	100	1293	216	87	1267	263	95	1264	261	94
	StdDev	178	75	7	113	75	8	119	88	9	157	84	5
2 BMS-477118 (2 mg/kg)	2201												
	2202												
	2203												
	Mean	1245	177	92	1346*	190	95	1356*	191	90	1314*	186	83
	StdDev	153	20	31	77	22	22	112	16	9	73	23	13
3 BMS-477118 (10 mg/kg)	3201												
	3202												
	3203												
	Mean	1475	235	107	1600*	206	140*	1826*	187	107	2044*	201	111*
	StdDev	325	84	24	432	58	35	539	3	18	700	12	8
4 BMS-477118 (30/20 mg/kg)	4201												
	4202												
	4203												
	4204												
	4205												
	Mean	1297	266	93	1562*	213	111*	NA	NA	NA	NA	NA	NA
	StdDev	204	80	25	157	25	9	NA	NA	NA	NA	NA	NA

Saxagliptin: One-Month Oral Toxicity Study in Monkeys
Summary of Absolute Number of Lymphocytes (10e³ /μL)
for Male Monkeys at Prestudy

Parameter	Group	N	Mean (SD)
CD2+CD20-	Control	5	3.66 (1.45)
	BMS-477118 2 mg/kg	3	3.52 (0.73)
	BMS-477118 10 mg/kg	3	2.38 (0.72)
	BMS-477118 30/20 mg/kg	5	3.78 (1.38)
CD20+CD2-	Control	5	0.63 (0.45)
	BMS-477118 2 mg/kg	3	0.61 (0.17)
	BMS-477118 10 mg/kg	3	0.68 (0.25)
	BMS-477118 30/20 mg/kg	5	0.67 (0.26)
CD4+CD8-	Control	5	1.80 (0.55)
	BMS-477118 2 mg/kg	3	1.43 (0.27)
	BMS-477118 10 mg/kg	3	1.08 (0.33)
	BMS-477118 30/20 mg/kg	5	1.67 (0.67)
CD8+CD4-	Control	5	2.43 (1.19)
	BMS-477118 2 mg/kg	3	2.58 (0.39)
	BMS-477118 10 mg/kg	3	1.65 (0.30)
	BMS-477118 30/20 mg/kg	5	2.67 (0.66)

Saxagliptin: One-Month Oral Toxicity Study in Monkeys
Summary of Absolute Number of Lymphocytes (10e³ /μL)
for Male Monkeys at Day 22

Parameter	Group	N	Mean (SD)
CD2+CD20-	Control	5	3.47 (0.87)
	BMS-477118 2 mg/kg	3	2.83 (0.63)
	BMS-477118 10 mg/kg	3	2.80 (1.04)
	BMS-477118 30/20 mg/kg	5	2.89 (1.54)
CD20+CD2-	Control	5	0.70 (0.23)
	BMS-477118 2 mg/kg	3	0.54 (0.24)
	BMS-477118 10 mg/kg	3	0.91 (0.13)
	BMS-477118 30/20 mg/kg	5	0.74 (0.20)
CD4+CD8-	Control	5	1.74 (0.39)
	BMS-477118 2 mg/kg	3	1.14 (0.11)
	BMS-477118 10 mg/kg	3	1.19 (0.40)
	BMS-477118 30/20 mg/kg	5	1.41 (0.73)
CD8+CD4-	Control	5	2.22 (0.56)
	BMS-477118 2 mg/kg	3	1.97 (0.70)
	BMS-477118 10 mg/kg	3	1.86 (0.56)
	BMS-477118 30/20 mg/kg	5	1.83 (0.80)

Female Monkeys at Prestudy

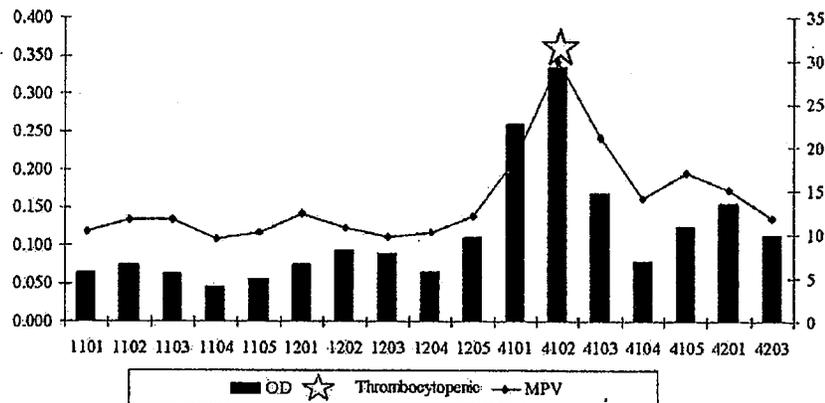
Parameter	Group	N	Mean (SD)
CD2+CD20-	Control	5	2.89 (0.88)
	BMS-477118 2 mg/kg	3	3.49 (0.56)
	BMS-477118 10 mg/kg	3	2.45 (0.75)
	BMS-477118 30/20 mg/kg	5	1.92 (0.57)
CD20+CD2-	Control	5	0.65 (0.25)
	BMS-477118 2 mg/kg	3	0.62 (0.14)
	BMS-477118 10 mg/kg	3	0.57 (0.28)
	BMS-477118 30/20 mg/kg	5	0.53 (0.14)
CD4+CD8-	Control	5	1.52 (0.63)
	BMS-477118 2 mg/kg	3	1.65 (0.31)
	BMS-477118 10 mg/kg	3	1.27 (0.58)
	BMS-477118 30/20 mg/kg	5	0.89 (0.32)
CD8+CD4-	Control	5	1.59 (0.28)
	BMS-477118 2 mg/kg	3	2.19 (0.77)
	BMS-477118 10 mg/kg	3	1.67 (0.50)
	BMS-477118 30/20 mg/kg	5	1.36 (0.26)

Female Monkeys at Day 21

Parameter	Group	N	Mean (SD)
CD2+CD20-	Control	5	3.89 (0.93)
	BMS-477118 2 mg/kg	3	3.40 (0.99)
	BMS-477118 10 mg/kg	3	3.06 (0.66)
CD20+CD2-	Control	5	0.76 (0.27)
	BMS-477118 2 mg/kg	3	0.60 (0.23)
	BMS-477118 10 mg/kg	3	0.51 (0.40)
CD4+CD8-	Control	5	2.01 (0.74)
	BMS-477118 2 mg/kg	3	1.51 (0.17)
	BMS-477118 10 mg/kg	3	1.79 (0.48)
CD8+CD4-	Control	5	2.01 (0.25)
	BMS-477118 2 mg/kg	3	2.17 (0.85)
	BMS-477118 10 mg/kg	3	1.52 (0.05)

No significant differences from the vehicle control group were observed, based on the Dunnett multiple-comparison t-test procedure (p≥0.05).

Platelet Reactive immunoglobulin



Evaluation of Platelet-Associated Ig at Day 29/30

Observed increases in PA-Ig correlates with increased mean platelet volume at Day 29/30. Thrombocytopenic monkey, 4102 (denoted with a star), had the greatest amount of PA-Ig. PA-Ig is expressed as optical density values on left 'y' axis; mean platelet volume indices are on the right 'y' axis.

Urinalysis: Only notable change was an increase in urinary protein in 1 MD female (1083 mg/dl) and 1 HD male (128 mg/dl) with glomerulopathy.

Organ weights:

- A drug-related increase in spleen weight was noted at 2 mg/kg (21 to 83%), 10 mg/kg (107% due to 1 female at week 4) and 30/20 mg/kg (25 to 31%). The significance of slightly higher kidney weight in the HD females is uncertain. The increase in spleen weight was correlated to lymphoid hyperplasia

Gross pathology:

- There were no macroscopic drug related finding at 2 mg/kg
- Macroscopic findings at 10 mg/kg (MD) included tail skin, focal or multifocal erosion and scabs on distal part of the tail, scrotal skin (focal erosion), skin erosion of the face (multifocal erosion), in the nose (multifocal erosion). These lesions were not seen in all MD animals but rather 1 or more and occasionally the same animal with more sites. The female monkey with glomerulopathy has subcutaneous edema (ascites) and hydrothorax secondary to urinary protein loss.
- Macroscopic findings in the HD animals were similar to MD animals except that tail lesion appeared necrotic in at least to males
- One HD male (4103, sacrificed on Day 39) had scrotal edema that was associated with ulceration/inflammation determined microscopically.

Histopathology:

In general histopathology findings noted at 2 mg/kg occurred in more animals at higher doses with increased severity and occasionally recruited new target organs

2 mg/kg

- Minimal to mild subacute to chronic inflammation and or mononuclear cell infiltration in the glandular organs (pituitary, parathyroid, mandibular, mammary, reproductive organs including testes, epididymides, seminal vesicles, prostate) and brain (choroid plexus), peripheral nerve (sciatic), liver, kidney and urinary bladder.
- Minimal subacute vasculitis in the skeletal muscle, minimal to slight lymphoid hyperplasia in the spleen and bone marrow.

10 mg/kg,

- Minimal to moderate subacute, chronic, and/or chronic-active inflammation and/or mononuclear-cell infiltration in glandular organs [pituitary, parathyroid, mandibular salivary gland, pancreas and mammary gland, skin (abdominal, dorsal, tail, and/or scrotum, nose), reproductive organs, including testes, epididymides, seminal vesicles, prostate, vagina, and uterus], brain (choroid plexus only), peripheral nerve (sciatic nerve), liver (3 of 3 females), kidneys, esophagus, tongue and urinary bladder.
- Minimal to moderate subacute vasculitis in the glandular organs (thyroid gland); skin (dorsal, face, and nose), skeletal muscle, diaphragm, urinary bladder, lung and intestine, vagina and cervix.

- There was also slight to marked interface inflammation and/or mononuclear-cell infiltration in the skin (abdominal, dorsal, tail, and face), vagina, and tongue, urinary bladder,
- Minimal to moderate ulceration and/or erosion in the skin, including tail, nose, face, and scrotum correlating with erosions, scabs, and/or the misshapen nostril (due to a healed ulcer)
- Minimal to mild lymphoid hyperplasia in the spleen and bone marrow
- Mild multifocal glomerulopathy of the kidneys with secondary pulmonary artery thrombus formation, subcutaneous and organ edema, ascites, and hydrothorax due to urinary protein loss in 1 female.

30/20 mg/kg

- Minimal to moderate subacute, chronic, and/or chronic-active inflammation and/or mononuclear-cell infiltrations in the glandular organs [pituitary, parathyroid gland; skin (tail, scrotum, and/or foot), testes, epididymides, and prostate; liver, kidneys, and urinary bladder
- Minimal to mild subacute vasculitis in the skin, including tail, foot; lung, testes and with fibrinoid necrosis of pulmonary arteries and foot microvasculature
- Moderate interface inflammation in the skin, including tail and/or scrotum
- Mild to marked ulceration with or without hemorrhage in the skin, tail, foot and scrotum
- Moderate to marked coagulative necrosis in the tail correlating with the hard and/or dark distal tails observed clinically (including 2 amputated tails)
- Minimal to slight lymphoid hyperplasia in the spleen
- Slight multifocal glomerulopathy of the kidneys with secondary thrombus formation of pulmonary arteries and small vessels in small intestine (jejunum) and foot due to urinary protein loss in 1 male

A summary of drug-related microscopic findings are presented in the table below.

Summary of Drug-related Microscopic Lesions in Affected Organ Systems							
		2 mg/kg/day (LD)		10 mg/kg/day (MD)		30/20 mg/kg/day (HD)	
Dosing Duration		13 wks (3M, 3F)		D29 (1F) 6 wks (3M, 2F)		D16 (1F); D39 (1M) 4 wks (2F, 2M) 6 wks (1M); 13 wks (1M) (acute deaths excluded)	
		M	F	M	F	M	F
Inflammation and/or Mononuclear-cell infiltrates							
Glandular*	Total	4	3	5	5	2	-
Skin*	Total	-	-	9	3	7	2
Reproductive*	Total	6	-	7	5	7	-
Solid*	Total	4	7	8	11	4	-
*Severity: LD=mostly minimal to mild; MD=minimal to moderate; HD =minimal to marked (more severe in skin than MD)							
Vasculitis							
Glandular**	Total	-	-	-	1	-	-
Skin**	Total	-	-	1	2	2	1
Reproductive**	Total	-	-	-	2	1	-
Solid**	Total	1	-	7	6	1	-
**Severity: LD= minimal; MD=minimal to moderate; HD =minimal to mild							
Interface Inflammation (Skin/Mucosa)							
Skin ⁺	Total	-	-	2	2	4	1
Vagina ⁺	Total	-	-	-	3	-	-
Solid ⁺	Total	-	-	2	4	-	-
⁺ Severity: LD= minimal; MD=minimal to moderate; HD =minimal to moderate							
Ulcers and/or Erosions							
Skin ^{^^}	Total	-	-	5	1	6	1
^{^^} Severity: MD= slight to moderate; HD = mild to marked							
Coagulative Necrosis							
Tail [#]	Total	-	-	-	-	3	1
[#] Severity: HD = moderate to marked							
Lymphoid Hyperplasia							
Spleen/Bone Marrow ⁺⁺	Total	3	4	3	4	4	1
⁺⁺ Severity: LD= minimal to slight; MD=slight to mild; HD =minimal to slight							
Glomerulopathy							
Kidney [^]	Total	-	-	-	1	1	-
[^] Severity: MD= mild; HD = slight							

- indicates absence of finding in group

Glandular Organs: pituitary gland, parathyroid gland, thyroid gland, salivary gland, pancreas, and/or mammary gland

Skin: routine (dorsal), tail, nose, distal appendages (hand/foot), scrotum, and/or face

Reproductive Organs: testes, epididymides, seminal vesicles, prostate, vagina, and/or uterus

Solid Organs: liver, kidneys, brain (choroid plexus), peripheral nerve (sciatic, cauda equina), urinary bladder, tongue, skeletal muscle, diaphragm, lung, intestine, and/or esophagus

Detailed Histopathology findings in monkeys at interim 4 week and 6 week and at the end of the study termination in monkeys treated with 0, 2, 10 and 30/20 mg/kg of saxagliptin (groups 1, 2, 3 and 4, respectively).

NUMBER OF ANIMALS WITH MICROSCOPIC FINDINGS BY ORGAN/GROUP/SEX Necropsy Status: Interim/Scheduled Necropsy (K1), Except Deaths 4 Week Interim Necropsy						
Sex		Males			Females	
Dose Group		1	3	4	1	3
No. Animals per Dose Group		2	-	2	2	1
ADRENAL GLANDS	No. Examined	2	-	2	2	1
	NAD	2	-	2	2	1
AORTA	No. Examined	2	-	2	2	1
	NAD	2	-	2	2	1
BONE MARROW, RIB	No. Examined	2	-	2	2	1
	NAD	2	-	2	2	1
BONE MARROW, STERNUM	No. Examined	2	-	2	2	1
	NAD	2	-	2	2	1
BONE, RIB	No. Examined	2	-	2	2	1
	NAD	2	-	2	2	1
BONE, STERNUM	No. Examined	2	-	2	2	1
	NAD	2	-	2	2	1
BRAIN	No. Examined	2	-	2	2	1
	NAD	2	-	2	2	1
- Infiltration: mononuclear cell	Grade 1	1	-	1	-	1
	Grade 1	1	-	1	-	1
CECUM	No. Examined	2	-	2	2	1
	NAD	2	-	2	2	1
- Giant cell: multinucleated	Grade 1	-	-	-	-	1
	Grade 1	-	-	-	-	1
- Inflammation: subacute	Grade 1	-	-	1	1	-
	Grade 1	-	-	1	1	-
CERVIX	No. Examined	-	-	-	2	1
	NAD	-	-	-	2	1
COLON	No. Examined	2	-	2	2	1
	NAD	2	-	2	2	1
- Inflammation: subacute	Grade 2	-	-	-	1	-
	Grade 2	-	-	-	1	-
- Giant cell: multinucleated	Grade 1	-	-	-	-	1
	Grade 1	-	-	-	-	1
DIAPHRAGM	No. Examined	2	-	2	2	1
	NAD	2	-	2	2	1
- Infiltration: mononuclear cell	Grade 1	1	-	-	1	1
	Grade 1	1	-	-	1	1
DUODENUM	No. Examined	2	-	2	2	1
	NAD	2	-	2	2	1
EPIDIDYMIDES	No. Examined	2	-	2	-	-
	NAD	2	-	2	-	-
- Infiltration: mononuclear cell	Grade 1	-	-	1	-	-
	Grade 1	-	-	1	-	-
ESOPHAGUS	No. Examined	2	-	2	2	1
	NAD	2	-	2	2	1
- Infiltration: mononuclear cell	Grade 1	-	-	-	1	-
	Grade 1	-	-	-	1	-
EYES	No. Examined	2	-	2	2	1
	NAD	2	-	2	2	1
- Infiltration: mononuclear cell	Grade 1	-	-	-	1	-
	Grade 1	-	-	-	1	-
GALLBLADDER	No. Examined	2	-	2	2	1
	NAD	2	-	2	2	1
HEART	No. Examined	2	-	2	2	1
	NAD	2	-	2	2	1
- Infiltration: mononuclear cell	Grade 1	1	-	1	1	-
	Grade 1	1	-	1	1	-
ILEUM	No. Examined	2	-	2	2	1
	NAD	2	-	2	2	1
JEJUNUM	No. Examined	2	-	2	2	1
	NAD	2	-	2	2	1

NAD = Nothing abnormal discovered
 Group 1, Control, males: Saxagliptin (0 mg/kg); females: Saxagliptin (0 mg/kg)
 Group 3, Mid Dose, males: Saxagliptin (10 mg/kg); females: Saxagliptin (10 mg/kg)
 Group 4, High Dose, males: Saxagliptin (20 mg/kg); females: Saxagliptin (20 mg/kg)

NUMBER OF ANIMALS WITH MICROSCOPIC FINDINGS BY ORGAN/GROUP/SEX Necropsy Status: Interim/Scheduled Necropsy (K1), Except Deaths 4 Week Interim Necropsy						
Sex		Males			Females	
Dose Group		1	3	4	1	3
No. Animals per Dose Group		2	-	2	2	1
KIDNEYS	No. Examined	2	-	2	2	1
	NAD	-	-	-	1	-
- Mineralization	Grade 1	-	-	1	-	-
	Grade 1	-	-	1	-	-
- Giant cell: multinucleated	Grade 1	2	-	-	-	-
	Grade 1	2	-	-	-	-
- Infiltration: mononuclear cell	Grade 1	2	-	1	1	-
	Grade 1	2	-	1	1	-
- Inflammation: subacute	Grade 2	-	-	-	-	1
	Grade 2	-	-	-	-	1
- Glomerulopathy	Grade 3	-	-	-	-	1
	Grade 3	-	-	-	-	1
LACRIMAL GLANDS	No. Examined	2	-	2	2	1
	NAD	2	-	2	2	1
LIVER	No. Examined	2	-	2	2	1
	NAD	-	-	1	-	2
- Leukocytosis: sinusoids	Grade 1	-	-	-	-	1
	Grade 1	-	-	-	-	1
- Inflammation: subacute	Grade 2	-	-	-	-	1
	Grade 2	-	-	-	-	1
- Infiltration: mononuclear cell	Grade 1	2	-	1	2	1
	Grade 1	2	-	1	2	1
LUNG	No. Examined	2	-	2	2	1
	NAD	1	-	2	-	2
- Histiocytosis: alveolar space	Grade 1	1	-	-	-	-
	Grade 1	1	-	-	-	-
- Infiltration: mononuclear cell	Grade 1	-	-	-	1	-
	Grade 1	-	-	-	1	-
- Inflammation: subacute	Grade 4	-	-	-	-	1
	Grade 4	-	-	-	-	1
- Thrombus: subacute	Grade 4	-	-	-	-	1
	Grade 4	-	-	-	-	1
- Inflammation: chronic	Grade 1	1	-	-	1	-
	Grade 1	1	-	-	1	-
	Grade 3	-	-	-	-	1
M. QUADRICEPS FEMORIS	No. Examined	2	-	2	2	1
	NAD	1	-	1	1	-
- Histiocytes	Grade 2	-	-	-	-	1
	Grade 2	-	-	-	-	1
- Infiltration: mononuclear cell	Grade 1	1	-	1	1	1
	Grade 1	1	-	1	1	1
MAMMARY GLAND	No. Examined	2	-	2	2	1
	NAD	2	-	2	2	1
- Infiltration: mononuclear cell	Grade 1	-	-	-	1	-
	Grade 1	-	-	-	1	-
MANDIB. LYMPH NODE	No. Examined	2	-	2	2	1
	NAD	2	-	2	2	1
- Red blood cells: sinuses	Grade 2	-	-	-	-	1
	Grade 2	-	-	-	-	1
- Inflammation: acute	Grade 4	-	-	-	-	1
	Grade 4	-	-	-	-	1
- Histiocytosis: sinus	Grade 2	-	-	-	1	-
	Grade 2	-	-	-	1	-
MANDIB. SALIVARY GLAND	No. Examined	2	-	2	2	1
	NAD	1	-	1	-	1
- Infiltration: mononuclear cell	Grade 1	1	-	1	2	-
	Grade 1	1	-	1	2	-
	Grade 2	1	-	-	1	-
MESENT. LYMPH NODE	No. Examined	2	-	2	2	1
	NAD	1	-	1	1	1
- Histiocytosis: sinus	Grade 2	1	-	1	1	-
	Grade 2	1	-	1	1	-
	Grade 4	-	-	1	-	-
NOSE	No. Examined	1	-	-	1	1
	NAD	1	-	-	1	-
- Inflammation: vascular	Grade 2	-	-	-	-	1
	Grade 2	-	-	-	-	1
- Erosion		-	-	-	-	1

NAD = Nothing abnormal discovered
 Group 1, Control, males: Saxagliptin (0 mg/kg); females: Saxagliptin (0 mg/kg)
 Group 3, Mid Dose, males: Saxagliptin (10 mg/kg); females: Saxagliptin (10 mg/kg)
 Group 4, High Dose, males: Saxagliptin (20 mg/kg); females: Saxagliptin (20 mg/kg)

NUMBER OF ANIMALS WITH MICROSCOPIC FINDINGS BY ORGAN/GROUP/SEX Necropsy Status: Interim/Scheduled Necropsy (R1), Except Deaths 4 Week Interim Necropsy						
Sex	Males			Females		
Dose Group No. Animals per Dose Group	1 2	3 -	4 2	1 2	3 1	4 2
NOSE	No. Examined	1	-	-	1	1
- Erosion	cont'd. Grade 2	-	-	-	1	-
OVARIES	No. Examined	-	-	-	2	1
- Mineralization	NAD Grade 2	-	-	-	1	1
PANCREAS	No. Examined	2	-	2	2	1
PARATHYROID GLANDS	No. Examined	2	-	2	2	1
- Cyst: multiloculated	NAD Grade 3	1	-	-	-	-
PERIPHER. NERVE(S)	No. Examined	2	-	2	2	1
PITUITARY GLAND	No. Examined	2	-	2	2	1
PROSTATE GLAND	No. Examined	2	-	2	-	-
- Infiltration: mononuclear cell	Grade 1 Grade 2	2	-	1	-	-
Dose Group No. Animals per Dose Group	1 2	3 -	4 2	1 2	3 1	4 2
SEMINAL VESICLES	No. Examined	2	-	2	-	-
SKIN/SUBCUTIS	No. Examined	2	-	2	2	1
- Infiltration: mononuclear cell	Grade 1 Grade 2	1	-	-	2	-
- Edema	Grade 4	-	-	-	1	-
SPINAL CORD, CERVIC.	No. Examined	2	-	2	2	1
- Axonal Spheroid	Grade 1	1	-	-	-	-
SPINAL CORD, LUMBAR	No. Examined	2	-	2	2	1
SPLEEN	No. Examined	2	-	2	2	1
- Hyperplasia: lymphoid	Grade 1	-	-	1	-	1
- Amyloid	Grade 1 Grade 2	2	-	-	2	-
STOMACH	No. Examined	2	-	2	2	1
- Inflammation: subacute	Grade 1 Grade 2 Grade 3	1	-	2	2	-
- Necrosis	Grade 1 Grade 2	-	-	-	1	-
TAIL	No. Examined	1	-	2	1	-
- Inflammation: subacute	Grade 2 Grade 4	-	-	1	-	1
- Inflammation: vascular	Grade 1 Grade 2	-	-	1	-	1
- Hemorrhage	Grade 4	-	-	2	-	-
- Inflammation: chronic-active	Grade 4	-	-	1	-	-
- Granuloma: foreign body	Grade 2	-	-	-	-	1
- Ulcer	Grade 1 Grade 3 Grade 4 Grade 5	1	-	2	-	-
- Necrosis: coagulative	Grade 4 Grade 5	-	-	2	-	-

NAD = Nothing abnormal discovered
 Group 1, Control, males: Saxagliptin (0 mg/kg); females: Saxagliptin (0 mg/kg)
 Group 3, Mid Dose, males: Saxagliptin (10 mg/kg); females: Saxagliptin (10 mg/kg)
 Group 4, High Dose, males: Saxagliptin (20 mg/kg); females: Saxagliptin (20 mg/kg)

NUMBER OF ANIMALS WITH MICROSCOPIC FINDINGS BY ORGAN/GROUP/SEX Necropsy Status: Interim/Scheduled Necropsy (R1), Except Deaths 4 Week Interim Necropsy						
Sex	Males			Females		
Dose Group No. Animals per Dose Group	1 2	3 -	4 2	1 2	3 1	4 2
TESTES	No. Examined	2	-	2	-	-
- Infiltration: mononuclear cell	Grade 1	-	-	1	-	-
- Atrophy: seminiferous tubule	Grade 1	-	-	1	-	-
THYMUS	No. Examined	2	-	2	2	1
THYROID GLAND	No. Examined	2	-	2	2	1
- Depletion: colloid	Grade 2	-	-	-	-	1
TONGUE	No. Examined	2	-	2	2	1
- Degeneration: skeletal muscle	cell Grade 1	1	-	-	-	1
- Infiltration: mononuclear cell	Grade 1	-	-	1	1	-
- Erosion	Grade 2	1	-	-	-	-
TRACHEA	No. Examined	2	-	2	2	1
URINARY BLADDER	No. Examined	2	-	2	2	1
- Infiltration: mononuclear cell	Grade 1	1	-	1	2	-
UTERUS	No. Examined	-	-	-	2	1
VAGINA	No. Examined	-	-	-	2	1
- Inflammation: subacute	Grade 1 Grade 2	-	-	-	2	2

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 Group 1, Control, males: Saxagliptin (0 mg/kg); females: Saxagliptin (0 mg/kg)
 Group 3, Mid Dose, males: Saxagliptin (10 mg/kg); females: Saxagliptin (10 mg/kg)
 Group 4, High Dose, males: Saxagliptin (20 mg/kg); females: Saxagliptin (20 mg/kg)

NUMBER OF ANIMALS WITH MICROSCOPIC FINDINGS BY ORGAN/GROUP/SEX Necropsy Status: Interim/Scheduled Necropsy (K2) 6 Week Interim Necropsy					
Sex		Males		Females	
Dose Group	No. Animals per Dose Group	3	4	3	4
ADRENAL GLANDS	No. Examined	3	1	2	-
	NAD	3	1	2	-
AORTA	No. Examined	3	1	1	-
	NAD	3	1	1	-
BONE MARROW, RIB	No. Examined	3	1	2	-
	NAD	3	1	2	-
BONE MARROW, STERNUM	No. Examined	3	1	2	-
	NAD	2	1	1	-
- Hyperplasia: lymphoid		1	-	1	-
	Grade 1	-	-	1	-
	Grade 2	1	-	-	-
BONE, RIB	No. Examined	3	1	2	-
	NAD	3	1	2	-
BONE, STERNUM	No. Examined	3	1	2	-
	NAD	3	1	2	-
BRAIN	No. Examined	3	1	2	-
	NAD	1	-	-	-
- Mineralization		-	-	1	-
	Grade 1	-	-	1	-
- Infiltration: mononuclear cell		2	1	2	-
	Grade 1	2	1	1	-
	Grade 2	-	-	1	-
CECUM	No. Examined	3	1	2	-
	NAD	2	1	1	-
- Inflammation: subacute		1	-	-	-
	Grade 1	1	-	-	-
- Inflammation: vascular		-	-	1	-
	Grade 2	-	-	1	-
CERVIX	No. Examined	-	-	2	-
	NAD	-	-	1	-
- Inflammation: vascular		-	-	1	-
	Grade 2	-	-	1	-
COLON	No. Examined	3	1	2	-
	NAD	1	-	1	-
- Parasite		1	-	-	-
- Inflammation: vascular		-	-	1	-
	Grade 2	-	-	1	-
- Giant cell: multinucleated		1	1	-	-
	Grade 1	1	-	-	-
	Grade 2	-	1	-	-
- Infiltration: mononuclear cell		1	-	-	-
	Grade 1	1	-	-	-
ESOPHAGUS	No. Examined	3	1	2	-
	NAD	-	-	1	-
- Inflammation: subacute cont'd.		-	-	1	-
	Grade 2	-	-	1	-
EYES	No. Examined	3	1	2	-
	NAD	2	1	1	-
- Infiltration: mononuclear cell		1	-	1	-
	Grade 1	1	-	1	-
GALLBLADDER	No. Examined	3	1	2	-
	NAD	2	1	1	-
- Infiltration: mononuclear cell		1	-	1	-
	Grade 1	1	-	-	-
	Grade 2	-	-	1	-
HEART	No. Examined	3	1	2	-
	NAD	3	1	2	-
- Infiltration: mononuclear cell		3	1	1	-
	Grade 1	3	1	1	-
	Grade 2	-	-	1	-
ILEUM	No. Examined	3	1	2	-
	NAD	3	1	2	-
JEJUNUM	No. Examined	3	1	2	-
	NAD	2	1	2	-
- Inflammation: vascular		1	-	-	-
	Grade 3	1	-	-	-

NUMBER OF ANIMALS WITH MICROSCOPIC FINDINGS BY ORGAN/GROUP/SEX Necropsy Status: Interim/Scheduled Necropsy (K2) 6 Week Interim Necropsy					
Sex		Males		Females	
Dose Group	No. Animals per Dose Group	3	4	3	4
KIDNEYS	No. Examined	3	1	2	-
	NAD	1	-	1	-
- Mineralization		1	-	-	-
	Grade 2	1	-	-	-
- Inflammation: chronic		-	-	1	-
	Grade 2	-	-	1	-
- Giant cell: multinucleated		-	1	-	-
	Grade 1	-	1	-	-
- Infiltration: mononuclear cell		2	-	1	-
	Grade 1	-	-	1	-
	Grade 2	2	-	-	-
- Fibroplasia/fibrosis		-	1	-	-
	Grade 1	-	1	-	-
LACRIMAL GLANDS	No. Examined	3	1	2	-
	NAD	3	1	2	-
LIVER	No. Examined	3	1	2	-
	NAD	-	-	1	-
- Vacuolation: hepatocyte		-	-	1	-
	Grade 2	-	-	1	-
- Inflammation: subacute		-	-	2	-
	Grade 1	-	-	1	-
	Grade 3	-	-	1	-
- Infiltration: mononuclear cell		3	1	2	-
	Grade 1	3	1	2	-
LUNG	No. Examined	3	1	2	-
	NAD	1	-	-	-
- Arteritis		1	-	-	-
	Grade 3	1	-	-	-
- Histiocytosis: alveolar space		-	1	-	-
	Grade 1	-	1	-	-
- Inflammation: subacute		-	-	1	-
	Grade 3	-	-	1	-
- Inflammation: chronic		2	-	1	-
	Grade 1	2	-	1	-
M. QUADRICEPS FEMORIS	No. Examined	3	1	2	-
	NAD	-	1	-	-
- Infiltration: mononuclear cell		-	1	-	-
	Grade 1	-	1	-	-
- Inflammation: vascular		3	-	2	-
	Grade 1	1	-	-	-
	Grade 2	2	-	1	-
	Grade 3	-	-	1	-
MAMMARY GLAND	No. Examined	3	1	2	-
	NAD	3	1	-	-
- Inflammation: subacute		-	-	1	-
	Grade 2	-	-	1	-
- Infiltration: mononuclear cell		-	-	1	-
	Grade 1	-	-	1	-
MANDIB. LYMPH NODE	No. Examined	3	1	2	-
	NAD	3	1	1	-
- Histiocytosis: sinus		-	-	1	-
	Grade 2	-	-	1	-
MANDIB. SALIVARY GLAND	No. Examined	3	1	2	-
	NAD	-	-	-	-
- Infiltration: mononuclear cell		2	1	1	-
	Grade 1	1	-	-	-
	Grade 2	1	1	1	-
- Inflammation: chronic		1	-	1	-
	Grade 2	-	-	1	-
	Grade 3	1	-	-	-
MESENT. LYMPH NODE	No. Examined	3	1	2	-
	NAD	3	1	1	-
- Histiocytosis: sinus		-	-	1	-
	Grade 2	-	-	1	-
NOSE	No. Examined	1	-	-	-
	NAD	-	-	-	-
- Ulcer		1	-	-	-
	Grade 4	1	-	-	-
- Inflammation: chronic		1	-	-	-
	Grade 3	1	-	-	-

NAD = Nothing abnormal discovered
 Group 3, Mid Dose, males: Saxagliptin (10 mg/kg); females: Saxagliptin (10 mg/kg)
 Group 4, High Dose, males: Saxagliptin (20 mg/kg); females: Saxagliptin (20 mg/kg)

NUMBER OF ANIMALS WITH MICROSCOPIC FINDINGS BY ORGAN/GROUP/SEX Necropsy Status: Interim/Scheduled Necropsy (K2) 6 Week Interim Necropsy					
Sex	Males		Females		
Dose Group No. Animals per Dose Group	3	4	3	4	
OVARIES No. Examined	-	-	2	-	-
- Mineralization	-	-	2	-	-
Grade 1	-	-	1	-	-
Grade 2	-	-	1	-	-
PANCREAS No. Examined	3	1	2	-	-
NAD	2	-	1	-	-
- Inflammation: subacute	1	-	1	-	-
Grade 1	1	-	1	-	-
- Infiltration: mononuclear cell	-	1	-	-	-
Grade 1	-	1	-	-	-
PARATHYROID GLANDS No. Examined	3	1	1	-	-
NAD	2	1	-	-	-
- Infiltration: mononuclear cell	1	-	1	-	-
Grade 1	1	-	1	-	-
PERIPHER. NERVE(S) No. Examined	3	1	2	-	-
NAD	1	1	1	-	-
- Infiltration: mononuclear cell	2	-	-	-	-
Grade 1	2	-	-	-	-
- Inflammation: subacute	-	-	1	-	-
Grade 2	-	-	1	-	-
PITUITARY GLAND No. Examined	3	1	2	-	-
NAD	1	-	1	-	-
- Infiltration: mononuclear cell	2	1	1	-	-
Grade 1	1	1	-	-	-
Grade 2	1	-	-	-	-
Grade 3	-	-	1	-	-
PROSTATE GLAND No. Examined	3	1	-	-	-
NAD	1	-	-	-	-
- Inflammation: subacute	2	1	-	-	-
Grade 2	-	1	-	-	-
Grade 3	2	-	-	-	-
SCROTUM No. Examined	1	1	-	-	-
- Ulcer	1	1	-	-	-
Grade 4	1	1	-	-	-
SEMINAL VESICLES No. Examined	3	1	-	-	-
NAD	2	1	-	-	-
- Inflammation: subacute	1	-	-	-	-
Grade 2	1	-	-	-	-
SKIN/SUBCUTIS No. Examined	3	1	2	-	-
NAD	1	-	-	-	-
- Inflammation: vascular	1	-	1	-	-
Grade 1	1	-	-	-	-
Grade 2	-	-	1	-	-
- Inflammation: subacute	2	1	1	-	-
Grade 1	1	1	-	-	-
Grade 2	-	-	1	-	-
Grade 4	1	-	-	-	-
- Ulcer	1	-	-	-	-
Grade 4	1	-	-	-	-
- Infiltration: mononuclear cell	-	-	1	-	-
Grade 1	-	-	1	-	-
SPINAL CORD, CERVIC. No. Examined	3	1	2	-	-
NAD	3	1	2	-	-
SPINAL CORD, LUMBAR No. Examined	3	1	2	-	-
NAD	3	1	2	-	-
SPLEEN No. Examined	3	1	2	-	-
NAD	1	-	-	-	-
- Hyperplasia: lymphoid	2	1	2	-	-
Grade 1	-	-	1	-	-
Grade 2	1	1	-	-	-
Grade 3	1	-	1	-	-
- Amyloid	-	1	-	-	-

NUMBER OF ANIMALS WITH MICROSCOPIC FINDINGS BY ORGAN/GROUP/SEX Necropsy Status: Interim/Scheduled Necropsy (K2) 6 Week Interim Necropsy					
Sex	Males		Females		
Dose Group No. Animals per Dose Group	3	4	3	4	
SPLEEN No. Examined	3	1	2	-	-
- Amyloid	cont'd.	1	-	-	-
Grade 1	-	1	-	-	-
STOMACH No. Examined	3	1	2	-	-
NAD	2	-	1	-	-
- Inflammation: subacute	1	1	1	-	-
Grade 1	1	-	-	-	-
Grade 2	-	1	-	-	-
Grade 3	-	-	1	-	-
TAIL No. Examined	2	1	2	-	-
NAD	-	-	1	-	-
- Inflammation: subacute	-	1	1	-	-
Grade 3	-	-	1	-	-
Grade 4	-	1	-	-	-
- Hemorrhage	-	1	-	-	-
Grade 4	-	1	-	-	-
- Inflammation: chronic-active	2	-	-	-	-
Grade 3	1	-	-	-	-
Grade 4	1	-	-	-	-
- Ulcer	2	1	-	-	-
Grade 3	1	-	-	-	-
Grade 4	1	-	-	-	-
Grade 5	-	1	-	-	-
- Necrosis: coagulative	-	1	-	-	-
TAIL No. Examined	2	1	2	-	-
- Necrosis: coagulative	cont'd.	1	-	-	-
Grade 4	-	1	-	-	-
TESTES No. Examined	3	1	-	-	-
NAD	2	1	-	-	-
- Infiltration: mononuclear cell	1	-	-	-	-
Grade 1	1	-	-	-	-
THYMUS No. Examined	3	1	2	-	-
NAD	3	1	2	-	-
THYROID GLAND No. Examined	3	1	2	-	-
NAD	2	-	1	-	-
- Infiltration: mononuclear cell	1	1	-	-	-
Grade 1	1	1	-	-	-
- Inflammation: vascular	-	-	1	-	-
Grade 2	-	-	1	-	-
TONGUE No. Examined	3	1	2	-	-
NAD	1	1	-	-	-
- Infiltration: mononuclear cell	2	-	1	-	-
Grade 1	-	-	1	-	-
Grade 2	2	-	-	-	-
- Erosion	1	-	-	-	-
Grade 1	1	-	-	-	-
- Inflammation: subacute	-	-	1	-	-
Grade 2	-	-	1	-	-
TRACHEA No. Examined	3	1	2	-	-
NAD	3	1	2	-	-
URINARY BLADDER No. Examined	3	1	2	-	-
- Inflammation: vascular	-	-	1	-	-
Grade 2	-	-	1	-	-
- Inflammation: subacute	2	-	2	-	-
Grade 1	1	-	1	-	-
Grade 2	1	-	1	-	-
- Infiltration: mononuclear cell	1	1	-	-	-
Grade 1	1	-	-	-	-
Grade 2	-	1	-	-	-
UTERUS No. Examined	-	-	2	-	-
- Infiltration: mononuclear cell	-	-	2	-	-
Grade 1	-	-	1	-	-
Grade 3	-	-	1	-	-
VAGINA No. Examined	-	-	2	-	-
- Inflammation: subacute	-	-	2	-	-
Grade 3	-	-	1	-	-
Grade 4	-	-	1	-	-
- Inflammation: vascular	-	-	1	-	-
Grade 4	-	-	1	-	-

NAD = Nothing abnormal discovered
 Group 3, Mid Dose, males: Saxagliptin (10 mg/kg); females: Saxagliptin (10 mg/kg)
 Group 4, High Dose, males: Saxagliptin (20 mg/kg); females: Saxagliptin (20 mg/kg)

NUMBER OF ANIMALS WITH MICROSCOPIC FINDINGS BY ORGAN/GROUP/SEX						
Necropsy Status: End-of-Dose Necropsy (R0), Except Deaths End-of-Dose Necropsy						
Sex	Males			Females		
Dose Group	1	2	4	1	2	4
No. Animals per Dose Group	3	3	1	3	3	-
ADRENAL GLANDS No. Examined	3	3	1	3	3	-
NAD	3	3	1	3	3	-
AORTA No. Examined	3	3	1	3	3	-
NAD	3	3	1	3	3	-
BONE MARROW, RIB No. Examined	3	3	1	3	3	-
NAD	3	2	1	3	1	-
- Hyperplasia: lymphoid	-	1	-	-	2	-
Grade 1	-	1	-	-	2	-
BONE MARROW, STERNUM No. Examined	3	3	1	3	3	-
NAD	3	3	1	3	3	-
- Hyperplasia: lymphoid	-	-	-	-	2	-
Grade 2	-	-	-	-	2	-
BONE, RIB No. Examined	3	3	1	3	3	-
NAD	3	3	1	3	3	-
BONE, STERNUM No. Examined	3	3	1	3	3	-
NAD	3	3	1	3	3	-
BRAIN No. Examined	3	3	1	3	3	-
NAD	2	2	1	2	2	-
- Infiltration: mononuclear cell	1	1	-	1	1	-
Grade 1	1	-	-	1	-	-
Grade 2	-	-	-	-	1	-
Grade 3	-	1	-	-	-	-
CECUM No. Examined	3	3	1	3	3	-
NAD	2	3	1	3	3	-
- Inflammation: subacute	1	-	-	-	-	-
Grade 2	1	-	-	-	-	-
- Parasite	1	-	-	-	-	-
CERVIX No. Examined	-	-	-	3	3	-
NAD	-	-	-	3	3	-
COLON No. Examined	3	3	1	3	3	-
NAD	2	3	1	3	3	-
- Parasite	1	-	-	-	-	-
- Inflammation: subacute	1	-	-	-	-	-
Grade 2	1	-	-	-	-	-
DIAPHRAGM No. Examined	3	3	1	3	3	-
NAD	3	2	1	3	2	-
- Infiltration: mononuclear cell	-	1	-	-	1	-
Grade 1	-	1	-	-	1	-
DUODENUM No. Examined	3	3	1	3	3	-
NAD	3	3	1	3	3	-
EPIDIDYMIDES No. Examined	3	3	1	-	-	-
NAD	3	1	1	-	-	-
- Infiltration: mononuclear cell	-	1	-	-	-	-
Grade 1	-	1	-	-	-	-
- Inflammation: subacute	-	1	-	-	-	-
Grade 2	-	1	-	-	-	-
ESOPHAGUS No. Examined	3	3	1	3	3	-
NAD	3	3	1	3	3	-
EYES No. Examined	3	3	1	3	3	-
NAD	3	3	1	3	3	-
GALLBLADDER No. Examined	3	3	1	3	3	-
NAD	2	2	-	3	1	-
- Infiltration: mononuclear cell	1	1	1	-	2	-
Grade 1	1	1	1	-	2	-
HEART No. Examined	3	3	1	3	3	-
NAD	2	1	-	-	-	-
- Inflammation: chronic	-	-	-	1	-	-
Grade 1	-	-	-	1	-	-
- Infiltration: mononuclear cell	1	2	1	2	3	-
Grade 1	-	2	1	2	3	-
Grade 2	1	-	-	-	-	-

NAD = Nothing abnormal discovered
 Group 1, Control, males: Saxagliptin (0 mg/kg); females: Saxagliptin (0 mg/kg)
 Group 2, Low Dose, males: Saxagliptin (2 mg/kg); females: Saxagliptin (2 mg/kg)
 Group 4, High Dose, males: Saxagliptin (20 mg/kg); females: Saxagliptin (20 mg/kg)

NUMBER OF ANIMALS WITH MICROSCOPIC FINDINGS BY ORGAN/GROUP/SEX						
Necropsy Status: End-of-Dose Necropsy (R0), Except Deaths End-of-Dose Necropsy						
Sex	Males			Females		
Dose Group	1	2	4	1	2	4
No. Animals per Dose Group	3	3	1	3	3	-
ILEUM No. Examined	3	3	1	3	3	-
NAD	3	3	1	3	3	-
JEJUNUM No. Examined	3	3	1	3	3	-
NAD	3	3	1	3	3	-
KIDNEYS No. Examined	3	3	1	3	3	-
NAD	-	-	1	2	3	-
- Mineralization	-	1	-	1	-	-
Grade 1	-	1	-	1	-	-
- Giant cell: multinucleated	1	1	-	-	1	-
Grade 1	1	1	-	-	1	-
- Infiltration: mononuclear cell	2	3	-	1	3	-
Grade 1	2	2	-	1	-	-
Grade 2	-	1	-	-	3	-
LACRIMAL GLANDS No. Examined	3	3	1	3	3	-
NAD	3	3	1	3	3	-
LIVER No. Examined	3	3	1	3	3	-
NAD	-	1	-	1	-	-
- Inflammation: subacute	-	-	-	-	2	-
Grade 1	-	-	-	-	2	-
- Infiltration: mononuclear cell	3	2	1	2	3	-
Grade 1	3	2	1	2	3	-
LUNG No. Examined	3	3	1	3	3	-
NAD	3	1	-	1	3	-
- Histiocytosis: alveolar space	-	-	1	-	-	-
Grade 1	-	-	1	-	-	-
- Inflammation: chronic	-	2	-	2	-	-
Grade 1	-	1	-	1	-	-
Grade 2	-	-	-	1	-	-
Grade 3	-	1	-	-	-	-
M. QUADRICEPS FEMORIS No. Examined	3	3	1	3	3	-
NAD	3	1	1	2	3	-
- Infiltration: mononuclear cell	-	1	-	1	-	-
Grade 1	-	1	-	1	-	-
- Inflammation: vascular	-	1	-	-	-	-
Grade 1	-	1	-	-	-	-
MAMMARY GLAND No. Examined	3	3	1	3	3	-
NAD	3	2	1	2	3	-
- Inflammation: subacute	-	-	-	-	2	-
Grade 1	-	-	-	-	2	-
- Infiltration: mononuclear cell	-	1	-	1	1	-
Grade 1	-	1	-	1	1	-
MANDIB. LYMPH NODE No. Examined	3	2	1	3	2	-
NAD	3	2	-	2	2	-
- Hyperplasia	-	-	1	1	-	-
Grade 1	-	-	1	1	-	-
Grade 2	-	-	1	-	-	-
MANDIB. SALIVARY GLAND No. Examined	3	3	1	3	3	-
NAD	2	-	-	3	-	-
- Infiltration: mononuclear cell	1	2	1	-	3	-
Grade 1	1	1	1	-	-	-
Grade 2	-	1	-	-	3	-
- Inflammation: chronic	-	1	-	-	-	-
Grade 2	-	1	-	-	-	-
MESENT. LYMPH NODE No. Examined	3	3	1	3	3	-
NAD	2	2	1	3	3	-
- Histiocytosis: sinus	1	1	-	-	-	-
Grade 2	1	1	-	-	-	-
OVARIES No. Examined	-	-	-	3	3	-
NAD	-	-	-	1	3	-
- Cyst: follicular	-	-	-	1	-	-
Grade 2	-	-	-	1	-	-
- Mineralization	-	-	-	1	-	-
Grade 1	-	-	-	1	-	-

NAD = Nothing abnormal discovered
 Group 1, Control, males: Saxagliptin (0 mg/kg); females: Saxagliptin (0 mg/kg)
 Group 2, Low Dose, males: Saxagliptin (2 mg/kg); females: Saxagliptin (2 mg/kg)
 Group 4, High Dose, males: Saxagliptin (20 mg/kg); females: Saxagliptin (20 mg/kg)

NUMBER OF ANIMALS WITH MICROSCOPIC FINDINGS BY ORGAN/GROUP/SEX Necropsy Status: End-of-Dose Necropsy (RO), Except Deaths End-of-Dose Necropsy						
Sex	Males			Females		
Dose Group No. Animals per Dose Group	1	2	4	1	2	4
PANCREAS No. Examined	3	3	1	3	3	-
NAD	2	3	1	2	3	-
- Fibrosis/fibrosis Grade 1	-	-	-	1	-	-
- Infiltration: mononuclear cell Grade 1	1	-	-	-	-	-
PARATHYROID GLANDS No. Examined	3	3	1	3	3	-
NAD	2	2	1	2	2	-
- Infiltration: mononuclear cell Grade 1	-	1	-	-	1	-
Grade 2	-	-	-	-	1	-
- Cyst: multiloculated Grade 1	1	-	-	1	-	-
PERIPHER. NERVE(S) No. Examined	3	3	1	3	3	-
NAD	3	3	1	3	2	-
- Infiltration: mononuclear cell Grade 1	-	-	-	-	1	-
PITUITARY GLAND No. Examined	3	3	1	3	3	-
NAD	3	1	1	3	3	-
- Infiltration: mononuclear cell Grade 1	-	2	-	-	-	-
Grade 2	-	2	-	-	-	-
PROSTATE GLAND No. Examined	3	3	1	-	-	-
NAD	2	-	1	-	-	-
- Infiltration: mononuclear cell Grade 1	1	2	-	-	-	-
Grade 2	1	1	-	-	-	-
Grade 2	-	1	-	-	-	-
- Inflammation: chronic Grade 3	-	1	-	-	-	-
SEMINAL VESICLES No. Examined	3	3	1	-	-	-
NAD	3	2	1	-	-	-
- Infiltration: mononuclear cell Grade 1	-	1	-	-	-	-
Grade 1	-	1	-	-	-	-
SKIN/SUBCUTIS No. Examined	3	3	1	3	3	-
NAD	2	3	1	3	2	-
- Inflammation: pyogranulomatous Grade 4	1	-	-	-	-	-
- Infiltration: mononuclear cell Grade 1	1	-	1	-	1	-
Grade 2	-	-	-	-	1	-
Grade 2	-	-	-	-	1	-
SPINAL CORD, CERVIC. No. Examined	3	3	1	3	3	-
NAD	3	3	1	3	3	-
SPINAL CORD, LUMBAR No. Examined	3	2	-	3	3	-
NAD	3	1	-	3	3	-
- Infiltration: mononuclear cell Grade 1	-	1	-	-	-	-
Grade 1	-	1	-	-	-	-
SPLEEN No. Examined	3	3	1	3	3	-
NAD	2	1	-	1	-	-
- Hyperplasia: lymphoid Grade 1	-	2	1	-	2	-
Grade 2	-	2	-	-	2	-
Grade 2	-	-	1	-	-	-
- Amyloid Grade 1	1	1	-	2	1	-
Grade 2	1	1	-	2	1	-
Grade 2	-	-	-	-	1	-
STOMACH No. Examined	3	3	1	3	3	-
NAD	2	-	1	3	3	-
- Inflammation: subacute Grade 1	1	3	-	-	-	-
Grade 2	1	1	-	-	-	-
Grade 2	-	2	-	-	-	-
TAIL No. Examined	-	1	1	-	1	-
- Scab Grade 2	-	-	1	-	-	-
Grade 2	-	-	1	-	-	-
- Ulcer Grade 2	-	1	-	-	1	-
Grade 2	-	1	-	-	1	-

NUMBER OF ANIMALS WITH MICROSCOPIC FINDINGS BY ORGAN/GROUP/SEX Necropsy Status: End-of-Dose Necropsy (RO), Except Deaths End-of-Dose Necropsy						
Sex	Males			Females		
Dose Group No. Animals per Dose Group	1	2	4	1	2	4
TESTES No. Examined	3	3	1	-	-	-
NAD	3	1	-	-	-	-
- Infiltration: mononuclear cell Grade 1	-	1	-	-	-	-
Grade 1	-	1	-	-	-	-
THYMUS No. Examined	3	3	1	3	3	-
NAD	3	3	1	3	3	-
THYROID GLAND No. Examined	3	3	1	3	3	-
NAD	2	3	1	3	3	-
- Infiltration: mononuclear cell Grade 1	1	-	-	-	-	-
Grade 1	1	-	-	-	-	-
TONGUE No. Examined	3	3	1	3	3	-
NAD	3	3	1	2	2	-
- Inflammation: subacute Grade 1	-	-	-	1	1	-
Grade 2	-	-	-	-	1	-
Grade 2	-	-	-	-	1	-
TRACHEA No. Examined	3	3	1	3	3	-
NAD	3	3	1	3	3	-
URINARY BLADDER No. Examined	3	3	1	2	3	-
NAD	3	2	-	1	1	-
- Inflammation: subacute Grade 1	-	1	1	-	-	-
Grade 2	-	1	-	-	-	-
Grade 2	-	-	1	-	-	-
- Infiltration: mononuclear cell Grade 1	-	-	-	1	2	-
Grade 1	-	-	-	1	2	-
UTERUS No. Examined	-	-	-	3	3	-
NAD	-	-	-	3	3	-
VAGINA No. Examined	-	-	-	3	3	-
NAD	-	-	-	1	-	-
- Inflammation: subacute Grade 1	-	-	-	2	3	-
Grade 1	-	-	-	2	3	-

NAD = Nothing abnormal discovered
 Group 1, Control, males: Saxagliptin (0 mg/kg); females: Saxagliptin (0 mg/kg)
 Group 2, Low Dose, males: Saxagliptin (2 mg/kg); females: Saxagliptin (2 mg/kg)
 Group 4, High Dose, males: Saxagliptin (20 mg/kg); females: Saxagliptin (20 mg/kg)

Electron Microscopy:

- Renal tissues from 2 animals (1 MD female #3203 and a HD male #4103) with nephropathy, hypoalbuminemia and a control with normal albumin levels were evaluated.
- In the two monkeys with low albumin with glomerulopathy, the degree of podocyte foot process fusion and effacement appeared exaggerated. Furthermore, in 1 of these monkeys (4103), portions of the glomerular basement membrane were unevenly expanded and contained sub-epithelial, intramembranous, and endothelial electron dense deposits along with the formation of podocyte-parietal epithelial cell synechiae. The ultrastructural pathology of renal glomeruli in the 2 drug-treated monkeys with low albumin was typical of glomerulopathy. These two monkeys had also significant proteinuria supporting changes in renal tissue noted by EM.

Toxicokinetics and pharmacodynamics:

- AUC exposure for saxagliptin and active metabolite, BMS-510849 increase in a dose-related manner with no notable gender differences.
- Repeated administration of saxagliptin resulted in about 2.2 fold higher exposure to both saxagliptin and BMS-510849.
- Exposure to metabolite was higher than parent (4.1 to 6 fold for males and 2.7 to 8.1 fold for females)

Toxicokinetic Summary

Saxagliptin					
Dose [mg/kg/day]	Study Day	Cmax [ng/mL]		AUC(0-T) ^a [ng•h/mL]	
		Males	Females	Males	Females
2	1	167	273	286	629
	28	422	327	607	804
	84 or 85 ^b	342	532	578	1367
10	1	656	717	1328	1225
	28	633	951	2857	1702
20	28	2072	3106 ^c	6013	4839 ^c
	85	2421 ^d	-	4956 ^d	-
30	1	3151	4956	8604	9409

^a Calculated from time zero to the time of last measurable concentration, ranging between 4 and 24 h.

^b Samples were collected for 24 hours post-dose on Day 85 for males and Day 84 for females.

^c n = 2 animals.

^d n = 1 animal.

BMS-510849 (Active Metabolite)							
Dose [mg/kg/day]	Study Day	Cmax [ng/mL]		AUC(0-T) ^a [ng•h/mL]		Molar AUC(0-T) ratio ^b	
		Males	Females	Males	Females	Males	Females
2	1	699	899	1962	3142	6.8	5.0
	28	835	814	2462	2892	4.1	3.5
	84 or 85 ^c	986	1138	2717	3837	4.5	2.7
10	1	2603	2680	8782	7675	6.0	6.1
	28	2852	3547 ^d	15811	12387 ^d	5.4	8.1 ^d
20	28	6620	9473	28207	26087	4.7	5.1
	85	9198 ^c	-	26698 ^c	-	5.1 ^c	-
30	1	10040	14451	44490	47855	5.0	5.1

^a Calculated from time zero to the time of last measurable concentration, ranging between 4 and 24 h.

^b Ratio of BMS-510849 to saxagliptin.

^c Samples were collected for 24 hours post-dose on Day 85 for males and Day 84 for females.

^d n = 2 animals.

^e n = 1 animal.

Investigative six-week oral comparative toxicity study in monkeys comparing saxagliptin (BMS-477118), vildagliptin (BMS-471211), and sitagliptin (BMS-730173)**Key study findings in saxagliptin group (10 mg/kg):**

- Skin abrasions/ulcerations on scrotum, skin, digits and feet, and tail. Necrosis of tail (1F) resulted in amputation (D16). Red and black skin discolorations, swelling/edema of scrotum, transient lameness (1 male, 2 females); and intermittent tremor
- Decrease in RBC parameters, platelets and increase in WBCs as noted in other monkey studies
- Increase in globulin (1.1 to 1.6x mean pretest) and decrease in albumin (65 to 88%)
- Glomerulopathy in 1 male and 1 female with increased urine protein
- Marked coagulative necrosis and/or ulceration of tail, moderate scab formation, mild to moderate chronic-active or chronic inflammation, and/or slight vascular hypertrophy (smooth muscle and endothelial-cell hypertrophy of microvasculature and small muscular arteries) with inflammatory-cell infiltration.
- In the nose/nasal cavity (2/3 M, F), slight to moderate erosion/ulceration, mild to moderate chronic-active inflammation and/or slight mononuclear-cell infiltration
- In the digits (2/3M, 3/3F), mild ulceration and/or scabbing, slight to mild chronic inflammation, and/or slight to mild vascular hypertrophy with inflammatory-cell infiltration
- In the scrotum (2/3M), perivulvar skin (2/3F), marked ulceration, slight to moderate subacute inflammation, slight to mild hemorrhage, mild to moderate vascular hypertrophy (smooth muscle and endothelial-cell hypertrophy of microvasculature and small muscular arteries) with inflammatory-cell infiltration, and/or minimal mononuclear-cell infiltration
- Minimal to moderate subacute inflammation and/or minimal to mild mononuclear cell infiltration (2/3M, 3/3F) in the pituitary gland (pars nervosa), mandibular salivary gland, esophagus, stomach, cecum, colon, skin (abdominal and dorsal skin), testes, epididymides, seminal vesicles, prostate, vagina, cervix, uterus, choroid plexus in brain, peripheral nerve, ciliary body in eyes, liver, kidneys, and/or urinary bladder
- In the skeletal muscle and/or diaphragm (2/2M, 3/3F), minimal to slight non-necrotizing vascular inflammation in all monkeys.
- Minimal to mild lymphoid hyperplasia (2/3M, 3/3F) in the spleen and/or bone marrow and mild lymphoid depletion in thymus (1/3 M, 1/3F)
- In the kidney, mild multifocal glomerulopathy (1/3M, 1/3F)

Key findings with vildagliptin group (Galvus®, 40/20/30/20 mg/kg)

- Findings in vildagliptin were comparable to saxagliptin
- Skin abrasions/ulcerations/scabs on digits and feet, and tail; and necrosis of tail (1/3M,1/3F) resulting in amputation on D16
- Swelling/edema of scrotum, hind foot and/or forefoot edema, and tail; decreased activity; red and black skin discolorations (3/3M,2/3F); transient lameness; and intermittent tremor (2/3M, 1/3F)
- Decreased red cell parameters and platelets and increase in reticulocytes and WBC similar to saxagliptin
- Decrease albumin, total protein and increase globulin similar to saxagliptin

- In the tail (1/3M, 1/3F), marked coagulative necrosis, moderate chronic inflammation, and/or slight hemorrhage correlating with necrosis observed clinically
- In the nose (1/3M), mild ulceration, vascular wall disruption of the microvasculature, and hemorrhage correlating with ulceration observed grossly.
- In the digits/feet(1/3M, 1/3F), moderate ulceration, slight to mild vascular wall disruption of the microvasculature, and slight to mild hemorrhage
- In the scrotum (2/3M)and sex skin (1/3F), mild ulceration, mild vascular wall disruption of the microvasculature, and/or mild hemorrhage,
- In the thoracic and abdominal subcutaneous tissue edema (1/3M),
- Mild lymphoid hyperplasia (1/3M) in spleen and bone marrow and minimal lymphoid depletion in thymus (1/3M)

Key findings with sitagliptin group (40 mg/kg)

- Reactive lymphocytes in 2/3 F
- Minimal to slight lymphoid hyperplasia in the spleen and/or bone marrow, minimal to slight lymphoid hyperplasia in 1/3M and 2/3 F
- No skin lesions were observed in any animal.

Study no.: DN06018

Volume #, and page #: eNDA

Conducting laboratory and location: BMS, One Squibb Drive, New Brunswick, NJ

Date of study initiation: March 15, 2006

GLP compliance: No (a mechanistic study)

QA report: yes () no (x)

Drug, lot #, and % purity: Saxagliptin batch 4K85994 (93.9%), Vildagliptin batch 003 (97%) and Sitagliptin batch 011 (79.4%)

Methods

The objective of this study was to compare skin lesions found in cynomolgus monkeys with saxagliptin to other DPP4 inhibitors, sitagliptin and vildagliptin. Vildagliptin (40 mg/kg) and sitagliptin (40 mg/kg) were selected to match the pharmacodynamics and or/pharmacokinetic parameters of 10 mg/kg saxagliptin in cynomolgus monkeys. Sitagliptin is more selective than saxagliptin and vildagliptin for the DPP4 enzyme. The dose of vildagliptin was reduced after 2 doses to 20 mg/kg due to edema then increased to 30 mg/kg on Day 8 when edema was resolving. However, 30 mg/kg vildagliptin worsened edema leading to another dose reduction to 20 mg/kg on Day 13. All three compounds were prepared in acidified water and administered orally to 3/sex/dose cynomolgus monkeys (2 to 4 ml/kg). Drug solutions were stable and within the target range ($\pm 10\%$) except for vildagliptin that was 10 to 13% lower than the target dose.

Treatment	Daily Dose (mg/kg/day)	Volume (mL/kg)	Concentration (mg/mL)	Number of Animals
Control	0	4	0	3M, 3F
Saxagliptin	10	4	2.5	3M, 3F
Vildagliptin ^{a,b}	20 to 40	2 to 4	5 to 10	3M, 3F
Sitagliptin	40	4	10	3M, 3F

^a Detailed description of dose, volume, and concentration adjustments are listed in Section 2.5.1.

^b Based on analytical results, vildagliptin doses were approximately 5% lower than targeted. However, dose levels are expressed as the target doses of 20 to 40 mg/kg in this report.

Vildagliptin Dose Adjustments

Study Day (males/females) ^a	Dose (mg/kg/day)	Volume (mL/kg)	Concentration (mg/mL)
1/NA	40	4	10
2/1	40	4	10
3/2	20	2	10
8/7	30	4	7.5
13/12	20	2	10
14/13 ^b	20	4	5

^a Represents the staggered start for males on 15-March-2006 and females on 16-March-2006.

^b Doses, volumes, and concentrations remained at these values for the duration of the study.

The K_i values for the products used in the monkey study were reported by the sponsor (BMS Study # 930021121).

Human K_i values at 37 °C

	DPP4 K _i (nM)	DPP8 K _i (nM)		DPP9 K _i (nM)	
	mean ± sd (N)	mean ± sd (N)	selectivity	mean ± sd (N)	selectivity
Saxagliptin	1.3 ± 0.31 (12)	508 ± 174 (13)	391	98 ± 44 (11)	75
BMS-510849	2.6 ± 1 (12)	2495 ± 727 (14)	948	423 ± 64 (12)	163
Vildagliptin	13 ± 2.8 (12)	5218 ± 2319 (14)	401	258 ± 93 (12)	20
Sitagliptin	18 ± 1.6 (12)	33780 ± 5532 (12)	1913	55142 ± 19414 (11)	3063
Alogliptin	13 ± 2.3 (12)	> 100000 (6)	> 7692	> 100000 (12)	> 7692

Species/strain: Cynomolgus monkeys from C

Route, formulation, volume, and infusion rate: oral, 2 to 4 ml/kg

Age: 25 to 29 months old

Weight: 2.1 to 3.8 kg

Sampling times: PK data for saxagliptin, vildagliptin and sitagliptin on during week 1, 3 and 6 at 1, 2, 4, 8 and 24 hrs post dose.

Unique study design or methodology: The study also examined the plasma DPP4 inhibition activity of each compound. Plasma DPP4 activity was measured before treatment on Day 1 as well as Day 42. Electron microscopy was performed on kidney samples of 1 control male and saxagliptin treated male (2103). Since maximum inhibition of DPP4 has always ranged between 80 to 85% of total plasma activity and the identity of enzyme(s) responsible for the residual 15 to 20% dipeptidylpeptidase activity is unknown suggests that the assay is not specific to DPP4 and thus the data will refer to inhibition as DPP activity rather than DPP4 activity.

b(4)

Results

Mortality and clinical signs: There were no deaths except for 1 male treated with vildagliptin which was euthanized on Day 17 (20 mg/kg) due to poor recovery from ketamine anesthesia.

NUMBER OF ANIMALS WITH NECROPSY FINDINGS BY ORGAN/GROUP/SEX
 STATUS AT NECROPSY: KO, INCL. DEATHS
 Unscheduled and End-of-Dose Necropsies

ORGAN/FINDING	DOSE GROUP:		1		2		3		4	
	SEX:		M	F	M	F	M	F	M	F
	ANIM. EXAM.:		3	3	3	3	3	3	3	3
NOSE	:									
- discharge, (nostril).	:	-	-	1	-	-	-	-	-	-
- discharge, red fluid, (nasal cavity).	:	-	-	-	1	-	-	-	-	-
- erosion, (nostril).	:	-	-	1	-	-	-	-	-	-
- ulcer, (nasal cavity).	:	-	-	-	1	-	-	-	-	-
- ulcer.	:	-	-	-	-	1	-	-	-	-
.....										
CERVIX	:									
- discoloration, red.	:	-	-	-	1	-	-	-	-	-
.....										
SKIN/SUBCUTIS	:									
- discoloration, 1-3 cm, red, (scrotum).	:	-	-	-	-	1	-	-	-	-
- edema, (scrotum).	:	-	-	1	-	-	-	-	-	-
- edema.	:	-	-	-	-	1	-	-	-	-
- erosion, (toe).	:	-	-	-	2	-	-	-	-	-
- erosion, 1-5 mm, (perineum).	:	-	-	-	1	-	-	-	-	-
- erosion, 1-5 mm, (toe).	:	-	-	-	1	1	-	-	-	-
- scab, (hindfoot).	:	-	-	-	-	1	-	-	-	-
- scab, (scrotum).	:	-	-	-	-	1	-	-	-	-
- scab, 1-5 mm, (toe).	:	-	-	2	-	-	-	-	-	-
- ulcer, 0.5-1 cm, (vulva).	:	-	-	-	1	-	-	-	-	-
- ulceration, (scrotum).	:	-	-	1	-	-	-	-	-	-
- ulceration, 1-5 mm, (perineum).	:	-	-	-	1	-	1	-	-	-
- ulceration, 1-5 mm, (toe).	:	-	-	-	-	-	1	-	-	-
.....										
TAIL	:									
- scab, 0.5-1 cm, (entire tail).	:	-	-	1	-	-	-	-	-	-
- scab, 0.5-1 cm.	:	-	-	-	1	-	-	-	-	-
- scab, 1-5 mm.	:	-	-	2	-	-	-	-	-	-
- scab.	:	1	-	-	-	-	-	-	-	-

Temp: 1, Control, males (9 mg/kg); females (9 mg/kg)
 Group 2, Saxagliptin (BMS 472137), males (10 mg/kg); females (10 mg/kg)
 Temp: 3, Vildagliptin (BMS 472137), males (20 mg/kg); females (20 mg/kg)
 Group 4, Sitagliptin (BMS 732137), males (40 mg/kg); females (40 mg/kg)

Body weights: No drug-related change in BW

Hematology:

Hematological change in cynomolgus monkeys relative to pretest values are shown in table below. Only change is sitagliptin was presence of reactive lymphocytes in 2/3 females on Day16. Most of the hematology findings in saxagliptin and vildagliptin were similar in nature, regenerative anemia and decreased platelet counts. It is highly possible that these changes were due to hemorrhage/ulceration noted in both saxagliptin and vildagliptin monkeys since sitagliptin treated monkeys with no skin lesions did not have any shift in RBC or WBC counts.

Dose	saxagliptin, 10 mg/kg	vildagliptin, 40/20 mg/kg	sitagliptin, 40 mg/kg
RBC count	-10 to -34%	-16 to -40%	-
Hgb	-9 to -35%	-16 to -40%	-
Hct	-10 to 35%	-17 to -43%	-
Reticulocytes	2.3 to 15.4x	4 to 14.4x	-
Neutrophil	3 to 6.1x	10.8x	-
Platelet	-23 to 58%	-63%	-
Monocytes	2.1 to 5.2x	10.6x	-
Lymphocytes	1.9 to 3.7x	decreased	-

Clinical chemistry: There were decreases in albumin and albumin/globulin ratio and increases in globulin and total protein. Clinical chemistry changes relative to pre-test values are shown in table below. Clinical chemistry changes along with WBC counts discussed above may have been due to inflammation and cell-infiltration in the skin and other target tissues, since they were absent in sitagliptin monkeys with no such lesions.

Dose	saxagliptin, 10 mg/kg	vildagliptin, 40/20 mg/kg	sitagliptin, 40 mg/kg
globulin	1.1 to 1.6x	1.2 to 1.4x	-
albumin,	-12 to -35%	-13 to -43%	-
albumin/globulin ratio	-12 to -69%	-40 to -52%	-
total protein,	1.1x	-14 to -27%*	-

*In a male on Day 17 vildagliptin

Urinalysis: Proteinuria in 1 male and 1 female in saxagliptin treated monkeys that appeared to be the result of glomerulopathy. Otherwise there was no change in other urinary parameters.

Organ weights: No drug-related change in organ weight in the short study

Gross pathology: Most of the gross findings in monkeys were limited to saxagliptin and vildagliptin treatment groups. No notable findings were seen in sitagliptin which is highly selective to DPP4.

Drug-related gross findings are presented in the following table.

Summary of Drug-Related Gross Lesions (Number of Animals with Findings)						
Drug	Saxagliptin (10 mg/kg/day)		Vildagliptin (40/20/30/20 mg/kg/day)		Sitagliptin (40 mg/kg/day)	
	M	F	M	F	M	F
Number of Animals	3	3	3	3	3	3
Tail						
Amputation	-	1	1	1	-	-
Scabs	3	1	-	-	-	-
Nose/Nasal Cavity						
Ulcer/Erosion	1	1	1	-	-	-
Red Discharge	1	1	-	-	-	-
Scrotum/Perivulvar/Sex Skin						
Ulcer/Erosion/Scab/Edema/ Red Discoloration	2	2	2	1	-	-
Digit/Foot Skin						
Ulcer/Scab-Multifocal	2	3	1	1	-	-
Thoracic/Abdominal Skin						
Edema	-	-	1	-	-	-
Thoracic/Peritoneal Cavities						
Ascites/Hydrothorax (Clear Fluid)	1	-	-	-	-	-
Cervix						
Red Discoloration		1		-		-
Animals with DR Lesions	3	3	2	1	0	0

- Indicates absence of finding in group

Histopathology:

Histopath findings were limited to saxagliptin and vildagliptin, two less selective DPP4 tested in the study. Sitagliptin, with 3000x more selectivity to DPP4, had minimal (lymphoid hyperplasia) or no effect on the target identified in the saxagliptin and vildagliptin treated monkeys.

Summary of Drug-Related Microscopic Lesions (Number of Animals with Findings)						
Drug	Saxagliptin (10 mg/kg/day)		Vildagliptin (40/20/30/20 mg/kg/day)		Sitagliptin (40 mg/kg/day)	
	M	F	M	F	M	F
Number of Animals	3	3	3	3	3	3
Tail						
Ulcer/necrosis/scab	1	2	1	1	-	-
Inflammation	3	1	-	1	-	-
Vascular hypertrophy with inflammatory-cell infiltration	3	1	-	-	-	-
Hemorrhage	-	-	-	1	-	-
Nose						
Ulcer/Erosion	1	-	1	-	-	-
Hemorrhage	-	-	1	-	-	-
Vascular wall disruption	-	-	1	-	-	-
Nasal Cavity						
Ulcer/inflammation	1	2	-	-	-	-
Scrotum/Perivulvar/Sex Skin						
Ulcer/Erosion/Scab	a	1	1	b	-	-
Hemorrhage	-	2	2	1	-	-
Inflammation/inflammatory-cell infiltrate	1	2	-	-	-	-
Vascular hypertrophy with inflammatory-cell infiltration	-	2	-	-	-	-
Vascular wall disruption	-	-	1	1	-	-
Digit/Foot Skin						
Ulcer/Scab	1	2	1	1	-	-
Inflammation	1	3	-	1	-	-
Hemorrhage	-	-	1	1	-	-
Vascular hypertrophy with inflammatory-cell infiltration	2	3	-	-	-	-

Summary of Drug-Related Microscopic Lesions (continued)						
(Number of Animals with Findings)						
Drug	Saxagliptin (10 mg/kg/day)		Vildagliptin (40/20/30/20 mg/kg/day)		Sitagliptin (40 mg/kg/day)	
	M	F	M	F	M	F
Digit/Foot Skin (continued)						
Vascular wall disruption	-	-	1	1	-	-
Thoracic/Abdominal Skin						
Edema	-	-	1	-	-	-
Bone Marrow						
Lymphoid Hyperplasia	2	3	1	-	-	1
Spleen						
Lymphoid Hyperplasia	2	3	1	-	1	2
Kidney						
Glomerulopathy	1	1	-	-	-	-
Other organs/tissues^c						
Inflammation/inflammatory-cell infiltrate	2	3	-	-	-	-
Skeletal muscle/diaphragm						
Non-necrotizing vascular inflammation	3	3	-	-	-	-
Thymus						
Lymphoid depletion	1	1	1	-	-	-
Animals with DR Lesions	3	3	2	1	1	2

-Indicates absence of finding in group

a - scrotum ulcer defined grossly

b - sex skin ulcer defined grossly

c - pituitary gland, salivary gland, skin (routine abdominal and dorsal), testes, epididymides, seminal vesicles, prostate, vagina, cervix, uterus, liver, kidneys, brain (choroid plexus), peripheral nerve (sciatic), eyes (ciliary body), urinary bladder, stomach, cecum, colon, and/or esophagus

Detailed histopath findings in male cynomolgus monkeys

NUMBER OF ANIMALS WITH MICROSCOPIC FINDINGS BY ORGAN/GROUP/SEX Necropsy Status: End-of-Dose Necropsy (KO), Incl. Deaths Unscheduled and End-of-Dose Necropsies				
Sex	Males			
Dose Group No. Animals per Dose Group	1	2	3	4
ADRENAL GLANDS No. Examined	3	3	3	3
NAD	3	3	3	3
AORTA No. Examined	3	3	3	3
NAD	3	3	3	3
BONE MARROW, RIB No. Examined	3	3	3	3
NAD	3	2	3	3
- Hyperplasia: lymphoid	-	1	-	-
Grade 1	-	1	-	-
BONE MARROW, SPERMUDNo. Examined	3	3	3	3
NAD	3	1	2	3
- Hyperplasia: lymphoid	-	2	1	-
Grade 1	-	1	-	-
Grade 3	-	1	1	-
BONE, RIB No. Examined	3	3	3	3
NAD	3	3	3	3
BONE, SPERMUM No. Examined	3	3	3	3
NAD	3	3	3	3
BRAIN No. Examined	3	3	3	3
NAD	1	-	2	3
- Infiltration: mononuclear cell	2	3	1	-
Grade 1	2	1	1	-
Grade 2	-	1	-	-
Grade 3	-	1	-	-
CECUM No. Examined	3	3	3	3
NAD	2	2	3	2
- Granuloma	1	-	-	-
Grade 2	1	-	-	-
- Parasite	-	-	-	1
- Infiltration: mononuclear cell	-	1	-	-
Grade 1	-	1	-	-
COLON No. Examined	3	3	3	3
NAD	3	3	3	3
DIAPHRAGM No. Examined	3	3	3	3
NAD	2	1	3	3
- Infiltration: mononuclear cell	1	-	-	-
Grade 1	1	-	-	-
- Inflammation: vascular: non-	-	2	-	-
necrotizing	-	2	-	-
Grade 1	-	1	-	-
Grade 2	-	1	-	-
DIGIT No. Examined	-	2	2	-
NAD	-	-	1	-
- Vascular Wall Disruption	-	-	1	-
Grade 2	-	-	1	-
- Hemorrhage	-	-	1	-
Grade 2	-	-	1	-
- Vascular Hypertrophy with	-	2	-	-
Inflammatory Cell Infiltration	-	2	-	-
Grade 2	-	1	-	-
Grade 3	-	1	-	-
- Inflammation: chronic	-	1	-	-
Grade 3	-	1	-	-
- Ulcer	-	1	1	-
Grade 3	-	1	-	-
Grade 4	-	-	1	-
DUODENUM No. Examined	3	3	3	3
NAD	3	3	3	3
EPIDIDYMIDES No. Examined	3	2	3	3
NAD	3	-	2	3
- Infiltration: mononuclear cell	-	-	1	-
Grade 1	-	-	1	-
- Inflammation: subacute	-	2	-	-
Grade 1	-	1	-	-

NAD = Nothing abnormal discovered
 Group 1, Control, males: (0 mg/kg)
 Group 2, Saxagliptin (BMS-47118), males: (10 mg/kg)
 Group 3, Wildagliptin (BMS-47121), males: (20 mg/kg)
 Group 4, Sitagliptin (BMS-730173), males: (40 mg/kg)
 One-Sided Exact Fisher Test: *) p<0.05; **) p<0.01; Control= 1,

NUMBER OF ANIMALS WITH MICROSCOPIC FINDINGS BY ORGAN/GROUP/SEX Necropsy Status: End-of-Dose Necropsy (KO), Incl. Deaths Unscheduled and End-of-Dose Necropsies				
Sex	Males			
Dose Group No. Animals per Dose Group	1	2	3	4
EPIDIDYMIDES No. Examined	3	2	3	3
- Inflammation: subacute cont'd.	-	2	-	-
Grade 2	-	1	-	-
ESOPHAGUS No. Examined	3	3	3	3
NAD	3	1	3	3
- Infiltration: mononuclear cell	-	2	-	-
Grade 1	-	1	-	-
Grade 2	-	1	-	-
EYES No. Examined	3	3	3	3
NAD	3	1	3	2
- Inflammation: subacute	-	2	-	-
Grade 1	-	1	-	-
Grade 2	-	1	-	-
- Infiltration: mononuclear cell	-	-	-	1
Grade 1	-	-	-	1
GALLBLADDER No. Examined	3	3	3	3
NAD	3	2	1	1
- Infiltration: mononuclear cell	-	1	2	2
Grade 1	-	-	2	1
Grade 2	-	1	-	1
HEART No. Examined	3	3	3	3
NAD	1	1	3	2
- Infiltration: mononuclear cell	2	2	-	1
Grade 1	2	1	-	1
Grade 2	-	1	-	-
ILEUM No. Examined	3	3	3	3
NAD	3	3	3	2
- Amyloid	-	-	-	1
Grade 1	-	-	-	1
JEJUNUM No. Examined	2	3	3	3
NAD	2	3	3	3
KIDNEYS No. Examined	3	3	3	3
NAD	1	-	-	2
- Fibroplasia/fibrosis	-	-	1	-
Grade 1	-	-	1	-
- Glomerulopathy	-	1	-	-
Grade 3	-	1	-	-
- Giant cell: multinucleated	-	2	-	-
Grade 1	-	1	-	-
Grade 2	-	1	-	-
- Mineralization	2	1	1	-
Grade 1	2	1	1	-
- Infiltration: mononuclear cell	1	2	2	1
Grade 1	1	1	1	1
Grade 2	-	1	1	-
- Dilatation: tubular lumen	-	-	1	-
Grade 2	-	-	1	-
- Inflammation: subacute	-	1	-	-
Grade 2	-	1	-	-
LACRIMAL GLANDS No. Examined	3	3	3	3
NAD	3	3	3	3
LIVER No. Examined	3	3	3	3
NAD	1	1	1	1
- Inflammation: subacute	-	2	-	-
Grade 2	-	2	-	-
- Infiltration: mononuclear cell	2	1	2	2
Grade 1	2	1	2	2
LUNG No. Examined	3	3	3	3
NAD	1	-	3	-
- Inflammation: subacute	1	3	-	2
Grade 1	1	2	-	2
Grade 2	-	1	-	-
- Inflammation: chronic	1	-	-	1
Grade 1	1	-	-	1
M. QUADRICEPS FEMORIS No. Examined	3	3	3	3
NAD	3	-	2	3
- Histiocytosis	-	-	1	-
Grade 1	-	-	1	-
- Inflammation: vascular: non-	-	3 *	-	-
necrotizing	-	3 *	-	-
Grade 1	-	1	-	-
Grade 2	-	2	-	-

NUMBER OF ANIMALS WITH MICROSCOPIC FINDINGS BY ORGAN/GROUP/SEX Necropsy Status: End-of-Dose Necropsy (KO), Incl. Deaths, Unscheduled and End-of-Dose Necropsies				
Sex	Males			
Dose Group No. Animals per Dose Group	1	2	3	4
HYPHARY GLAND No. Examined NAD	3	3	3	3
MANDIB. LYMPH NODE No. Examined NAD	3	2	3	3
MANDIB. SALIVARY GLAND No. Examined NAD	3	3	3	3
- Inflammation: subacute Grade 1	-	2	-	-
Grade 2	-	1	-	-
- Infiltration: mononuclear cell Grade 1	1	-	2	3
Grade 2	1	-	2	2
MESENT. LYMPH NODE No. Examined NAD	3	3	3	3
NASAL CAVITY No. Examined NAD	1	1	-	-
- Ulcer: respiratory epithelium Grade 4	-	1	-	-
- Inflammation: chronic-active Grade 4	-	1	-	-
NOSE No. Examined	-	2	1	-
- Hemorrhage Grade 3	-	-	1	-
- Infiltration: mononuclear cell Grade 2	-	2	-	-
- Erosion Grade 2	-	1	-	-
- Ulcer Grade 3	-	-	1	-
- Vascular Wall Disruption Grade 3	-	-	1	-
PANCREAS No. Examined NAD	3	3	3	3
- Infiltration: mononuclear cell Grade 1	1	1	1	-
Grade 2	1	-	1	-
PARATHYROID GLANDS No. Examined NAD	2	2	3	3
- Infiltration: mononuclear cell Grade 1	-	1	-	-
- Cyst Grade 3	-	-	1	-
PITUITARY GLAND No. Examined NAD	3	3	3	3
- Infiltration: mononuclear cell Grade 1	1	2	1	1
Grade 2	1	-	1	1
PROSTATE GLAND No. Examined NAD	3	3	3	2
- Inflammation: subacute Grade 2	-	2	-	-
Grade 4	-	1	-	-
- Infiltration: mononuclear cell Grade 1	1	1	3	2
Grade 2	1	1	2	1
Grade 2	-	-	1	1
SCIATIC NERVE No. Examined NAD	3	3	3	3
- Inflammation: subacute Grade 2	-	1	-	-
Grade 2	-	1	-	-
SCROTUM No. Examined	-	1	2	-
- Infiltration: mononuclear cell Grade 1	-	1	-	-
- Vascular Wall Disruption Grade 3	-	-	1	-
- Ulcer Grade 3	-	-	1	-
- Hemorrhage Grade 3	-	-	2	-
SEMPINAL VESICLES No. Examined NAD	3	3	3	3
- Inflammation: subacute Grade 1	-	1	-	-
Grade 2	-	1	-	-
- Infiltration: mononuclear cell Grade 1	-	-	1	-
Grade 1	-	-	1	-

NUMBER OF ANIMALS WITH MICROSCOPIC FINDINGS BY ORGAN/GROUP/SEX Necropsy Status: End-of-Dose Necropsy (KO), Incl. Deaths, Unscheduled and End-of-Dose Necropsies				
Sex	Males			
Dose Group No. Animals per Dose Group	1	2	3	4
SKIN/SUBCUTIS No. Examined NAD	3	3	3	3
- Edeema Grade 2	-	-	1	-
- Inflammation: subacute Grade 2	-	1	-	-
- Infiltration: mononuclear cell Grade 1	2	-	1	1
SPINAL CORD, CERVIC. No. Examined NAD	3	3	3	3
SPINAL CORD, LUMBAR No. Examined NAD	3	3	3	3
SPLLEEN No. Examined NAD	3	3	3	3
- Hyperplasia: lymphoid Grade 2	-	2	1	1
Grade 3	-	2	1	1
- Amyloid Grade 1	1	-	1	2
TESTES No. Examined NAD	3	3	3	3
- Inflammation: subacute Grade 2	-	1	-	-
Grade 2	-	1	-	-
THYMUS No. Examined NAD	3	3	3	3
- Depletion: lymphoid Grade 1	-	1	1	-
Grade 3	-	-	1	-
THYROID GLAND No. Examined NAD	3	3	3	3
- Cyst Grade 2	2	1	2	-
Grade 2	2	1	2	-
TONGUE No. Examined NAD	3	3	3	3
- Erosion Grade 1	-	1	-	-
- Inflammation: subacute Grade 1	-	2	1	1
Grade 2	-	-	1	1
TRACHEA No. Examined NAD	3	3	3	3
URINARY BLADDER No. Examined NAD	3	3	3	3
- Inflammation: subacute Grade 2	-	2	-	-
Grade 3	-	1	-	-
- Infiltration: mononuclear cell Grade 1	3	-	3	2
Grade 2	3	-	2	2
Grade 2	-	-	1	-

NAD = Nothing abnormal discovered
 Group 1, Control, females: (0 mg/kg),
 Group 2, Saxagliptin (BMS-477119), females: (10 mg/kg)
 Group 3, Vildagliptin (BMS-472111), females: (20 mg/kg)
 Group 4, Sitagliptin (BMS-730173), females: (40 mg/kg)
 One-Sided Exact Fisher Test: * p<0.05; ** p<0.01; Control= 1.

Detailed histopath findings in female cynomolgus monkeys

NUMBER OF ANIMALS WITH MICROSCOPIC FINDINGS BY ORGAN/GROUP/SEX Necropsy Status: End-of-Dose Necropsy (KO), Incl. Deaths Unscheduled and End-of-Dose Necropsies					
Sex	Females				
Dose Group No. Animals per Dose Group	1	2	3	4	
ADRENAL GLANDS No. Examined NAD	3	3	3	3	3
AORTA No. Examined NAD	3	3	3	3	3
BONE MARROW, RIB No. Examined NAD	3	2	3	3	3
BONE MARROW, STERNUM No. Examined NAD	3	3	3	3	3
- Hyperplasia: lymphoid Grade 1 Grade 2 Grade 3	-	3*	-	1	1
BONE, RIB No. Examined NAD	3	3	3	3	3
BONE, STERNUM No. Examined NAD	3	3	3	3	3
BRAIN No. Examined NAD	3	3	3	3	3
- Infiltration: mononuclear cell Grade 1 Grade 2	-	3*	1	1	1
- Mineralization Grade 1	-	-	1	2	1
BRAIN No. Examined	3	3	3	3	3
- Mineralization cont'd. Grade 3	-	-	1	2	1
CECUM No. Examined NAD	3	3	3	3	3
- Infiltration: mononuclear cell Grade 1	-	2	-	-	-
CERVIX No. Examined NAD	3	3	3	3	3
- Infiltration: mononuclear cell Grade 1	3	-*	-*	-*	-*
- Inflammation: subacute Grade 1 Grade 2	-	3*	-	-	-
COLON No. Examined NAD	3	3	3	3	3
- Parasite	-	-	-	1	-
- Infiltration: mononuclear cell Grade 1	-	1	-	-	-
DIAPHRAGM No. Examined NAD	3	3	3	3	3
- Inflammation: vascular: non- necrotizing Grade 1 Grade 2	-	3*	-	-	-
DIGIT No. Examined	-	3	1	-	-
- Vasculer Wall Disruption Grade 3	-	-	1	-	-
- Hemorrhage Grade 3	-	-	1	-	-
- Vasculer Hypertrophy with Inflammatory Cell Infiltration Grade 2 Grade 3	-	3	-	-	-
- Inflammation: subacute Grade 3	-	-	1	-	-
- Inflammation: chronic Grade 2 Grade 3	-	3	-	-	-
- Ulcer Grade 3 Grade 4	-	1	1	-	-

NUMBER OF ANIMALS WITH MICROSCOPIC FINDINGS BY ORGAN/GROUP/SEX Necropsy Status: End-of-Dose Necropsy (KO), Incl. Deaths Unscheduled and End-of-Dose Necropsies					
Sex	Females				
Dose Group No. Animals per Dose Group	1	2	3	4	
- Scab Grade 3	-	1	-	-	-
DUODENUM No. Examined NAD	3	3	3	3	3
ESOPHAGUS No. Examined NAD	3	3	3	3	3
- Infiltration: mononuclear cell Grade 1	-	1	-	-	-
EYES No. Examined NAD	3	3	3	3	3
GALLBLADDER No. Examined NAD	3	3	3	3	3
- Infiltration: mononuclear cell Grade 1	1	2	-	-	-
HEART No. Examined NAD	3	3	3	3	3
- Infiltration: mononuclear cell Grade 1	1	2	1	1	1
ILEUM No. Examined NAD	3	3	3	3	3
JEJUNUM No. Examined NAD	3	3	3	3	3
- Vacuolation Grade 3	-	-	1	-	-
- Infiltration: mononuclear cell Grade 1	-	1	-	-	-
KIDNEYS No. Examined NAD	3	3	3	3	3
- Glomerulopathy Grade 3	-	1	-	-	-
- Cast: proteinaceous Grade 1	-	-	1	-	-
- Giant cell: multinucleated Grade 1	-	-	1	-	-
- Deposition Grade 2	-	-	1	-	-
- Mineralization Grade 1	-	1	-	-	1
- Infiltration: mononuclear cell Grade 1	2	2	-	1	1
- Inflammation: subacute Grade 2	-	1	-	-	-
LACRIMAL GLANDS No. Examined NAD	3	3	3	3	3
LIVER No. Examined NAD	3	3	3	3	3
- Infiltration: mononuclear cell Grade 1	2	2	3	-	-
LUNG No. Examined NAD	3	3	3	3	3
- Inflammation: subacute Grade 1	2	2	1	1	1
- Inflammation: chronic Grade 1	-	-	-	1	1
M. QUADRICEPS FEMORIS No. Examined NAD	3	3	3	3	3
- Inflammation: vascular: non- necrotizing Grade 2	-	3*	-	-	-

NAD = Nothing abnormal discovered
 Group 1, Control, females: (0 mg/kg),
 Group 2, Saxagliptin (BMS-477118), females: (10 mg/kg)
 Group 3, Vildagliptin (BMS-471211), females: (20 mg/kg)
 Group 4, Sitagliptin (BMS-730173), females: (40 mg/kg)
 One-Sided Exact Fisher Test: *) p<0.05; **) p<0.01; Control= 1.

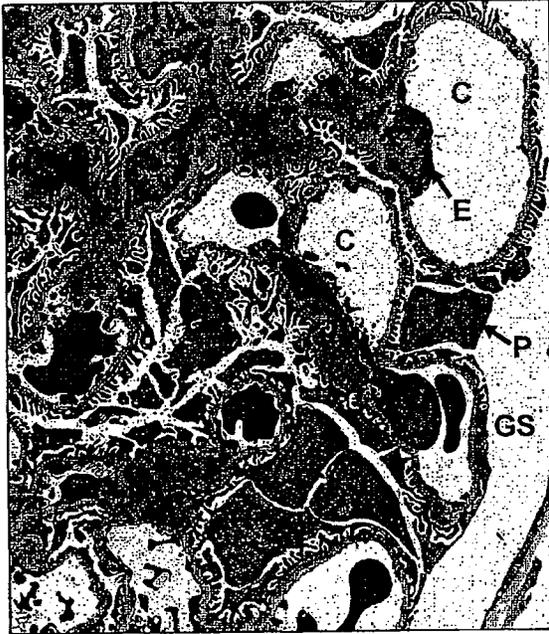
NUMBER OF ANIMALS WITH MICROSCOPIC FINDINGS BY ORGAN/GROUP/SEX Necropsy Status: End-of-Dose Necropsy (NO), Incl. Deaths Unscheduled and End-of-Dose Necropsies				
Sex	Females			
Dose Group No. Animals per Dose Group	1	2	3	4
MAMMARY GLAND No. Examined	3	3	3	3
NAD	2	1	3	-
- Infiltration: mononuclear cell Grade 1	-	1	-	-
- Inflammation: acute Grade 1	1	-	-	-
- Inflammation: subacute Grade 1	-	1	-	2
Grade 2	-	1	-	-
- Fibrosis/fibrosis Grade 1	-	-	-	1
MANDIB. LYMPH NODE No. Examined	3	3	3	3
NAD	3	3	3	3
MANDIB. SALIVARY GLAND No. Examined	3	3	3	3
NAD	-	1	3	1
- Inflammation: subacute Grade 1	-	2	-	-
Grade 2	-	1	-	-
- Mineralization Grade 1	1	-	-	-
Grade 2	1	-	-	-
- Infiltration: mononuclear cell Grade 1	3	-	-	2
Grade 2	2	-	-	2
Grade 3	1	-	-	-
MESENT. LYMPH NODE No. Examined	3	3	3	3
NAD	3	3	2	3
- Histiocytosis: sinus Grade 3	-	-	1	-
NASAL CAVITY No. Examined	-	2	-	-
- Ulcer: respiratory epithelium Grade 3	-	2	-	-
Grade 4	-	1	-	-
- Inflammation: chronic-active Grade 3	-	2	-	-
Grade 4	-	1	-	-
NOSE No. Examined	-	2	-	-
NAD	-	2	-	-
OVARIES No. Examined	3	3	3	3
NAD	3	3	2	3
- Mineralization Grade 2	-	-	1	-
PANCREAS No. Examined	3	3	3	3
NAD	3	3	3	3
PARATHYROID GLANDS No. Examined	3	2	3	3
NAD	3	2	3	3
PENILEM No. Examined	-	2	1	-
- Vascular Wall Disruption Grade 3	-	-	1	-
- Hemorrhage Grade 2	-	2	1	-
Grade 3	-	1	1	-
- Vascular Hypertrophy with Inflammatory Cell Infiltration Grade 3	-	2	-	-
Grade 4	-	1	-	-
- Inflammation: subacute Grade 2	-	2	-	-
Grade 3	-	1	-	-
Grade 4	-	1	-	-
- Ulcer Grade 5	-	1	-	-
PITUITARY GLAND No. Examined	3	3	3	3
NAD	3	2	3	3
- Infiltration: mononuclear cell Grade 1	-	1	-	-
SCIATIC NERVE No. Examined	3	3	3	3
NAD	3	2	3	3
- Inflammation: subacute Grade 2	-	1	-	-

NAD - Nothing abnormal discovered
 Group 1, Control, females: (0 mg/kg)
 Group 2, Saxagliptin (NMS-477119), females: (10 mg/kg)
 Group 3, Vildagliptin (NMS-471211), females: (20 mg/kg)
 Group 4, Saxagliptin (NMS-730173), females: (40 mg/kg)
 One-Sided Exact Fisher Test: * p<0.05; ** p<0.01; Control= 1.

NUMBER OF ANIMALS WITH MICROSCOPIC FINDINGS BY ORGAN/GROUP/SEX Necropsy Status: End-of-Dose Necropsy (NO), Incl. Deaths Unscheduled and End-of-Dose Necropsies				
Sex	Females			
Dose Group No. Animals per Dose Group	1	2	3	4
SKIN/SUBCUTIS No. Examined	3	3	3	3
NAD	2	1	2	3
- Inflammation: subacute Grade 3	-	1	-	-
- Ulcer Grade 5	-	1	-	-
- Infiltration: mononuclear cell Grade 1	1	1	1	-
SPINAL CORD, CERVIC. No. Examined	3	3	3	3
NAD	3	3	3	3
SPINAL CORD, LUMBAR No. Examined	3	3	3	3
NAD	3	2	3	3
- Infiltration Grade 1	-	1	-	-
SPLEEN No. Examined	3	3	3	3
NAD	2	-	3	-
- Hyperplasia: lymphoid Grade 1	-	3*	-	2
Grade 2	-	1	-	-
Grade 3	-	1	-	-
- Amyloid Grade 1	1	1	-	3
Grade 2	1	-	-	3
Grade 3	-	1	-	-
STOMACH No. Examined	3	3	3	3
NAD	2	1	-	2
- Inflammation: subacute Grade 2	1	-	3	1
Grade 3	1	-	2	-
- Infiltration: mononuclear cell Grade 2	-	2	-	-
TAIL No. Examined	3	3	3	3
NAD	3	-	2	3
- Infiltration: mononuclear cell Grade 1	-	2	-	-
- Necrosis: coagulative Grade 5	-	1	1	-
- Inflammation: chronic Grade 3	-	1	1	-
Grade 4	-	-	1	-
- Hemorrhage Grade 2	-	-	1	-
- Scab Grade 4	-	1	-	-
- Vascular Hypertrophy with Inflammatory Cell Infiltration Grade 2	-	1	-	-
THYRUS No. Examined	3	3	3	3
NAD	3	2	3	3
- Depletion: lymphoid Grade 3	-	1	-	-
THYROID GLAND No. Examined	3	3	3	3
NAD	3	2	2	1
- Cyst Grade 2	-	1	1	2
Grade 3	-	1	-	2
Grade 4	-	-	1	-
TONGUE No. Examined	3	3	3	3
NAD	2	2	3	3
- Degeneration: skeletal muscle cell Grade 1	-	1	-	-
- Inflammation: subacute Grade 1	1	-	-	-
TONGUE No. Examined	3	3	3	3
NAD	3	3	3	3
URINARY BLADDER No. Examined	3	3	3	3
NAD	1	-	1	1
- Infiltration: mononuclear cell Grade 1	2	3	2	2
Grade 2	1	2	2	1
Grade 3	1	1	-	1
UTERUS No. Examined	3	3	3	3
NAD	2	1	3	3
- Inflammation: subacute Grade 2	-	1	-	-
- Infiltration: mononuclear cell Grade 1	1	1	-	-
VAGINA No. Examined	3	3	3	3
- Inflammation: subacute Grade 1	2	3	3	-
Grade 2	-	-	2	-
Grade 3	2	-	1	-
Grade 4	-	3	-	-
- Infiltration: mononuclear cell Grade 1	1	-	-	3
Grade 2	1	-	-	3

Electron Microscopy:

- A control male (#1103) and saxagliptin treated male renal tissue was evaluated by EM due to the presence of fluid in abdominal and thoracic cavity and potential glomerulopathy in the treated male (#2103).
- Control male was unremarkable
- Renal glomerular ultrastructural lesions in the treated male included fusion and effacement of many podocyte foot processes, accumulation of numerous cytoplasmic granules (morphologically typical of heterophagosomes and phagolysosomes) within many podocytes, and accumulation of many mononuclear leukocytes within glomerular capillaries.
- Phagolysosomes contained electron dense amorphous material and were generally single membrane-bound. One mononuclear leukocyte had extended a pseudopod through an endothelial pore to contact and partially envelop a portion of the basement membrane.



Glomerulus, kidney, monkey 1103 (control).

Podocyte foot processes are generally uniform and well developed. Only erythrocytes are evident within glomerular capillary lumens.

C = Capillary lumens; E = Endothelial cell;
P = Podocyte; GS = Glomerular space.



Glomerulus, kidney, monkey 2103 (given 10 mg/kg/day BMS-477118).

Ultrastructural lesions include widespread effacement and fusion of podocyte foot processes (curved black arrow), and the accumulation of heterophagosomes and phagolysosomes within the cytoplasm of a podocyte (white arrow). A mononuclear leukocyte is evident within a glomerular capillary lumen (straight black arrow).



Glomerulus, kidney, monkey 2103 (given 10 mg/kg/day BMS-477118).

Podocytes contain numerous phagosomes and phagolysosomes within their cytoplasm (straight black arrows), and portions of 3 mononuclear leukocytes are apparent within glomerular capillary lumens (at the top left corner, and top and right edges of electron micrograph).

Toxicokinetics and pharmacodynamics:

- There was no gender effect on TK parameters of any of the compounds
- Repeated administration of saxagliptin resulted in 2.1 to 2.6 fold increase in AUC
- There was no apparent accumulation with either vildagliptin or sitagliptin.

DPP-4 Inhibitor	Dose (mg/kg/day)	Study Day	C _{max} (ng/mL)		AUC(0-T) ^a [ng·h/mL]	
			Males	Females	Males	Females
Saxagliptin (BMS-477118)	10	1	1306	1229	2500	1948
	10	15	1173	1285	2093	1985
	10	42	1391	1672	4448	5254
Vildagliptin (BMS-471211)	40	1	5876	8822	16874	18951
	20	15	3981	4224	8959	7870
	20	42	3578	4309	8837	8153
Sitagliptin (BMS-730173)	40	1	7663	9523	34278	33790
	40	15	9806	8884	36614	32110
	40	42	11426	10219	45250	41649

^a Calculated from time zero to the time of last measurable concentration, ranging between 8 and 24 h.

Three-Month Oral Toxicity Study in Cynomolgus Monkeys administered Saxagliptin**Key study findings:**

- There were no drug-related deaths at any dose level
- There were no notable findings at 0.03 and 0.3 mg/kg
- Macroscopic and microscopic findings were noted at 3 mg/kg in 1/4 males and 4/4 females during the treatment phase.
- Macroscopic findings included rough skin on tail and scabs on foot correlated with vascular hypertrophy (smooth muscle and endothelial cells) of microvasculature and small arteries and associated inflammatory-cell infiltration (intramural and perivascular mononuclear cells) with minimal to mild epithelial hyperplasia (secondary reparative change).
- Additional findings included minimal chronic inflammation in the pancreas in 1/4 males
- Increased incidence and severity of slight mononuclear-cell infiltration (perivascular and/or periglandular) in the mammary gland (3 of 4 females) and minimal non-necrotizing vascular inflammation in skeletal muscles in 3/4 females
- Minimal lymphoid hyperplasia (1/4 M, 2/4 F) in the spleen in 1/4 males and 1/4 females, thymus in 1 of 4 females, and/or in the bone marrow in 2 of 4 females.
- Saxagliptin related skin changes healed in 2/2 monkeys during the recovery, Day 68. There were no drug-related histopath findings at the end of the recovery
- Based on adverse skin findings at 3 mg/kg, the 0.3 mg/kg saxagliptin was selected as NOAEL in monkeys. The exposure multiple at NOAEL was close to unity in females and 2.5 fold in males. Since skin lesions were seen at 3 mg/kg at exposure multiples of 20 to 27x the clinical dose, the risk to humans is relatively reduced.

Study no.: DN06061

Volume #, and page #: eNDA

Conducting laboratory and location: BMS, One Squibb Drive, New Brunswick, NJ

Date of study initiation: Oct 24, 2006

GLP compliance: yes

QA report: yes (x) no ()

Drug, lot #, and % purity: Batch 4K85994 with free base purity of 93.5%

Methods

Prior studies identified relatively mild and self-resolving lesions at 3mg/kg in cynomolgus monkeys. This study was conducted to determine the NOAEL in monkeys; therefore, saxagliptin doses of 0.03, 0.3 and 3 mg/kg were administered to cynomolgus monkeys for 3 months with a 4-week recovery phase. Concentrations of saxagliptin in solution were measured at several time points and were within the acceptable 10% range. Saxagliptin was stable within 3% of the solution concentrations. Analysis of the vehicle found no saxagliptin in the control samples.

Doses: 0.03, 0.3 and 3 mg/kg, (free base used in the study)

Species/strain: cynomolgus monkeys from

Number/sex/group or time point (main study): 4/sex/dose +3/sex/dose for recovery

Route, formulation, volume, and infusion rate: oral

Satellite groups used for toxicokinetics or recovery: No

Age: 25 to 32 months old

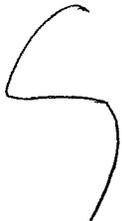
Weight: 2.6 to 3.5 kg for males and 2.4 to 3.1 kg for females

b(4)

Sampling times: PK data for saxagliptin and active metabolite was collected on Day 1, 36 and 85 at 0.5, 1, 2, 4, 8 and 24 hrs post dose. Prior to freezing, an aliquot of plasma was taken for DPP4 inhibition evaluation. Plasma samples were analyzed at

b(4)

Unique study design or methodology:



b(4)

Observations and times:

- Mortality: daily
- Clinical signs: twice daily
- Body weights: weekly
- Food consumption: daily
- Ophthalmoscopy: before and at the end of treatment phase (Day 78 to 80)
- ECG: before and at the end of treatment phase
- Hematology: before and at Days 8, 22, 50, 78 and 99 from femoral or cephalic vein.
- Clinical chemistry: before and at Days 8, 22, 50, 78 and 99 from femoral or cephalic vein.
- Urinalysis: before and at Days 8, 22, 50, 78 and 99
- Gross pathology: at the end of each phase (treatment and recovery).
- Organ weights: standard list with greater emphasis on the skin lesions to determine NOAEL.
- Histopathology: Adequate Battery: yes (x)
Peer review: yes (x)

Results

Mortality: There was no drug-related mortality in the study; however, one control female was removed from the study due to accidental injury.

Clinical signs:

- Drug-related skin lesions were seen primarily on the feet and or tail of 2 HD males (4103 and 4105) and 2 HD females (4204 and 4205) as early as Day 50 and lasting through the treatment phase. It should be noted that skin lesions in left foot of 1 HD female healed by Day 88.
- Skin lesions were characterized by scabs and healed during the recovery phase. There were no open sores compared to lesions seen in the earlier monkey study when open sores were seen at 10 mg/kg. Please see table below for more detailed list.

Skin lesions/scabs observed in individual monkeys				
Saxagliptin dose (mg/kg)	Monkey ID	Observation	Drug related	Explanation
0.03	2106	Multifocal scabs on nose	No	Transient; no histopathologic correlate
0.03	2203	Lesions in middle of tail; Multifocal scabs on middle and tip of tail	No	Lesions and scabs considered secondary to accidental injury
0.3	3102	Lesion on left foot; Multifocal scabs on tail	No	Foot lesion considered secondary to accidental injury; tail scabs indistinguishable from those seen in vehicle controls
3	4103	Multifocal scabs on tail and both feet	Yes	Persistent scabs present on both tail and feet
3	4105	Multifocal scabs on tail	Yes	Persistent scabs; corresponding histopathologic findings
3	4106	Multifocal scabs on abdomen	No	Transient (<2 weeks); location not consistent with observations at higher doses ²
3	4204	Lesion on distal tail; multifocal scabs on both feet	Yes	Seen on both tail and feet; tail lesions different from anything seen in vehicle controls
3	4205	Multifocal scabs on tail and left foot	Yes	Persistent scabs present on both tail and foot

- Slight increase in body temperature noted in males at 0.3 mg/kg and males and female at 3 mg/kg on Day 79/80 were within the normal body temperature range in cynomolgus monkeys
- Alopecia was seen in some animals including controls.

Body weights: No drug-related change in BW

Food consumption: No drug-related change in food intake

Ophthalmoscopy: No drug-related change

EKG and arterial oxygenation:

- There was no drug-related change in QT intervals relative to pre-treatment values.
- Statistically significant differences in QRS duration in males at 0.03 mg/kg and 3 mg/kg males were within the normal limits.
- There was no drug-related change in heart rate.
- There was no relevant drug-related change in respiratory function or arterial oxygenation saturation.

Hematology: No drug-related change or trend in hematology or coagulation values.

Clinical chemistry: No drug-related change or trend in serum chemistry

Urinalysis: No change

Organ weights: No notable drug-related change in organ weights. The significance of slightly higher kidney weight in the HD females is uncertain. It seems that all the treated females had slightly heavier ($p > 0.05$) than controls. The liver in the treated males were slightly smaller than controls which appeared to be due to slightly lower BW.

Gross pathology:

- There were no notable macroscopic findings in the monkeys treated with 0.03 and 0.3 mg/kg
- Macroscopic findings at 3 mg/kg were limited to skin on tail and or foot of 1 male and 1 female. These changes were characterized by multifocal rough areas on the skin in male and 4 small ulcers on the tip of the tail and scabs on the food of the female. Histologically, they were marked by vascular hypertrophy (smooth muscle and endothelial cell of micro vessels and arteries) and inflammatory cells infiltration (intramural and perivascular mononuclear cells) and minimal to mild epithelial hyperplasia. Skin lesions caused by accidental trauma such as minor ulcers, erosions and laceration were distinguished from drug-related changes by examining the severity and potential cause of a lesion and microscopic changes such as vascular hypertrophy of the microvasculature and small arterioles
- There were no such findings during the recovery phase

Histopathology: Adequate Battery: yes (x) with peer review

- Drug-related histopath findings were seen at 3 mg/kg in 1/4 male and 4/4 females
- Skin (tail of 1 male, tail and foot of 1 female), vascular hypertrophy with inflammatory-cell infiltration of the microvasculature and small caliber arteries and minimal to mild epithelial hyperplasia (a secondary reparative change) correlating with the multifocal areas of a roughened surface on the tail of the male and 4 small ulcers of the tail tip and 2 scabs of the foot of the female
- Skeletal muscle (quadriceps femoris), minimal non-necrotizing vascular inflammation in 3 females. Muscle fibers were not affected.
- Pancreas minimal chronic inflammation (1 male)
- Mammary gland, increased incidence and severity of slight mononuclear-cell infiltration (perivascular and/or periglandular) in 3 HD females
- Minimal lymphoid hyperplasia (spleen of 1 M, 1 F, Thymus, 1 F, bone marrow 2 F)
- Inflammatory cells infiltration and inflammation is not uncommon in cynomolgus monkeys thus increased incidence and severity was used to distinguish them from normal inflammatory findings in control and in general in monkeys.
- There were no histopath findings in the recovery monkeys suggesting that saxagliptin induced lesions (when mild) are reversible.
- Slides were also evaluated by a peer-reviewer, Karyn Colman. Her analysis was in basic agreement with the original pathology report. Slides quality and tissue accountability was acceptable according to her report.

End-of-Dose Monkeys

Incidence of Saxagliptin-Related Microscopic Findings

Dose (mg/kg/day):	0	0.03	0.3	3
No. of (M/F):	4/3	4/4	4/4	4/4
Sex:	M/F	M/F	M/F	M/F
<u>Tail Skin:</u>				
Vascular hypertrophy with inflammatory cell infiltration				
Minimal	-/-	-/-	-/-	1/0
Slight	-/-	-/-	-/-	0/1
<u>Foot Skin:</u>				
Vascular hypertrophy with inflammatory cell infiltration				
Minimal	-/-	-/-	-/-	0/1
<u>Skeletal Muscle:</u>				
Vascular inflammation, non-necrotizing				
Minimal	-/-	-/-	-/-	0/3
<u>Mammary Gland:</u>				
Infiltration: mononuclear cell				
Slight	-/-	-/-	-/-	0/3
<u>Pancreas:</u>				
Inflammation: chronic				
Minimal	-/-	-/-	-/-	1/0
<u>Spleen/Bone Marrow/Thymus:</u>				
Hyperplasia: lymphoid				
Minimal	-/-	-/-	-/-	1/2

A dash (-) indicates absence of finding in group

Toxicokinetics and pharmacodynamics:

- Saxagliptin and BMS-510849 exposures (AUC) generally increased in a dose-proportional manner
- Saxagliptin and BMS-510849 exposures in male and female monkeys were generally similar except for slightly higher exposure in females at 0.3 mg/kg
- Repeated administration of saxagliptin resulted in 2.4 to 2.8x higher saxagliptin exposure on Day 85 than Day 1, suggesting a potential for saxagliptin accumulation with increasing dose in monkeys. Higher exposure to parent drug would suggest reduced metabolism or enhanced absorption. Since the most prominent metabolite was not affected, it is likely that metabolic pathway for smaller inactive metabolites were inhibited.
- There was no evidence of accumulation for active metabolite, BMS-510849. The mean AUC values were combined for safety margin calculations.

- 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%).

Toxicokinetics Summary

Dose (mg/kg/day)	Study Day	Saxagliptin			
		Cmax (ng/mL)		AUC(0-T) ^a [ng•h/mL]	
		Males	Females	Males	Females
0.03	1	4.1	4.4	9.1	8.3
	36	4.1	3.3	8.7	8.9
	85	3.4	3.6	8.6	8.9
0.3	1	30.0	32.1	70.6	78.7
	36	33.8	30.3	88.3	79.1
	85	58.0	29.1	200.2	79.2
3	1	316.3	359.7	667.4	852.2
	36	474.1	533.0	1137.2	1761.6
	85	537.3	657.0	1592.4	2196.2
BMS-510849					
		Cmax (ng/mL)		AUC(0-T) ^a [ng•h/mL]	
		Males	Females	Males	Females
0.03	1	9.4	11.6	51.1	53.1
	36	10.6	11.5	53.3	58.8
	85	8.5	12.0	53.9	61.6
0.3	1	117.1	136.9	412.6	480.3
	36	117.8	149.6	386.4	502.6
	85	130.3	140.9	479.7	504.0
3	1	1258.9	1381.1	3901.4	4794.9
	36	1240.7	1318.6	3669.4	4805.5
	85	1474.4	1361.6	4647.4	4825.2

^a Calculated from time zero to the time of last measurable concentration, ranging between 8 and 24 hours.

Plasma DPP4 inhibition:

Saxagliptin doses 0.3 and 3 mg/kg inhibited DPP4 levels near maximal plasma DPP4 activity inhibition (Emax of 57 to 70%). Saxagliptin dose of 0.03 mg/kg also inhibited plasma DPP4 activity but to a slightly less extent (Emax 49 to 54%). Overall, the analysis suggests that DPP4 in plasma was being inhibited at NOAEL dose of 0.3 mg/kg which was near clinical exposure (5 mg dose). DPP4 inhibition also occurred at doses as low as 0.03 mg/kg. Earlier studies in monkeys have shown a maximal DPP4 inhibition between 70 and 80%. There were no apparent gender differences regarding DPP4 inhibition and Day 1 values were similar to Day 85 suggesting that there is no decrement in drug pharmacodynamic effect. 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.

Pharmacodynamic Summary

Dose (mg/kg/day)	Study Day	Emax (% Inhibited) ^{a,b}		Emin (% Inhibited) ^{a,c}	
		Males	Females	Males	Females
0	1	15.3	26.2	1.7	15.4
	36	14.9	23.0	4.3	13.3
	85	16.3	16.0	5.2	13.6
0.03	1	53.8	53.3	9.7	12.3
	36	52.8	52.0	12.1	11.7
	85	49.3	52.2	12.8	28.0
0.3	1	68.6	60.0	45.1	22.0
	36	69.4	58.1	47.5	17.4
	85	69.8	56.6	51.6	19.5
3	1	64.2	61.0	31.0	44.5
	36	62.5	61.1	31.3	47.9
	85	64.4	61.3	29.9	48.4

^aMaximum effect (Emax) and minimum effect (Emin) are arithmetic means.

^bEmax was generally achieved 1 to 2 hours after dosing.

^cEmin values represent DPP inhibition 24 hours after dosing.

Toxicology studies with active metabolite, BMS-510849

Rats were given 300 and 600 mg/kg of active metabolite for 5 days by subcutaneous route and plasma levels of BMS-510849 were measured on Day 5. There was a dose-proportional increase in BMS-510849 levels in plasma with no notable gender differences. BMS-510849 doses up to 600 mg/kg for 5 days produced no notable clinical signs or gross-pathologic changes at the injection sites.

Plasma Concentrations of BMS-510849

Dose (mg/kg)	300		600	
	M	F	M	F
Concentration (µg/ml)	221 ± 35.8	268 ± 13.6	516 ± 28.0	590 ± 37.0

To determine the potential central nervous system (CNS) toxicity of BMS-510849 (an active metabolite) at doses producing systemic exposures similar to those achieved at doses of the parent compound (BMS-477118) in which brain lesions were observed after 1 month of dosing, solutions of BMS-510849 (a metabolite of BMS-477118) in water were administered subcutaneously to rats once daily for 1 month at doses of 10, 50, or 200 mg/kg. Blood levels of cyanide and serum and urine thiocyanate levels were determined. The doses used represent 3X (M-F), 20-35X (M-F) and 115-119X (M-F) MRHD based on AUC of metabolite only. Body weight gain decreased dose-dependently in females (18-52%) with no corresponding decrease in food consumption. In males, body weight gain decrement (36%) occurred only at 10 mg/kg and was not associated with decreased food consumption as well. Urine volume decreased by 23-28% in males at doses ≥ 50 mg/kg. Blood cyanide and serum thiocyanate levels increased with dose, were higher in males than females and higher levels were noted at week 1 relative to week 4. Urine thiocyanate levels increased by 26-154X in males at doses ≥ 50 mg/kg. Macroscopic lesions were mainly confined to the injection sites (red discoloration) which correlated microscopically to hemorrhage and inflammation. Granuloma and scabs were also

observed microscopically. These lesions were attributed to physical trauma of the repeated subcutaneous injections and not due to drug. An increased incidence of progressive nephropathy and alveolar histiocytosis was observed at 10 mg/kg relative to control. Males at 10 mg/kg showed an increased incidence of mononuclear cell infiltration of the prostate gland relative to control. The target organs of toxicity include kidney (nephropathy), lung (histiocytosis), liver (coagulative necrosis), prostate gland (mononuclear cell infiltration), spleen (lymphoid hyperplasia) and the stomach (inflammation, hemorrhage). NOAEL is < 10 mg/kg (< 3X, metabolite only) for females based on the dose-related body weight gain decrements noted at doses \geq 10 mg/kg. NOAEL for males is 50 mg/kg (20X, metabolite only) based on the target organ pathology at 200 mg/kg. Systemic exposures (AUC) to BMS-510849 attained in males in this study (165 to 283 $\mu\text{g}\cdot\text{h}/\text{ml}$) were similar to those in rats given 1800 mg/kg (155 $\mu\text{g}\cdot\text{h}/\text{ml}$) of saxagliptin (BMS-477118). In a previous 1-month oral investigative escalating-dose study in male rats, the parent compound, BMS-477118, was associated with brain lesions in the corpus callosum, thalamus, piriform cortex, and caudate putamen at a dose of 1800 mg/kg. The results of this study indicate that BMS-510849 is not the cause of the brain lesions observed in the 1-month study with saxagliptin.

Chronic Investigational Central Nervous System Toxicity Study in Rats

This study was originally designed to assess the potential carcinogenicity of BMS-477118 (saxagliptin) in rats. Male and female SD rats (60 rats/sex/group) were assigned to 6 groups. Two groups received water as control, and 4 groups received BMS-477118 in a vehicle of acidified water at doses of 25, 75, 150, or 300 mg/kg. All rats were dosed once daily by oral gavage at a dose volume of 5 ml/kg/day.

The study design was revised to characterize microscopic findings in the brain as initially detected in males given 300 mg/kg that died or were sacrificed in a moribund condition. At Week 54, an interim necropsy was conducted for all surviving rats in one control and the 300 mg/kg groups and 20 rats/sex/group in the 25, 75, and 150 mg/kg groups. Remaining rats continued on study. Because only 15 males given 150 mg/kg remained at Week 77, a terminal sacrifice was performed at Week 81/82 to ensure adequate numbers of rats for evaluation.

Key study findings:

- Survival of males given \leq 150 mg/kg and females given \leq 300 mg/kg was similar to that of controls through Week 54. Males given 300 mg/kg had reduced survival (73%) relative to the average of the 2 control groups (93%) at Week 54. At Week 80/81 (prior to the scheduled terminal sacrifice), survival of males given 150 mg/kg was lower than the remaining male control group [42% (Group 5) vs. 62% (Group 1)]. At Week 80/81, survival of males given \leq 75 mg/kg and of females given \leq 150 mg/kg was similar to that of controls.
- Drug-related microscopic findings were observed only in the brain of male rats at doses \geq 150 mg/kg. No drug-related microscopic findings were noted in any other tissues examined in rats at all doses. 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 BMS-477118 and BMS-510849 was not extensive (brain:plasma concentration ratio ≤ 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 (0.33, 0.48, or 1.2 $\mu\text{g/ml}$) in only 3 males given 150 mg/kg. 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.
 - Animal No C60366 (150 mg/kg group) with whole blood cyanide concentration of 1.2 $\mu\text{g/ml}$, had microscopic findings in the brain which included minimal degeneration of myelin in the corpus callosum with associated myelin loss (identified with LFB stain), intracytoplasmic PAS-positive material in glial cells in the corpus callosum and caudate-putamen, and minimal focal/multifocal gliosis in the caudate-putamen.
 - The brain of the second male (Animal No C60379 – 150 mg/kg group) with a measurable whole blood cyanide concentration (0.33 $\mu\text{g/ml}$) was microscopically unremarkable.
 - The third male (Animal No C60382) that had brain lesions did not have a measurable whole blood cyanide but did have a serum thiocyanate concentration of 8.4 $\mu\text{g/ml}$. This animal had slight degeneration/rarefaction in the corpus callosum, degeneration of myelin in the corpus callosum with associated myelin loss (identified with LFB stain), intracytoplasmic PAS-positive material in glial cells in the corpus callosum and caudate-putamen, and focal/multifocal gliosis in the caudate-putamen.

Study no: DN03101.

Volume # and page #: Vol. 1, pg 005.

Conducting laboratory and location:

Date of study initiation: December 22, 2003.

GLP compliance: Yes.

QA report: yes (X) no ()

Drug, lot #, radiolabel, and % purity: Lot #s 3D65913 (91.6% pure), 3D65912 (90.8-91.4% pure), 4E84589-02 (94.5% pure) and 4J88286 (92.6% pure).

Formulation/vehicle: BMS 477118 in acidified water.

b(4)

Methods (unique aspects):

Dosing:

Species/strain: Rat/SD.

#/sex/group or time point (main study): 60/sex/group.

Satellite groups used for toxicokinetics or recovery: None.

Age: 46-52 days old (M); 47-53 days old (F).

Weight: 172-235 g (M); 131-185 (F).

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

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

Group ^a	No of Rats		Dose Level (mg/kg/day)	Dose Concentration (mg/mL)
	Male	Female		
1 (Control 1) ^b	60 ^{c,d}	60	0	0
2 (Control 2) ^b	60	60	0	0
3 (Low)	60	60	25	5
4 (Mid-Low)	60	60	75	15
5 (Mid-High)	60	60	150	30
6 (High)	60	60	300	60

a All surviving Group 2 and 6 rats and 20 rats/sex/group in Groups 3, 4, and 5 were necropsied in Week 54. With the exception of rats transferred to other studies (noted below), all other surviving rats were necropsied in Weeks 81 or 82.

b Rats in the control groups received reverse osmosis water only.

c Ten (10) Group 1 males (Animal Nos C60093, C60095, C60096, C60097, C60098, C60099, C60100, C60101, C60102, and C60104) were transferred during Week 62 to another study () 6108-501.

d Twenty-one (21) Group 1 males (Animal Nos. C60105, C60106, C60107, C60108, C60109, C60110, C60111, C60113, C60114, C60115, C60116, C60117, C60118, C60119, C60120, C60121, C60122, C60123, C60124, C60125, and C60127) were transferred during Week 69 to () 6108-521.

b(4)

EXPERIMENTAL DESIGN

Observations and times:

Clinical signs: Daily.

Body weights: Recorded prior to dosing, weekly for weeks 1- 14, and at 4-week intervals thereafter.

Food consumption: Recorded weekly for weeks 1-13, and at 4-week intervals thereafter.

Ophthalmoscopy: Not conducted.

EKG: Not conducted.

Hematology: Blood samples for hematology evaluation were collected from fasted animals in Weeks 54 and 79/80.

Clinical chemistry: Blood samples for clinical chemistry evaluation were collected from fasted animals in weeks 54 and 79/80.

Urinalysis: Not conducted.

Gross pathology: List in the histopath section

Organs weighed: None

Histopathology:

Unscheduled Deaths through Week 54: Tissues listed in the histopathology table collected from each rat necropsied before the Week 54 sacrifice was processed for microscopic examination. Histology slides of brain, heart, kidney, liver and lung were prepared and stained for evaluation from 12 males given 300 mg/kg [#C60400, C60402, C60407, C60412, C60423, C60428, C60429, C60431, C60444, C60445, C60447, and C60450 (unscheduled deaths prior to Week 33) 1 and 2 Group 2 control males [#C60162 and C60172 (unscheduled deaths prior to Week 40)].

Week 54 Sacrifice: For all rats in the control (group 2) and HD groups, and the first 10 rats/sex/group in the LD, MD and HMD sacrificed during Week 54, the brain, spinal cord, optic nerve, spleen, thymus, and lymph nodes (mandibular and mesenteric) were collected for microscopic examination.

Unscheduled Deaths Week 54 to Day 540/539 [Week 78/77 (males/females)]: After the Week 54 sacrifice and up to Day 540/539, brain, spinal cord (including cervical, thoracic,

and lumbar regions), spleen, thymus, and mandibular and mesenteric lymph nodes were collected, preserved, and examined from rats that died or were sacrificed in a moribund condition.

Unscheduled Deaths Day 540/539 [Week 78/77 (males/females)] to Week 82/81

Terminal Sacrifice: Between Day 540/539 and the scheduled terminal sacrifice, all tissues listed in the histopathology table were collected, preserved, and examined microscopically from rats that died or were sacrificed in a moribund condition.

Week 82/81 Terminal Sacrifice: For the 6 HMD males with the highest whole blood cyanide concentration or, if cyanide was not measurable, the highest serum thiocyanate concentration, the left cervical and lumbar dorsal root ganglia and the left sciatic and left tibial nerves were processed for microscopic examination. The right dorsal root ganglia, right sciatic and right tibial nerves, and all other preserved tissues (as appropriate) from each rat were embedded in paraffin, sectioned, and stained with hematoxylin and eosin. PAS- and LFB (Luxol Fast Blue)-stained slides (separate slides, 1 stain/slide) of right cervical and lumbar dorsal root ganglia, right sciatic and right tibial nerves, brain, and spinal cord were also prepared. Sciatic and tibial nerves, cervical and lumbar dorsal root ganglia, brain, spinal cord, thyroid with parathyroid (parathyroid was on same section but not examined), spleen, and mandibular and mesenteric lymph nodes were evaluated microscopically.

Toxicokinetics: Blood samples for TK were collected from the first 9 rats/sex/group at 0.5, 1, 2, 4, 8 and 24 hours post-dose during weeks 26, 54 and 81. At Week 54, brain samples were collected for measurement of saxagliptin and active metabolite, BMS-510849 from the same rats used for blood drug analysis. Whole Blood Cyanide and Serum Thiocyanate Analysis: Because in vitro studies (pending BMS report) suggested that the male-specific enzyme, CYP2C11, could metabolize BMS-477118 to its des-cyano form with the concurrent release of cyanide, whole blood cyanide and serum thiocyanate concentrations were measured in this study. Thiocyanate is a metabolic product of cyanide. In Week 78, blood for analysis of whole blood cyanide and serum thiocyanate concentrations was collected from 20 Group 1 control males and 15 Group 5 (150 mg/kg) males. These rats were not fasted prior to sample collection.

Results:

Mortality: Survival of males given ≤ 150 mg/kg and females given ≤ 300 mg/kg was similar to that of controls through Week 54. Males given 300 mg/kg had reduced survival rate (73%) compared with the average of the 2 control groups (93%) at Week 54. At Week 80/81 (prior to the scheduled terminal sacrifice), survival of males given 150 mg/kg was lower than the remaining male control group [42% (Group 5) vs. 62% (Group 1)]. At Week 80/81, survival of males given ≤ 75 mg/kg and of females given ≤ 150 mg/kg was similar to that of controls.

The deaths of 8 rats in various dose groups (Animal Nos. C60172, C60220, C60450, C60531, C60636, C60648, C60674, and C60735) throughout the study were considered accidental deaths associated with the dosing procedure and unrelated to BMS-477118. All 8 of these rats had evidence of gavage-related injury upon macroscopic and/or microscopic examination. One (1) additional male (Animal No C60447) given 300 mg/kg had a fractured skull noted at necropsy. The clinical observations (swollen nose, red oral discharge, hypoactivity, and labored respiration) for this rat were not consistent with other males given 300 mg/kg and the death of this rat was considered not drug-related.

Dose (mg/kg)	0	0	25	75	150	300
Sex	M	M	M	M	M	M
Week 30	60/60 (100%)	60/60 (100%)	59/60 (98%)	60/60 (100%)	57/60 (95%)	51/60 (85%)
Week 54	57/60 (95%)	*54/59 (92%)	27/30 (90%)	30/31 (97%)	27/31 (87%)	*43/59 (73%)
Week 80	18/29 (62%)	*	17/30 (57%)	19/31 (61%)	13/31 (42%)	*

* animals sacrificed at week 54; for the LD, MD and HMD groups, 30 rats/group were sacrificed at Week 54 – hence survival is based on 30 rats.

Dose (mg/kg)	0	0	25	75	150	300
Sex	F	F	F	F	F	F
Week 30	59/60 (98%)	59/60 (98%)	60/60 (100%)	58/59 (98%)	59/59 (100%)	56/60 (93%)
Week 54	57/60 (95%)	*54/59 (92%)	30/31 (97%)	27/29 (93%)	28/30 (93%)	*57/59 (97%)
Week 80	45/60 (75%)	* (0%)	24/31 (77%)	20/28 (71%)	23/30 (77%)	* (0%)

* all animals sacrificed at week 54; for the LD, MD and HMD groups, 30 or 31 rats/group were sacrificed at Week 54 – hence survival is based on 30 rats.

Unscheduled Sacrifices (Through Week 54):

For all groups, 36 male and 24 female rats were found dead or sacrificed in a moribund condition prior to or during Week 54. Of these unscheduled deaths, 17 males and 7 females were from the 300 mg/kg group. The causes of death for most of the rats could not be determined based on the pathology findings alone. The deaths of 2 male rats (# C60412 and C60427) dosed with 300 mg/kg were attributed to drug-related non-neoplastic brain lesions.

Neoplasms were the apparent causes of death for 10 control rats and 1 BMS-477118-dosed rat as follows:

- Thymus neoplasm - One (1) control male (animal # C60146)
- Brain neoplasms - Two (2) control males (# C60103 and C60174) and 1 male given 300 mg/kg (# C60397)
- Mammary neoplasms - Six (6) control females (# C60456, C60471, C60520, C60536, C60537, and C60549)
- Hematopoietic neoplasm - One (1) control female (# C60510)

Unscheduled Sacrifices (Weeks 55 through 81):

45 male and 30 female rats had unscheduled deaths during the period between the interim and terminal sacrifices (Weeks 55 through 81); 16 of the males and 5 of the females were from the 150 mg/kg group. Sponsor stated that because of the limited tissue collection procedure utilized during this interval, the cause of death for most rats could not be determined. However, a brain neoplasm was the apparent cause of death for 1 male (Animal No C60325) dosed with 75 mg/kg, hematopoietic neoplasms were causes of death for 1 control female (Animal No C60469) and 1 female (Animal No C60736) dosed with 150 mg/kg, and an apparent gavage-related death was noted for 1 female (Animal No C60674) dosed with 75 mg/kg.

Clinical signs:

Dose (mg/kg)	0		0		25		75		150		300	
Sex	M	F	M	F	M	F	M	F	M	F	M	F
n	60	60	60	60	60	60	60	60	60	60	60	60
Thin	2	2	3	1	0	0	0	1	2	1	9	6
Hypoactive	0		2		1		0		1		6	
Rough hair coat	1	0	4	1	1	3	1	10	2	23	11	35
Red Genital discharge		6		5		1		3		6		13
Pale body		0		1		1		4		3		8
Yellow hair coat – perineal area		1		0		1		5		17		19

Body weights: (g)

Dose (mg/kg)	0		0		25		75		150		300	
Sex	M	F	M	F	M	F	M	F	M	F	M	F
Week 1	203		203		204		205		204		203	
Week 54	583		584		579		558		541		499(14%↓)	
Week 82	547		*		524		542		479(12%↓)		*	

* Animals were sacrificed at week 54

Dose (mg/kg)	0		0		25		75		150		300	
Sex	F	M	F	M	F	M	F	M	F	M	F	
Week 1	156		157		158		157		157		156	
Week 54	315		311		300		301		290		278(12%↓)	
Week 82	329		*		297(10%↓)		312		281(15%↓)		*	

* Animals were sacrificed at week 54

Food consumption: (g) – No significant decreases in food consumption of treated rats relative to controls.

Dose (mg/kg)	0		0		25		75		150		300	
Sex	M	F	M	F	M	F	M	F	M	F	M	F
Week 1	186		193		183		183		183		180	
Week 53	199		200		195		195		189		181	
Week 80	182		*		171		172		167		*	

* Animals were sacrificed at week 54

Dose (mg/kg)	0		0		25		75		150		300	
Sex	F	M	F	M	F	M	F	M	F	M	F	
Week 1	138		139		135		131		132		134	
Week 53	150		144		142		139		134		138	
Week 80	137		*		137		138		126		*	

* Animals were sacrificed at week 54

Ophthalmoscopy: No data.

Electrocardiography: No data.

Hematology: Hematology results for treated females were unremarkable. Notable results for males are shown in the following table.

Dose (mg/kg)	PLT (10 ³ /UL)		APTT (sec)	
	Week 54	Week 80	Week 54	Week 80
0		1157		16.5
0	1059	*	16.2	*
25	1101	918	16.7	15.9
75	988	869	15.3	16.8
150	896	956	15.8	15.8
300	817	*	14.8	*

* Animals were sacrificed at week 54

Clinical chemistry: Clinical chemistry results for treated females were unremarkable. Notable results for males are shown in the following table.

Dose (mg/kg)	BUN (mg/dl)		Creatinine (mg/dl)		ALK PHOS (IU/L)	
	Week 54	Week 80	Week 54	Week 80	Week 54	Week 80
0		31		1.3		57
0	19	*	0.9	*	64	*
25	27	33	1.1	1.5	72	94
75	28	44	1.2	1.6	77	96
150	18	39	0.8	1.6	90	96
300	57	*	1.6	*	120	*

Urinalysis: No data.

Organ weights: No data.

Gross pathology:

Unscheduled deaths (Through Week 54):

36 males and 24 females from all dose groups were unscheduled deaths (i.e., rats found dead or those sacrificed in a moribund condition) prior to or during Week 54. Of these unscheduled deaths, 17 males and 7 females were from the 300 mg/kg group. The causes of death for most of the rats could not be determined based on the gross pathology findings alone. Therefore no drug-related macroscopic findings were observed in any unscheduled deaths through Week 54.

Interim Sacrifice (Week 54):

No drug-related macroscopic findings were observed in males or females given \leq 300 mg/kg at the interim sacrifice.

Unscheduled Sacrifices (Weeks 55 through 81): No drug-related macroscopic findings were observed in any rats that died or were sacrificed in a moribund condition during this interval.

Terminal Sacrifice (Weeks 81 and 82): No drug-related macroscopic findings were observed in rats examined at the terminal sacrifice.

Histopathology:

Unscheduled deaths (Through Week 54): 36 males and 24 females from all dose groups were unscheduled deaths (i.e., rats found dead or those sacrificed in a moribund condition) prior to or during Week 54. Of these unscheduled deaths, 17 males and 7 females were from the 300 mg/kg group. The causes of death for most of the rats could not be determined based on the pathology findings alone. The deaths of 2 male rats (# C60412 and C60427) dosed with 300 mg/kg, were attributed to drug-related non-neoplastic brain lesions.

Neoplasms were the apparent causes of death for 10 control rats and 1 saxagliptin-dosed rat as follows:

- Thymus neoplasm - One (1) control male (# C60146)
- Brain neoplasms - Two (2) control males (# C60103 and C60174) and 1 male given 300 mg/kg (# C60397)
- Mammary neoplasms - Six (6) control females (# C60456, C60471, C60520, C60536, C60537, and C60549)
- Hematopoietic neoplasm - One (1) control female (# C60510)

Brain Lesions: No drug-related brain findings were seen in males at doses \leq 150 mg/kg that were unscheduled deaths prior to or during Week 54. Drug-related brain lesions were noted microscopically in 11 of 17 males at 300 mg/kg that were unscheduled deaths prior to or during Week 54. The incidence of microscopic brain lesions is presented in the table below.

Incidence and Severity of Selected Microscopic Brain Findings in Males, Unscheduled Deaths through Week 54

Group	1	2	3	4	5	6
Dose (mg/kg)	0	0	25	75	150	300
No. Examined (n)	3	7	4	1	4	17
Corpus Callosum, Degeneration/Rarefaction	0	0	0	0	0	9 3(1) 3(2) 3(3)
Corpus Callosum, Degeneration, Myelin, with associated Myelin loss (LFB stain)	0	0	0	0	0	7 2(1) 1(2) 3(3) 1(4)
Caudate-putamen, Necrosis, Focal/Multifocal	0	0	0	0	0	4 2(2) 2(4)
Piriform/Temporal Cortex, Necrosis, Focal/Multifocal	0	0	0	0	0	3 3(2)
Thalamus, Necrosis, Focal/Multifocal	0	0	0	0	0	1 1(2)
Thrombus/Microthrombus	0	0	0	0	0	1 1(1)

1 = minimal, 2 = slight, 3 = moderate, 4 = marked

Brain lesions in these unscheduled-death males given 300 mg/kg were seen most commonly in the corpus callosum, but lesions in the caudate-putamen, thalamus, and/or piriform/temporal cortex were also seen in 1 or more males. Drug-related findings included degeneration/rarefaction in the corpus callosum and focal or multifocal necrosis in the caudate-putamen, thalamus, and the piriform/temporal cortex. One (1) of the affected males also had a fully organized and fibrosed thrombus closely associated with one of the areas of brain necrosis. An additional brain finding seen in some sections stained with LFB was myelin degeneration and loss in the corpus callosum. Minimal or slight focal or multifocal necrosis of the temporal/piriform cortex was seen in 1 Group 2 (control) male and in 1 male given 25 mg/kg. The minimal necrosis in these 2 males was considered a spontaneous, agonal change.

Interim Sacrifice (Week 54): No drug-related brain findings were seen in males given \leq 75 mg/kg and sacrificed during Week 54. Microscopic lesions observed are indicated in the table below.

**Incidence of Selected Microscopic Brain Findings in Males
Interim Sacrifice at Week 54**

Group	2	3	4	5	6
Dose (mg/kg)	0	25	75	150	300
No. Examined (n)	7	4	1	4	17
Corpus Callosum, Attenuation	0	0	0	0	5 2(3) 3(4)
Corpus Callosum, Degeneration/Rarefaction	0	0	0	0	15 8(1) 5(2) 1(3) 1(4)
Corpus Callosum, PAS Positive Material, Extracellular & Intracytoplasmic, Glial/Gitter Cells	0	0	0	1 1(P)	15 15(P)
Caudate-putamen, Gliosis, Focal/Multifocal	0	0	0	1 1(1)	8 3(1) 4(2) 1(3)
Caudate-putamen, Increased Vascularization, Focal/Multifocal	0	0	0	0	5 3(1) 2(2)
Caudate-putamen, Necrosis, Focal/Multifocal	0	0	0	0	2 1(1) 1(2)
Caudate-putamen, PAS Positive Material, Extracellular & Intracytoplasmic, Glial/Gitter Cells	0	0	0	1 1(P)	20 20(P)
Piriform/Temporal Cortex, Necrosis, Focal/Multifocal	0	0	0	0	2 1(1) 1(3)
Piriform/Temporal Cortex, PAS Positive Material, Extracellular & Intracytoplasmic, Glial/Gitter Cells	0	0	0	0	2 2(P)
Thalamus, Necrosis, Focal/Multifocal	0	0	0	0	1 1(2)
Thalamus, PAS Positive Material, Extracellular & Intracytoplasmic, Glial/Gitter Cells	0	0	0	0	3 3(P)
No. Examined (n)	43	10	10	10	34
Corpus Callosum, Increased GFAP Immunoreactivity	0	0	0	1 1(1)	10 2(2) 3(3) 4(4) 1(5)
Caudate-putamen, Increased GFAP Immunoreactivity	0	0	0	0	11 1(1) 6(2) 4(3)
Thalamus, Increased GFAP Immunoreactivity	0	0	0	0	6 6(2)

1 = minimal, 2 = slight, 3 = moderate, 4 = marked, 5 - severe

Overall, drug-related brain lesions were seen in 2 of 29 males given 150 mg/kg and in 22 of 43 males given 300 mg/kg. Lesions in males given 150 mg/kg were restricted to the corpus callosum or caudate-putamen, while lesions in males given 300 mg/kg were seen most commonly in the corpus callosum but also in the caudate-putamen, thalamus, and/or piriform/temporal cortex.

In the brain of the 2 affected males given 150 mg/kg, 1 had corpus callosum findings (increased GFAP immunoreactivity and intracytoplasmic PAS-positive material in glial/gitter cells), and 1 male had caudate-putamen findings (focal/multifocal gliosis and intracytoplasmic PAS-positive material in glial/gitter cells).

Drug-related findings in males given 300 mg/kg 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/gitter cells in the corpus callosum, caudate-putamen, piriform/temporal cortex, and thalamus; and increased GFAP immunoreactivity in the corpus callosum, caudate-putamen, and thalamus. Drug-related microscopic findings were not seen in the spinal cord of any saxagliptin-treated male or female.

In the spleen, an increase in the incidence and mean severity of increased extramedullary hematopoiesis was seen in females given 300 mg/kg. This was not considered toxicologically important or drug-related because correlating clinical pathology findings were not observed, and this finding was not present in males in any dose group.

Unscheduled Sacrifices (Weeks 55 through 81): Drug-related brain lesions were noted microscopically in 5 of 16 males given 150 mg/kg that died or were sacrificed in a moribund condition during the period between the interim and terminal sacrifices. No drug-related brain findings were seen in females sacrificed at an unscheduled interval or in males in the lower dose groups. Brain lesions in males given 150 mg/kg were seen most commonly in the caudate-putamen, but lesions in the corpus callosum, frontal cortex, and/or cerebellum were also seen in 1 or more males (see table below).

**Incidence and Severity of Selected Microscopic Brain Findings in Males
Unscheduled Deaths - (Weeks 55 through 81)**

Group	1	3	4	5
Dose (mg/kg)	0	0	25	75
No. Examined (n)	8	10	11	16
Frontal Cortex, Degeneration/Rarefaction, Focal	0	0	0	1 1(2)
Frontal Cortex, PAS Positive Material, Extracellular & Intracytoplasmic, Glial/Gitter Cells	0	0	0	1 1(P)
Corpus Callosum, Attenuation	0	0	0	2 1(1) 1(2)
Corpus Callosum, Degeneration/Rarefaction	0	0	0	2 2(1)
Corpus Callosum, Degeneration, Myelin, with associated Myelin loss (LFB stain)	0	0	0	2 1(1) 1(2)
Corpus Callosum, PAS Positive Material, Extracellular & Intracytoplasmic, Glial/Gitter Cells	0	0	0	2 2(P)
Caudate-putamen, Gliosis, Focal/Multifocal	0	0	0	1 1(1)
Caudate-putamen, Increased Vascularization, Focal/Multifocal	0	0	0	1 1(2)
Caudate-putamen, Mineralization, Focal/Multifocal	0	0	0	1 1(2)
Caudate-putamen, PAS Positive Material, Extracellular & Intracytoplasmic, Glial/Gitter Cells	0	0	0	3 3(P)
Cerebellum, Necrosis, Focal/Multifocal	0	0	0	1
Cerebellum, PAS Positive Material, Extracellular & Intracytoplasmic, Glial/Gitter Cells	0	0	0	1

1 = minimal, 2 = slight, 3 = moderate, 4 = marked, P = present

Drug-related findings included the following: 1 male had degeneration/rarefaction and PAS-positive material that was present extracellularly and in the cytoplasm of glial/gitter cells in the frontal cortex; 2 males had attenuation, degeneration/rarefaction, PAS-positive material in the

cytoplasm of glial/gitter cells, and myelin degeneration with associated myelin loss (identified with LFB stain) in the corpus callosum; 3 males had PAS-positive material in the cytoplasm of glial/gitter cells in the caudate-putamen, and 1 of these 3 males also had gliosis, increased vascularization, and mineralization in the caudate-putamen; and 1 male had focal/multifocal necrosis and PAS-positive material in the cytoplasm of glial/gitter cells in the cerebellum.

Terminal Sacrifice (Weeks 81 and 82): Drug-related microscopic findings were observed in the brains of 2 of 11 males dosed with 150 mg/kg, but no definitive drug-related findings were observed in males given 25 or 75 mg/kg or in females at any dose. Lesions observed in the 2 affected males given 150 mg/kg were restricted to the corpus callosum or caudate-putamen (see table below).

Incidence of Selected Microscopic Brain Findings in Males, Terminal Sacrifice - (Weeks 81 and 82)

Group	1	3	4	5
Dose (mg/kg)	0	0	25	75
No. Examined (n)	18	17	19	11
Corpus Callosum, Degeneration/Rarefaction	0	0	0	1 1(2)
Corpus Callosum, Degeneration, Myelin, with associated Myelin loss (LFB stain)	0	0	0	2 1(1) 1(2)
Corpus Callosum, PAS Positive Material, Extracellular & Intracytoplasmic, Glial/Gitter Cells	0	0	0	2 2(P)
Caudate-putamen, Gliosis, Focal/Multifocal	0	0	0	2 1(1) 1(2)
Caudate-putamen, PAS Positive Material, Extracellular & Intracytoplasmic, Glial/Gitter Cells	0	0	0	2 2(P)
Visual Cortex Necrosis, Focal	0	0	1 1(2)	0
Visual Cortex, PAS Positive Material, Intracytoplasmic, Glial/Gitter Cells	0	0	1 1(P)	0
Thrombus/Microthrombus	0	0	1 1(1)	0

1 = minimal, 2 = slight, 3 = moderate, 4 = marked, P = present

One (1) of the affected males dosed with 150 mg/kg had degeneration/rarefaction in the corpus callosum, and both had degeneration of myelin in the corpus callosum with associated myelin loss (identified with LFB Stain), intracytoplasmic PAS-positive material in glial/gitter cells in the corpus callosum and caudate-putamen, and focal/multifocal gliosis in the caudate-putamen. It also should be noted that 1 male dosed with 75 mg/kg had a single microthrombus in a vessel in the visual cortex with associated focal necrosis and intracytoplasmic PAS-positive material in glial/gitter cells. This lesion was not observed in any other rats in this study (including rats from all sacrifices and all unscheduled deaths) and was not present in rats given higher doses of BMS-477118; therefore the visual cortex lesion in this rat was probably unrelated to drug administration.

Toxicokinetics:

- Systemic exposure (as C_{max} and AUC values) saxagliptin and to BMS-510849 generally increased in a dose-related manner at 25 to 300 mg/kg for 26 weeks or 54 weeks, and at 25 to 150 mg/kg for 81 weeks.
- At ≤ 150 mg/kg, accumulation of saxagliptin and BMS-510849 in males and females was evident based upon higher AUC values (generally Week 81 saxagliptin and BMS-510849 AUC values in males were ~3-6x and in females were ~1.2-1.7x those in Week 26) with longer duration of dosing. At 300 mg/kg, from Week 26 to Week 54, there was no evidence of accumulation for saxagliptin or BMS-510849.

Dose [mg/kg/day]	Week ^a	C _{max} [µg/mL]				AUC _(0-T) [µg•h/mL] ^b			
		Males		Females		Males		Females	
		BMS-477118	BMS-510849	BMS-477118	BMS-510849				
25	26	1.61	2.05	0.50	0.58	2.95	6.50	1.06	2.16
	54	2.37	1.92	1.03	0.51	4.57	6.40	1.82	2.02
	81	4.51	6.68	1.84	1.41	7.82	9.16	6.31	3.63
75	26	3.19	9.38	0.95	1.78	8.78	23.94	2.90	9.57
	54	8.64	7.44	2.59	1.32	13.82	17.96	6.67	7.00
	81	23.41	24.66	8.15	3.95	47.92	33.67	62.89	10.93
150	26	6.26	18.86	1.91	3.00	20.62	58.81	6.53	15.52
	54	13.11	20.43	8.93	2.81	27.26	49.11	25.58	13.75
	81	28.36	50.86	9.99	4.69	54.38	74.64	37.89	19.20
300	26	13.59	26.85	2.98	10.95	71.89	139.51	22.69	35.88
	54	15.05	58.79	11.68	5.79	55.56	131.52	42.04	32.46
	81	--	--	--	--	--	--	--	--
Nominal Dose Ratio		C _{max} Ratio				AUC _(0-T) Ratio			
1:3:6:12	26	1:2:4:8	1:5:9:13	1:2:4:6	1:3:5:19	1:3:7:24	1:4:9:21	1:3:6:21	1:4:7:17
1:3:6:12	54	1:4:6:6	1:4:11:31	1:3:9:11	1:3:5:11	1:3:6:12	1:3:8:21	1:4:14:23	1:3:7:16
1:3:6	81	1:5:6	1:4:8	1:4:5	1:3:3	1:6:7	1:4:8	1:10:6	1:3:5

a No toxicokinetic samples were available at Week 81 for the 300 mg/kg/day group as all surviving rats in this group were necropsied in Week 54.

b Calculated from time 0 to the time of last measurable concentration, ranging between 8 and 24 h.

- Systemic exposure to saxagliptin was generally higher (~1.8x) in females than males at Weeks 26, 54, and 81 while systemic exposure to BMS-5 10849 in males and females was similar over the course of the study. Systemic exposure values for the parent were generally higher, at least 2-3 x, than those of the metabolite, BMS-5 10849 for each dose, sex and study interval evaluated.
- Brain:Plasma Concentration Ratios: For selected rats in Week 54, the individual saxagliptin (BMS-477118) and BMS-510849 plasma and brain concentrations were used to generate the brain:plasma concentration ratios.
- Generally, brain penetration of saxagliptin and BMS-510849 was not extensive (brain:plasma concentration ratio ≤ 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. The saxagliptin brain:plasma concentration ratios at the 24 h time point for females given 150 or 300 mg/kg were 23 or 4.5, respectively. The reason for the higher brain:plasma concentration ratios for saxagliptin in these groups at this single time point is unknown.

Dose [mg/kg/day]	Hours After Dosing	Brain:Plasma Concentration Ratio in Week 54 ^a			
		Males	Females	Males	Females
		BMS-477118	BMS-477118	BMS-510849	BMS-510849
25	4	_ ^b	_ ^b	_ ^b	_ ^b
	8	_ ^b	_ ^b	_ ^b	_ ^b
	24	_ ^c	_ ^b	_ ^c	_ ^c
75	4	0.08	0.15	_ ^b	_ ^b
	8	_ ^b	0.15	_ ^b	_ ^b
	24	_ ^b	_ ^b	_ ^c	_ ^c
150	4	0.08	0.07	0.05	_ ^b
	8	0.13	0.16	0.07	_ ^b
	24	_ ^b	23.12	_ ^b	_ ^b
300	4	0.06	0.07	0.04	0.04
	8	0.11	0.14	0.09	_ ^b
	24	_ ^b	4.48	_ ^b	_ ^b

a The lower limits of quantitation (LLQ) for BMS-477118 or BMS-510849 were 5 or 10 ng/mL, respectively, in both plasma and brain. Data are mean (n ≤ 3/time point) of individual brain:plasma concentration ratios. Values <LLQ were treated as missing for the calculation of mean values.

b No brain:plasma concentration ratio was calculated as all brain concentrations were <LLQ

c No brain:plasma concentration ratio was calculated as all brain and plasma concentrations were <LLQ

Whole Blood Cyanide and Serum Thiocyanate Analysis:

Cyanide was not measurable in any control male samples and was measurable (0.33, 0.48, or 1.2 µg/ml) in only 3 males given 150 mg/kg. Thiocyanate concentrations ranged from 1.8 to 3.4 µg/ml in control rats and 3.8 to 12 µg/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.

By the time the selection of rats for whole body perfusion occurred, there were only 2 surviving males (# C60366 and C60379) given 150 mg/kg with measurable whole blood cyanide concentrations (1.2 and 0.33 µg/ml, respectively). Because no other males had measurable cyanide concentrations, 4 males given 150 mg/kg (# C60364, C60380, C60383, and C60386) were selected (for whole body perfusion) based on the highest serum thiocyanate concentrations.

Of the 6 males given 150 mg/kg that were subjected to whole body perfusion, drug-related microscopic findings in the brain were as follows:

- Animal No C60366 had a measurable whole blood cyanide concentration of 1.2 µg/ml, and microscopic findings in the brain included minimal degeneration of myelin in the corpus callosum with associated myelin loss (identified with LFB stain), intracytoplasmic PAS-positive material in glial/gitter cells in the corpus callosum and caudate-putamen, and minimal focal/multifocal gliosis in the caudate-putamen.
- The brain of the second male (Animal No C60379) with a measurable whole blood cyanide concentration (0.33 µg/ml) was microscopically unremarkable.
- The third male (Animal No C60382) that had brain lesions did not have a measurable whole blood cyanide but did have a serum thiocyanate concentration of 8.4 µg/ml. Animal No C60382 had slight degeneration/rarefaction in the corpus callosum, degeneration of myelin in the corpus callosum with associated myelin loss (identified with LFB stain), intracytoplasmic PAS-positive material in glial/gitter cells in the corpus callosum and caudate-putamen, and focal/multifocal gliosis in the caudate-putamen.

2.6.6.4 Genetic toxicology

The genotoxicity of initial saxagliptin (BMS-477118) product with impurities Γ used in toxicology and clinical studies was tested in the 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. Saxagliptin and its' major active metabolite (BMS-510849) were not genotoxic under the assay conditions. However, in a cytogenetics study using human lymphocytes, the initial saxagliptin product with impurities was clastogenic in vitro at the highest concentration tested (1000 $\mu\text{g}/\text{mL}$), in the absence of S9. 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 appeared 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 new to-be-marketed saxagliptin manufacture by process D and its' major active metabolite (BMS-510849) were not genotoxic under the assay conditions.

b(4)

A brief summary of genotoxicity studies previously reviewed by Dr. John Colerangle is described below.

Exploratory Ames reverse-mutation study in Salmonella Study #DS00162, Nov 20, 2000

In an exploratory assay, the sponsor investigated the genotoxicity of two DPP4 inhibitors, BMS-465980 and BMS-4771 18 (saxagliptin) using *Salmonella* strain only. BMS-465980 and BMS-4771 18 were tested in duplicate cultures both in the presence and absence of S-9 metabolic activation. The positive-control articles were tested in duplicate cultures and prepared in DMSO, with the exception of sodium azide which was dissolved in Milli QT® water. The negative (vehicle) controls were tested in replicates of five cultures.

In this exploratory study, BMS-465980 exhibited cytotoxicity to strains TA98 and TA100 at concentrations $\geq 500 \mu\text{g}/\text{plate}$. Saxagliptin exhibited cytotoxicity to strain TA 100 only, and only at the highest concentration(s) tested. Cytotoxicity ranging from minimal to complete annihilation was determined by reductions in mean revertant number and/or reductions in the bacterial background lawn density. The histidine+ revertant values seen in this assay were not elevated significantly in any of the BMS-465980 or saxagliptin treated cultures with respect to the negative-control cultures. As expected significant increases in the histidine+ revertant numbers were observed in the cultures treated with the positive-control articles. In conclusion both BMD-465980 and saxagliptin (BMS-477118) were not mutagenic.

Saxagliptin (BMS-477118): Ames reverse-mutation study in Salmonella and Escherichia coli Study No. DS01143, July 25, 2002

The mutagenicity potential (frameshift or base-pair substitution mutations) of saxagliptin and an anticipated degradant Γ was evaluated in using *Salmonella typhimurium* (histidine-) tester strains TA98, TA100, TA1535, and TA1537, and *Escherichia coli* (tryptophan-) tester strain WP2 *uvrA*. Saxagliptin was spiked to contain 0.98% (w/w) of the degradant Γ and tested with each of the five strains in a range-finding assay and subsequently in a full

b(4)

mutation assay in presence and in the absence of S-9 metabolic activation, up to the maximum concentration of 5000 µg/plate. In the full mutation assay, minimal cytotoxicity was observed in strain TA98 at the highest concentration tested. No cytotoxicity was observed in any other strain in either assay. The mean histidine+ and tryptophan+ revertant values observed in these assays were not elevated significantly in any of the saxagliptin treated cultures with respect to the negative-control cultures. In conclusion, saxagliptin at concentrations of up to 5000 µg/plate containing 0.98% of degradant [redacted] was not mutagenic in this study. b(4)

Study title: Qualifying Reverse Mutation Study in Salmonella typhimurium and E. coli
Study#DS03126, September 16, 2003.

The mutagenicity of saxagliptin (BMS-477118) containing [redacted] (impurities observed in early batches but not present in final drug substance) was investigated in a qualifying bacterial reverse-mutation study in Salmonella typhimurium and E. coli. Cytotoxicity was noted in strains TA100 (≥ 2500 µg/plate) and TA1537 (5000 µg/plate) with and without S9 activation. BMS-477118 containing [redacted] tested negative under the conditions of the study. b(4)

BMS-477118 : Cytogenetics study in primary human lymphocytes
Study # D501178, July 25, 2002

An *in-vitro* cytogenetics study was conducted to determine the potential of saxagliptin (BMS-477118) containing [redacted] of the degradant [redacted] to induce chromosome aberrations in primary human lymphocytes. Based on the solubility of saxagliptin in DMSO and the cytotoxicity results of a range-finding study, concentrations of 125 to 1000 µg/ml were evaluated for the 24-hr exposure without and 5-hr exposure with Aroclor 1254-induced rat liver enzyme (S-9 fraction) activation. b(4)

In the 24-hr exposure without S-9 metabolic activation, there was no significant increase in chromosome aberration frequencies at any concentration. However, at the highest concentration, 1000 µg/ml, a minimal elevation in the frequency of chromosome aberrations, 6% vs. 1% in the vehicle control was observed. At this concentration, there was a reduction in the mitotic index of approximately 40% when compared to the vehicle control.

To further investigate this response, the assay was repeated. In trial 2, there was a significant increase in the chromosome aberration frequency at the highest concentration, 1000 µg/ml. The frequency of chromosome aberrations was 9.5% compared to 3.5% for the vehicle control and the mitotic index was reduced by 53% compared to the vehicle-control level.

In the 5-hr exposure to saxagliptin with S-9, there were no significant increases in the chromosome aberration frequencies at any concentration. There were minimal reductions in the mitotic indices of approximately 3 to 9% in saxagliptin-treated cultures relative to vehicle control. Saxagliptin containing [redacted] of the degradant [redacted] was clastogenic to dividing human lymphocytes when tested to the maximum concentration (the concentration that caused at least 50% toxicity i.e. reduction in mitotic index). b(4)

BMS-510849: Reverse-mutation study in salmonella typhimurium and E.coli
Study # DS03079, Sept 24, 2003

In a non-GLP range-finding assay, BMS-510849 was evaluated in duplicate cultures at concentrations of up to 5000 µg/plate. BMS-510849 was tested in *Salmonella typhimurium* strains TA98, TA100, TA1535, and TA1537 and in *Escherichia coli* strain WP2 *uvrA*, with and without S9 metabolic activation. The metabolic activation system was the S9 fraction of a rat liver homogenate from animals treated previously with Aroclor 1254 (500 mg/kg). Cytotoxicity was

observed in only one of the strains (*S. typhimurium* TA1537) at the highest concentration tested, with S9 activation only. A reduction in the bacterial background lawn density demonstrated cytotoxicity. The mean histidine+ and tryptophan+ revertant counts observed in this assay did not meet the criteria for a positive response in any of the BMS-510849-treated cultures.

In a full mutation assay, BMS-510849 was tested in triplicate cultures at concentrations of 250, 500, 1000, 1600, 3000, and 5000 µg/plate in *S. typhimurium* strains TA98, TA100, TA1535, and TA1537 and in *E. coli* strain WP2 *uvrA*, with and without S9 metabolic activation. Cytotoxicity was observed in two *S. typhimurium* strains, TA100 and TA1537, at the highest concentration tested with and without S9 activation. A reduction in the bacterial background lawn density demonstrated cytotoxicity. The mean histidine+ and tryptophan+ revertant counts observed did not meet the criteria for a positive response in any of the BMS-510849-treated cultures. As expected, significant increases in the histidine+/tryptophan+ revertant counts were observed in cultures treated with the positive-control articles. In conclusion, active metabolite of saxagliptin, BMS-510849 at concentrations up to 5000µg/plate was not mutagenic in this study.

Study title: Oral micronucleus study in male rats

Study # DS01130

Genotoxicity of saxagliptin alone and spiked with 0.07% degradant () was tested in an *in-vivo* male rat micronucleus assay. Saxagliptin doses up to 2000mg/kg were given to male rats. Mortality was seen in 1/5 males at 1500 mg/kg and 3/5 at 2000 mg/kg of saxagliptin+degradant. There were 1/5 Administration of 1500 mg/kg. Drug-related clinical signs of toxicity at both doses included chromorrhinorrhea, haircoat soiling, and salivation. No bone-marrow toxicity (decreases in PCE) was observed in the surviving rats. There were no increases in MN-PCE. At 1500 mg/kg, the mean frequency of MN-PCE in the bone marrow of rats was 0.09%, and the negative-control value was 0.09%. At 2000 mg/kg, the mean frequency of MN-PCE was 0.07% for the two surviving rats. The low survival at 2000 mg/kg precluded use of this dose level for meaningful genotoxic evaluation.

When saxagliptin was given alone up to 1000 mg/kg, there were no deaths. Salivation was observed at 500 and 1000 mg/kg. Additional clinical signs at 1000 mg/kg included decreased activity, haircoat soiling, lack of feces, and rales. No bone-marrow toxicity occurred in any drug-treated animals. The frequency of MN-PCE in the bone marrow of drug-treated rats was not increased when compared to the negative-control group. At 500 and 1000 mg/kg, the mean frequencies of MN-PCE in the bone marrow were 0.13 and 0.12%, respectively, and the negative-control value was 0.13%. Analyses of bone-marrow smears obtained from CP-treated rats approximately 24-hr after the third dose from both assays revealed positive micronucleus responses (1.92 and 1.90% MN-PCE, respectively), as expected for this known clastogen. In conclusion, BMS-477118 (plus 0.07% ()) was non-genotoxic in the oral rat bone-marrow micronucleus test when tested up to 1500 mg/kg under the conditions of the study.

BMS-477118: One-month oral in vivo/in vitro cytogenetics study in rat peripheral blood lymphocytes Study # DS05037, July 5, 2005

The potential for BMS-477118 to cause chromosomal aberration was tested in rats used for 1-month toxicology study using peripheral blood lymphocytes at () facility. Rats (10/sex/dose) were treated with 75, 150, 300 and 500 mg/kg of BMS-477118 in water for 1-month and the exposure to both BMS-477118 (saxagliptin) and its metabolite, BMS-510849 were acquired. Single dose of Cyclophosphamide (CP, 60 mg/kg) was administered to 10 rats per sex approximately 24 hrs prior to euthanasia and served as the positive control.

There were 2 drug-related deaths at 500 mg/kg. The first male was found dead on Day 1 and was replaced as indicated by the protocol. The second male was found dead on Day 2 with clinical signs of tremors, recumbency, labored respiration, red discharge - nasal/oral, and yellow hair coat prior to death. Observations at 500 mg/kg in 2 males and 1 female included tremors, recumbency, squinted eyes, irregular/labored respiration, and red hair coat. The clinical signs observed and mortality in males is consistent with effects observed in previous studies.

Toxicokinetic Summary

Dose [mg/kg/day]	Study Day	C _{max} [ng/mL]			
		BMS-477118		BMS-510849	
		Male	Female	Male	Female
150	28	6731	49797	2057	4644
300	28	9438	77466	2721	7921
500	28	19026	138898	5148	10804
1:2:3:3 Dose ratio	28	1:1.4:2.8	1:1.6:2.8	1:1.3:2.5	1:1.7:2.3
		AUC ^a [ng.h/mL]			
		BMS-477118		BMS-510849	
		Male	Female	Male	Female
150	28	23191	90844	7009	14852
300	28	46831	201157	15251	37408
500	28	98667	357588	27961	52272
1:2:3:3 Dose ratio	28	1:2.0:4.3	1:2.2:3.9	1:2.2:4.0	1:2.5:3.5

^a Calculated from time zero to the time of last measurable concentration, ranging between 8 and 24 h.

There was no statistically significant increase in the frequency of cells with structural aberrations in BMS-477118-treated rats. In addition, the frequency of structural aberrations values were within the historical vehicle control range for BMS studies at this laboratory (0 to 2%). In conclusion, the *in-vivo/in-vitro* chromosomal aberration assay in rats found no BMS-477118 related clastogenicity in peripheral blood lymphocytes of male or female rats administered BMS-477118 at doses as high as 500 mg/kg (AUC \leq 98667 ng.h/mL for males and AUC \leq 357588 ng.h/mL for females). Since rats were also exposed to the active metabolite, BMS-510849, one can conclude BMS-510849 was not clastogenic in this assay.

Study title: Oral DNA Repair Study In Male Rats (Unscheduled DNA Synthesis)

Study #DS02118, June 14, 2002

Genotoxicity of saxagliptin was repeated in an oral DNA repair study in male rats. Male rats were given single oral doses of saxagliptin up to 1500 mg/kg.

In the 2-4 hr exposure, there was one death (1/6) at 1500 mg/kg. At the HD, there was mortality in 1/5 rats. The mean net nuclear grain count for the negative control group was -0.7. The means of the net nuclear grain counts for the 250, 500, 1000 and 1500 mg/kg groups were -0.9, -1.6, -1.6 and -1.3, respectively. None of the test article doses caused a significant increase in the mean net nuclear counts. The mean net nuclear grain count for the positive control group was 35.4. The positive control compound, DMN, at 35 mg/kg, induced an increase in the average mean net nuclear grain counts of 36.1 over that of the negative control.

In the 12-16 hr Exposure, there were 2 deaths out of 5 animals in the negative controls and 1/5 at 680 mg/kg and 2/6 at 1500 mg/kg with no clinical signs. The mean net nuclear grain count for the negative control group was -1.9. The means of the net nuclear grain counts for the 340, 680, 1360 and 1500 mg/kg groups were -1.7, -1.0, -1.5 and -1.7, respectively. None of the test article doses caused a significant increase in the mean net nuclear counts. The mean net nuclear grain count for the positive control group was 27.1. The positive control compound, DMN, at 35

mg/kg, induced an increase in the average mean net nuclear grain counts of 29.0 over that of the negative control. Saxagliptin containing () did not induce a significant increase in the mean number of net nuclear grain counts (i.e., an increase of at least 5 counts over the negative control) in hepatocytes isolated from treated animals. The test article was judged to be negative in the unscheduled DNA synthesis (UDS) test with mammalian liver cells in vivo under the conditions of the study. b(4)

Study title: One Month Oral *In-vivo* /*In-vitro* Cytogenetics Study in Rat Peripheral Blood Lymphocytes (DS05037).

An in vivo/in vitro cytogenetics study was conducted to determine the potential of saxagliptin to induce chromosome aberrations in rat peripheral blood lymphocytes following daily oral gavage dosing for 1 month. Additionally, plasma drug concentrations were determined in vehicle control and saxagliptin-treated animals to evaluate systemic exposure to BMS-477118 and its active metabolite, BMS-510849. Saxagliptin was administered to 4 groups of 10 male and 10 female rats at doses of 75, 150, 300, and 500 mg/kg. An additional group (10 rats/sex) was administered reverse osmosis water (hereafter referred to as RO water) pH 5.5 ± 0.5 on the same schedule and served as the vehicle control. Cyclophosphamide (CP) was administered as a single oral dose of 60 mg/kg to 10 rats/sex approximately 24 hrs prior to euthanasia and served as the positive control.

Key findings:

- Saxagliptin (BMS-477118), was not clastogenic in peripheral blood lymphocytes of male or female rats administered BMS-477118 orally for 1 month under the conditions of the study.

Study no: DS05037.

Study type (if not reflected in title): Chromosome aberration study (clastogenicity).

Volume # and page #: Vol. 1, pg. 104.

Conducting laboratory and location: () b(4)

Date of study initiation: July 13, 2005.

GLP compliance: Yes.

QA reports: yes (X) no ()

Drug, lot #, radiolabel, and % purity: Batch # 4K85994, 94.2% pure.

Methods

Strains/species/cell line: Rat peripheral blood lymphocytes.

Doses used in definitive study: 75, 150, 300 and 500 mg/kg.

Basis of dose selection: The doses were selected based on a 2-week oral TK study (DN03033) in rats. 12/rats/sex/group was given daily oral doses of BMS-477118 in acidified water at 300, 600 or 1200 mg/kg. No deaths occurred at 300 mg/kg, however 1 of 12 males showed transient signs of toxicity (decreased activity, collapse, dyspnea) after the first dose. Deaths occurred after the first dose of 600 (3 of 12 males) or 1200 mg/kg (9 of 12 males) and were preceded by clinical signs including decreased activity, collapse, dyspnea and tremors. Therefore, a HD of 500 mg/kg was selected for this study. Additional doses of 75, 150, and 300 mg/kg were selected to allow for the observation of any dose-related responses.

Negative controls: Reverse osmosis water (RO water).

Positive controls: Cyclophosphamide).

Incubation and sampling times: Peripheral blood lymphocytes were obtained from rats treated with the test article (1 month) and appropriate control agents. The Peripheral blood lymphocytes were incubated at 37°C for approximately 46 hours. Approximately 2.5 hours before the harvest of the lymphocytes, colcemid was added to the cultures.

Results

Clinical Observations Summary Table

Target Dose Level (per day)	Sex	Animal Number	Day 1	Day 2	Day 16	Day 20	Day 22	Day 23	Day 27	Day 28
300 mg/kg	M	1913					1			
	F	1976							7, 11, 12, 13	7, 10, 11, 12, 13, 14
500 mg/kg		1920	6		2, 3, 4, 5					
	M	1924	2, 3, 6,	3, 6, 7, 8, 9→16						
		1926				2, 3				
	F	1980						15		

Key: 1: missing teeth, 2: tremors, 3: recumbency, 4: squinted eyes, 5: irregular respiration, 6: labored respiration, 7: red discharge-nasal, 8: red discharge-oral, 9: yellow hair coat, 10: swollen area, 11: thin, 12: light-red eye discoloration, 13: red discharge-ocular, 14: rough hair coat, 15: red hair coat, 16: found dead → = followed by.

Observations were also taken on Days 3-15, 17-19, 21, and 24-26. All animals appeared normal at these observations. Animals not included on the tables appeared normal at all observation intervals. For detailed analysis of the clinical observations, please see Appendix 5.

Chromosome Aberrations in Rat Peripheral Blood Lymphocytes - Male – Summary

Assay No.: 27398-0-444		Lab No.: CY080905									
Test Article: BMS-477118		Initiation of Dosing: 07/13/05									
Treatment	Dose Level	Harvest Time (~ hr after culture initiation)	Number of Animals	Total Number of Cells Analyzed for Aberrations	% -g Group Mean ± S.E.	% +g Group Mean ± S.E.	Judgment (+/-) ^a	% Polyploidy Group Mean ± S.E.	% Endoreduplication Group Mean ± S.E.	Judgment (+/-) ^b	% Mitotic Index Group Mean ± S.E.
Controls											
Water pH 5.5 ± 0.5	0.0 mg/kg/day	46	5	500	0.2 ± 0.20	1.8 ± 0.37	-	0.0 ± 0.00	0.0 ± 0.00	-	4.1 ± 0.88
Cyclophosphamide	60 mg/kg ^c	46	5	251	71.4 ± 4.55	75.7 ± 3.79	+	0.0 ± 0.00	0.0 ± 0.00	-	1.3 ± 0.27 ^d
Test Article											
	150 mg/kg/day	46	5	500	0.2 ± 0.20	1.4 ± 0.51	-	0.0 ± 0.00	0.0 ± 0.00	-	5.0 ± 0.59
	300 mg/kg/day	46	5	500	0.8 ± 0.37	2.4 ± 0.75	-	0.0 ± 0.00	0.0 ± 0.00	-	5.2 ± 0.72
	500 mg/kg/day	46	5	500	0.0 ± 0.00	0.8 ± 0.58	-	0.0 ± 0.00	0.0 ± 0.00	-	4.8 ± 0.98

% -g = % of cells with chromosome aberrations.
 % +g = % of cells with chromosome aberrations + % of cells with gaps.
 Water = reverse osmosis water
^a Significantly greater in -g than the corresponding vehicle control, p ≤ 0.05.
^b Significantly greater in polyploidy and endoreduplication than the corresponding vehicle control, p ≤ 0.05.
^c The positive control group was dosed on the next to last day with a single oral gavage administration.
^d Mitotic index significantly less than the corresponding vehicle control, p ≤ 0.05.

Chromosome Aberrations in Rat Peripheral Blood Lymphocytes - Female – Summary

Assay No.: 27398-0-444		Lab No.: CY080905									
Test Article: BMS-477118		Initiation of Dosing: 07/13/05									
Treatment	Dose Level	Harvest Time (~ hr after culture initiation)	Number of Animals	Total Number of Cells Analyzed for Aberrations	% -g Group Mean ± S.E.	% +g Group Mean ± S.E.	Judgment (+/-) ^a	% Polyploidy Group Mean ± S.E.	% Endoreduplication Group Mean ± S.E.	Judgment (+/-) ^b	% Mitotic Index Group Mean ± S.E.
Controls											
Water pH 5.5 ± 0.5	0.0 mg/kg/day	46	5	500	0.2 ± 0.20	1.6 ± 0.68	-	0.0 ± 0.00	0.0 ± 0.00	-	4.8 ± 0.57
Cyclophosphamide	60 mg/kg ^c	46	5	250	62.8 ± 7.96	65.6 ± 8.54	+	0.2 ± 0.20	0.0 ± 0.00	-	2.6 ± 0.81
Test Article											
	150 mg/kg/day	46	5	500	0.2 ± 0.20	1.0 ± 0.55	-	0.0 ± 0.00	0.0 ± 0.00	-	5.9 ± 0.86
	300 mg/kg/day	46	5	500	0.8 ± 0.49	2.0 ± 0.63	-	0.2 ± 0.20	0.0 ± 0.00	-	5.9 ± 0.80
	500 mg/kg/day	46	5	500	0.6 ± 0.24	2.0 ± 0.45	-	0.0 ± 0.00	0.0 ± 0.00	-	4.2 ± 0.66

% -g = % of cells with chromosome aberrations.
 % +g = % of cells with chromosome aberrations + % of cells with gaps.
 Water = reverse osmosis water
^a Significantly greater in -g than the corresponding vehicle control, p ≤ 0.05.
^b Significantly greater in polyploidy and endoreduplication than the corresponding vehicle control, p ≤ 0.05.
^c The positive control group was dosed on the next to last day with a single oral gavage administration.

Toxicokinetic Summary

Dose [mg/kg/day]	Study Day	C _{max} [ng/mL]			
		BMS-477118		BMS-510849	
		Male	Female	Male	Female
150	28	6731	49797	2057	4644
300	28	9438	77466	2721	7921
500	28	19026	138898	5148	10804
1:2:3:3 Dose ratio	28	1:1.4:2.8	1:1.6:2.8	1:1.3:2.5	1:1.7:2.3
		AUC ^a [ng·h/mL]			
		BMS-477118		BMS-510849	
		Male	Female	Male	Female
150	28	23191	90844	7009	14852
300	28	46831	201157	15251	37408
500	28	98667	357588	27961	52272
1:2:3:3 Dose ratio	28	1:2.0:4.3	1:2.2:3.9	1:2.2:4.0	1:2.5:3.5

^a Calculated from time zero to the time of last measurable concentration, ranging between 8 and 24 h.

Exposure of rats to BMS-477118 and its active metabolite, BMS-510849 was dose-related and systemic exposure to both analytes was higher in females relative to males.

Study validity:

5 slides per dose group were evaluated. Counting was done microscopically. The test article is considered to be positive (clastogenic) if statistically significant increases in the frequency of metaphases with aberrant chromosomes (excluding gap-type aberrations) are observed at one or more dose levels with evidence of dose-response relationship. The test article is considered to be negative for inducing chromosome aberrations if no significant increase in number of cells with chromosome aberrations was observed at any dose level. The study is valid because the positive control results were significantly higher than the negative controls. The increase in cells with chromosome aberrations in the positive controls were reproducible between replicate cultures and between tests and were not associated with significant changes in pH or osmolarity of the treatment medium or extreme toxicity. The doses evaluated included at least three analyzable doses. The negative control cultures contain less than ~ 5% cells with aberration.

Study outcome:

- There was no statistically significant increase in the frequency of cells with structural aberrations in either males or females receiving saxagliptin 150, 300, or 500 mg/kg for 28 days. The mean frequency of structural aberrations in the saxagliptin treatment groups ranged from 0.0 to 0.8% for males and from 0.2 to 0.8% for females. The frequency of structural aberrations in the vehicle control group was 0.2% for males and 0.2% for females.
- The positive control, cyclophosphamide, induced a statistically significant increase in mean frequency of structural aberrations relative to vehicle control, with a mean and standard error of $71.4 \pm 4.55\%$ and $62.8 \pm 7.96\%$, for the males and females, respectively. In addition, the mitotic index group mean for the males dosed with the positive control was significantly less than the corresponding vehicle control, with a group mean and standard error of $1.3 \pm 0.27\%$ and $4.1 \pm 0.88\%$, respectively (Section 12 Data Tables).
- In summary, saxagliptin was not clastogenic in peripheral blood lymphocytes of male or female rats administered saxagliptin orally for 1 month up to a maximum dose of 500 mg/kg.

2.6.6.5 Carcinogenicity

Note: Complete review of the carcinogenicity studies in mice and rats were submitted to the DFS record for NDA 22-350 as a separate file.

BMS-477118: 104-Week Oral Gavage Carcinogenicity Study in Mice

Study Summary

Male and female Crl:CD-1@(ICR)BR mice (60 /sex/group) were treated with 50, 250 and 600 mg/kg of BMS-477118 for 104 weeks. Two control groups were dosed with the vehicle (acidified water, 5 ml/kg/day) by oral gavage. Toxicokinetic animals were dosed similarly and evaluated for BMS-477118 and its prominent active metabolite BMS-510849 after 6 months of treatment. Due to extensive early deaths at 250 and 600 mg/kg in males, necropsies were conducted in mid-dose (MD) and high dose (HD) males on week 90 ($\geq 25\%$ survival) and all males at week 100. Female mice of all dose groups had greater than 25% survival and were necropsied as scheduled on WK 104. There were no drug-related clinical signs or change in body weight, food consumption, or hematology parameters. A statistically significant, dose-dependent increase in mortality was observed in male mice at 250 and 600 mg/kg. As noted earlier, MD (250 mg/kg) and HD (600 mg/kg) male mice were terminated on WK 90 when survival reached $\sim 25\%$. There was no statistically significant difference in the incidence of neoplasms at any dose level, compared to controls, in males or females. The non-neoplastic findings in groups treated with BMS-477118 were similar to controls. There was no evidence of target organ toxicity at any dose level. Cmax and AUC values for BMS-477118 and BMS-510849 appeared to increase in greater than dose-proportional manner. Between the 50- to 600-mg/kg doses, exposure (AUC) to metabolite BMS-510849 was 1.4- to 3.9-times greater than parent drug, BMS-477118 (saxagliptin). Although there was a significant increase in mortality in male mice treated with 250 and 600 mg/kg, there was no notable gender effect on exposure. Steady-state AUC exposures for BMS-477118 and BMS-510849 at 600 mg/kg were 70436 and 147802 (males) and 94,393 and 131,654 ng•h/mL (females), respectively.

In conclusion, BMS-477118 was not carcinogenic when tested up to 600 mg/kg for 2 years in mice. The study did not identify a target organ of toxicity although a dose related increase in mortality was noted in male mice at 250 and 600 mg/kg. There was sufficient number of animals in the study surviving to week 90 to allow adequate statistical analysis. The exposure multiples at 600 mg/kg in mice were 869 and 1165 times the parent clinical dose of 5 mg, based on AUC. Highest exposure to metabolite BMS-510849 in mice was 337 and 300 fold human exposure. If the exposure to the metabolite is reduced by 39%, exposure at the top dose in mice is still in excess of 150 fold.

Adequacy of the carcinogenicity study and appropriateness of the test model: The carcinogenicity protocol and saxagliptin doses were reviewed by toxicology reviewer (John Colerangle) and doses were concurred by the Executive Carcinogenesis Assessment Committee (ECAC). Based on a maximally tolerated dose of ≥ 600 mg/kg in the 3-month mouse study, eCAC recommended 50, 250 and 600 mg/kg in contrast to the sponsor's original recommendation of 50, 150 and 500 mg/kg.

Evaluation of tumor findings:

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. The ECAC agreed that there were no statistically significant differences in the incidences of tumors between control and saxagliptin treated mice.

**Ascribed cause of death/morbidity in Males
(Dying or euthanatized during the course of the study)**

	Group	Males				
		1	2	3	4	5
BMS-477118 (mg/kg/day)	0	0	50	250	600	
No. Examined		32 ^a	39 ^a	38 ^a	45 ^a	45 ^b
Undetermined		2	2	4	5	22
Neoplasia		13	13	10	9	4
Other non-neoplastic lesions		14	23	24	28	15
Accidental (gavage-related/trauma/other)		3	1	0	3	4

a Terminal necropsy occurred during Week 100.

b Terminal necropsy occurred during Week 90.

**Ascribed cause of death/morbidity in Males
(Dying or euthanatized during the course of the study)**

	Group	Males				
		1	2	3	4	5
BMS-477118 (mg/kg/day)	0	0	50	250	600	
No. Examined		32 ^a	39 ^a	38 ^a	45 ^a	45 ^b
Undetermined		2	2	4	5	22
Neoplasia		13	13	10	9	4
Other non-neoplastic lesions		14	23	24	28	15
Accidental (gavage-related/trauma/other)		3	1	0	3	4

a Terminal necropsy occurred during Week 100.

b Terminal necropsy occurred during Week 90.

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
104-week Mouse Carci Study	50	M: 1605, F: 2615	M:6246, F:7643	M:20, F:32	M:14, F:17
	250	M:34661, F: 30483	M:76123, F:49443	M:428, F:376	M:174, F:113
	600	M: 70436, F:94393	M:147802, F:131654	M:870, F:1165	M:337, F:301
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 metabolite, 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

BMS-477118: 104-Week Oral Gavage Carcinogenicity Study in Rats

Sprague-Dawley rats (60/sex/group) were treated with 25, 75, 150 and 300 mg/kg of BMS-477118 plus 2 vehicle control groups in the 2 year carcinogenicity study. The dose selection was based on the maximally tolerated dose identified in a 3-month rat study (300, 600 and 1200 mg/kg, PO). The study protocol was reviewed by the Division and eCAC.

BMS-477118 was prepared in water acidified by hydrochloric acid (5 ml/kg). There was a significant increase in mortality in the high dose (HD) males with survival rate of 23%. The HD animals were therefore sacrificed prematurely during week 68. The remaining male rats were sacrificed during week 99 due to unexpected decrease in survival rate in one of the control groups. The final survival rates for males during week 99 were 22%, 15%, 35%, 27%, and 27% for the dual control groups, 25, 75, and 150 mg/kg groups, respectively. The final survival rates for females during week 105 were 43%, 42%, 45%, 50%, 47%, and 50% for the dual control groups, 25, 75, 150, and 300 mg/kg groups, respectively. Clinical signs noted in HD males noted as early as day 16 included tremors, breathing difficulty (labored) and recumbency. Similar signs were noted in other tox studies due to cyanide toxicity. There were no notable findings at 25 mg/kg.

The target organs for non-neoplastic histological changes were brain, lung, Harderian gland, epididymis, urinary bladder and liver. Notable findings at 75 mg/kg were occasional decreases in BW in both male and females and increased incidence of pulmonary inflammation and alveolar macrophage infiltrates in female rats. At ≥ 150 mg/kg, there were occasional decreases in BW at some intervals in both male and female rats which correlated with sporadic decrease in food intake. The decrease in BW was greatest in HD male (-17%). Increased incidence and severity of mononuclear cell infiltrates were seen in female urinary bladder at ≥ 150 mg/kg and male urinary bladder at 300 mg/kg. Brain associated lesions seen at ≥ 150 mg/kg were localized to corpus callosum thalamus and caudate putamen in males at ≥ 150 . Brain lesions limited to males at 300 mg/kg were further extended to piriform/temporal cortex. These lesions were marked by myelin breakdown products stained by PAS and cellular debris. It should be noted that tremors and changes in respiratory activity were more prominent in the HD males suggesting that brain lesions likely caused these clinical signals. The brain lesions were caused by liberation of cyanide from BMS-477118 by CYP2C11, as demonstrated in separate mechanistic studies.

There were no drug-related increases in incidence of tumors in either male or female rats treated with BMS-477118. The toxicokinetic animals were evaluated for parent (BMS-477118) and active metabolite (BMS-510849) at 6 months. Exposure in female rats to BMS-477118 and BMS-510849 were 1.6 to 2.9 fold greater than in males except at 300 mg/kg where exposure in both genders appeared similar. Exposure to active metabolite, BMS-510849 was 17 to 47% greater than parent, BMS-477118. Interestingly, female rats with higher exposure to both parent and metabolite had better survival than males.

In summary, BMS-477118 was not considered carcinogenic in rats. The incidence of tumors between saxagliptin and controls was similar. The AUC exposure for BMS-477118 and its metabolite were 28742 and 9204 ng.h/ml in males and 179606 and 29730 ng.h/ml in females, 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).

Adequacy of the carcinogenicity study and appropriateness of the test model: The rat carcinogenicity protocol was reviewed by the Division and concurred by the eCAC. The study design for the animal model was considered appropriate for testing the carcinogenicity of saxagliptin.

Evaluation of tumor findings:

The ECAC agreed that there were no statistically significant differences in the incidences of tumors between control and saxagliptin treated rats. However, there were non-neoplastic microscopic findings in the treated groups (brain, harderian gland, epididymis, urinary bladder and liver).

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
104-week Rat Study,	25	M: 3492, F:8763	M:1174, F:2658	M:43, F: 108	M:3, F:6
	75	M: 13993, F:30808	M:3843, F:7672	M:173, F:380	M:9, F:18
	150	M: 28742, F: 81962	M:9204, F:15226	M: 355, F: 1012	M:21, F:35
	300	M:68568, F: 179606	M:28569, F:29730	M:847, F:2217	M:65, F:68
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 metabolite, thus all the submitted AUC values for BMS-510849 in mice, rats, pregnant rabbits, dogs, Cyno 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.

2.6.6.6 Reproductive and developmental toxicology

Fertility and early embryonic development

Oral study of fertility and early embryonic developments in rats

(J. Colerangle review revised by F. Alavi)

Key study findings:

- 2/25 males and 2/25 females in the LD group were euthanized moribund or found dead due to gavage error. 4/25 HD males were found dead (2/25 shortly after dosing). The remaining 2/25 HD males died due to gavage error. Except for 1/25 HD females euthanized moribund, 12/25 HD females were found dead between dosing days 6 – 32. All of these HD female rats had the following necropsy findings: spleen (enlarged), mouth (perioral substance, brown), snout (perinasal substance, brown), eyes (periocular substance, brown) and spleen (enlarged).
- Body weight of treated males decreased by 6% (dosing day 15) and by 8% (dosing day 29) relative to control.
- Weight of the gravid uterus decreased dose-dependently in treated females being significant (24%↓) in HD females.
- Food consumption was decreased by 10% in treated males. Gestational food consumption decreased by 8% in HD females.
- Though not significant, fertility index was decreased in untreated females (81%) mated with HD treated males relative to controls (96%). Similarly fertility index was decreased in HD treated females (83%) mated with untreated males relative to controls (92%).
- The duration of proestrus decreased while the duration of estrus increased in HD females during the treatment period.
- Dose dependent decreases (not SS) in corpora lutea (12%↓ - HD) and implantations (21%↓ - HD) were observed in treated females mated with untreated males. Pre-implantation loss increased dose-dependently (not SS) in treated females (5X – MD; 7X – HD) mated with untreated males. Post-implantation loss increased 4X (MD) and 3X (HD) in treated females mated with untreated males. This was significant in the MD treated females. Though not significant, early resorptions increased 3X (MD) and 2X (HD) in treated females mated with untreated males. Viable embryos (litter size) decreased dose-dependently (not SS) in treated females (15%↓ - MD; 29%↓ - HD) mated with untreated males.
- Relative weight of the epididymis increased by 8% in treated males relative to control. Relative weight of the right testis increased by 13% relative to control.
- The drug did not have any adverse effect on sperm morphology or motility in treated males.
- NOAEL for reproductive effects is 200 mg/kg (604x clinical dose of 5 mg- based on AUC) for males (mortality, decreased fertility at 400 mg/kg) and 125 mg/kg (775x clinical dose of 5 mg-AUC) for females (increased embryoletality, prolonged estrous, abbreviated proestrus, decreased fertility, reductions in corpora lutea and implantations).
- In summary, 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.

- BMS-477118 caused effects on reproductive function only at 400 mg/kg (1113x MRHD, AUC) in males (reduced fertility) and increased embryoletality in females at 300 (2069x MRHD, AUC). BMS-477118 affected reproductive function in rats only at doses that also caused overt toxicity. The NOAEL for reproductive effects is 200 mg/kg (604x MRHD) in males and 125 mg/kg (776x MRHD) in females.

Study no.: DN03043

Volume # and page #: Vol. 1, pg. 001.

Conducting laboratory and location: Bristol-Myers Squibb Pharmaceutical Research Institute, Department of Reproductive Toxicology, New Brunswick, New Jersey.

Date of study initiation: September 15, 2003.

GLP compliance: Yes (USA).

QA reports: yes (X) no ()

Drug, lot #, radiolabel, and % purity: Batch # 3D65912, 90.8% pure.

Formulation/vehicle: BMS-477118 (free base) dissolved in water containing HCl at concentrations equimolar to those of BMS-477118.

Methods:

Species/strain: Rat/SD.

Doses employed: 125, 300 and 750 mg/kg (females) and 100, 200 and 400 mg/kg (males).

Route of administration: Oral (gavage).

Study design: Saxagliptin was prepared in acidified water and administered orally by gavage. Following 2 weeks of dosing, treated female rats were placed in cohabitation with untreated males for a maximum of 21 days. Daily dosing continued until day 7 of gestation (GD7) in female rats, which were euthanized and cesarean-sectioned on day 15 of gestation. After the completion of cohabitation with treated females, the untreated males were dosed for at least 2 weeks prior to the initiation of cohabitation with untreated females for a maximum of 14 days. Treatment of the male rats continued until the day before scheduled necropsy (total of 29 to 32 daily doses).

Group Number	Daily Dose		Concentration BMS-477118 (mg/ml)	Number of Female Rats Assigned to Study
	BMS-477118 (mg/kg/day)	Volume (ml/kg)		
1 (Control)	0	10	0	25
2	125	10	12.5	25
3	300	10	30	25
4	750	10	75	25

Group Number	Daily Dose		Concentration BMS-477118 (mg/ml)	Number of Male Rats Assigned to Study
	BMS-477118 (mg/kg/day)	Volume (ml/kg)		
5 (Control)	0	5	0	25
6	100	5	20	25
7	200	5	40	25
8	400	5	80	25

Number/sex/group: 25/sex/group.

Parameters and endpoints evaluated:

Clinical signs: Daily.

Mortality: Twice daily.

Body weight: Twice Weekly.

Food consumption: Weekly.

Estrous cycling: In treated female rats, estrous cycling was evaluated by examination of vaginal smears for at least 14 consecutive days prior to the start of the dosing period and for the first 14 days of dosing beginning with the day after the first dose. In both treated and untreated female rats, estrous cycling data were recorded during cohabitation until mating was confirmed; cohabitation estrous cycling data were not tabulated or analyzed.

Terminal examination: Males were killed and discarded without examination after completing the mating period. All study females were killed on Day 20 after mating (GD 15) and cesarean-sectioned. For pregnant females, the number of corpora lutea, number and distribution of implantations in uterine horns, classified as early resorptions, late resorptions, dead fetuses or live fetuses. Live fetuses and their placenta were removed and the uterus and ovaries retained in neutral buffered formaldehyde. Placental weights, fetal weights (live fetuses), fetal sex (live fetuses) and external fetal abnormalities were recorded.

Sperm Evaluations in Male Rats: Following completion of the cohabitation period with untreated females, male rats were weighed, euthanatized and examined for gross lesions. The right testis, left testis, right epididymis, left epididymis, and prostate (with seminal vesicles) were weighed. The left epididymis was frozen for caudal epididymal sperm counts. One-half of the right caudal epididymis was used to prepare slides for sperm morphology. Samples of spermatozoa were collected from the left vas deferens, and sperm motility was videotaped. Sperm counts, morphology, and motility were evaluated in control and high-dose (400 mg/kg) groups; evaluations of the lower-dose groups were not performed because no drug-related changes in sperm parameters were noted at the high dose.

Toxicokinetics: yes

Results:

In-life observations:

Mortality: n = 25/sex/group

Dose (mg/kg)	0		100		125		200		300		400		750	
Sex	M	F	M	F	M	F	M	F	M	F	M	F	M	F
Total Mortality	0	0	2	2	0	0	0	0	4	13				
Found Dead	0	0	1	0	0	0	0	0	2a	12				
Intubation Accident	0	0	2	2	0	0	0	0	2	0				
Euthanized	0	0	0	0	0	0	0	0	0	1 ^a				

The 2/25 LD and 2/25 HD males that died of intubation error were observed to have discolored and/or mottled lungs. One of the two animals that died in the LD and HD groups had perforated lungs.

a = occurred shortly after first dose (within 2 hr of dosing)

Necropsy Findings in Treated Male Rats found dead, euthanized or died of intubation error.

Dose (mg/kg)	Animal # and Sex	Day of Necropsy	Necropsy Findings
100	6M0129	DD8	Euthanized moribund on day 8; esophagus (tear), lungs (dark red), R. axilla (filled with food-like substance).
	6M0138	DD5	Found dead on day 5; esophagus (tear), lungs (Right, perforated, mottled and dark brown), thoracic cavity (fluid filled, clear).
400	8M0187	DD22	Found dead on day 22; lungs (mottled and discolored, dark red), white foamy perioral and perinasal substance.
	8M0189	DD1	Found dead on day 1 shortly after dosing; all tissues appeared normal.
	8M0194	DD1	Found dead on day 1 shortly after dosing; all tissues appeared normal.
	8M0198	DD14	Found dead on day 13; lungs (mottled, left perforated), thoracic cavity (fluid filled, clear), mouth (perioral substance, white).

DD = dosing day

Necropsy findings in treated female rats found dead, euthanized or died of intubation error.

Dose (mg/kg)	Animal # and Sex	Day of Necropsy	Necropsy Findings
125	2F0029	GD5	Euthanized moribund on GD5; thoracic cavity (fluid filled, red), lungs (dark red, right perforated).
	2F0041	DD15	Euthanized moribund on day 15 of dosing; Axilla (right, fluid filled, clear, filled with feed-like substance), esophagus (tear).
750	4F0079	DD10	Found dead on day 10; spleen (enlarged), mouth (perioral substance, brown), snout (perinasal substance, brown).
	4F0080	DD6	Found dead on day 6; spleen (enlarged), mouth (perioral substance, brown), snout (perinasal substance, brown), eyes (periocular substance, brown).
	4F0083	DD25	Euthanized on day 25; spleen (enlarged, misshapen), liver (enlarged, mottled).
	4F0085	DD6	Found dead on day 6; spleen (enlarged), mouth (perioral substance, brown), snout (perinasal substance, brown), eyes (periocular substance, brown).
	4F0086	DD7	Found dead on day 7; spleen (enlarged), snout (perinasal substance, brown), eyes (periocular substance, brown).
	4F0088	DD32	Found dead on day 32; spleen (mottled), salivary glands (enlarged, hardened), thymus (enlarged, discolored, red), adrenal glands (enlarged, discolored, red).
	4F0089	DD15	Found dead on day 15; mouth (perioral substance, brown), snout (perinasal substance, brown), eyes (periocular substance, brown), spleen (enlarged, mottled), salivary glands (enlarged), thymus (enlarged), axillary lymph nodes (enlarged).
	4F0092	DD12	Found dead on day 12; mouth (perioral substance, brown), snout (perinasal substance, brown), spleen (enlarged, pale, mottled), mesenteric lymph nodes (enlarged).
	4F0093	DD8	Found dead on day 8; mouth (perioral substance, brown), snout (perinasal substance, brown), spleen (enlarged).
	4F0094	DD6	Found dead on day 6; mouth (perioral substance, brown), snout (perinasal substance, brown), eyes (periocular substance, brown), spleen (enlarged).
	4F0095	DD8	Found dead on day 8; spleen (enlarged).
	4F0099	DD8	Found dead on day 8; mouth (perioral substance, brown), snout (perinasal substance, brown), spleen (enlarged).
4F0100	DD7	Found dead on day 7; mouth (perioral substance, brown), snout (perinasal substance, brown), eyes (periocular substance, brown), spleen (enlarged).	

DD = dosing day; GD = gestation day

Clinical signs:

Dose (mg/kg)	0	0	100	125	200	300	400	750
Sex	M	F	M	F	M	F	M	F
Perioral substance ^b	1	0	5(2)	3(2)	16	5	22(2)	20(11)
Perinasal substance ^b	8	2	10(2)	10(2)	17	11	21(2)	19(12)
Decreased motor activity	0	0	0	2(2)	0	0	6(2)	4(4)
Urine stained coat	1	0	1	6(1)	3	15	13(2)	19(11)
Collapse	0	0	0	0	0	0	6 ^c (3)	0
Decreased feces	0	3	0	4(2)	0	7	0	7(2)
Periocular substance ^b	0	0	0	1(1)	0	0	0	11(11)
Ungroomed coat	0	0	0	1	0	0	0	11(8)

a = occurred shortly after first dose; b = brown, clear, red, and/or white and foam-like; c = noted on the ventral/dorsal surface and/or forelimb(s)/hindlimb(s); d = noted on the head, lip, ventral surface, and/or snout. () = # or rats with the observation that died or were euthanized prior to scheduled termination.

Body weight: Males (g)

Dose (mg/kg)	0	100	200	400
Sex	M	M	M	M
Day 1	557	555	553	549
Day 15	570	561	555	536*(6%↓)
cohabitation with untreated female rats occurred between days 15 and 29				
Day 29	593	580	572	543**(8%↓)

* p<0.05; ** p<0.01; Data excludes animals found dead or euthanized due to intubation error

Body weight: Pre-mating Body weight – Treated Females (g)

Dose (mg/kg)	0	125	300	750
Sex	F	F	F	F
Days 1	258	260	258	255
Days 15	265	264	262	264
% decrease in b. wt.	-	0.4	1	0.4

Data excludes animals found dead or euthanized due to intubation error

Summary of Gestation Body weights: (g) – Treated Females

Dose (mg/kg)	0	125	300	750
Sex	F	F	F	F
Days 0	271	272	263	263
Days 15	336	332	321	315
% decrease in b. wt.	-	1	5	6
Gravid Uterus	21	22	19	16* (24%↓)
Carcass wt.	315	310	302	299

* p<0.05; ** p<0.01

Food consumption: (g consumed/days/interval) - Males

Dose (mg/kg)	0	100	200	400
Sex	M	M	M	M
Days 1 - 8	30	29	29	28*(7%↓)
Days 8 - 15	31	31	31	29
Days 1 - 15	31	30	31	28**(10%↓)

* p<0.05; ** p<0.01

Pre-mating Food consumption: (g consumed/days/interval) – Treated Females

Dose (mg/kg)	0	125	300	750
Sex	M	M	M	M
Days 1 – 8	19	19	18	18
Days 8 - 15	20	20	19	19
Days 1 - 15	20	19	19	18

Gestation Food consumption: (g consumed/days/interval) – Treated Females

Dose (mg/kg)	0	125	300	750
Sex	M	M	M	M
Days 0 - 4	23	21	24	21
Days 4 - 8	25	25	22	23
Days 8 - 10	26	27	25	24
Days 10 - 13	26	27	27	27
Days 13 – 15	25	25	24	25
Days 0 - 15	25	25	24	23(8%↓)

For fertility studies:

In-life observations:

Dose (mg/kg)	0	125	300	750
Sex	F	F	F	F
Rats Cohabited	25	24	25	12
Rats Mated	25	24	24	12
Rats Pregnant	23	22	23	10

Summary of Mating and Fertility – Treated Males Mated With Untreated Females

GROUP TREATMENT MALE DAILY DOSE (mg/kg/day)		5 Control 0	6 RMS-477118 100	7 BMS-477118 200	8 BMS-477118 400
MALE RATS COHABITED	N	24 ^a	23	25	21 ^b
RATS WITH CONFIRMED MATING DATES	N	24	21	24	21
RATS MATING DAYS 1-7	N	24	21	24	21
DAYS IN COHABITATION PRIOR TO MATING	MEAN SD	2.6 1.1	2.8 1.4	2.5 1.4	2.6 1.6
MATING INDEX (MATED/COHABITED)	N/N %	24/24 100.0	21/23 91.3	24/25 96.0	21/21 100.0
FERTILITY INDEX (PREGNANT/MATED)	N/N %	23/24 95.8	19/21 90.5	22/24 91.7	17/21 81.0

Statistical Analyses: Fisher's Exact test was used for proportion data.
Kruskal-Wallis with Dunn's procedure was used for enumeration data.

- a - Excludes male rat 5N0103 which had necropsy findings consistent with an intubation accident. The rat mated on day 4 of cohabitation and sired a litter.
- b - Excludes male rat 5N0187 which was found dead following an intubation accident on day 22. The rat mated on day 1 of cohabitation but failed to sire a litter.

Summary of Estrous-Cycling Evaluations in Treated Female Rats – Pretreatment Period

GROUP TREATMENT DAILY DOSE (mg/kg/day)		1 Control 0	2 BMS-477118 125	3 BMS-477118 300	4 BMS-477118 750
<u>PRETREATMENT PERIOD</u>					
RATS EVALUATED	N	25	25	25	25
NUMBER (PERCENT) OF DAYS IN ESTROUS CYCLE STAGE					
PROESTRUS	MEAN SD	3.1 (22.0) 0.4 (2.9)	3.1 (22.0) 0.5 (3.5)	3.2 (23.1) 0.5 (3.8)	3.3 (23.7) 0.5 (3.4)
ESTRUS	MEAN SD	3.4 (24.3) 0.5 (3.6)	3.2 (23.1) 0.4 (3.1)	3.4 (24.6) 0.5 (3.6)	3.3 (23.7) 0.5 (3.4)
METESTRUS	MEAN SD	3.8 (27.2) 0.5 (3.6)	3.7 (26.6) 0.5 (3.3)	3.6 (26.0) 0.5 (3.5)	3.6 (25.7) 0.6 (4.1)
DIESTRUS	MEAN SD	3.7 (26.6) 0.6 (4.4)	4.0 (28.3) 0.7 (5.3)	3.7 (26.3) 0.7 (5.0)	3.8 (26.9) 0.5 (3.8)
RATS WITH 3 OR MORE CONSECUTIVE DAYS OF P/E	N %	0 0	0 0	0 0	0 0
RATS WITH 3 OR MORE CONSECUTIVE DAYS OF DIESTRUS	N %	0 0	0 0	0 0	0 0

NOTE: Analyses based on a 14-day evaluation period prior to treatment.
P/E: Proestrus and/or Estrus

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

Summary of Estrous-Cycling Evaluations in Treated Female Rats – Treatment Period

GROUP		1	2	3	4
TREATMENT		Control	BMS-477118	BMS-477118	BMS-477118
DAILY DOSE (mg/kg/day)		0	125	300	750
<u>TREATMENT PERIOD</u>					
RATS EVALUATED	N	25	25	25	14 ^a
NUMBER (PERCENT) OF DAYS IN ESTROUS CYCLE STAGE					
PROESTRUS	MEAN	3.7 (26.3)	3.8 (26.9)	3.3 (23.4)	2.8* (19.9)**
	SD	0.7 (5.0)	0.4 (3.1)	0.7 (5.3)	1.1 (7.5)
ESTRUS	MEAN	4.0 (28.3)	3.7 (26.6)	4.1 (29.1)	4.9* (34.7)
	SD	1.9 (13.9)	0.6 (4.4)	1.1 (8.0)	1.7 (12.2)
METESTRUS	MEAN	2.9 (20.8)	3.0 (21.4)	2.9 (20.8)	2.4 (17.3)
	SD	0.8 (5.8)	0.3 (2.1)	0.6 (4.6)	1.0 (7.3)
DIESTRUS	MEAN	3.4 (24.6)	3.5 (25.1)	3.7 (26.6)	3.9 (28.1)
	SD	1.1 (8.0)	0.8 (5.5)	0.9 (6.4)	1.7 (12.1)
RATS WITH 6 OR MORE CONSECUTIVE DAYS OF P/E	N	1 ^b	0	2 ^c	3 ^d
	%	4.0	0	8.0	21.4
RATS WITH 4 CONSECUTIVE DAYS OF DIESTRUS	N	0	1	1	1
	%	0	4.0	4.0	7.1

NOTE: Analyses based on a 14-day evaluation period prior to cohabitation and restricted to rats surviving to the end of the evaluation.
P/E: Proestrus and/or Estrus

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

- * Significantly different from control at P ≤ 0.05.
- ** Significantly different from control at P ≤ 0.01.

- a - Excludes eleven rats (4F0079, 4F0080, 4F0085, 4F0086, 4F0089, 4F0092, 4F0093, 4F0094, 4F0095, 4F0099, and 4F0100) which were found dead on days 6 to 15 of dosing.
- b - Noted in rat 1F0007 which had 14 consecutive days of P/E.
- c - Noted in rats 3F0055 and 3F0071 which had 6 and 8 consecutive days of P/E, respectively.
- d - Noted in rat 4F0076 which had 10 consecutive days of P/E, and rats 4F0082 and 4F0098 which had two episodes of prolonged P/E (each episode lasted 4 to 6 consecutive days).

Summary of Mating and Fertility – Treated Females Mated With Untreated Male

GROUP TREATMENT DAILY DOSE (mg/kg/day)		1 Control 0	2 BMS-477118 125	3 BMS-477118 300	4 BMS-477118 750
FEMALE RATS COHABITED	N	25	24	25	12 ^a
RATS WITH CONFIRMED MATING DATES	N	25	24	25	12
RATS MATING DAYS 1-7	N	24	22	23	12
RATS MATING DAYS 8-14	N	0	0	1	0
RATS MATING DAYS 15-21	N	1	2	1	0
DAYS IN COHABITATION PRIOR TO MATING	MEAN SD	3.1 3.0	4.1 3.9	3.0 3.8	2.4 1.4
MATING INDEX (MATED/COHABITED)	N/N %	25/25 100.0	24/24 100.0	25/25 100.0	12/12 100.0
FERTILITY INDEX (PREGNANT/MATED)	N/N %	23/25 92.0	22/24 91.7	23/25 92.0	10/12 83.3

Statistical Analysis: Fisher's Exact test used for proportion data.
Kruskal-Wallis with Dunn's procedure used for enumeration data.

a - Excludes two rats (4F0083 and 4F0088) which were found dead or euthanatized during cohabitation.

Terminal and necroscopic evaluations:

Summary of Cesarean-Sectioning Observations in Untreated Females Mated With Treated Males

GROUP TREATMENT MALE DAILY DOSE (mg/kg/day)		5 Control 0	6 BMS-477118 100	7 BMS-477118 200	8 BMS-477118 400
RATS COHABITED	N	25	23	25	22
RATS MATED ^a	N	25	21	24	22
RATS PREGNANT ^b	N(%)	24 (96.0)	19 (90.5)	22 (91.7)	17 (77.3)
PREGNANT RATS SURVIVING TO DAY 15 CESAREAN-SECTIONING	N	23 ^c	19	22	17
CORPORA LUTEA	MEAN	17.4	16.6	17.0	16.4
	SD	1.5	2.5	2.2	2.2
IMPLANTATIONS	MEAN	16.7	15.4	15.8	15.6
	SD	1.7	4.0	1.8	2.2
PREIMPLANTATION LOSS ^d	MEAN†	4.1	9.0	6.8	4.2
	SD	4.1	17.0	7.0	6.1
POSTIMPLANTATION LOSS ^e	MEAN†	5.7	5.8	6.1	5.2
	SD	4.9	7.0	6.6	4.8
VIABLE EMBRYOS (LITTER SIZE)	MEAN	15.7	14.4	14.8	14.9
	SD	1.8	3.6	2.1	2.5
EARLY RESORPTIONS	N	22	19	21	13
	MEAN	1.0	1.0	1.0	0.8
	SD	0.8	1.2	1.0	0.7
DAMS WITH ANY RESORPTIONS	N(%)	16 (69.6)	9 (47.4)	13 (59.1)	11 (64.7)
DAMS WITH NO VIABLE CONCEPTUSES	N(%)	0	0	0	0

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

- a - Restricted to rats with a confirmed date of mating.
- b - Restricted to pregnant rats with a confirmed date of mating.
- c - Excludes female rat P-0103 for which the corresponding male rat (5M0103) had necropsy findings consistent with an intubation accident. The female was pregnant and had one early resorption and 17 live embryos.
- d - Preimplantation loss calculated as: $\{(\text{Corpora lutea} - \text{implantations}) / \text{corpora lutea}\} \times 100$.
- e - Postimplantation loss calculated as: $(\text{Early resorptions} / \text{implantations}) \times 100$.

Summary of Cesarean-Sectioning Observations in Treated Females Mated With Untreated Males

GROUP TREATMENT DAILY DOSE (mg/kg/day)		1 Control 0	2 BMS-477118 125	3 BMS-477118 300	4 BMS-477118 750
RATS COHABITED	N	25	24	25	12
RATS MATED ^a	N	25	24	25	12
RATS PREGNANT ^b	N(%)	23(92.0)	22(91.7)	23(92.0)	10(83.3)
PREGNANT RATS SURVIVING TO DAY 15 CESAREAN-SECTIONING					
	N	23	21	23	10
CORPORA LUTEA					
	MEAN	14.7	15.9	14.5	12.9
	SD	1.8	1.6	2.9	2.7
IMPLANTATIONS					
	MEAN	14.5	15.6	13.7	11.5
	SD	1.8	1.9	3.9	3.2
PREIMPLANTATION LOSS ^c					
	MEAN%	1.5	1.9	7.8	11.0
	SD	2.9	4.2	17.6	13.4
POSTIMPLANTATION LOSS ^d					
	MEAN%	5.0	5.6	19.8*	17.9
	SD	6.4	6.0	26.3	24.2
VIABLE EMBRYOS (LITTER SIZE)					
	MEAN	13.8	14.8	11.7	9.8
	SD	2.1	2.0	4.9	4.4
EARLY RESORPTIONS					
	N	16	18	45	17
	MEAN	0.7	0.9	2.0	1.7
	SD	0.9	1.0	2.1	1.9
DAMS WITH ANY RESORPTIONS	N(%)	11(47.8)	13(61.9)	17(73.9)	7(70.0)
DAMS WITH NO VIABLE CONCEPTUSES	N(%)	0	0	1(4.3) ^e	0

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

* Significantly different from control at P ≤ 0.05.

- a - Restricted to rats with a confirmed date of mating.
- b - Restricted to pregnant rats with a confirmed date of mating.
- c - Preimplantation loss calculated as: [(Corpora lutea - implantations)/corpora lutea] x 100.
- d - Postimplantation loss calculated as: (Early resorptions/implantations) x 100.
- e - Noted in rat 3F0070 which had eight corpora lutea and two early resorptions. Exclusion of this rat from group calculations will alter the group mean value for the following parameters: corpora lutea (14.8), implantations (14.2), preimplantation loss (4.7%), postimplantation loss (16.1%), and viable embryos (12.2).

Summary of Reproductive Organ Weights in Treated Males

Dose (mg/kg)	0	100	200	400
Sex	M	M	M	M
Terminal Body wt.	599	585	574	546*(6%↓)
R. Testis (%)	0.30	0.32	0.32	0.34** (13%↑)
R. Epididymis (%)	0.12	0.13*(8%↑)	0.13*(8%↑)	0.13*(8%↑)
L. Epididymis (%)	0.12	0.12	0.13** (8%↑)	0.13** (8%↑)

* p≤0.05; ** p≤0.01; (%) = Relative organ wt (relative to body wt.)

Summary of Caudal Epididymal Sperm Morphology and Sperm Motility in Treated Males

GROUP TREATMENT DAILY DOSE (mg/kg/day)		5 Control 0	6 BMS-477118 100	7 BMS-477118 200	8 BMS-477118 400
RATS SURVIVING TO SCHEDULED TERMINATION	N	24 ^a	23	25	21
<u>CAUDA EPIDIDYMAL SPERM COUNTS</u>					
CAUDA EPIDIDYMIS WEIGHTS (g)	MEAN SD	0.29 0.03	.b	.b	0.30 0.03
COUNT PER CAUDA EPIDIDYMIS (10 ⁶)	MEAN SD	209.8 20.5	.b	.b	205.1 34.1
COUNT PER GRAM CAUDA EPIDIDYMIS ^c (10 ⁶ /g)	MEAN SD	777.1 75.5	.b	.b	695.4 114.8
<u>SPERM MORPHOLOGY</u>					
PERCENT ABNORMAL SPERM ^d	MEAN SD	0.4 0.2	.b	.b	0.5 0.3
<u>SPERM MOTILITY</u>					
PERCENT MOTILE SPERM ^e	MEAN SD	96.2 1.9	.b	.b	96.6 1.9

Statistical Analysis: Analysis of Variance with Dunnett's procedure used for continuous data.

- a - Excludes rat 5M0103 which had necropsy findings consistent with an incubation accident.
- b - Evaluations at 100 and 200 mg/kg/day were not required because no drug-related changes in sperm parameters (counts, morphology, and motility) were noted at 400 mg/kg/day.
- c - Sperm Count Per Cauda Epididymis calculated as: Cauda epididymis sperm count/Cauda epididymis weight in grams.
- d - Percent abnormal sperm calculated as: [Abnormal sperm/(Abnormal + Normal sperm)] x 100.
- e - Percent motile sperm calculated as: { Motile sperm/(Motile + Nonmotile sperm) } x 100.

Summary of Necropsy Observations in Treated Males

GROUP TREATMENT DAILY DOSE (mg/kg/day)		5 Control 0	6 BMS-477118 100	7 BMS-477118 200	8 BMS-477118 400
RATS EVALUATED	N	25	25	25	25
<u>SPLEEN</u>					
Enlarged	N	0	0	2	3
<u>KIDNEYS</u>					
Pale or pelvis, dilated	N	0	0	1	1
<u>STOMACH LINING</u>					
Foci, discolored red	N	0	0	0	1
<u>TESTES</u>					
Discolored red areas present	N	0	0	0	1
Small and soft	N	1 ^a	0	0	0
<u>EPIDIDYMIDES</u>					
Discolored red areas present	N	0	0	0	1
Small	N	1 ^a	0	0	0
<u>LUNGS</u>					
Discolored and/or mottled	N	0	2(2)	0	2(2)
Perforated	N	0	1(1)	0	1(1)
<u>THORACIC CAVITY</u>					
Fluid-filled, clear	N	0	1(1)	0	1(1)
<u>PROSTATE AND SEMINAL VESICLES</u>					
Small	N	1 ^a	0	0	0
<u>STOMACH</u>					
Gas-filled	N	0	1(1)	0	0
<u>LARGE INTESTINES</u>					
Gas-filled	N	0	1(1)	0	0
<u>ESOPHAGUS</u>					
Tear	N	0	2(2)	0	0

() = Number of rats with the observation that died or were euthanized prior to scheduled termination.

a - Noted in rat 5F0103.

Summary of Necropsy Observations in Treated Males... continued

GROUP TREATMENT DAILY DOSE (mg/kg/day)		5 Control 0	6 BMS-477118 100	7 BMS-477118 200	8 BMS 477118 400
RATS EVALUATED	N	25	25	25	25
MESENTERY					
Nodules, discolored white	N	1	0	0	0
VENTRAL/DORSAL SURFACE					
Mass, fluid-filled with feed-like substance	N	1 ^a	0	0	0
PELVIS					
Mass, attached to prostate	N	1 ^a	0	0	0
AXILLA					
Filled with feed-like substance	N	0	1(1)	0	0
EXTERNAL OBSERVATION^b					
Perioral substance ^c	N	0	1(1)	0	2(2)
Perinasal substance ^c	N	0	1(1)	0	1(1)
Periocular substance ^c	N	0	1(1)	0	0
Coat, urine-stained	N	0	1(1)	0	0

() = Number of rats with the observation that died or were euthanized prior to scheduled termination.

a - Noted in rat SF0103.

b - Gross external observation noted at necropsy, but not noted as an in-life clinical sign on the day of necropsy; see Appendix 1 for external observations confirmed at necropsy.

c - Brown, white, and/or foamy.

Exposure to saxagliptin and its active metabolite, BMS-510849 was measured in lactating rats (LD4) to provide data for potential use of saxagliptin in women of childbearing cage. Effect of saxagliptin on gestation, parturition, lactation, maternal behavior (F0 generation) and developmental and reproductive functions in F1-generatoion were evaluated. The stability of saxagliptin was determined previously. Saxagliptin was stable for 7 days after preparation when refrigerated. Solution concentrations were within $\pm 3\%$ of the target dose.

Parameters and endpoints evaluated: Effect of saxagliptin on gestation, parturition, lactation, maternal behavior (F0 generation) and developmental and reproductive functions in F1-generatoion were evaluated. . Standard reproductive parameters for both F0 and F1 generations were collected at specific time points in the study. F0 and some of the F1 generation were necropsies on LD-21. Maternal behavior was evaluated once daily from LDs 1 to 21. Body weights were recorded at least weekly during acclimation, on GD 0, once daily during the dosing period, and at scheduled euthanasia (terminal weight). Food consumption values were recorded on GD 0 and once daily from GD 6 through LD 14. For F1 generation, natural delivery parameters were recorded during and at the completion of parturition, including adverse clinical signs during parturition, duration of gestation, litter sizes, and pup viability at birth. Post weaning observation of F1 generation included acoustic startle habituation (PNDs 48-50), motor activity (PND58-62), water filled M-maze for overt coordination (PND69-80) and reproductive capacity (PND88-112).

Dose Levels, Concentrations, Dose Volume, and Assigned Rat Numbers

Dose Group	Mated Rats Assigned to Study	Dose (mg/kg/day)	Concentration (mg/mL)	Dose Volume (mL/kg)	Assigned Rat Numbers
Maternal Toxicity and Pre-/Postnatal Development Evaluations					
I	25	0 (R.O. Deionized Water)	0	5	29001 - 29025
II	25	40	8	5	29026 - 29050
III	25	100	20	5	29051 - 29075
IV	25	250	50	5	29076 - 29100
V	25	500	100	5	29101 - 29125
Maternal Toxicokinetic Evaluations					
VI	10	0 (R.O. Deionized Water)	0	5	29126 - 29135
VII	10	40	8	5	29136 - 29145
VIII	10	100	20	5	29146 - 29155
IX	10	250	50	5	29156 - 29165
X	10	500	100	5	29166 - 29175

All doses and concentrations were adjusted for saxagliptin "as is" purity.

Results

F₀ in-life:

Body weight:

- No change in maternal BW at 40 or 100 mg/kg
- Significant decrease in maternal BW gain (~40%) at 250 and 500 mg/kg on LD (lactation Day) 1 to LD7

Food consumption:

- There was no change in maternal food intake at 40 and 100 mg/kg
- The decrease in BW gain at ≥ 250 mg/kg was associated with 11 to 13% decrease in food intake

F₀ necropsy:

- There were no apparent drug-related deaths at saxagliptin doses up to 250 mg/kg and only death (euthanized) of 1 dam at 500 mg/kg was considered drug-related. She had severe BW loss (84 g) with no food intake and was euthanized on LD9. Clinical signs included dehydration, hunched posture, ptosis and cold to the touch and ungroomed coat. Necropsy found only a pale appearance of pancreas. Microscopic analysis did not identify the cause of death and pale appearance of pancreas had no histopathological correlates.
- Six other deaths observed in the study which occurred without a dose-dependence were not considered drug related.
- There were no drug-related adverse postmortem or microscopic observations in dams that survived to LD21.

SAXAGLIPTIN (BMS-477118): ORAL STUDY OF PRE- AND POSTNATAL DEVELOPMENT IN RATS
(SPONSOR'S REFERENCE NUMBER: DN06067)

MORTALITY/SURVIVAL AND CLINICAL OBSERVATIONS - SUMMARY - F₀ GENERATION FEMALE RATS

DOSE GROUP DOSE (MG/KG/DAY) a	0 (R.O. DEIONIZED WATER)	I/VI	II/VII 40	III/VIII 100	IV/IX 250	V/X 500
MORTALITY AND SURVIVAL INCIDENCES						
TOTAL RATS ASSIGNED TO STUDY	N	35	35	35	35	35
TOTAL UNSCHEDULED NECROPSIES	N	0	1b	3c,d	0	3c,d,f
TOTAL SCHEDULED NECROPSIES	N	35	34	32	35	32
DISPOSITION AND/OR CAUSE OF DEATH						
RATS ASSIGNED TO PRE- AND POSTNATAL DEVELOPMENT EVALUATIONS (GROUPS I TO V)						
	N	25	25	25	25	25
UNSCHEDULED NECROPSIES						
MORIBUND EUTHANIZED	N	0	0	0	0	1f
THYMIC LYMPHOMA OR MASTITIS	N	0	0	1c	0	1c
DOSING ACCIDENT	N	0	0	1d	0	1d
SCHEDULED NECROPSIES						
GESTATION DAY 25 (NOT PREGNANT)	N	0	1	2	4	0
LACTATION DAY 17 (NO LIVE PUPS)	N	0	0	0	1e	0
LACTATION DAY 21	N	25	24	21	20	22
RATS ASSIGNED TO TOXICOKINETIC EVALUATIONS (GROUPS VI TO X)						
	N	10	10	10	10	10
UNSCHEDULED NECROPSIES						
SEVERE UROLITHIASIS	N	0	1b	0	0	0
DOSING ACCIDENT	N	0	0	1d	0	0
SCHEDULED NECROPSIES						
GESTATION DAY 25 (NOT PREGNANT)	N	0	0	0	0	2g
LACTATION DAY 13	N	10	9	9	10	8

a. Dosed on Gestation Day 6 through Lactation Day 20 (or Day 24 of presumed gestation for rats that were not pregnant).
 b. Rat 29139 was euthanized on Gestation Day 17; severe urolithiasis was noted at necropsy.
 c. Rats 29071 (Group III) and 29111 (Group V) were euthanized on Lactation Day 14; thymic lymphoma and mastitis were determined at microscopic evaluation, respectively.
 d. Rats 29074 (Group III), 29106 (Group V), and 29150 (Group VIII) were euthanized on Lactation Days 9 and 11, and Gestation Day 19, respectively; signs consistent with intubation trauma were noted at necropsy and/or microscopic evaluations.
 e. Rat 29096 was euthanized on Lactation Day 17 following complete loss of her pups.
 f. Rat 29125 was euthanized in moribund condition on Lactation Day 9; death was considered drug-related.
 g. Rats 29169 and 29175 did not deliver a litter and were presumed not pregnant; the rats were not examined for pregnancy status or gross lesions at euthanasia on Day 25 of presumed gestation.

MORTALITY/SURVIVAL AND CLINICAL OBSERVATIONS - SUMMARY - F₃ GENERATION FEMALE RATS

DOSE GROUP DOSE (MG/KG/DAY) ^a	I 0 (R.O. DEIONIZED WATER)	II 40	III 100	IV 250	V 500
CLINICAL OBSERVATIONS					
<u>PRESUMED GESTATION:</u>					
MAXIMUM POSSIBLE INCIDENCE ^b	401/ 25	404/ 25	411/ 25	415/ 25	400/ 25
URINE-STAINED ABDOMINAL FUR	0/ 0	0/ 0	6/ 1c	6/ 2	0/ 0
CHROMORRHINORRHEA	0/ 0	0/ 0	0/ 0	4/ 1	0/ 0
LOCALIZED ALOPECIA: LIMB(S)	16/ 1	0/ 0	0/ 0	0/ 0	0/ 0
<u>LACTATION:</u>					
MAXIMUM POSSIBLE INCIDENCE ^b	525/ 25	504/ 24	464/ 23	437/ 21	496/ 25
DEHYDRATION	0/ 0	0/ 0	0/ 0	0/ 0	4/ 3e,f,g
CHROMODACRYORRHEA	0/ 0	0/ 0	2/ 1d	1/ 1	2/ 2e,f
URINE-STAINED ABDOMINAL FUR	0/ 0	0/ 0	1/ 1c	1/ 1	2/ 2e
PTOSIS	0/ 0	0/ 0	1/ 1d	0/ 0	2/ 2e,g
MOUTH (RIGHT UPPER LIP): YELLOW OR TIP OF TAIL: RED	0/ 0	0/ 0	1/ 1d	0/ 0	1/ 1e
CHROMORRHINORRHEA	0/ 0	0/ 0	2/ 1d	3/ 1	2/ 1e
PALE EXTREMITIES	0/ 0	0/ 0	0/ 0	0/ 0	1/ 1e
NECK, FACE, MOUTH (RIGHT UPPER LIP), LEFT AND/OR RIGHT AXILLA: SWOLLEN	0/ 0	0/ 0	4/ 2c,d	2/ 1	3/ 1e
NECK: MASS	0/ 0	0/ 0	2/ 1d	0/ 0	0/ 0
CHEST AND/OR RIGHT/LEFT FLANK: MASS	0/ 0	0/ 0	0/ 0	0/ 0	2/ 1f
NIPPLES: BLACK OR SCAB PRESENT	0/ 0	0/ 0	0/ 0	2/ 1	1/ 1f
UNGROOMED COAT	0/ 0	0/ 0	1/ 1d	0/ 0	1/ 1g
HUNCHED POSTURE	0/ 0	0/ 0	0/ 0	0/ 0	2/ 1g
COLD TO TOUCH	0/ 0	0/ 0	0/ 0	0/ 0	1/ 1g
RED PERINASAL AND PERIORAL SUBSTANCE	0/ 0	0/ 0	0/ 0	0/ 0	1/ 1e
CLEAR PERIVAGINAL SUBSTANCE	0/ 0	0/ 0	0/ 0	0/ 0	1/ 1f
LABORED BREATHING	0/ 0	0/ 0	2/ 2c,d	0/ 0	0/ 0
HYPERPNEA	0/ 0	0/ 0	1/ 1c	0/ 0	0/ 0
LOCALIZED ALOPECIA: LIMB(S)	21/ 1	0/ 0	0/ 0	0/ 0	0/ 0

MAXIMUM POSSIBLE INCIDENCE = (DAYS x RATS)/NUMBER OF RATS EXAMINED PER GROUP
 N/N = TOTAL NUMBER OF OBSERVATIONS/NUMBER OF RATS WITH OBSERVATION

- a. Dosed on Gestation Day 6 through Lactation Day 20 (or Day 24 of presumed gestation for rats that were not pregnant).
- b. Restricted to rats assigned to pre- and postnatal development evaluations.
- c. Noted in rat 29071 which had lesions consistent with thymic lymphoma and was euthanized on Lactation Day 14.
- d. Noted in rat 29074 which had lesions consistent with dosing accident and was euthanized on Lactation Day 9.
- e. Noted in rat 29106 which had lesions consistent with dosing accident and was euthanized on Lactation Day 11.
- f. Noted in rat 29111 which had lesions consistent with mastitis and was euthanized on Lactation Day 14.
- g. Noted in rat 29125 which was euthanized in moribund condition on Lactation Day 9.

NECROPSY OBSERVATIONS - SUMMARY - F₁ GENERATION FEMALE RATS

DOSE GROUP		I	II	III	IV	V
DOSE (MG/KG/DAY) ^a	0 (R.O. DEIONIZED WATER)	40	100	250	500	
RATS EXAMINED ^b	N	25	25	25	25	25
UNSCHEDULED EUTHANATIZED	N	0	0	2c,d	0	3e-g
APPEARED NORMAL	N	24	25	22	22	20
LEFT SIDE OF SNOCT: SWOLLEN	N	0	0	1c	0	0
NECK: WHITE, TAN AND RED LOBULAR MASS	N	0	0	0	0	1e
LYMPH NODES (LNs), LARGE AND/OR DISCOLORED (RED/DARK RED/YELLOW)						
MEDIASTINAL LNs:	N	0	0	1d	1h	2e,f
SCHEMATICULAR AND AXILLARY LNs:	N	0	0	0	1h	1f
MESENTERIC LNs:	N	0	0	0	1h	0
LUMBAR AND EXTERNAL ILIAC LNs:	N	0	0	0	0	1e
THYMES: LARGE; ADHERED TO BACK OF THORACIC CAVITY	N	0	0	1c	0	0
EDEMATOUS	N	0	0	0	0	1e
SPLEEN: LARGE AND/OR NUMEROUS TAN AREAS	N	0	0	0	1	1e
ADRENALS: BILATERAL, PURPLE, DARK RED, GREY AND/OR LARGE	N	1	0	2c,d	1h	3e,f
LUNGS: ALL LOBES, PALE	N	0	0	0	0	1e
RIGHT LOBES, ENCASED IN A TAN, CLEAR THIN MEMBRANE AND RED	N	0	0	0	0	1f
THORACIC CAVITY: THIN BROWN FLUID	N	0	0	0	0	1f
HEART: RIGHT ATRIUM, LARGE	N	0	0	0	0	1e
PERICARDIUM, ADHERED TO RIGHT SIDE OF THORACIC WALL	N	0	0	0	0	1f
STOMACH: ENITRE MUCOSAL SURFACE, RED, 3 ULCERATIONS OR NUMEROUS RED FOCI	N	0	0	2d	0	0
RED MATERIAL	N	0	0	0	0	1e
LIVER: ALL LOBES, ROUGH	N	0	0	1d	0	0
LEFT MEDIAL LOBE, TAN AREA	N	0	0	0	0	1f
PANCREAS: PALE	N	0	0	0	0	2e,g
CECCUM: ENTIRE MUCOSAL SURFACE, DARK RED	N	0	0	0	0	1e
LEFT INGUINAL NIPPLE: SUBCUTANEOUSLY, TAN FIRM MASS	N	0	0	0	1h	0
MAMMARY TISSUES: THICK	N	0	0	0	1h	0
NUMEROUS TAN FOCI, EXTENDED THROUGH THE TISSUE	N	0	0	0	0	1
LEFT UTERINE HORN: APPROXIMATELY 1.7 CM FROM VAGINA, CONSTRICTION	N	0	0	0	1	0

a. Dosed on Gestation Day 6 through Lactation Day 20 (or Day 24 of presumed gestation for rats that were not pregnant).
b. Restricted to rats assigned to pre- and postnatal development evaluations. Refer to the individual clinical observations table (Appendix 1) for external observations confirmed at necropsy.
c. Noted in rat 29071 which had lesions consistent with thymic lymphoma and was euthanized on Lactation Day 14.
d. Noted in rat 29074 which had lesions consistent with dosing accident and was euthanized on Lactation Day 9.
e. Noted in rat 29106 which had lesions consistent with dosing accident and was euthanized on Lactation Day 11.
f. Noted in rat 29111 which had lesions consistent with mastitis and was euthanized on Lactation Day 14.
g. Noted in rat 29125 which was euthanized in moribund condition on Lactation Day 9.
h. Noted in rat 29097.

NATURAL DELIVERY OBSERVATIONS - SUMMARY - F₁ GENERATION FEMALE RATS

DOSE GROUP DOSE (MG/KG/DAY) a		I 0 (R.O. DEIONIZED WATER)	II 40	III 100	IV 250	V 500
RATS ASSIGNED TO NATURAL DELIVERY	N	25	25	25	25	25
PREGNANT	N	25 (100.0)	24 (96.0)	23 (92.0)	21 (84.0)	25 (100.0)
DELIVERED LITTERS	N(%)	25 (100.0)	24 (100.0)	23 (100.0)	21 (100.0)	25 (100.0)
DURATION OF GESTATION b	MEAN±S.D.	22.8 ± 0.4	22.6 ± 0.5	22.8 ± 0.4	22.8 ± 0.5	23.0 ± 0.2
IMPLANTATION SITES PER DELIVERED LITTER	N MEAN±S.D.	372 14.9 ± 2.2	351 14.6 ± 2.3	350 c 15.9 ± 1.4	298 d 14.9 ± 2.9	377 15.1 ± 2.5
DAMS WITH STILLBORN PUPS	N(%)	7 (28.0)	3 (12.5)	3 (13.0)	2 (9.5)	3 (12.0)
DAMS WITH NO LIVEBORN PUPS	N	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
GESTATION INDEX e	̄ N/N	100.0 25/ 25	100.0 24/ 24	100.0 23/ 23	100.0 21/ 21	100.0 25/ 25
DAMS WITH ALL PUPS DYING POSTNATAL DAYS 1-4	N(%)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
DAMS WITH ALL PUPS DYING POSTNATAL DAYS 5-21	N	0 (0.0)	0 (0.0)	0 (0.0)	1 (4.8) f	0 (0.0)

Statistical Analysis: Continuous data - Bartlett's test and, if not significant at p<0.001, analysis of variance with Dunnett's procedure. If Bartlett's test was significant, Kruskal-Wallis test with Dunn's procedure.
 Enumeration data - If number of ties at one value was less than 75% of total, Kruskal-Wallis with Dunn's procedure, otherwise Fisher's exact test.
 Proportion data - Fisher's exact test.

- a. Dosed on Gestation Day 6 through Lactation Day 20.
- b. Calculated (in Days) as the time elapsed between confirmed mating (arbitrarily defined as Day 0 of gestation) and the time the first pup was delivered.
- c. Excludes the value from dam 29059; it was incorrectly recorded.
- d. Excludes the value from dam 29093; the left uterine horn was inadvertently not examined for implantation sites.
- e. Number of rats with live offspring/number of pregnant rats.
- f. Observed in dam 29096 on Lactation Day 17.

Toxicokinetics in F₀ generation:

- Systemic exposures to saxagliptin and its active metabolite, S-510849 in F₀-generation dams were dose related. The increase in saxagliptin dose produced greater dose-proportional increase in AUC and C_{max} for saxagliptin and AUC for BMS-510849, however, C_{max} for BMS-510849 was less than dose-proportional.
- AUC exposures to BMS-510849 were 13% to 25% of those to saxagliptin.
- 100 mg/kg was considered NOAEL. The maternal exposure at NOAEL was 490x the clinical dose of 5 mg based on AUC. The metabolite exposure at NOAEL was 22x the clinical AUC for BMS-510849 metabolite.
- The AUC and C_{max} exposure in lactating rats (20 daily doses, GD6 to LD 4) are shown in table below.
- Reanalysis of the BMS-510849 peak was found to be overestimated in table below by 42.7% in rats. Correcting safety margin for lower BMS-510849 exposure yield a safety margin of 14x the clinical BMS-510849 exposure at saxagliptin dose of 5 mg.

Toxicokinetic Summary - Lactation Day 4

Dose (mg/kg/day)	C _{max} (ng/mL)		AUC ^a (ng·h/mL)	
	Saxagliptin	BMS-510849	Saxagliptin	BMS-510849
40	4702	1059	14100	3427
100	14475	2330	38061	9573
250	33800	4744	131985	23293
500	91300	5960	301680	37728

a - Calculated from time zero to the time of last measurable concentration, ranging between 8 and 24 h.

F₁ physical development:

- There were no adverse drug-related postmortem or microscopic observations.
- Stillbirths decreased (0.7 to 1.1%) at all doses of saxagliptin, most likely due to an unusually high stillbirth rate in the control group.

Body weight in F1 generation

- Decrease in BW and BW gain on PND 4(-7 and -11%), PND 21 (-15 and -19%) at 250 and 500 mg/kg during preweaning period
- Decrease in BW in females on PND22 (18-12%) at 250 mg/kg and PND85 (5-18%) at 500 mg/kg during the postweaning period
- Decrease in BW in males on PND22 (5-16%) at 250 and 500 mg/kg during postweaning period

Changes in Mean Pup Weights/Litter during the Preweaning Period ^a			
Dose Group Dose (mg/kg/day)	I 0 (Control)	IV 250	V 500
PNDs 1 to 4	+2.2	+1.9	+1.6
PNDs 4 to 7	+4.1	+3.2	+3.0
PNDs 7 to 14	+13.8	+11.3	+11.4
PNDs 14 to 21	+15.6	+13.4	+12.2
PNDs 1 to 21	+35.7	+29.8	+28.2

a. Grams/interval.

F₁ behavioral evaluation:

- There was no drug-related effect on behavior of F1 generation
- Increased frequency of not nesting, cold to touch, dehydration and pale in appearance and no milk band were noted in 1 dam at 500 mg/kg that was euthanized. Findings in pups may have been due to maternal toxicity at this dose since they preceded changes in her pups
- There was no drug-related mortality at any dose level. One male at 250 mg/kg was euthanized on PND53 due to broken palate. One female at 259 mg/kg was also found dead on PND23 with chromorhinorrhea, dehydration and decreased BW prior to death. With no histopath finding, the cause of death was not determined. Since there were no such findings at 500 mg/kg, they were not considered drug-related.
- There were no clinical signs or necropsy observation in F1 generation at any dose level
- There was no drug-related change in motor activity

F₁ reproduction:

- The absolute left epididymis and right testis at 500 mg/kg were 8 and 6% lower than control. The significance of this is not clear since the contralateral organs were normal.
- There were no drug-related effects on the age of sexual maturation (preputial separation for males and vaginal patency for females) in the F₁-generation rats at any dose tested except for slight increase in preputial separation age at 100, 250, and 500 mg/kg (47.7 to 48.4 vs. 46.6 in controls). Since this was within the historical range at \leq $>$, it was not considered a meaningful change.
- Although there was a slightly lower trend for fertility at 40, 250 and 500 mg/kg (68, 75 and 76% vs. 84% for control), the lower fertility rate was within historical range (64-100%) and not dose-dependent
- There was no drug-related effect on mating or fertility parameters except for slight

b(4)

- Values for corpora lutea, implantations, litter sizes, live or dead fetuses, resorptions, fetal sex ratios, and fetal body weights in the treated groups were comparable to the controls.

LITTER OBSERVATIONS (NATURALLY DELIVERED PUPS) - SUMMARY - F. GENERATION LITTERS

DOSE GROUP DOSE (MG/KG/DAY) ^a		I 0 (R.O. DEIONIZED WATER)	II 40	III 100	IV 250	V 500
DELIVERED LITTERS WITH ONE OR MORE LIVEBORN PUPS	N	25	24	23	21	25
PUPS DELIVERED (TOTAL)	N	351	330	341	286	363
	MEAN±S.D.	14.0 ± 1.9	13.8 ± 2.1	14.8 ± 1.1	13.6 ± 2.6	14.5 ± 2.5
LIVEBORN	MEAN±S.D. N(%)	13.5 ± 2.1 337(96.0)	12.6 ± 2.1 327(99.1)*	14.7 ± 1.3 338(99.1)*	13.5 ± 2.6 283(99.0)*	14.4 ± 2.5 359(98.9)*
STILLBORN	MEAN±S.D. N(%)	0.6 ± 1.1 14(4.0)	0.1 ± 0.3 2(0.9)*	0.1 ± 0.3 3(0.9)*	0.1 ± 0.3 2(0.7)*	0.2 ± 0.5 4(1.1)*
UNKNOWN VITAL STATUS	N	0	0	0	1	0
PUPS FOUND DEAD OR PRESUMED CANNIBALIZED						
DAY 1	N/N(%)	4/337(1.2)	2/327(0.6)	2/338(0.6)	2/283(0.7)	4/359(1.1)
DAYS 2-4	N/N(%)	6/333(1.8)	5/325(1.5)	7/336(2.1)	10/281(3.6)	12/355(3.4)
DAYS 5-7	N/N(%)	1/327(0.3)	0/320(0.0)	3/329(0.9)	2/271(0.7)	4/343(1.2)
DAYS 8-14	N/N(%)	0/326(0.0)	0/320(0.0)	0/299(0.0) ^b	4/269(1.5)	4/317(1.3) ^c
DAYS 15-21	N/N(%)	1/326(0.3)	0/320(0.0)	0/299(0.0)	2/265(0.8)	1/300(0.3) ^d
VIABILITY INDEX ^e	% N/N	97.0 327/337	97.8 320/327	97.3 329/338	95.8 271/283	95.5 343/359
LACTATION INDEX ^f	% N/N	99.4 325/327	100.0 320/320	99.0 299/302 ^b	97.0 263/271	97.1 299/308 ^{c,d}

Statistical Analysis: Continuous data - (except for pup weights/litter which were analyzed by analysis of covariance with t-tests) - Bartlett's test and, if not significant at p≤0.001, analysis of variance with Dunnett's procedure. If Bartlett's test was significant, Kruskal-Wallis test with Dunn's procedure. Enumeration data - If number of ties at one value was less than 75% of total, Kruskal-Wallis with Dunn's procedure, otherwise Fisher's exact test. Proportion data - Fisher's exact test. * Significantly different from the control group value (p≤0.05).

DAY(S) = POSTNATAL DAY(S)

- a. Dosed on Gestation Day 6 through Lactation Day 20.
- b. Excludes 12 and 15 pups from litters 29074 and 29071, respectively. These pups were euthanized as required by the protocol following maternal death on Lactation Days 9 and 14, respectively.
- c. Excludes 9 and 13 pups from litters 29125 and 29106, respectively. These pups were euthanized as required by the protocol following maternal death on Lactation Days 9 and 11, respectively.
- d. Excludes 13 pups from litter 29111. These pups were euthanized as required by the protocol following maternal death on Lactation Day 14.
- e. Number of live pups on postnatal Day 4/number of liveborn pups on Postnatal Day 1.
- f. Number of live pups on postnatal Day 21/number of live pups on Postnatal Day 4.

CLINICAL OBSERVATIONS FROM BIRTH TO POSTNATAL DAY 21 - SUMMARY - F. GENERATION PUPS

MATERNAL DOSE GROUP ^a MATERNAL DOSE (MG/KG/DAY) ^a LITTERS EXAMINED (N)		I 0 (R.O. DEIONIZED WATER)	II 40	III 100	IV 250	V 500
		25	24	22 ^b	21	24 ^b
CLINICAL OBSERVATIONS:		TOTAL FREQUENCY (DAYS X PUPS)/LITTERS WITH OBSERVATIONS				
NOT NESTING	N/N	1/1	2/2	0/0	5/2	33/3 ^c
COLD TO TOUCH	N/N	0/0	2/2	0/0	4/1	32/2 ^c
DEHYDRATION	N/N	1/1	15/2	0/0	17/3	33/2 ^c
PALE IN APPEARANCE	N/N	0/0	0/0	0/0	0/0	13/2 ^c
NO MILK BAND PRESENT	N/N	0/0	0/0	0/0	1/1	31/1 ^c
BACK OR LOWER MIDLINE, SCAB	N/N	0/0	1/1	0/0	0/0	18/1 ^d
BACK, HEAD, TAIL, NOSE, SNOOT, AND/OR LOWER MIDLINE, BLACK OR PURPLE ^e	N/N	17/4	8/3	8/4	7/1	0/0
NOT NURSING	N/N	0/0	0/0	0/0	4/1	0/0
LEFT EYE, OPACITY	N/N	0/0	0/0	0/0	6/1	0/0
FORESHOULDERS, DARK SUBCUTANEOUS AREA	N/N	0/0	0/0	1/1	0/0	0/0
WHOLE BODY, EDEMA	N/N	1/1	0/0	0/0	0/0	0/0

- a. Dosed on Gestation Day 6 through Lactation Day 20.
- b. Excludes litters 29074 (Group III) and 29106 (Group V); the dams had lesions consistent with dosing accident and were euthanized on Lactation Days 9 and 11, respectively.
- c. Mostly (92-100% the total frequency) observed in litter 29125; the dam was euthanized in moribund condition on Lactation Day 9.
- d. Noted in 3 pups from litter 29116 on Postnatal Days 14 to 19.
- e. Color was not recorded for one pup in litters 29070 and 29072.

MORTALITY AND CLINICAL OBSERVATIONS - POSTWEANING PERIOD - SUMMARY - F₂ GENERATION MALE RATS

MATERNAL DOSE GROUP	I	II	III	IV	V
MATERNAL DOSE (MG/KG/DAY)	0 (R.O. DEIONIZED WATER)	40	100	250	500
RATS EXAMINED	N	25	25	25	25
MORTALITY	N	0	0	0	1a
CLINICAL OBSERVATIONS:					
MAXIMUM POSSIBLE INCIDENCE	2921/ 25	2923/ 25	2936/ 25	2841/ 25	2927/ 25
TAIL BENT	0/ 0	111/ 1	418/ 5	544/ 8	617/ 7
TAIL, BACK, NOSE, ABOVE LEFT EYE, OR HINDPAWS: SCAB	0/ 0	2/ 1	19/ 1	3/ 1	13/ 4
INCISOR(S): TOTAL	47/ 1	130/ 2	70/ 3	133/ 4	67/ 4
MISSING/BROKEN	0/ 0	7/ 2	7/ 2	72/ 4	48/ 4
MISALIGNED	47/ 1	128/ 2	66/ 2	117/ 2	39/ 2
CHROMORRHINORRHEA	2/ 2	1/ 1	6/ 3	9/ 4b	12/ 2
TIP OF TAIL: LACERATION	0/ 0	0/ 0	0/ 0	0/ 0	7/ 2
CHROMODACRYORRHEA	41/ 1	7/ 1	6/ 1	84/ 2b	10/ 1
SPARSE HAIR COAT: TOTAL	0/ 0	58/ 3	36/ 3	71/ 3	2/ 1
LIMB(S)	0/ 0	58/ 3	33/ 2	71/ 3	2/ 1
NECK	0/ 0	0/ 0	3/ 1	0/ 0	0/ 0
NECK, BACK, RIGHT FOREPAW OR LEFT EAR: ABRASION	0/ 0	0/ 0	2/ 1	15/ 3	7/ 1
CRINE-STAINED ABDOMINAL FUR	0/ 0	0/ 0	1/ 1	5/ 2	7/ 1
RED SUBSTANCE IN CAGE	0/ 0	1/ 1	0/ 0	2/ 2b	2/ 2
UNGROOMED COAT	1/ 1	0/ 0	0/ 0	0/ 0	1/ 1
SNOUT: SWOLLEN	0/ 0	0/ 0	0/ 0	0/ 0	3/ 1
RECOHABITATION (POSTWEANING DAY 1 TO THE FIRST DAY OF COHABITATION):					
MAXIMUM POSSIBLE INCIDENCE	1746/ 25	1749/ 25	1758/ 25	1685/ 25	1756/ 25
SPARSE HAIR COAT: TOTAL	27/ 2	48/ 2	19/ 1	21/ 2	106/ 6
LIMB(S)	27/ 2	47/ 2	19/ 1	21/ 2	90/ 5
UNDERSIDE	0/ 0	27/ 1	0/ 0	0/ 0	16/ 1
TAIL BENT	0/ 0	161/ 3	286/ 5	272/ 6	175/ 5
NECK, LEFT FOREPAW, MOUTH OR NOSE (TO THE RIGHT): SCAB	7/ 1	7/ 1	15/ 2	17/ 2	6/ 1
DEHYDRATION	3/ 1	0/ 0	0/ 0	3/ 2b	1/ 1
LOCALIZED ALOPECIA: TOTAL	19/ 2	23/ 1	0/ 0	0/ 0	43/ 1
LIMB(S)	11/ 1	23/ 1	0/ 0	0/ 0	43/ 1
NECK	8/ 1	0/ 0	0/ 0	0/ 0	0/ 0
INCISOR(S): TOTAL	8/ 1	33/ 2	11/ 1	5/ 1	0/ 0
MISSING/BROKEN	8/ 1	4/ 1	11/ 1	5/ 1	0/ 0
MISALIGNED	0/ 0	29/ 1	0/ 0	0/ 0	0/ 0
CHROMODACRYORRHEA	0/ 0	24/ 1	0/ 0	3/ 1	0/ 0
LEFT EAR: TORN	0/ 0	0/ 0	0/ 0	51/ 1	0/ 0
SCANT FECES	0/ 0	0/ 0	0/ 0	2/ 1	0/ 0

MAXIMUM POSSIBLE INCIDENCE = (DAYS x RATS)/NUMBER OF RATS EXAMINED PER GROUP
 N/N = TOTAL NUMBER OF OBSERVATIONS/NUMBER OF RATS WITH OBSERVATION
 a. Rat 23297 was found dead on Day 2 postweaning.
 b. Observed on rat 23297.

MORTALITY AND CLINICAL OBSERVATIONS - POSTWEANING PERIOD - SUMMARY - F₂ GENERATION FEMALE RATS

MATERNAL DOSE GROUP MATERNAL DOSE (MG/KG/DAY)	I 0 (R.O. DEIONIZED WATER)	II 40	III 100	IV 250	V 500
CLINICAL OBSERVATIONS (CONTINUED):					
PRECOHABITATION (POSTWEANING DAY 1 TO THE FIRST DAY OF COHABITATION):					
MAXIMUM POSSIBLE INCIDENCE	1746/ 25	1749/ 25	1758/ 25	1685/ 25	1756/ 25
CHROMORHINORRHEA	0/ 0	0/ 0	0/ 0	1/ 1a	0/ 0
SMOCT: SWOLLEN	0/ 0	2/ 1	1/ 1	0/ 0	0/ 0
NECK OR LEFT FOREPAW: ABRASION	7/ 1	13/ 2	0/ 0	0/ 0	0/ 0
MOUTH: LACERATION	0/ 0	4/ 1	0/ 0	0/ 0	0/ 0
PRESUMED GESTATION:b					
MAXIMUM POSSIBLE INCIDENCE	525/ 25	525/ 25	525/ 25	483/ 23c	525/ 25
SPARSE HAIR COAT: TOTAL	25/ 4	36/ 3	37/ 3	44/ 4	83/ 6
LIMB(S)	21/ 3	36/ 3	30/ 3	44/ 4	81/ 6
UNDERSIDE	4/ 2	21/ 1	11/ 2	0/ 0	16/ 1
TAIL BENT	0/ 0	42/ 2	105/ 5	126/ 6	105/ 5
LOCALIZED ALOPECIA: LIMB(S)	12/ 1	23/ 2	26/ 2	16/ 2	39/ 3
LEFT EAR: TORN	0/ 0	0/ 0	0/ 0	21/ 1	0/ 0
INCISOR(S): TOTAL	0/ 0	23/ 2	19/ 2	0/ 0	0/ 0
MISSING/BROKEN	0/ 0	2/ 1	19/ 2	0/ 0	0/ 0
MISALIGNED	0/ 0	21/ 1	0/ 0	0/ 0	0/ 0
CHROMODACRYORRHEA	0/ 0	21/ 1	6/ 1	0/ 0	0/ 0
SOFT OR LIQUID FECES	0/ 0	0/ 0	2/ 1	0/ 0	0/ 0
MOUTH: SCAB	0/ 0	7/ 1	0/ 0	0/ 0	0/ 0

N/N = TOTAL NUMBER OF OBSERVATIONS/NUMBER OF RATS WITH THE OBSERVATION
 MAXIMUM POSSIBLE INCIDENCE = (DAYS x RATS)/NUMBER OF RATS EXAMINED PER GROUP
 a. Observed on rat 23297.
 b. Restricted to rats with a confirmed mating date.
 c. Excludes rat 23297 which was found dead on Day 2 postweaning and rat 23277 which did not have a confirmed mating date.

F₂ findings:

- Saxagliptin had no effect on F1-generaton females and their litters.
- There were no drug-related gross external alterations in the F2-generation fetuses.

LITTER OBSERVATIONS (CAESAREAN-DELIVERED FETUSES) - SUMMARY - F₂ GENERATION LITTERS

MATERNAL DOSE GROUP MATERNAL DOSE (MG/KG/DAY)	I 0 (R.O. DEIONIZED WATER)	II 40	III 100	IV 250	V 500	
LITTERS WITH ONE OR MORE LIVE FETUSES	N	21	17	21	18	19
IMPLANTATIONS	MEAN±S.D.	14.3 ± 3.5	15.8 ± 2.0	14.8 ± 3.3	15.3 ± 1.6	15.1 ± 2.5
LIVE FETUSES	N	291	253	294	262	272
	MEAN±S.D.	13.8 ± 3.5	14.9 ± 2.4	14.0 ± 3.8	14.6 ± 1.6	14.3 ± 2.6
LIVE MALE FETUSES	N	155	123	145	131	122
‡ LIVE MALE FETUSES/LITTER	MEAN±S.D.	51.0 ± 15.9	48.1 ± 11.4	49.6 ± 16.5	49.7 ± 13.9	43.9 ± 16.4
LIVE FETAL BODY WEIGHTS (GRAMS)/LITTER	MEAN±S.D.	3.62 ± 0.36	3.68 ± 0.27	3.52 ± 0.26	3.66 ± 0.21 [17]a	3.65 ± 0.23
MALE FETUSES	MEAN±S.D.	3.73 ± 0.35 [20]b	3.78 ± 0.30	3.63 ± 0.24	3.76 ± 0.23 [17]a	3.78 ± 0.25
FEMALE FETUSES	MEAN±S.D.	3.52 ± 0.37	3.59 ± 0.26	3.41 ± 0.32 [20]c	3.56 ± 0.21 [17]a	3.56 ± 0.22
‡ RESORBED CONCEPTUSES/LITTER (POSTIMPLANTATION LOSS)	MEAN±S.D.	3.0 ± 4.8	6.2 ± 6.4	6.5 ± 12.6	4.9 ± 7.0	5.4 ± 5.6

Statistical Analysis: Continuous data - Bartlett's test and, if not significant at p≤0.001, analysis of variance with Dunnett's procedure. If Bartlett's test was significant, Kruskal-Wallis test with Dunn's procedure.
 Enumeration data - If number of ties at one value was less than 75% of total, Kruskal-Wallis with Dunn's procedure, otherwise Fisher's exact test.

POSTIMPLANTATION LOSS = [(DEAD + RESORBED CONCEPTUSES)/IMPLANTATIONS] x 100
 [] = NUMBER OF VALUES AVERAGED
 a. Excludes values for litter 23277; the dam did not have a confirmed mating date.
 b. Excludes values for litter 23213 which had no male fetuses.
 c. Excludes values for litter 23259 which had no female fetuses.

Study title: Saxagliptin (BMS-4771 18) and Metformin (BMS-2071 50): Oral combination Study of Embryo-Fetal Development in Rats

The aim of this study was to support the use of fixed dose combination of saxagliptin with metformin.

Key study findings:

- There were no adverse findings or malformations at 5/200 mg/kg/d of saxagliptin/metformin (20x/4x the maximum therapeutic dose of saxagliptin/metformin, based on AUC). However, malformations were found at 25/200 mg/kg/d of saxagliptin/metformin (109x/4x the maximum therapeutic dose of saxagliptin/metformin, based on AUC).
- Malformations were characterized as incomplete closure of the skull (craniorachischisis) and absent renal papilla(e) in 2 fetuses from a single dam (litter). One of the two fetuses had cleft palate. The fetal and litter incidence of craniorachischisis was 0.7% and 4.5%, respectively, which exceed historical incidence data for the conducting laboratory.
- Malformations were seen in two additional fetuses from another dam, described as absent or shortened digits of forepaws/hindpaws (ectrodactyly, brachydactyly). The fetal (but not litter) incidence of these malformations exceeds the historical data for the conducting laboratory.
- Since malformations were found when the dose of saxagliptin was increased from 5 to 25 mg/kg/d with no change in metformin dose, the data seems to point to saxagliptin as the potential cause possibly by a pharmacodynamic interaction with metformin.

Study no.: DN08072

Volume #, and page #: electronic

Conducting laboratory and location: ()

Date of study initiation: Nov 17, 08 (necropsy on Dec 04-05, 2008)

b(4)

GLP compliance: Yes (USA).

QA reports: Yes (X) no ()

Drug, lot #, radiolabel, and % purity: BMS-477118 Batch #s 2J66264, 67.6% pure free base but provided as benzoate salt, Metformin (BMD-207150) batch #8E35072, purity of 99.5%

Formulation/vehicle: saxagliptin in 1.25% Avicel (1ml/kg) and metformin in water (4 ml/kg)

Methods:

Species/strain: pregnant CrI:CD(SD) Rat

Doses employed: 5/200 and 25/200 mg/kg/d of saxagliptin/metformin

Route of administration: Oral (gavage).

Number/sex/group: 22 pregnant females/group plus 12/group for TK.

Mortality: Twice daily.

Clinical signs: Each rat was examined daily for abortion, premature delivery, and clinical signs.

Body weight: Daily

Food consumption: Daily.

Hematology and clinical chemistry: blood samples were collected from TK animals and analyzed for standard hematology and blood chemistry parameters

Toxicokinetics: yes

Study design: Time-mated pregnant SD rats were treated with combination of saxagliptin and metformin from gestation day 6 to 15. Hematology, clinical chemistry and drug levels were measured from the TK animals on gestation day 14-15. Dams in the main study were evaluated for food intake and changes in BW until cesarean section and necropsy on GD 21. At necropsy, the reproductive tract was dissected and ovaries were removed and the corpora lutea was counted. The uterine weight (gravid and non gravid), uterine contents including placentas were examined. The numbers and position of live fetuses, dead fetuses, and resorptions were recorded. Each fetus was weighted, sexed and examined for external and internal (soft tissue and/or skeletal) alterations. The two products prepared weekly were stable and within the target concentrations ($\pm 10\%$).

Group Number Identification	Saxagliptin/Metformin (or Vehicles for Group 1)			Female Numbers	
	Dose Level (mg/kg/day)	Concentration (mg/mL)	Dose Volume (mL/kg/day)	Main Study ^f	Satellite ^d
1/ Control ^a	0/0	0/0	1/4	1501-1506, 1508-1522, 1607	1523-1534
2/ Saxagliptin + Metformin ^b	5/200	5/50	1/4	2501-2508, 2510-2517, 2519-2522, 2609, 2618	2523-2534
3/ Saxagliptin + Metformin ^b	25/200	25/50	1/4	3501-3522	3523-3534

a - 1.25% Avicel was dosed first and immediately followed by deionized water.

b - Saxagliptin formulation was administered first and immediately followed by metformin formulation.

c - Assigned to maternal and developmental toxicity evaluations.

d - Assigned to maternal clinical-pathology and toxicokinetic evaluations.

Results:

Mortality and clinical signs:

- All dams survived to the end of the study.
- Saxagliptin combination with metformin was well tolerated.

Body weight and food intake:

- There was no drug-related change in maternal BW or BW gain.
- Food intake was slightly decreased on GD 6 and 7 for 5/200 and 25/200 mg/kg/d of saxagliptin/metformin but since it lasted only 1 day and had no effect on maternal weight, it was considered irrelevant.

Hematology:

- A dose-dependent decrease in platelet counts (0.84 to 0.87x control) was noted in both treated groups. Since this has been seen with saxagliptin alone, the changes were likely due to saxagliptin treatment.
- Elevated lymphocyte count (1.3x control), leukocyte count (1.24x control) were noted at 25/200 mg/kg/d of saxagliptin/metformin. This was also likely due to saxagliptin.

Clinical Chemistry:

- Minimal decrease in serum albumin (0.96 to 0.94x the controls). Since saxagliptin was shown to decrease serum albumin, the slightly lower albumin was likely related to saxagliptin.
- Slight increases in ALP and serum P in the 5/200 mg/kg/d were considered coincidental

Toxicokinetic:

- In pregnant rats, AUC exposure for saxagliptin and its metabolite, BMS-510849 increased in a dose-proportional manner. Maternal exposure to metformin was similar in the two groups suggesting no notable drug-drug pharmacokinetic interaction
- The NOAEL for saxagliptin/metformin was 5/200 mg/kg/d due to malformation at 25/200 mg/kg/d.
- The exposure at 5/200 mg/kg/d saxagliptin/metformin dose was 20x/4x the maximum therapeutic doses of saxagliptin and metformin based on AUC.
- The AUC for clinical dose of saxagliptin and metformin were 81 ng.h/ml and 20544 ng.h/ml, respectively.

Analytes	Saxagliptin/Metformin Dose (mg/kg/day)	C _{max} (ng/mL)	AUC (ngxh/mL)	Human Exposure Multiple ^{a,b}
Saxagliptin	5/200	513	1630	21 x
	25/200	2790	8860	114 x
BMS-510849	5/200	170	658	3 x
	25/200	732	3510	16 x
Metformin	5/200	11400	85200	4 x
	25/200	10500	89300	4 x

a - Relative to human AUC values for saxagliptin and BMS-510849 (78 and 214 ng·h/mL, respectively) associated with a therapeutic dose of 5 mg daily¹.

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

Summary of Observations at Necropsy:

- There were no drug-related gross findings in dams.

Embryo-fetal development studies:**In-life observations:**

- Offspring: The reproductive parameters [i.e. corpora lutea, implantations, live and dead fetuses, sex ratio, resorption indices (early, middle and late), and fetal body weights] were similar among three groups.

Terminal and necroscopic evaluations:**Offspring:**

- There were no drug-related malformations in fetuses at 5/200 mg/kg/d of saxagliptin/metformin combination dose group.
- Major malformations were seen in the 25/200 mg/kg/d saxagliptin/metformin group.
 1. Incidence of craniorachischisis and forelimb flexure in 2 fetuses in one litter with fetal and litter incidences of 0.7 and 4.5%, respectively. One of the fetuses had cleft palate.
 2. Digital malformations in 2 fetuses from another litter that exceeded the historical fetal incidence data.
 3. The noted single incidence of sinus inversus and absent kidney was considered spontaneous and within or slightly greater than historical range of the testing facility.

- Minor external, visceral and skeletal abnormalities
 - A) There were no minor abnormalities at 5/200 mg/kg/d of saxagliptin/metformin group
 - B) At 25/200 mg/kg/d, renal papillae was absent from the kidneys of the 2 fetuses with craniorachischisis noted above
 - C) There were no drug-related increases in fetuses/litters with sternbrae or thoracic centrum variants.

Group Incidence of Fetal External, Visceral and Skeletal Findings - Major Malformations, Minor Anomalies, and Common Variants

	Group 1 - Vehicle control		Group 3 - Saxagliptin/Metformin 25/200 mg/kg/day			
	Group 2 - Saxagliptin/Metformin 5/200 mg/kg/day		Group 2		Group 3	
	L/E	F/E	L/E	F/E	L/E	F/E
External (EXT)	22	277	21	250	22	280 ^a
Visceral (VIS)	22	138	21	126	22	142 ^b
Skeletal (SKE)	22	139	21	124	22	141 ^{a,c}
Technique of Wilson (WT)	22	138	21	126	22	140
	L/A	F/A	L/A	F/A	L/A	F/A
Major Malformations (Total)	0	0	2	2	2	4
Head						
Cleft palate (WT)	0	0	0	0	1	1 ^c
Gross Exam						
Craniorachischisis (EXT, confirmed by SKE and/or WT)	0	0	0	0	1	2 ^b
General						
Situs inversus (VIS)	0	0	1	1	0	0
Kidneys						
Kidney(s) absent (VIS)	0	0	1	1	0	0
Ureter(s)						
Ureter(s) absent (VIS)	0	0	1	1	0	0
Limbs						
Digit(s) of forepaws and hindpaws absent (ectrodactyly) (EXT, confirmed by SKE)	0	0	0	0	1	2 ^d
Digit(s) of forepaws shortened (brachydactyly) (EXT, confirmed by SKE)	0	0	0	0	1	2 ^d
Abnormal flexure of forelimb(s) (EXT)	0	0	0	0	1	2 ^b

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

Group Incidence of Fetal External, Visceral and Skeletal Findings - Major Malformations, Minor Anomalies, and Common Variants

	Group 1 - Vehicle control		Group 3 - Saxagliptin/Metformin 25/200 mg/kg/day			
	Group 2 - Saxagliptin/Metformin 5/200 mg/kg/day		Group 2		Group 3	
	L/A	F/A	L/A	F/A	L/A	F/A
Major Malformations (Cont'd)						
Tail						
Tail shortened (microcaudia) (EXT, confirmed by SKE)	0	0	0	0	1	2 ^a
Minor External and Visceral Anomalies (Total)	4	4	3	4	3	4
Kidneys						
Renal papilla(e) absent (VIS)	0	0	0	0	1	2 ^b
Minor External and Visceral Anomalies (Cont'd)	L/A	F/A	L/A	F/A	L/A	F/A
Liver						
Supernumerary liver lobe (VIS)	0	0	1	1	0	0
Discoloration pale (VIS)	0	0	0	0	1	1
Ureter(s)						
Ureter(s) dilated (megaureter) (VIS)	4	4	2	3	2	2
Convoluted ureter(s) (VIS)	0	0	0	0	1	1
Skull						
Parietal bone(s): Incomplete ossification	1	1	5	6	4	4
Frontal bone(s): Incomplete ossification	0	0	1	1	0	0
Interparietal bone: Incomplete ossification	10	23	12	31	8	17
Supraoccipital bone: Incomplete ossification	0	0	2	2	0	0
Hyoid bone: Incomplete ossification	7	11	9	16	5	6

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

Group Incidence of Fetal External, Visceral and Skeletal Findings - Major Malformations, Minor Anomalies, and Common Variants

	Group 1 - Vehicle control		Group 3 - Saxagliptin/Metformin 25/200 mg/kg/day			
	Group 2 - Saxagliptin/Metformin 5/200 mg/kg/day		Group 2		Group 3	
	L/A	F/A	L/A	F/A	L/A	F/A
Minor Skeletal Anomalies (Cont'd)						
Vertebral Column						
Ossification center(s) on 1st lumbar vertebra or 14th thoracic vertebra	10	19	8	19	12	23
Ossification center(s) on 4th lumbar vertebra	1	1	0	0	0	0
Lumbar centrum semi-bipartite	1	1	0	0	1	1
Sacral vertebral centrum: Fused	0	0	0	0	1	2
Thoracic vertebra(e) centrum: Irregular ossification	0	0	0	0	1	1
Lumbar vertebral arch(es): Incomplete ossification	0	0	0	0	1	1
Ribs						
Notched rib(s)	0	0	2	2	1	1
Rudimentary 14th rib(s)	0	0	1	1	0	0
Ossification center(s) on 7th cervical vertebra	1	1	0	0	0	0
Rib(s) on 7th cervical vertebra	1	1	0	0	0	0
Limbs						
Femur incomplete ossification	0	0	3	3	1	1

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

