Study Summary

Pregnant SD rats (22/group for main study and 12/dose for TK) were treated with combination of saxagliptin/metformin dose of 5/200 and 25/200 mg/kg/d from gestation day (GD) 6 though 15. There were no separate arms for saxagliptin or metformin in the study. Saxagliptin was prepared in 0.125% Avicel/water (1 ml/kg) while metformin was prepared in water (4 ml/kg) for oral gavage delivery. Blood samples for hematology and clinical chemistry and TK were collected from the TK group on GD 14-15. The combination saxagliptin/metformin was well tolerated and there were no deaths. 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, at higher dose of saxagliptin (25/200 mg/kg/d dose of saxagliptin/metformin), neural tube defect was observed in 2 fetuses from one litter. Neural tube defect also known as craniorachischisis was marked by incomplete closure of the skull and spinal column. The same two fetuses had missing renal papilla(e), microcaudia (short tail), and one of them had cleft palate. The fetal and litter incidence of craniorachischisis was 0.7% and 4.5%, respectively, which greatly exceeds historical experience. Two additional fetuses from a second litter showed an increased incidence of missing or absent digits of the paws/hindlimbs. The cause of malformation in the combination study is not clear since both drugs were not teratogenic in embryofetal development studies in rats and rabbits. Since malformations were seen when the dose of saxagliptin was raised from 5 to 25 mg/kg/d with no change in metformin dose, the data implicate saxagliptin as the potential cause of malformations, possibly via a pharmacodynamic interaction with metformin. However, the sponsor believes that malformations were due to metformin since at least in one publication, 500 mg/kg/d of metformin produced an incidence of neural tube defect (craniorachischisis) in pregnant rats (GD 1 to 12). The author of the paper had considered metformin not to be strongly teratogenic. The sponsor also stated that metformin treated patients have been shown to have low circulating levels of folate and Vit B12, two cofactors needed for synthesis of methionine. Since methionine is essential for normal fetal development, the malformations in the combination study were likely due to metformin and not saxagliptin. Spontaneous incidence of craniorachischisis is very rare. According to the sponsor the historical control data collected from 2005 to 2007 found only one incidence of craniorachischisis. This low historical background further highlights the need for repeating the study in rats as well as a new study in rabbits. The study should include separate arms for saxagliptin and metformin in addition to the combination. The sponsor has agreed to do the studies but the results of the two studies are likely to become available post approval (late 2009).

Historical control Data from

b(4)

HISTORICAL CONTROL DATA
RAT - CD® IGS (Crl:CD[SD]), 1996-2007

ALL ROUTES OF ADMINISTRATION

EMBRYO-FETAL DEVELOPMENT STUDIES		
FETAL FINDINGS - SUMMARY DATA		
Parameter	Minimum	Maximum
GESTATION DAY 20		
Fetal Weight (G) - Males	3.49	4.21
Fetal Weight (G) - Females	3.28	3.93
Fetal Weight (G) - Total	3.38	4.11
GESTATION DAY 21		
Fetal Weight (G) - Males	5.02	6.14
Fetal Weight (G) - Females	4.80	5.82
Fetal Weight (G) - Total	4.94	5.97
COMBINATION OF GESTATION DAY 20 AND 21		
Major Malformations - Litters Affected (%)	0.0	13.6
Major Malformations - Fetuses Affected (%)	0.0	1.0
Minor External and Visceral Anomalies - Litters Affected (%)	0.0	33.3
Minor External and Visceral Anomalies - Fetuses Affected (%)	0.0	4.4
GESTATION DAY 20		
Minor Skeletal Anomalies - Litters Affected (%)	27.3	95.8
Minor Skeletal Anomalies - Fetuses Affected (%)	6.6	50.0
Vertebral Centrum Variants - Fetuses Affected (%)	9.7	40.7
Sternebral Variants 1 to 4 - Fetuses Affected (%)	1.3	26.9
Sternebral Variants 5 and 6 - Fetuses Affected (%)	49.9	96.7
GESTATION DAY 21		
Minor Skeletal Anomalies - Litters Affected (%)	45.5	95.0
Minor Skeletal Anomalies - Fetuses Affected (%)	9.3	46.7
Vertebral Centrum Variants (unossified, incomplete ossification, bipartite, semi-bipartite) - Fetuses Affected (%)	1.1	39.1
Sternebral Variants 1 to 4 (unossified, incomplete ossification, bipartite, semi-bipartite) - Fetuses Affected (%)	0.0	2.5
Sternebral Variants 5 and 6 (unossified, incomplete ossification, bipartite, semi-bipartite) - Fetuses Affected (%)	5.1	45.8

HISTORICAL CONTROL DATA
RAT - CD® IGS (Crl:CD(SD)), 1996-2007

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ALL ROUTES OF ADMINISTRATION

EMBRYO-FETAL DEVELOPMENT STUDIES				
GROUP INCIDENCE OF FETAL EXTERNAL, VISCERAL AND SKELETAL FINDINGS MAJOR MALFORMATIONS AND MINOR ANOMALIES				
	Litters examined			
EXTERNAL (EXT)	1814	Total no. of studies used		
VISCERAL (VIS)	1813	82		
SKELETAL (SKE)	1812			
TECHNIQUE OF WILSON (WT)	1812			
	Litters affected			
	SUM	AVERAGE %	MIN %	MAX %
MAJOR MALFORMATIONS (TOTAL)	37	2.09	0.00	13.64
Cranium: Auditory/vestibular system; absent (WT)	1	0.06	0.00	4.00
Cranium: Auditory/vestibular system; reduced/ incomplete formation (WT)	2	0.11	0.00	4.76
Cranium: Cleft palate/lip (EXT,WT)	3	0.17	0.00	4.76
Cranium: Microtia (EXT)	1	0.06	0.00	4.76
Cranium: Microcephaly (EXT,WT)	2	0.11	0.00	5.26
Cranium: Exencephaly (EXT,SKE)	1	0.06	0.00	5.00
Brain: Hydrocephaly (EXT,WT)	4	0.22	0.00	9.09
Brain: Cerebrum, cyst-like formation (WT)	1	0.06	0.00	4.00
Brain: Lateral ventricles reduced (WT)	1	0.06	0.00	4.00
Eye(s): Anophthalmia (EXT,WT)	6	0.33	0.00	5.26
Eye(s): Exophthalmia (EXT,WT)	1	0.06	0.00	4.00
Eye(s): Microphthalmia (WT)	2	0.11	0.00	4.00
Eye(s): Open (EXT,WT)	1	0.06	0.00	4.00
Eye(s): Retinal folding (WT)	2	0.11	0.00	4.35
Eye(s): Aphakia (WT)	1	0.06	0.00	4.35
Face: Aglossia (EXT,WT)	3	0.17	0.00	8.33
Face: Agnathia (EXT,WT)	5	0.28	0.00	8.33
Face: Astomia (EXT,WT)	3	0.17	0.00	5.26
Face: Micrognathia (EXT,WT)	1	0.06	0.00	4.17
Face: Mandibular micrognathia (EXT)	5	0.28	0.00	5.00
Face: Microstomia (EXT,WT)	1	0.06	0.00	4.00
Face: Nares; reduced (EXT,WT)	1	0.06	0.00	4.00
Face: Nares opening; reduced (WT)	1	0.06	0.00	4.17
Face: Nasal septum/turbinate formation; reduced (WT)	1	0.06	0.00	4.17
Face: Nasal septum; lack of turbinate formation (WT)	1	0.06	0.00	4.76
Face: Palate; absent (WT)	3	0.17	0.00	8.33
Face: Split tongue (WT)	1	0.06	0.00	4.76
Face: Upper jaw absent (EXT)	2	0.11	0.00	5.26
Face: Nares absent (EXT)	1	0.06	0.00	5.26
Face: Probosis (EXT,WT)	1	0.06	0.00	5.26
Heart: Interventricular septal defect (VIS)	2	0.11	0.00	5.00
Heart: Dilatation of ascending aorta (VIS)	1	0.06	0.00	4.17
Heart: Right descending aorta (VIS)	1	0.06	0.00	5.00

HISTORICAL CONTROL DATA

ALL ROUTES OF ADMINISTRATION

b(4)

EMBRYO-FETAL DEVELOPMENT STUDIES GROUP INCIDENCE OF FETAL EXTERNAL, VISCERAL AND SKELETAL FINDINGS MAJOR MALFORMATIONS AND MINOR ANOMALIES				
	Litters affected			
MAJOR MALFORMATIONS (CONT'D)	SUM	AVERAGE %	MIN %	MAX %
Heart: Stenosis of ascending aorta (VIS)	1	0.06	0.00	5.00
Heart: Transposition of major vessels (VIS)	4	0.22	0.00	5.00
Heart: Globular heart (VIS)	1	0.06	0.00	5.00
Heart: Ringed aorta (VIS)	1	0.06	0.00	4.76
Diaphragm: Diaphragmatic hernia (VIS)	1	0.06	0.00	4.35
Lungs and thymus: Lung lobes; absent (VIS)	2	0.11	0.00	4.76
Lungs and thymus: Lung lobes; reduced (VIS)	1	0.06	0.00	4.76
Vertebral column: Multiple fusion and anomalies in vertebral column (SKE)	3	0.17	0.00	4.35
Thorax: Trunk shortened (EXT)	1	0.06	0.00	4.35
Abdomen: Anal atresia (EXT,VIS)	4	0.22	0.00	4.55
Abdomen: Gastroschisis (EXT)	1	0.06	0.00	4.00
Abdomen: Situs inversus (VIS)	1	0.06	0.00	5.00
Abdomen: Omphalocele (EXT)	5	0.28	0.00	5.00
Abdomen: Urogenital region fissure (EXT)	1	0.06	0.00	4.76
Abdomen: Abdominal muscles herniated (EXT)	1	0.06	0.00	4.17
Abdominal cavity: Colon blind (VIS)	1	0.06	0.00	4.00
Abdominal cavity: Malposition stomach/ pancreas/spleen (VIS)	1	0.06	0.00	5.00
Abdominal cavity: Intestine; stenosis (VIS)	1	0.06	0.00	4.55
Tail: Acaudia (EXT)	2	0.11	0.00	4.00
Tail: Microcaudia (EXT)	4	0.22	0.00	4.76
Limb(s): Ectrodactyly (EXT)	2	0.11	0.00	4.76
Limb(s): Brachydactyly (EXT)	2	0.11	0.00	4.76
Limb(s): Abnormal flexure of hindlimb(s) (EXT)	2	0.11	0.00	4.76
Limb(s): Hindpaw(s) absent (SKE)	1	0.06	0.00	4.00
Skull: Mandible shortened (SKE)	1	0.06	0.00	5.00
Skull: Maxilla shortened/Incisive bone (SKE)	1	0.06	0.00	5.00
Gross exam: Anasarca (EXT)	5	0.28	0.00	9.52
General: Situs inversus (VIS)	4	0.22	0.00	4.55
MINOR VISCERAL AND EXTERNAL ANOMALIES (TOTAL)	132	7.22	0.00	33.33
Cranium: Pinna(e); displaced (EXT)	1	0.06	0.00	4.17
Cranium: Subcutaneous hematoma (WT)	1	0.06	0.00	4.17
Cranium: Cutis aplasia (EXT,WT)	1	0.06	0.00	4.00
Cranium: Cyst-like formation subcutaneously (WT)	1	0.06	0.00	4.00
Cranium: Moderate dilatation of the third ventricle (WT)	3	0.17	0.00	4.55
Cranium: Moderate dilatation of the lateral ventricles (WT)	1	0.06	0.00	4.55
Eye(s): Lens(es) oval (WT)	8	0.44	0.00	9.52
Eye(s): Hematoma adjacent to eye(s) (WT)	2	0.11	0.00	5.00
Face: Protruding tongue (EXT)	1	0.06	0.00	5.00
Nasal septum: Reduction in turbinate formation (WT)	1	0.06	0.00	4.00
Heart: Innominate artery absent (VIS)	13	0.72	0.00	10.53
Heart: Innominate artery reduced (VIS)	1	0.06	0.00	5.00
Heart: Innominate artery malpositioned (VIS)	1	0.06	0.00	4.76
Liver: Discoloration pale (VIS)	1	0.06	0.00	4.17
Liver: Supernumery lobes (VIS)	7	0.39	0.00	10.00
Liver: Vestigial lobe (VIS)	1	0.06	0.00	5.00
Spleen: Small (VIS)	1	0.06	0.00	4.55
Kidney(s): Reduction of renal papilla(e) (VIS)	9	0.50	0.00	16.67
Kidney(s): Reduced (VIS)	2	0.11	0.00	5.00
Ureter(s): Dilation (VIS)	69	3.81	0.00	27.27
Ureter(s): Megaureter (VIS)	22	1.21	0.00	13.64
Adrenal gland(s): Hemorrhage (VIS)	1	0.06	0.00	4.76
Testes: Malpositioned (VIS)	1	0.06	0.00	4.76
Skin: Pale (EXT)	1	0.06	0.00	4.17
Tail: Kinked (EXT)	3	0.17	0.00	4.55
General: Subcutaneous hematoma (EXT)	2	0.11	0.00	8.00

HISTORICAL CONTROL DATA
RAT - CD® IGS (Crl:CDISD) 1996-2007

ALL ROUTES OF ADMINISTRATION
GESTATION DAY 20*

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EMBRYO-FETAL DEVELOPMENT STUDIES GROUP INCIDENCE OF FETAL EXTERNAL, VISCERAL AND SKELETAL FINDINGS MAJOR MALFORMATIONS AND MINOR ANOMALIES				
SKELETAL (SKE)	Litters examined 806		Total no. of studies used 36	
	Litters affected			
MINOR SKELETAL ANOMALIES (TOTAL)	SUM 619	AVERAGE % 76.80	MIN % 27.27	MAX % 95.83
SKULL				
Frontal bone(s): Reduced ossification	5	0.62	0.00	5.26
Frontal bone(s): Irregular ossification	1	0.12	0.00	4.17
Parietal bone(s): Reduced ossification	52	6.45	0.00	23.81
Parietal bone(s): Irregular ossification	5	0.62	0.00	9.09
Supraoccipital bone: Reduced ossification	110	13.65	0.00	42.86
Supraoccipital bone: Irregular ossification	121	15.01	0.00	55.00
Interparietal bone: Reduced ossification	170	21.09	0.00	77.27
Interparietal bone: Irregular ossification	224	27.79	0.00	75.00
Hyoid bone: Absent	6	0.74	0.00	9.52
Hyoid bone: Reduced ossification	365	45.29	0.00	80.00
Hyoid bone: Irregular ossification	4	0.50	0.00	12.50
Extra suture(s) in frontal/parietal bone(s)	1	0.12	0.00	4.00
VERTEBRAL COLUMN				
Extra pre-sacral vertebra(e)	7	0.87	0.00	5.00
25 pre-sacral vertebrae	6	0.74	0.00	10.53
Lumbar centrum: Bipartite	1	0.12	0.00	4.17
Lumbar centrum: Semi-bipartite	6	0.74	0.00	8.33
Lumbar vertebral centrum: Absent	1	0.12	0.00	4.76
Lumbar vertebral arch(es): Reduced ossification	1	0.12	0.00	5.00
Lumbar vertebral arch(es): Irregular ossification	0	0.00	0.00	0.00
Ossification center on 1st lumbar vertebra or 14th thoracic vertebra	170	21.09	4.17	50.00
Sacral vertebra(e): Reduced no.	1	0.12	0.00	4.17
Sacral vertebral centrum: Absent	2	0.25	0.00	4.76
Sacral vertebral centrum: Reduced ossification	3	0.37	0.00	5.26
Sacral vertebral arch(es): Reduced ossification	23	2.85	0.00	24.00
Caudal vertebra(e): Reduced no.	12	1.49	0.00	5.26
Thoracic centrum: Misaligned	1	0.12	0.00	4.76
Thoracic centrum: Reduced ossification	2	0.25	0.00	4.55
Thoracic vertebral centra: Misaligned	1	0.12	0.00	4.17
Thoracic vertebral centrum: Displaced	2	0.25	0.00	4.55
Thoracic vertebral arch(es): Fused	1	0.12	0.00	4.17
Thoracic vertebral arch(es): Absent	2	0.25	0.00	4.55
Thoracic vertebral arch(es): Reduced ossification	1	0.12	0.00	4.17

* = Last study conducted in 2003

HISTORICAL CONTROL DATA
RAT - CD® IGS (Crl:CD[SD]), 1996-2007

ALL ROUTES OF ADMINISTRATION
GESTATION DAY 20*

b(4)

EMBRYO-FETAL DEVELOPMENT STUDIES GROUP INCIDENCE OF FETAL EXTERNAL, VISCERAL AND SKELETAL FINDINGS MAJOR MALFORMATIONS AND MINOR ANOMALIES				
	Litters affected			
MINOR SKELETAL ANOMALIES (CONT'D)	SUM	AVERAGE %	MIN %	MAX %
STERNEBRAL COLUMN				
Extra	0	0.00	0.00	0.00
Fused	2	0.25	0.00	4.76
Misaligned	3	0.37	0.00	5.00
Sternebrae: All absent	4	0.50	0.00	5.26
RIBS				
Absent	2	0.25	0.00	4.55
Fused	1	0.12	0.00	4.17
Reduced	28	3.47	0.00	13.64
Nodule(s)	4	0.50	0.00	5.26
Extra 14th rib	7	0.87	0.00	15.00
Rudimentary 14th rib	60	7.44	0.00	35.00
Wavy	4	0.50	0.00	5.00
Ossification center(s) on 7th cervical vertebra(e)	12	1.49	0.00	19.05
Rib(s) on 7th cervical vertebra(e)	8	0.99	0.00	16.67
Extra 14th rib with contralateral rudimentary rib	1	0.12	0.00	5.26
Rudimentary 14th rib with contralateral ossification center	16	1.99	0.00	22.73
PELVIC GIRDLE				
Pubic bone(s): Absent	5	0.62	0.00	5.26
Pubic bone(s): Reduced ossification	146	18.11	0.00	45.83
Pubic bone(s): Irregular ossification	2	0.25	0.00	9.09
Ischial bone(s): Absent	1	0.12	0.00	3.85
Ischial bone(s): Reduced ossification	37	4.59	0.00	20.00
Ischial bone(s): Irregular ossification	1	0.12	0.00	4.55
Ilial bone(s): Reduced ossification	1	0.12	0.00	4.55
LIMBS				
Reduced no. of phalange(s) in forepaw(s)	2	0.25	0.00	4.76
Reduced no. of phalange(s) in hindpaw(s)	2	0.25	0.00	4.76

* = Last study conducted in 2003

HISTORICAL CONTROL DATA
RAT - CD® IGS (Crl:CD[SD]), 1996-2007

b(4)

ALL ROUTES OF ADMINISTRATION
GESTATION DAY 21

EMBRYO-FETAL DEVELOPMENT STUDIES GROUP INCIDENCE OF FETAL EXTERNAL, VISCERAL AND SKELETAL FINDINGS MAJOR MALFORMATIONS AND MINOR ANOMALIES				
SKELETAL (SKE)	Litters examined 1006		Total no. of studies used 46	
	Litters affected			
MINOR SKELETAL ANOMALIES (TOTAL)	SUM 756	AVERAGE % 75.15	MIN % 45.45	MAX % 95.45
SKULL				
Frontal bone(s): Incomplete ossification	28	2.78	0.00	19.05
Parietal bone(s): Incomplete ossification	121	12.03	0.00	47.83
[Parietal bone(s): Reduced ossification]	1	0.10	0.00	4.17
[Parietal bone(s): Irregular ossification]	1	0.10	0.00	4.17
Supraoccipital bone: Incomplete ossification	43	4.27	0.00	19.05
[Supraoccipital bone: Reduced ossification]	1	0.10	0.00	4.17
Interparietal bone: Incomplete ossification	317	31.51	0.00	72.73
[Interparietal bone: Reduced ossification]	10	0.99	0.00	41.67
Hyoid bone: Unossified	3	0.30	0.00	9.09
Hyoid bone: Incomplete ossification	399	39.66	0.00	68.18
[Hyoid bone: Reduced ossification]	9	0.89	0.00	37.50
Extra suture(s) in frontal/parietal bone(s)	0	0.00	0.00	0.00
Nasal bone(s): Incomplete ossification	2	0.20	0.00	4.55
VERTEBRAL COLUMN				
Cervical vertebral arch(es): Fused	1	0.10	0.00	4.17
Cervical vertebral arch(es): Incomplete ossification	1	0.10	0.00	4.17
Cervical vertebral arch(es): Absent	2	0.20	0.00	8.33
Cervical 7th and thoracic 1st vertebral arch(es): Fused	1	0.10	0.00	4.17
Extra pre-sacral vertebra(e)	11	1.09	0.00	8.70
25 pre-sacral vertebrae	2	0.20	0.00	4.00
Lumbar centrum: Bipartite	1	0.10	0.00	4.55
Lumbar centrum: Semi-bipartite	4	0.40	0.00	4.76
Lumbar vertebral centrum: Absent	0	0.00	0.00	0.00
Lumbar vertebral arch(es): Irregular ossification	1	0.10	0.00	5.00
Ossification center on 1st lumbar vertebra or 14th thoracic vertebra	371	36.88	8.33	72.73
Sacral vertebra(e): Reduced no.	0	0.00	0.00	0.00
Caudal vertebra(e): Reduced no.	0	0.00	0.00	0.00
Caudal vertebra(e): Bipartite	1	0.10	0.00	4.76
Thoracic centrum: Misaligned	1	0.10	0.00	4.55
Thoracic vertebral centra: Misaligned	0	0.00	0.00	0.00
Thoracic vertebral centrum: Displaced	1	0.10	0.00	5.00
Thoracic vertebral centrum: Fused	1	0.10	0.00	4.17

[ ] = Terminology/grading changed in 2002/2003

HISTORICAL CONTROL DATA
RAT - CD® IGS (Crl:CD(SD)), 1996-2007

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ALL ROUTES OF ADMINISTRATION
GESTATION DAY 21

EMBRYO-FETAL DEVELOPMENT STUDIES GROUP INCIDENCE OF FETAL EXTERNAL, VISCERAL AND SKELETAL FINDINGS MAJOR MALFORMATIONS AND MINOR ANOMALIES				
	Litters affected			
MINOR SKELETAL ANOMALIES (CONT'D)	SUM	AVERAGE %	MIN %	MAX %
VERTEBRAL COLUMN (CONT'D)				
Thoracic vertebral arch(es): Fused	2	0.20	0.00	4.17
Thoracic vertebral arch(es): Absent	2	0.20	0.00	4.76
Thoracic vertebral arch(es): Reduced ossification	1	0.10	0.00	4.17
Thoracic vertebral arch(es): Incomplete ossification	1	0.10	0.00	4.76
STERNEBRAL COLUMN				
Extra	4	0.40	0.00	8.00
Fused	3	0.30	0.00	4.76
Misaligned	0	0.00	0.00	0.00
Sternebrae: All absent	0	0.00	0.00	0.00
RIBS				
Absent	3	0.30	0.00	4.76
Fused	5	0.50	0.00	5.00
Reduced	3	0.30	0.00	8.33
Nodule(s)	14	1.39	0.00	9.09
Extra 14th rib	5	0.50	0.00	9.52
Rudimentary 14th rib	58	5.67	0.00	31.82
Wavy	9	0.89	0.00	8.33
Ossification center(s) on 7th cervical vertebra(e)	20	1.99	0.00	20.00
Ossification center(s) on 5th cervical vertebra(e)	1	0.10	0.00	4.17
Rib(s) on 7th cervical vertebra(e)	5	0.50	0.00	12.00
Rib(s) Incomplete ossification	19	1.89	0.00	9.52
Extra 14th rib with contralateral rudimentary rib	4	0.40	0.00	4.76
Rudimentary 14th rib with contralateral ossification center	28	2.78	0.00	12.50
PELVIC GIRDLE				
Pubic bone(s): Incomplete ossification	4	0.40	0.00	9.09
Ischial bone(s): Incomplete ossification	4	0.40	0.00	4.76
LIMBS				
Reduced no. of phalange(s) in forepaw(s)	0	0.00	0.00	0.00
Reduced no. of phalange(s) in hindpaw(s)	0	0.00	0.00	0.00

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HISTORICAL CONTROL DATA
RAT - CD® IGS (Crl:CD[SD]), 1996-2007

ALL ROUTES OF ADMINISTRATION

EMBRYO-FETAL DEVELOPMENT STUDIES GROUP INCIDENCE OF FETAL EXTERNAL, VISCERAL AND SKELETAL FINDINGS MAJOR MALFORMATIONS AND MINOR ANOMALIES				
	Fetuses examined			
EXTERNAL (EXT)	25228	Total no. of studies used		
VISCERAL (VIS)	12781	82		
SKELETAL (SKE)	12762			
TECHNIQUE OF WILSON (WT)	12630			
	Fetuses affected			
MAJOR MALFORMATIONS (TOTAL)	SUM 39	AVERAGE % 0.16	MIN % 0.00	MAX % 1.00
Cranium: Auditory/vestibular system; absent (WT)	1	0.01	0.00	0.53
Cranium: Auditory/vestibular system; reduced/ incomplete formation (WT)	2	0.02	0.00	0.67
Cranium: Cleft palate/lip (EXT,WT)	3	0.01	0.00	0.34
Cranium: Microtia (EXT)	1	0.00	0.00	0.33
Cranium: Microcephaly (EXT,WT)	2	0.01	0.00	0.41
Cranium: Exencephaly (EXT,SKE)	1	0.00	0.00	0.36
Brain: Hydrocephaly (EXT,WT)	4	0.02	0.00	0.66
Brain: Cerebrum, cyst-like formation (WT)	1	0.01	0.00	0.53
Brain: Lateral ventricles reduced (WT)	1	0.01	0.00	0.53
Eye(s): Anophthalmia (EXT,WT)	6	0.02	0.00	0.41
Eye(s): Exophthalmia (EXT,WT)	1	0.00	0.00	0.27
Eye(s): Microphthalmia (WT)	2	0.02	0.00	0.58
Eye(s): Open (EXT,WT)	1	0.00	0.00	0.27
Eye(s): Retinal folding (WT)	2	0.02	0.00	0.65
Eye(s): Aphakia (WT)	1	0.01	0.00	0.65
Face: Aglossia (EXT,WT)	3	0.01	0.00	0.64
Face: Agnathia (EXT,WT)	5	0.02	0.00	0.64
Face: Astomia (EXT,WT)	3	0.01	0.00	0.41
Face: Micrognathia (EXT,WT)	1	0.00	0.00	0.32
Face: Mandibular micrognathia (EXT)	5	0.02	0.00	0.36
Face: Microstomia (EXT,WT)	1	0.00	0.00	0.27
Face: Nares; reduced (EXT,WT)	1	0.00	0.00	0.27
Face: Nares opening; reduced (WT)	1	0.01	0.00	0.63
Face: Nasal septum/turbinate formation; reduced (WT)	1	0.01	0.00	0.63
Face: Nasal septum; lack of turbinate formation (WT)	1	0.01	0.00	0.67
Face: Palate; absent (WT)	3	0.02	0.00	1.27
Face: Split tongue (WT)	1	0.01	0.00	0.86
Face: Upper jaw absent (EXT)	2	0.01	0.00	0.41
Face: Nares absent (EXT)	1	0.00	0.00	0.32
Face: Probosis (EXT,WT)	1	0.00	0.00	0.41
Heart: Interventricular septal defect (VIS)	2	0.02	0.00	0.73
Heart: Dilatation of ascending aorta (VIS)	1	0.01	0.00	0.63
Heart: Right descending aorta (VIS)	1	0.01	0.00	0.66

QA'd 20-Feb-09
20Feb09

HISTORICAL CONTROL DATA
RAT - CD® IGS (Crl:CD(SD)) 1996-2007

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ALL ROUTES OF ADMINISTRATION

EMBRYO-FETAL DEVELOPMENT STUDIES GROUP INCIDENCE OF FETAL EXTERNAL, VISCERAL AND SKELETAL FINDINGS MAJOR MALFORMATIONS AND MINOR ANOMALIES				
	Fetuses affected			
MAJOR MALFORMATIONS (CONT'D)	SUM	AVERAGE %	MIN %	MAX %
Heart: Stenosis of ascending aorta (VIS)	1	0.01	0.00	0.73
Heart: Transposition of major vessels (VIS)	4	0.03	0.00	0.73
Heart: Globular heart (VIS)	1	0.01	0.00	0.73
Heart: Ringed aorta (VIS)	1	0.01	0.00	0.69
Diaphragm: Diaphragmatic hernia (VIS)	1	0.01	0.00	0.64
Lungs and thymus: Lung lobes; absent (VIS)	3	0.02	0.00	1.35
Lungs and thymus: Lung lobes; reduced (VIS)	1	0.01	0.00	0.68
Vertebral column: Multiple fusion and anomalies in vertebral column (SKE)	3	0.02	0.00	0.65
Thorax: Trunk shortened (EXT)	1	0.00	0.00	0.32
Abdomen: Anal atresia (EXT,VIS)	4	0.02	0.00	0.33
Abdomen: Gastroschisis (EXT)	1	0.00	0.00	0.27
Abdomen: Situs inversus (VIS)	1	0.01	0.00	0.73
Abdomen: Omphalocele (EXT)	5	0.02	0.00	0.36
Abdomen: Urogenital region fissure (EXT)	1	0.00	0.00	0.38
Abdomen: Abdominal muscles herniated (EXT)	1	0.00	0.00	0.31
Abdominal cavity: Colon blind (VIS)	1	0.01	0.00	0.53
Abdominal cavity: Malposition stomach/ pancreas/spleen (VIS)	1	0.01	0.00	0.66
Abdominal cavity: Intestine; stenosis (VIS)	1	0.01	0.00	0.66
Tail: Acaudia (EXT)	2	0.01	0.00	0.31
Tail: Microcaudia (EXT)	4	0.02	0.00	0.36
Limb(s): Ectrodactyly (EXT)	2	0.01	0.00	0.33
Limb(s): Brachydactyly (EXT)	2	0.01	0.00	0.33
Limb(s): Abnormal flexure of hindlimb(s) (EXT)	2	0.01	0.00	0.33
Limb(s): Hindpaw(s) absent (SKE)	1	0.01	0.00	0.61
Skull: Mandible shortened (SKE)	1	0.01	0.00	0.70
Skull: Maxilla shortened/Incisive bone (SKE)	1	0.01	0.00	0.70
Gross exam: Anasarca (EXT)	5	0.02	0.00	0.66
General: Situs inversus (VIS)	4	0.03	0.00	0.69
MINOR VISCERAL AND EXTERNAL ANOMALIES (TOTAL)	169	0.67	0.00	4.41
Cranium: Pinna(e); displaced (EXT)	1	0.00	0.00	0.32
Cranium: Subcutaneous hematoma (WT)	1	0.01	0.00	0.63
Cranium: Cutis aplasia (EXT,WT)	1	0.00	0.00	0.27
Cranium: Cyst-like formation subcutaneously (WT)	1	0.01	0.00	0.53
Cranium: Moderate dilatation of the third ventricle (WT)	3	0.02	0.00	0.66
Cranium: Moderate dilatation of the lateral ventricles (WT)	1	0.01	0.00	0.64
Eye(s): Lens(es) oval (WT)	10	0.08	0.00	2.07
Eye(s): Hematoma adjacent to eye(s) (WT)	2	0.02	0.00	0.67
Face: Protruding tongue (EXT)	1	0.00	0.00	0.36
Nasal septum: Reduction in turbinate formation (WT)	1	0.01	0.00	0.53
Heart: Innominate artery absent (VIS)	14	0.11	0.00	2.07
Heart: Innominate artery reduced (VIS)	1	0.01	0.00	0.72
Heart: Innominate artery malpositioned (VIS)	1	0.01	0.00	0.68
Liver: Discoloration pale (VIS)	1	0.01	0.00	0.57
Liver: Supernumery lobes (VIS)	8	0.06	0.00	1.74
Liver: Vestigial lobe (VIS)	1	0.01	0.00	0.69
Spleen: Small (VIS)	1	0.01	0.00	0.64
Kidney(s): Reduction of renal papilla(e) (VIS)	10	0.08	0.00	2.58
Kidney(s): Reduced (VIS)	2	0.02	0.00	0.66
Ureter(s): Dilation (VIS)	93	0.73	0.00	8.00
Ureter(s): Megaureter (VIS)	25	0.20	0.00	2.76
Adrenal gland(s): Hemorrhage (VIS)	1	0.01	0.00	0.69
Testes: Malpositioned (VIS)	1	0.01	0.00	0.69
Skin: Pale (EXT)	1	0.00	0.00	0.28
Tail: Kinked (EXT)	3	0.01	0.00	0.32
General: Subcutaneous hematoma (EXT)	2	0.01	0.00	0.54

HISTORICAL CONTROL DATA
RAT - CD® IGS (Crl:CD[SD]), 1996-2007

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ALL ROUTES OF ADMINISTRATION
GESTATION DAY 20*

EMBRYO-FETAL DEVELOPMENT STUDIES GROUP INCIDENCE OF FETAL EXTERNAL, VISCERAL AND SKELETAL FINDINGS MAJOR MALFORMATIONS AND MINOR ANOMALIES				
SKELETAL (SKE)	Fetuses examined 5951		Total no. of studies used 36	
	Fetuses affected			
MINOR SKELETAL ANOMALIES (TOTAL)	SUM 1721	AVERAGE % 28.92	MIN % 6.63	MAX % 50.00
SKULL				
Frontal bone(s): Reduced ossification	6	0.10	0.00	1.28
Frontal bone(s): Irregular ossification	1	0.02	0.00	0.54
Parietal bone(s): Reduced ossification	65	1.09	0.00	5.74
Parietal bone(s): Irregular ossification	7	0.12	0.00	1.81
Supraoccipital bone: Reduced ossification	179	3.01	0.00	11.51
Supraoccipital bone: Irregular ossification	178	2.99	1.27	12.67
Interparietal bone: Reduced ossification	309	5.19	0.00	23.02
Interparietal bone: Irregular ossification	438	7.36	0.00	25.33
Hyoid bone: Absent	7	0.12	0.00	2.03
Hyoid bone: Reduced ossification	816	13.71	8.92	36.00
Hyoid bone: Irregular ossification	5	0.08	0.00	2.29
Extra suture(s) in frontal/parietal bone(s)	1	0.02	0.00	0.54
VERTEBRAL COLUMN				
Extra pre-sacral vertebra(e)	7	0.12	0.00	0.70
25 pre-sacral vertebrae	6	0.10	0.00	1.28
Lumbar centrum: Bipartite	1	0.02	0.00	0.56
Lumbar centrum: Semi-bipartite	6	0.10	0.00	1.12
Lumbar vertebral centrum: Absent	1	0.02	0.00	0.65
Lumbar vertebral arch(es): Reduced ossification	1	0.02	0.00	0.70
Lumbar vertebral arch(es): Irregular ossification	0	0.00	0.00	0.00
Ossification center on 1st lumbar vertebra or 14th thoracic vertebra	239	4.02	0.54	10.43
Sacral vertebra(e): Reduced no.	1	0.02	0.00	0.56
Sacral vertebral centrum: Absent	2	0.03	0.00	0.65
Sacral vertebral centrum: Reduced ossification	3	0.05	0.00	0.82
Sacral vertebral arch(es): Reduced ossification	33	0.55	0.00	6.13
Caudal vertebra(e): Reduced no.	14	0.24	0.00	2.03
Thoracic centrum: Misaligned	1	0.02	0.00	0.65
Thoracic centrum: Reduced ossification	2	0.03	0.00	0.69
Thoracic vertebral centra: Misaligned	1	0.02	0.00	0.64
Thoracic vertebral centrum: Displaced	2	0.03	0.00	0.69
Thoracic vertebral arch(es): Fused	1	0.02	0.00	0.64
Thoracic vertebral arch(es): Absent	2	0.03	0.00	0.69
Thoracic vertebral arch(es): Reduced ossification	1	0.02	0.00	0.64

* = Last study conducted in 2003

QA'd 20-Feb-09
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HISTORICAL CONTROL DATA
RAT - CD® IGS (Crl:CD(SD)) 1996-2007

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ALL ROUTES OF ADMINISTRATION

EMBRYO-FETAL DEVELOPMENT STUDIES GROUP INCIDENCE OF FETAL EXTERNAL, VISCERAL AND SKELETAL FINDINGS MAJOR MALFORMATIONS AND MINOR ANOMALIES				
	Fetuses affected			
MAJOR MALFORMATIONS (CONT'D)	SUM	AVERAGE %	MIN %	MAX %
Heart: Stenosis of ascending aorta (VIS)	1	0.01	0.00	0.73
Heart: Transposition of major vessels (VIS)	4	0.03	0.00	0.73
Heart: Globular heart (VIS)	1	0.01	0.00	0.73
Heart: Ringed aorta (VIS)	1	0.01	0.00	0.69
Diaphragm: Diaphragmatic hernia (VIS)	1	0.01	0.00	0.64
Lungs and thymus: Lung lobes; absent (VIS)	3	0.02	0.00	1.35
Lungs and thymus: Lung lobes; reduced (VIS)	1	0.01	0.00	0.68
Vertebral column: Multiple fusion and anomalies in vertebral column (SKE)	3	0.02	0.00	0.65
Thorax: Trunk shortened (EXT)	1	0.00	0.00	0.32
Abdomen: Anal atresia (EXT,VIS)	4	0.02	0.00	0.33
Abdomen: Gastroschisis (EXT)	1	0.00	0.00	0.27
Abdomen: Situs inversus (VIS)	1	0.01	0.00	0.73
Abdomen: Omphalocele (EXT)	5	0.02	0.00	0.36
Abdomen: Urogenital region fissure (EXT)	1	0.00	0.00	0.38
Abdomen: Abdominal muscles herniated (EXT)	1	0.00	0.00	0.31
Abdominal cavity: Colon blind (VIS)	1	0.01	0.00	0.53
Abdominal cavity: Malposition stomach/ pancreas/spleen (VIS)	1	0.01	0.00	0.66
Abdominal cavity: Intestine; stenosis (VIS)	1	0.01	0.00	0.66
Tail: Acaudia (EXT)	2	0.01	0.00	0.31
Tail: Microcaudia (EXT)	4	0.02	0.00	0.36
Limb(s): Ectrodactyly (EXT)	2	0.01	0.00	0.33
Limb(s): Brachydactyly (EXT)	2	0.01	0.00	0.33
Limb(s): Abnormal flexure of hindlimb(s) (EXT)	2	0.01	0.00	0.33
Limb(s): Hindpaw(s) absent (SKE)	1	0.01	0.00	0.61
Skull: Mandible shortened (SKE)	1	0.01	0.00	0.70
Skull: Maxilla shortened/Incisive bone (SKE)	1	0.01	0.00	0.70
Gross exam: Anasarca (EXT)	5	0.02	0.00	0.66
General: Situs inversus (VIS)	4	0.03	0.00	0.69
MINOR VISCERAL AND EXTERNAL ANOMALIES (TOTAL)	169	0.67	0.00	4.41
Cranium: Pinna(e); displaced (EXT)	1	0.00	0.00	0.32
Cranium: Subcutaneous hematoma (WT)	1	0.01	0.00	0.63
Cranium: Cutis aplasia (EXT,WT)	1	0.00	0.00	0.27
Cranium: Cyst-like formation subcutaneously (WT)	1	0.01	0.00	0.53
Cranium: Moderate dilatation of the third ventricle (WT)	3	0.02	0.00	0.66
Cranium: Moderate dilatation of the lateral ventricles (WT)	1	0.01	0.00	0.64
Eye(s): Lens(es) oval (WT)	10	0.08	0.00	2.07
Eye(s): Hematoma adjacent to eye(s) (WT)	2	0.02	0.00	0.67
Face: Protruding tongue (EXT)	1	0.00	0.00	0.36
Nasal septum: Reduction in turbinate formation (WT)	1	0.01	0.00	0.53
Heart: Innominate artery absent (VIS)	14	0.11	0.00	2.07
Heart: Innominate artery reduced (VIS)	1	0.01	0.00	0.72
Heart: Innominate artery malpositioned (VIS)	1	0.01	0.00	0.68
Liver: Discoloration pale (VIS)	1	0.01	0.00	0.57
Liver: Supernumery lobes (VIS)	8	0.06	0.00	1.74
Liver: Vestigial lobe (VIS)	1	0.01	0.00	0.69
Spleen: Small (VIS)	1	0.01	0.00	0.64
Kidney(s): Reduction of renal papilla(e) (VIS)	10	0.08	0.00	2.58
Kidney(s): Reduced (VIS)	2	0.02	0.00	0.66
Ureter(s): Dilation (VIS)	93	0.73	0.00	8.00
Ureter(s): Megaureter (VIS)	25	0.20	0.00	2.76
Adrenal gland(s): Hemorrhage (VIS)	1	0.01	0.00	0.69
Testes: Malpositioned (VIS)	1	0.01	0.00	0.69
Skin: Pale (EXT)	1	0.00	0.00	0.28
Tail: Kinked (EXT)	3	0.01	0.00	0.32
General: Subcutaneous hematoma (EXT)	2	0.01	0.00	0.54

HISTORICAL CONTROL DATA
RAT - CD® IGS (Crl:CD[SD]), 1996-2007

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ALL ROUTES OF ADMINISTRATION
GESTATION DAY 20*

EMBRYO-FETAL DEVELOPMENT STUDIES				
GROUP INCIDENCE OF FETAL EXTERNAL, VISCERAL AND SKELETAL FINDINGS MAJOR MALFORMATIONS AND MINOR ANOMALIES				
SKELETAL (SKE)	Fetuses examined 5951		Total no. of studies used 36	
	Fetuses affected			
MINOR SKELETAL ANOMALIES (TOTAL)	SUM 1721	AVERAGE % 28.92	MIN % 6.63	MAX % 50.00
SKULL				
Frontal bone(s): Reduced ossification	6	0.10	0.00	1.28
Frontal bone(s): Irregular ossification	1	0.02	0.00	0.54
Parietal bone(s): Reduced ossification	65	1.09	0.00	5.74
Parietal bone(s): Irregular ossification	7	0.12	0.00	1.81
Supraoccipital bone: Reduced ossification	179	3.01	0.00	11.51
Supraoccipital bone: Irregular ossification	178	2.99	1.27	12.67
Interparietal bone: Reduced ossification	309	5.19	0.00	23.02
Interparietal bone: Irregular ossification	438	7.36	0.00	25.33
Hyoid bone: Absent	7	0.12	0.00	2.03
Hyoid bone: Reduced ossification	816	13.71	8.92	36.00
Hyoid bone: Irregular ossification	5	0.08	0.00	2.29
Extra suture(s) in frontal/parietal bone(s)	1	0.02	0.00	0.54
VERTEBRAL COLUMN				
Extra pre-sacral vertebra(e)	7	0.12	0.00	0.70
25 pre-sacral vertebrae	6	0.10	0.00	1.28
Lumbar centrum: Bipartite	1	0.02	0.00	0.56
Lumbar centrum: Semi-bipartite	6	0.10	0.00	1.12
Lumbar vertebral centrum: Absent	1	0.02	0.00	0.65
Lumbar vertebral arch(es): Reduced ossification	1	0.02	0.00	0.70
Lumbar vertebral arch(es): Irregular ossification	0	0.00	0.00	0.00
Ossification center on 1st lumbar vertebra or 14th thoracic vertebra	239	4.02	0.54	10.43
Sacral vertebra(e): Reduced no.	1	0.02	0.00	0.56
Sacral vertebral centrum: Absent	2	0.03	0.00	0.65
Sacral vertebral centrum: Reduced ossification	3	0.05	0.00	0.82
Sacral vertebral arch(es): Reduced ossification	33	0.55	0.00	6.13
Caudal vertebra(e): Reduced no.	14	0.24	0.00	2.03
Thoracic centrum: Misaligned	1	0.02	0.00	0.65
Thoracic centrum: Reduced ossification	2	0.03	0.00	0.69
Thoracic vertebral centra: Misaligned	1	0.02	0.00	0.64
Thoracic vertebral centrum: Displaced	2	0.03	0.00	0.69
Thoracic vertebral arch(es): Fused	1	0.02	0.00	0.64
Thoracic vertebral arch(es): Absent	2	0.03	0.00	0.69
Thoracic vertebral arch(es): Reduced ossification	1	0.02	0.00	0.64

* = Last study conducted in 2003

QA'd 20-Feb-09
Lam 25Feb09

HISTORICAL CONTROL DATA
RAT - CD® IGS (Crl:CD(SD)). 1996-2007

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ALL ROUTES OF ADMINISTRATION
GESTATION DAY 20*

EMBRYO-FETAL DEVELOPMENT STUDIES GROUP INCIDENCE OF FETAL EXTERNAL, VISCERAL AND SKELETAL FINDINGS MAJOR MALFORMATIONS AND MINOR ANOMALIES				
	Fetuses affected			
MINOR SKELETAL ANOMALIES (CONT'D)	SUM	AVERAGE %	MIN %	MAX %
STERNEBRAL COLUMN				
Extra	0	0.00	0.00	0.00
Fused	2	0.03	0.00	0.65
Misaligned	3	0.05	0.00	0.73
Sternebrae: All absent	4	0.07	0.00	0.82
RIBS				
Absent	2	0.03	0.00	0.69
Fused	1	0.02	0.00	0.64
Reduced	30	0.50	0.00	1.92
Nodule(s)	8	0.13	0.00	3.50
Extra 14th rib	7	0.12	0.00	2.08
Rudimentary 14th rib	81	1.36	0.00	5.71
Wavy	4	0.07	0.00	0.72
Ossification center(s) on 7th cervical vertebra(e)	14	0.24	0.00	3.60
Rib(s) on 7th cervical vertebra(e)	10	0.17	0.00	2.15
Extra 14th rib with contralateral rudimentary rib	1	0.02	0.00	0.64
Rudimentary 14th rib with contralateral ossification center	19	0.32	0.00	2.82
PELVIC GIRDLE				
Pubic bone(s): Absent	5	0.08	0.00	0.82
Pubic bone(s): Reduced ossification	243	4.08	0.00	15.05
Pubic bone(s): Irregular ossification	2	0.03	0.00	1.20
Ischial bone(s): Absent	1	0.02	0.00	0.63
Ischial bone(s): Reduced ossification	41	0.69	0.00	2.15
Ischial bone(s): Irregular ossification	1	0.02	0.00	0.60
Ilial bone(s): Reduced ossification	1	0.02	0.00	0.60
LIMBS				
Reduced no. of phalange(s) in forepaw(s)	2	0.03	0.00	0.65
Reduced no. of phalange(s) in hindpaw(s)	2	0.03	0.00	0.65

* = Last study conducted in 2003

QA'd 20-Feb-09

lam 20Feb09

HISTORICAL CONTROL DATA
RAT - CD® IGS (Crl:CDISD), 1996-2007

ALL ROUTES OF ADMINISTRATION
GESTATION DAY 21

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EMBRYO-FETAL DEVELOPMENT STUDIES GROUP INCIDENCE OF FETAL EXTERNAL, VISCERAL AND SKELETAL FINDINGS MAJOR MALFORMATIONS AND MINOR ANOMALIES				
SKELETAL (SKE)	Fetuses examined 6811		Total no. of studies used 46	
	Fetuses affected			
MINOR SKELETAL ANOMALIES (TOTAL)	SUM 1925	AVERAGE % 28.26	MIN % 9.32	MAX % 46.67
SKULL				
Frontal bone(s): Incomplete ossification	37	0.54	0.00	7.41
Parietal bone(s): Incomplete ossification	178	2.61	0.00	14.07
[Parietal bone(s): Reduced ossification]	2	0.03	0.00	1.08
[Parietal bone(s): Irregular ossification]	1	0.01	0.00	0.54
Supraoccipital bone: Incomplete ossification	50	0.73	0.00	4.17
[Supraoccipital bone: Reduced ossification]	1	0.01	0.00	0.54
Interparietal bone: Incomplete ossification	629	9.24	0.00	30.00
[Interparietal bone: Reduced ossification]	20	0.29	0.00	10.81
Hyoid bone: Unossified	3	0.04	0.00	1.42
Hyoid bone: Incomplete ossification	762	11.19	0.00	22.15
[Hyoid bone: Reduced ossification]	16	0.23	0.00	8.65
Extra suture(s) in frontal/parietal bone(s)	0	0.00	0.00	0.00
Nasal bone(s): Incomplete ossification	3	0.04	0.00	1.08
VERTEBRAL COLUMN				
Cervical vertebral arch(es): Fused	1	0.01	0.00	0.63
Cervical vertebral arch(es): Incomplete ossification	1	0.01	0.00	0.63
Cervical vertebral arch(es): Absent	2	0.03	0.00	1.27
Cervical 7th and thoracic 1st vertebral arch(es): Fused	1	0.01	0.00	0.63
Extra pre-sacral vertebra(e)	16	0.23	0.00	3.95
25 pre-sacral vertebrae	2	0.03	0.00	0.59
Lumbar centrum: Bipartite	1	0.01	0.00	0.65
Lumbar centrum: Semi-bipartite	4	0.04	0.00	0.70
Lumbar vertebral centrum: Absent	0	0.00	0.00	0.00
Lumbar vertebral arch(es): Irregular ossification	1	0.01	0.00	0.72
Ossification center on 1st lumbar vertebra or 14th thoracic vertebra	639	9.38	2.16	24.82
Sacral vertebra(e): Reduced no.	0	0.00	0.00	0.00
Caudal vertebra(e): Reduced no.	0	0.00	0.00	0.00
Caudal vertebra(e): Bipartite	1	0.01	0.00	0.69
Thoracic centrum: Misaligned	1	0.01	0.00	0.71
Thoracic vertebral centra: Misaligned	0	0.00	0.00	0.00
Thoracic vertebral centrum: Displaced	1	0.01	0.00	0.70
Thoracic vertebral centrum: Fused	1	0.01	0.00	0.63

[ ] = Terminology/grading changed in 2002/2003

QA'd 20-Feb-09

HISTORICAL CONTROL DATA
RAT - CD® IGS (Crl:CD[SD]), 1996-2007

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GESTATION DAY 21

EMBRYO-FETAL DEVELOPMENT STUDIES GROUP INCIDENCE OF FETAL EXTERNAL, VISCERAL AND SKELETAL FINDINGS MAJOR MALFORMATIONS AND MINOR ANOMALIES				
SKELETAL (SKE)	Fetuses examined 6811		Total no. of studies used 46	
	Fetuses affected			
MINOR SKELETAL ANOMALIES (TOTAL)	SUM 1925	AVERAGE % 28.26	MIN % 9.32	MAX % 46.67
SKULL				
Frontal bone(s): Incomplete ossification	37	0.54	0.00	7.41
Parietal bone(s): Incomplete ossification	178	2.61	0.00	14.07
[Parietal bone(s): Reduced ossification]	2	0.03	0.00	1.08
[Parietal bone(s): Irregular ossification]	1	0.01	0.00	0.54
Supraoccipital bone: Incomplete ossification	50	0.73	0.00	4.17
[Supraoccipital bone: Reduced ossification]	1	0.01	0.00	0.54
Interparietal bone: Incomplete ossification	629	9.24	0.00	30.00
[Interparietal bone: Reduced ossification]	20	0.29	0.00	10.81
Hyoid bone: Unossified	3	0.04	0.00	1.42
Hyoid bone: Incomplete ossification	762	11.19	0.00	22.15
[Hyoid bone: Reduced ossification]	16	0.23	0.00	8.65
Extra suture(s) in frontal/parietal bone(s)	0	0.00	0.00	0.00
Nasal bone(s): Incomplete ossification	3	0.04	0.00	1.08
VERTEBRAL COLUMN				
Cervical vertebral arch(es): Fused	1	0.01	0.00	0.63
Cervical vertebral arch(es): Incomplete ossification	1	0.01	0.00	0.63
Cervical vertebral arch(es): Absent	2	0.03	0.00	1.27
Cervical 7th and thoracic 1st vertebral arch(es): Fused	1	0.01	0.00	0.63
Extra pre-sacral vertebra(e)	16	0.23	0.00	3.95
25 pre-sacral vertebrae	2	0.03	0.00	0.59
Lumbar centrum: Bipartite	1	0.01	0.00	0.65
Lumbar centrum: Semi-bipartite	4	0.04	0.00	0.70
Lumbar vertebral centrum: Absent	0	0.00	0.00	0.00
Lumbar vertebral arch(es): Irregular ossification	1	0.01	0.00	0.72
Ossification center on 1st lumbar vertebra or 14th thoracic vertebra	639	9.38	2.16	24.82
Sacral vertebra(e): Reduced no.	0	0.00	0.00	0.00
Caudal vertebra(e): Reduced no.	0	0.00	0.00	0.00
Caudal vertebra(e): Bipartite	1	0.01	0.00	0.69
Thoracic centrum: Misaligned	1	0.01	0.00	0.71
Thoracic vertebral centra: Misaligned	0	0.00	0.00	0.00
Thoracic vertebral centrum: Displaced	1	0.01	0.00	0.70
Thoracic vertebral centrum: Fused	1	0.01	0.00	0.63

[ ] = Terminology/grading changed in 2002/2003

QA'd 20-Feb-09

KAm 20Feb09

HISTORICAL CONTROL DATA
RAT - CD® IGS (Crl:CD(SD)). 1996-2007

C 7 b(4)

ALL ROUTES OF ADMINISTRATION
GESTATION DAY 21

EMBRYO-FETAL DEVELOPMENT STUDIES
GROUP INCIDENCE OF FETAL EXTERNAL, VISCERAL AND SKELETAL FINDINGS
MAJOR MALFORMATIONS AND MINOR ANOMALIES

MINOR SKELETAL ANOMALIES (CONT'D)	Fetuses affected			
	SUM	AVERAGE %	MIN %	MAX %
VERTEBRAL COLUMN (CONT'D)				
Thoracic vertebral arch(es): Fused	2	0.03	0.00	0.63
Thoracic vertebral arch(es): Absent	2	0.03	0.00	0.71
Thoracic vertebral arch(es): Reduced ossification	1	0.01	0.00	0.54
Thoracic vertebral arch(es): Incomplete ossification	1	0.01	0.00	0.71
STERNEBRAL COLUMN				
Extra	4	0.06	0.00	1.08
Fused	3	0.04	0.00	0.71
Misaligned	0	0.00	0.00	0.00
Sternebrae: All absent	0	0.00	0.00	0.00
RIBS				
Absent	3	0.04	0.00	0.71
Fused	5	0.07	0.00	0.71
Reduced	3	0.04	0.00	1.08
Nodule(s)	17	0.25	0.00	2.04
Extra 14th rib	5	0.07	0.00	1.39
Rudimentary 14th rib	73	1.06	0.00	7.09
Wavy	11	0.16	0.00	1.43
Ossification center(s) on 7th cervical vertebra(e)	20	0.29	0.00	2.53
Ossification center(s) on 5th cervical vertebra(e)	1	0.01	0.00	0.63
Rib(s) on 7th cervical vertebra(e)	5	0.07	0.00	1.61
Rib(s) Incomplete ossification	21	0.31	0.00	1.61
Extra 14th rib with contralateral rudimentary rib	5	0.07	0.00	1.32
Rudimentary 14th rib with contralateral ossification center	33	0.48	0.00	3.47
PELVIC GIRDLE				
Pubic bone(s): Incomplete ossification	5	0.07	0.00	1.48
Ischial bone(s): Incomplete ossification	8	0.12	0.00	3.70
LIMBS				
Reduced no. of phalange(s) in forepaw(s)	0	0.00	0.00	0.00
Reduced no. of phalange(s) in hindpaw(s)	0	0.00	0.00	0.00

QA'd 20-Feb-09

20Feb09

2.6.6.7 Local tolerance

Bovine Corneal Opacity and Permeability Assay (2002)

The effect of saxagliptin on corneal opacity and permeability was tested in vitro using corneal tissue from animals from slaughter house. Saxagliptin (20%) had no notable effect on corneal opacity or permeability. The positive control, imidazole (20%) significantly increased corneal opacity.

BCOP Results of the Test Article

Date	IIVS Test Article Number	Sponsor's Designation	Conc. (w/v)	Exposure Time	Opacity Value	OD_{490} Value	*In Vitro* Score	pH (dosing solution)
5/23/02	02AD03	BMS 477118-08	20%	4 hours	-1.3	-0.001	-1.3	6.0

BCOP Results of the Positive Control

Date	Positive Control	Conc. (w/v)	Exposure Time	Opacity Value	OD_{490} Value	*In Vitro* Score
5/23/02	Imidazole	20%	4 hours	57.3	2.033	87.8

in vitro score:

from 0 to 25	= mild irritant
from 25.1 to 55	= moderate irritant
from 55.1 and above	= severe irritant

Local Lymph Node assay in Mouse:

Skin sensitivity to saxagliptin (99.4% batch purity) was tested using local lymph node assay in mouse. Repeated exposures of the skin to certain chemicals have been shown to produce immunologically-mediated delayed-onset hypersensitivity. This assay has the potential to assess any delayed contact hypersensitivity by examining lymphocyte proliferation at lymph nodes close to the topical application sites (i.e. ear). For the assay, auditory pinnae in the ear were painted for 3 days with saxagliptin (0.5, 1, 2.5, 5, 10 and 25% in dimethylformamide), or vehicle. On day 6, 20 µCi of tritiated thymidine was injected by IV route and local lymph nodes were biopsied 5 hours post injection. The proliferative indices were determined (the ratio of the scintillation count per lymph node obtained from a test group relative to the corresponding scintillation count from controls). The threshold level for the Proliferation Index to be considered a positive indicator of moderate to severe potential to cause skin sensitization is 3.0. The assay showed that saxagliptin has the potential to cause sensitization (>3.0) when applied topically to skin.

Animals dosed 12-14 June 2002	Concentration of test article in applied formulation (%m/v)		
	0.5%	1.0%	2.5%
Proliferation Index	3.2	7.0	4.1

Animals dosed 3-5 July 2002	Concentration of test article in applied formulation (%m/v)		
	5%	10%	25%
Proliferation Index	9.1	19.1	14.4

Skin Irritation Study in the rabbit, May 2002

The potential for saxagliptin to cause skin irritation was tested at the request of the sponsor at ( ) Intact rabbit skin was exposed to single (4-hour), semi-occluded topical application of saxagliptin (500 mg). Briefly, 500 mg of saxagliptin was topically applied to shaved skin (30x20 mm area) on the dorsum of New Zealand white rabbits on Day 1 of the test. Reaction to the topical drug application was evaluated for four hours. There was no reaction following single semi-occluded, topical application of saxagliptin to the intact rabbit skin for 4 hours. Based on this study, saxagliptin did not appear to be an irritant to rabbit skin. Since this study was very short in duration, the significance of a negative finding is relatively limited. b(4)

Individual dermal reactions (single patch test)

Animal number and sex	Dermal changes	Time after completion of exposure				
		0 hr	1 hr	24 hr	48 hr	72 hr
38♀ (sentinel)	Erythema	0	0	0	0	0
	Oedema	0	0	0	0	0
41♀	Erythema	NA	0	0	0	0
	Oedema		0	0	0	0
42♀	Erythema	NA	0	0	0	0
	Oedema		0	0	0	0

NA Not applicable

Group mean values for dermal reactions

Time of observation	Erythema	Oedema
1 hr	0	0
24 hr	0	0
48 hr	0	0
72 hr	0	0
Mean of 24, 48 and 72 hour scores	0	0

2.6.6.8 Special toxicology studies

One-Month Oral Study of T-Cell-Dependent Antibody Response in Rats (Immunotoxicology, Study # DS02082)

Key study findings:

- Antibody response to the T-cell-dependent antigen (KLH) decreased notably (but not significantly) in males by 51-56% at doses ≥ 50mg/kg. The antibody response to KLH in females was far less effected (8-13% lower vs. control). By comparison, the positive control dexamethasone suppressed immune responses by 95% in males and 82% in females.
- Changes in T-cell subsets were variable and not statistically significant. At the highest dose of 200mg/kg, there was a trend for an increased proportion of $CD3^+$, $CD4^+$, and $CD8^+$ subsets without a change in CD45RA.
- Target organs of toxicity include the spleen (lymphoid hyperplasia), mesenteric lymph node (lymphoid hyperplasia), and mandibular lymph node (lymphoid and plasma cell hyperplasia).
- Saxagliptin at 10mg/kg had minimal or no effect on the primary immune response to KLH. A reduced immune response appears to have occurred in males at doses ≥50mg/kg, or approximately 100-fold higher than human exposure for the parent and 10-fold higher for the metabolite.

Study no: DS02082
Volume #, and page #: Vol. 2, pg. 001 (Serial # 012).
Conducting laboratory and location: Bristol-Myers Squibb Pharmaceutical Research Institute Departments of Toxicology and Pathology Syracuse, New York USA
Date of study initiation: May 23, 2002.
GLP compliance: Yes (USA).
QA reports: Yes (X) no ( ):
Drug, lot #, radiolabel, and % purity: BMS-477118 Batch # 2D52273, 66.9% pure.
Formulation/vehicle: Suspension of BMS-477118 in 1.25% Avicel.

Methods:
Dosing: Suspensions of BMS-477118 in 1.25% Avicel® or 1.25% Avicel® alone (vehicle control) were administered by oral garage once daily for 28 days. On days 20 - 28, dexamethasone (immunosuppressant) was administered to the positive control group (Group 5) by oral garage. All rats were dosed according to the following experimental design:

Experimental Design

Group Number	Test Article [a]	Daily Dose (mg/kg)	Dose Volume (ml/kg)	Concentration (mg/ml)	Number of Animals
1	Vehicle control [b]	0	10	0	10 M, 10 F
2	BMS-477118	10	10	1	10 M, 10 F
3	BMS-477118	50	10	5	10 M, 10 F
4	BMS-477118	200	10	20	10 M, 10 F
5	Positive control [c]	5	2.5	2	10 M, 10 F

[a] All animals administered 1 mg KLH prior to test article administration on day 23.
[b] 1.25% Avicel®.
[c] Dexamethasone, oral gavage.

On day 23, all animals were administered 1 mg of Keyhole Limpet Hemocyanin (KLH) immunogen (0.2 ml of a 5 mg/ml suspension) subcutaneously prior to administration of vehicle, test article or positive control. On day 29, 6 days following KLH administration, blood samples were obtained from each animal (Groups 1 - 5) just prior to necropsy. The whole blood was processed for serum, to quantitate KLH-specific antibodies. The KLH-specific antibody responses were measured by enzyme-linked immunosorbent assays (ELISA).

Species/strain: Rat/Sprague-Dawley Crl:CD®(SD)IGS.
#/sex/group or time point (main study): 10/sex/group
Satellite groups used for toxicokinetics or recovery: Not applicable.
Age: 6-8 weeks old.
Weight: 213-239 g (M); 151-175 G (F).
Doses in administered units: 10, 50, 200 mg/kg.
Route, form, volume, and infusion rate: Oral (gavage), 10 ml/kg.

Observations and times:
Clinical signs: Daily.
Body weights: Predose, then twice weekly.
Food consumption: Predose, then weekly.

Hematology: On day 26 of treatment, blood samples for hematology evaluation were obtained from all surviving fasted animals in the 0, 10, 50 and 200 mg/kg dose groups, prior to a daily dose on day 26.

Clinical chemistry: On day 26 of treatment, blood samples for clinical chemistry evaluation were obtained from all surviving fasted animals in the 0, 10, 50 and 200 mg/kg dose groups, prior to a daily dose on day 26.

Urinalysis: Not conducted.

Gross pathology: Examinations were made on all animals in the 0, 10, 50 and 200 mg/kg dose groups including the one female that was euthanatized on day 26 because of poor physical condition. Limited necropsy was conducted on all animals in the 0, 10, 50 and 200 mg/kg dose groups and included gross examination of lymph nodes (mandibular and mesenteric), spleen, thymus, and gross lesions.

Organs weighed: The spleen and thymus were weighed for all animals in the 0, 10, 50 and 200 mg/kg dose groups that survived to scheduled necropsy.

Histopathology: Representative samples of lymph nodes (mandibular and mesenteric), spleen, and thymus and all gross lesions were fixed in 10% neutral buffered formalin. All tissues collected from high-dose, including the one moribund-euthanatized animal, and vehicle-control animals were examined by light microscopy for drug-related or spontaneous lesions. Only target organs, defined microscopically at the high dose, and gross lesions were examined at lower doses.

Clinical Immunology:

Antibody Response to T-Cell-Dependent Antigen (KLH = Keyhole Limpet Hemocyanin):
To assess the effects of BMS-477118 on the T-cell-dependent humoral immune response, KLH-specific IgM antibodies in serum obtained on day 29 were measured by ELISA and expressed as antibody titers.

Microtiter plates were coated with KLH to capture KLH-specific antibodies in serially diluted (three-fold) test samples. KLH-specific rat antibodies were detected with alkaline phosphatase-conjugated goat anti-rat IgM antibody followed by substrate (1 mg/ml para-nitrophenyl phosphate in diethanolamine buffer, pH 9.8). After the reactions were stopped with 3N NaOH, the absorbance (dual wavelength: 405 and 550 nm) was recorded using a microtiter plate reader. Results were expressed as antibody titer, defined as the reciprocal of the interpolated dilution that resulted in an absorbance reading equal to five-fold the mean plate-background absorbance for KLH antibodies. Plate background was determined as the absorbance measurement recorded in the absence of serum.

Splenic-Lymphocyte Phenotyping: At scheduled necropsy, a portion of the spleen from the first five surviving rats/sex/group in Groups 1 - 4 was placed in a tube containing Hank's Balanced Salt Solution (HBSS) with phenol-red. For each animal, the weight of the entire spleen and the splenic portion used for phenotyping was recorded and a single-cell suspension of splenocytes was prepared. Splenic lymphocytes were stained directly with fluorochrome-conjugated anti-rat antibodies to CD3 (pan-T cell), CD4+CD8- (T-helper cell), CD8+CD4' (T-cytotoxic cell), and CD45RA (B cell) cell-surface markers. The percentage of lymphocytes expressing a given cell-surface marker was determined by flow cytometric analysis. The absolute numbers of lymphocytes per total spleen expressing an individual marker were then calculated as the product of the total lymphocyte count, the percentage of lymphocytes expressing a given marker, and the weight of the total spleen.

Results:
Mortality: 1/10 females dosed 200 mg/kg was sacrificed moribund (day 26). Necropsy revealed general pallor, increased size of salivary gland, spleen and various lymph nodes; discoloration of thymus and mesenteric lymph nodes; and subcutaneous tissue masses at the neck and left forelimb. Microscopic evaluation: bacterial infection and septicemia, multifocal subacute inflammation of lymph nodes.

Clinical signs:

Animal #	Dose (mg/kg)	Clinical signs
4205 F	200	Chromorhinorrhea, chromodacryorrhea, swollen forelimb (L), swollen ventral neck, moribund, sacrificed on day 26.
4209 F	200	Pale body discoloration, swollen forelimb (R), swollen ventral neck on days 27-28

In the positive control group (dexamethasone) clinical signs included hunched whole body, rough hair coat, and red urine in 1 male on day 27.

Body weights: (g)

Dose (mg/kg)	0		10		50		200		Positive Control (Dexamethasone)	
Sex	M	F	M	F	M	F	M	F	M	F
Day 1	223	163	225	161	224	163	224	164	224	162
Day 28	362	233	369	242	355	235	339*	224	252**	197**
% decrease relative to control	-	-	-	-	2	-	6	4	30	15

* $p < 0.05$; ** $p < 0.01$

Food consumption: No data.

Hematology:

Dose (mg/kg)	0		10		50		200	
Sex	M	F	M	F	M	F	M	F
RBC (10^6/ul)	7.8		8.0		8.3* (6%↑)		8.5**(9%↑)	
HGB (g/dl)	15.2		15.4		15.8**(4%↑)		16.2**(7%↑)	
HCT %	43.8		44.6		45.5*(4%↑)		45.8**(5%↑)	
MCV (fl)	56.2	55.9	55.7	55.5	55.2	53.8*(4%↓)	54.2*(4%↓)	53.9*(4%↓)
Platelet (10^3/ul)	1284	1209	1189	1195	1142* (11%↓)	1223	1001** (14%↓)	957* (21%↓)
Eosinophils (10^3/ul)		0.118		0.089		0.086		0.059* (50%↓)

Clinical chemistry:

Dose (mg/kg)	0		10		50		200	
Sex	M	F	M	F	M	F	M	F
ALB (g/dl)		3.67		3.62		3.45		3.34*(9%↓)
GLOB (g/dl)		3.12		3.24		3.18		3.58**(15%↑)
A/G ratio		1.18		1.12		1.10		0.96**(19%↓)
PHOS (mg/dl)		7.77		6.93*		7.74		6.99*(10%↓)

* $p < 0.05$; ** $p < 0.01$

Organ weights: (g) – only spleen and thymus were weighed.

Dose (mg/kg)	0		10		50		200	
Sex	M	F	M	F	M	F	M	F
Spleen (g)	0.83	0.61	0.82	0.63	0.82	0.67	1.14**(37%↑)	0.79*(30%↑)
Spleen (% B.wt.)	0.23	0.26	0.22	0.26	0.23	0.29	0.33**(44%↑)	0.36**(39%↑)
Thymus (g)		0.50		0.51		0.46		0.39*(22%↓)
Thymus (% B.wt.)		0.21		0.21		0.19		0.17*(19%↓)

* $p < 0.05$; ** $p < 0.01$

Gross pathology: lymph nodes (mandibular and mesenteric), spleen, thymus, and gross lesions were examined in all dose groups.

Dose (mg/kg)	0		10		50		200	
Sex	M	F	M	F	M	F	M	F
Spleen Increased size							2/10	2/10
Mandibular LN Increased size						1/10	2/10	3/10

Histopathology: Only target organs, defined microscopically at the high dose, and gross lesions were examined at lower doses.

Dose (mg/kg)	0		10		50		200	
Sex	M	F	M	F	M	F	M	F
Mandibular LN Lymphoid hyperplasia	3/10(1)	3/10(1)	2/10(1)	6/10(1)	3/10(1)	5/10 3/10(1) 2/10(2)	8/10 5/10(1) 2/10(2)	7/10 3/10(1) 2/10(2) 2/10(3)
Hyperplasia, plasma cell	1/10(2)	2/10(1)	0/10	3/10 2/10(1) 1/10(2)	1/10(1)	2/10 1/10(1) 1/10(2)	4/10 2/10(1) 2/10(2)	7/10 5/10(1) 2/10(2)
Inflammation								1/10(4)*
Bacteria, multifocal								1/10(2)*
Necrosis								1/10(2)*
Mesenteric LN Lymphoid hyperplasia	0/10	0/10	0/10	0/10	0/10	2/10(1)	8/10(1)	4/10 2/10(1) 2/10(2)
Inflammation								1/10(2)*
Hemorrhage								1/10(2)*
Cervical LN Inflammation								1/10(3)*
Mediastinal LN Inflammation								1/10(2)*
Hemorrhage								1/10(2)*
Iliac LN Inflammation								1/10(3)*
Other LN (not specified) Inflammation								1/10(4)*
Spleen Lymphoid hyperplasia	2/10(1)	2/10(1)	2/10(1)	5/10 3/10(1) 2/10(2)	8/10 7/10(1) 1/10(2)	10/10 6/10(1) 4/10(2)	10/10 3/10(1) 7/10(2)	8/10 2/10(1) 5/10(2) 1/10(3)
Salivary gland Inflammation								1/10(3)*
Thymus Lymphoid depletion	0/10	0/10	0/10	0/10	0/10	0/10	0/10	2/10(3) *

1 = minimal, 2 = mild, 3 = moderate, 4 = marked, * = same animal (#4205)

Clinical Immunology:

Antibody Response to T-Cell-Dependent Antigen (KLH)

Group	KLH-Specific Antibody Titers[a] Male	Female
1 Vehicle Control	2356 + 2581	2405 ± 1599
2 BMS-477118 (10 mg/kg)	3552 ± 4567	1936 ± 1537
3 BMS-477118 (50 mg/kg)	1162 ± 693	2221 ± 2323
4 BMS-477118 (200 mg/kg)	1045 ± 430	2087 ± 1379[b]
5 Positive Control	114 ± 151[c]	439 ± 315[c]

[a] Values represent mean ± standard deviation for 10 animals/sex/group.
[b] Values represent mean ± standard deviation for 9 females in Group 4.
[c] Values are significantly different from vehicle control, based on Welsh t-test ($p < 0.05$).

Splenic-Lymphocyte Phenotype, Male

Group	Absolute Count ($x10^7$ per total spleen) of Splenic-Lymphocytes Expressing[a] CD3	$CD4^+CD8^-$	$CD8^+CD4^-$	CD45RA
1 0 mg/kg	35.06 ± 12.37	20.68 ± 8.27	17.26 ± 7.22	42.79 ± 8.54
2 10 mg/kg	26.98 ± 10.04	18.00 ± 7.36	14.45 ± 3.82	32.24 ± 4.63
3 50 mg/kg	30.40 ± 11.00	18.52 ± 6.41	15.03 ± 4.24	36.55 ± 8.52
4 200 mg/kg	53.44 ± 24.21	30.04 ± 10.21	24.79 ± 12.38	42.27 ± 14.53

[a] Values represent mean ± standard deviation for 5 animals.
No significant differences between BMS-477118-treated and vehicle-control groups ($p > 0.05$).

Splenic-Lymphocyte Phenotype, Female

Group	Absolute Count ($x10^7$ per total spleen) of Splenic-Lymphocytes Expressing[a] CD3	$CD4^+CD8^-$	$CD8^+CD4^-$	CD45RA
1 0 mg/kg	32.29 ± 12.99	20.34 ± 8.94	15.38 ± 4.72	42.47 ± 18.15
2 10 mg/kg	25.27 ± 9.89	15.70 ± 5.89	12.40 ± 5.02	27.80 ± 11.12
3 50 mg/kg	23.52 ± 6.85	15.98 ± 4.38	11.16 ± 2.81	29.93 ± 7.22
4 200 mg/kg	41.47 ± 18.76	23.99 ± 8.88	18.78 ± 9.88	36.54 ± 9.01

[a] Values represent mean ± standard deviation for 5 animals.
No significant differences between BMS-477118-treated and vehicle-control groups ($p > 0.05$).

Intermittent Dose Oral Immunotoxicity Study in Monkeys with BMS-477118 (saxagliptin)

This study was conducted to evaluate potential acute clinical and hematologic (lymphocyte) changes following once weekly administration of saxagliptin to monkeys. In clinical drug-interaction studies, saxagliptin (100 mg) was administered on Days 1 and 9. In one of these studies, 5 of 15 subjects reported flu-like symptoms (myalgia, body aches and fever) within 14 hours of the second dose of 100 mg, and all 15 subjects had transiently decreased absolute lymphocyte counts (approximately 66%). Similar decreases in lymphocyte counts had not been observed in previous repeat daily dose toxicity studies in mice or rats, and only a transient decrease (25%) was observed at 10 mg/kg (at 6 months) in a 1-year dog study.
In previous single-dose toxicity studies in monkeys (DN01106) using the benzoate salt of BMS-477118, 1 monkey died approximately 22 hours after a dose of 50 mg/kg. No drug-related findings were noted after a dose of 5 or 25 mg/kg. For this study, the initial dose of 3 mg/kg was selected because this dose was associated with transient side effects (increased body temperature and transiently decreased lymphocytes) in humans (based on a comparison of monkey and human exposures).

Key study findings:

- No deaths or drug-related effects on body temperature were noted.
- Drug-related skin lesions (scabs and open wounds) on the tip of the tail and/or back were noted in one male (2102) and one female (2201) in the drug-treated group beginning on Days 20 or 21 following the second challenge dose of 10 mg/kg administered on Day 15. One additional drug-treated female (2203) developed a tail lesion on Day 27.
- Lymphocyte count decreased 30-60% in males and females (the latter were more affected) starting primarily after the second dose of 3mg/kg and continuing through the subsequent two doses of 10mg/kg. The decrease was observed at both 5hr and 24hrs post-dose, which effectively excludes a 'stress-response' as a possible explanation.
- T-cell subsets decreased by saxagliptin in females included CD8+, CD4+, (CD2+CD20-), and (CD20+CD2-) populations. Males were less affected, as only the (CD20+CD2-) population declined.

Study no: DN05054.
Volume # and page #: Vol. 1, pg. 86.
Conducting laboratory and location: Bristol-Myers Squibb Pharmaceutical Research Institute, New Brunswick, NJ.
Date of study initiation: June 1, 2005.
GLP compliance: No.
QA reports: yes ( ) no (x):
Drug, lot #, radiolabel, and % purity: Batch #4F70817 (5 mg) and 4E88951 (40 mg); purity was not provided.
Formulation/vehicle: Clinical formulations of the 5 and 40 mg tablets suspended in water.

Methods:
Suspensions of the clinical formulation (5 and 40 mg tablets) of saxagliptin (BMS-477118) in water were administered orally by gavage to cynomolgus monkeys (3/sex) as single doses. An initial dose of 3 mg/kg was given on Day 1 followed by challenge doses on Days 8 (3 mg/kg), 15 (10 mg/kg), and 22 (10 mg/kg). The duration of this study was 27 days.

Experimental Design

Group	Initial BMS-477118 Dose (mg/kg)	Challenge BMS-477118 Dose (mg/kg)	Volume (mL/kg)	Concentration BMS-477118 (mg/mL)	Number of Monkeys
1	0 (water)	0	1.0	0	3 M, 3 F
2	3	3 (Day 8) 10 (Days 15 and 22)	1.0	3 (Days 1 and 8) 10 (Days 15 and 22)	3 M, 3 F

Dosing: 3 mg/kg (Days 1 and 8) and 10 mg/kg (Days 15 and 22).

Hematology
Blood samples for hematology tests were obtained from non-fasted animals pretest and at the following approximate times on Day 1 (5-hours post-dose), Day 2 (24-hours post-dose), Day 3 (48-hours post-dose), Day 8 (predose and 5-hours post-dose), Day 9 (24-hours post-dose), Day 10 (48-hours post-dose), Day 15 (predose and 5-hours post-dose), Day 16 (24-hours post-dose), Day 17 (48-hours post-dose), Day
22 (predose and 5-hours post-dose), Day 23 (24-hours post-dose), and Day 24 (48-hours post-dose).

Clinical Immunology (Peripheral-Blood Lymphocyte Phenotyping)
An aliquot of anticoagulated blood was collected from each monkey pretest and at the following approximate times on Day 2 (24-hours post-dose), Days 8 and 9 (predose and 24-hours post-dose. Respectively), and Days 15 and 16 (predose and 24-hours post-dose, respectively) and assessed by flow cytometric analysis. The whole blood was analyzed for the absolute number of lymphocytes that were $CD2^{+}CD20^{-}$ (T cells), $CD4^{+}CD8^{-}$ (T-helper cells), and $CD8^{+}CD4^{-}$ (T-cytotoxic cells), and $CD20^{+}CD2^{-}$ (B cells) using a validated method.

Observations and times: Observations for moribund or dead animals were made twice daily. Each monkey was observed once after dosing for changes in condition and behavior. Each monkey was weighed pretest and on Days 7, 14, and 20.

Results:
Mortality and Clinical observations:

- There were no deaths or drug-related effects on body temperature.
- One male (2102) and one female (2201) monkey in the drug-treated group developed lesions (scabs and open wounds) on the tip of the tail and/or back beginning on Days 20 or 21 following the second challenge dose of 10 mg/kg that was administered on Day 15. The sponsor stated that these monkeys were separated from their cage mates on Day 23 for a period of 4 days to determine if these lesions were inflicted by their cage mates. The lesions did not resolve during the separation period and these monkeys were reunited with the cage mates on Day 27. One additional drug-treated female (2203) developed a tail lesion on Day 27. Based on these findings and the results from a 1- to 3-month oral study of BMS-477118 in monkeys in which similar skin lesions were noted following daily dosing, the skin lesions were considered to be drug related. Following completion of this study (Day 27) and despite continued veterinary treatment, the tip of the tail of monkey 2102 became necrotic requiring amputation of approximately 3 centimeters of the tail tip 10 days following the end of the study.

Body weight: (kg) – No treatment-related effect on body weight.

Group	Day numbers relative to Start Date				
Sex	-2	7	14	20	27
0 mg/kg (M)	2.80	2.90	2.80	2.77	2.90
10 mg/kg (M)	2.70	2.77	2.80	2.70	2.80
0 mg/kg (F)	2.73	2.83	2.77	2.70	2.80
10 mg/kg (F)	2.63	2.70	2.63	2.57	2.63

Hematology:

Saxagliptin was administered on Day 1 at a dose of 3 mg/kg. Following the first challenge dose of 3 mg/kg on Day 8 (5 hr postdose), hematology changes included a decrease in lymphocytes (~ 33-46% relative to control) and slight but significant increases in MCHC in females. The sponsor argued that the decreased lymphocyte counts were due to stress and not considered to be drug related since similar changes were noted in control monkeys based on a comparison of pre-dose and post-dose values. However, the decrement in lymphocytes was more pronounced following treatment and the reviewer believes it may be drug-related. A second challenge dose of 10 mg/kg on Day 15 caused a decrease in lymphocytes by 32% (not SS) and 36% in males and females respectively relative to controls (5 hr postdose). Prior to dosing on Day 15, the decrement in lymphocytes was 17% and 19% for males and females respectively. A third challenge dose of 10 mg/kg on Day 22 caused a decrease in lymphocytes by 28% in females relative to control (5 hr postdose). The highest decrement (62%) occurred on Day 16 (24 hr postdose) in treated females relative to control females. By Day 24, the lymphocytes were decreased by 29% in treated females. Sporadic increases in neutrophils were observed in treated females on Days 9, 16, and 24.

Day -6 (pre-dose)				
Parameters	MCH	MCH	MCHC	Lymphocytes
Dose	Picogram	Picogram	g/dl	10^3/ul
0 mg/kg (M)				3.10
3 mg/kg (M)				3.82
0 mg/kg (F)				3.91
3 mg/kg (F)				3.95

empty cells = no significant difference between control and treated group

Day 1 (5 hr postdose)				
Parameters	MCH	MCH	MCHC	Lymphocytes
Dose	Picogram	Picogram	g/dl	10^3/ul
0 mg/kg (M)				2.76
3 mg/kg (M)				3.75
0 mg/kg (F)		19.40		5.14
3 mg/kg (F)		21.13* (9%↑)		4.11 (20%↓)

* $p<0.01$; empty cells = no significant difference between control and treated group

	Day 8 (0 hr postdose)		Day 8 (5 hr postdose)		
Parameters	MCH	Lymphocytes	MCH	MCHC	Lymphocytes
Dose	Picogram	10^3/ul	Picogram	g/dl	10^3/ul
0 mg/kg (M)		5.96			3.90
3 mg/kg (M)		4.96 (17%↓)			2.63* (23%↓)
0 mg/kg (F)	19.77	7.74	19.73	27.97	4.68
3 mg/kg (F)	21.53** (9%↑)	5.44* (30%↓)	21.53* (9%↑)	29.37* (5%↑)	2.52* (46%↓)

	Day 9 (24 hr postdose)				
Parameters	WBC	Monocytes	MCH	Neutrophils	Lymphocytes
Dose	10^3/ul	10^3/ul	Picogram	10^3/ul	10^3/ul
0 mg/kg (M)	12.43	0.57			5.74
10 mg/kg (M)	8.34** (33%↓)	0.29* (49%↓)			3.69 (36%↓)
0 mg/kg (F)				3.38	7.85
10 mg/kg (F)				9.93** (194%↑)	3.36** (57%↓)

	Day 15 (0 hr postdose)		Day 15 (5 hr postdose)		
Parameters	MCH	Lymphocyte	HGB	MCHC	Lymphocyte
Dose	Picogram	10^3/ul	g/dl	g/dl	10^3/ul
0 mg/kg (M)		5.33			3.63
10 mg/kg (M)		4.45 (17%↓)			2.47 (32%↓)
0 mg/kg (F)	20.37	7.10	11.77		4.77
10 mg/kg (F)	22.03* (8%↑)	5.72 (19%↓)	13.30* (13%↑)		3.07* (36%↓)

	Day16 (24 hr postdose)				
Parameters	MCH	Monocytes	HGB	Neutrophils	Lymphocytes
Dose	Picogram	10^3/ul	g/dl	10^3/ul	10^3/ul
0 mg/kg (M)					4.33
10 mg/kg (M)					2.92 (33%↓)
0 mg/kg (F)	20.43			4.32	6.49
10 mg/kg (F)	21.90* (7%↑)			10.11** (134%↑)	2.46* (62%↓)

** p<0.05; * p<0.01; empty cells = no significant difference between control and treated group

	Day 22 (0 hr postdose)		Day 22 (5 hr postdose)		
Parameters	MCH	Lymphocyte	HGB	MCHC	Lymphocyte
Dose	Picogram	10^3/ul	g/dl	g/dl	10^3/ul
0 mg/kg (M)		4.27			4.39
10 mg/kg (M)		4.74			4.00 (9%↓)
0 mg/kg (F)		6.62			5.78
10 mg/kg (F)		5.93 (10%↓)			4.15* (28%↓)

** p<0.05; * p<0.01; empty cells = no significant difference between control and treated group

	Day 24 (48 hr postdose)				
Parameters	WBC	Neutrophils	HGB	MCHC	Lymphocyte
Dose	10^3/ul	10^3/ul	g/dl	g/dl	10^3/ul
0 mg/kg (M)					4.43
10 mg/kg (M)					4.44
0 mg/kg (F)	15.01	7.82			6.24
10 mg/kg (F)	24.98* (40%↑)	19.38* (60%↑)			4.43 (29%↓)

** p<0.05; * p<0.01; empty cells = no significant difference between control and treated group

Clinical Immunology:

Although there were no significant decreases in the lymphocyte subsets of treated animals relative to controls, the decreases in treated females on Day 16 appear to be biologically significant (58-68%). The decreases in the lymphocyte subsets of treated females correlated with the decrease in lymphocyte counts observed in treated females on Day 16. In treated males, while there were no significant decreases in the lymphocyte subsets, a biologically significant decrease (57%) was noted in $CD20^+CD2^-$ (T cells) on Day 16. Overall, while the observed decreases in total lymphocyte counts correlated with decreases in peripheral-blood lymphocyte subsets of treated females, decreases in total lymphocyte counts of treated males were not associated with any clear change in a specific peripheral-blood lymphocyte subset except $CD20^+CD2^-$ (T cells) on Day 16.

Peripheral-Blood Lymphocyte Phenotyping - Male

Group	Pretest	Day 2[a]	Day 8[b]	Day 9[a]	Day 15[b]	Day 16[a]
	Absolute Number of Lymphocytes (10e3/µl) Expressing $CD2^+CD20^-$					
1- 0 mg/kg	1.91 ± 0.13	3.16 ± 1.07	3.95 ± 1.54	3.80 ± 1.48	3.52 ± 1.25	2.79 ± 0.95
2- 3 mg/kg[c]	2.35 ± 0.81	2.92 ± 0.33	3.53 ± 0.85	2.83 ± 0.64	3.01 ± 0.27	2.00 ± 0.95
	Absolute Number of Lymphocytes (10e3/µl) Expressing $CD20^+CD2^-$					
1- 0 mg/kg	0.59 ± 0.16	0.97 ± 0.33	0.93 ± 0.44	1.08 ± 0.50	0.91 ± 0.39	0.81 ± 0.31
2- 3 mg/kg[c]	0.48 ± 0.10	0.59 ± 0.06	0.49 ± 0.02	0.36 ± 0.15	0.52 ± 0.03	0.35 ± 0.08
	Absolute Number of Lymphocytes (10e3/µl) Expressing $CD4^+CD8^-$					
1- 0 mg/kg	0.81 ± 0.11	1.48 ± 0.34	1.73 ± 0.52	1.73 ± 0.55	1.62 ± 0.42	1.27 ± 0.40
2- 3 mg/kg[c]	0.90 ± 0.30	1.53 ± 0.20	1.73 ± 0.41	1.65 ± 0.44	1.40 ± 0.12	1.01 ± 0.47
	Absolute Number of Lymphocytes (10e3/µl) Expressing $CD8^+CD4^-$					
1- 0 mg/kg	1.29 ± 0.21	1.90 ± 0.66	2.48 ± 0.83	2.26 ± 0.75	2.18 ± 0.68	1.74 ± 0.40
2- 3 mg/kg[c]	2.09 ± 0.78	1.87 ± 0.26	2.18 ± 0.53	1.32 ± 0.44	2.04 ± 0.12	1.26 ± 0.65

[a] Samples collected approximately 24 hours postdose.

[b] Samples collected predose.

[c] 3 mg/kg on Days 1 and 8; 10 mg/kg on Day 15.

Peripheral-Blood Lymphocyte Phenotyping – Female

Group	Pretest	Day 2[a]	Day 8[b]	Day 9[a]	Day 15[b]	Day 16[a]
	Absolute Number of Lymphocytes (10e3/µl) Expressing $CD2^+CD20^-$					
1- 0 mg/kg	2.30 ± 0.46	4.37 ± 0.29	5.46 ± 0.42	5.38 ± 0.85	4.97 ± 0.37	4.59 ± 0.59
2- 3 mg/kg[c]	2.57 ± 0.82	3.61 ± 0.38	3.85 ± 0.52	2.63 ± 0.43	3.91 ± 0.69	1.71 ± 1.09
	Absolute Number of Lymphocytes (10e3/µl) Expressing $CD20^+CD2^-$					
1- 0 mg/kg	0.69 ± 0.38	1.00 ± 0.45	0.96 ± 0.37	1.31 ± 0.56	0.95 ± 0.44	1.02 ± 0.45
2- 3 mg/kg[c]	0.69 ± 0.49	0.91 ± 0.47	0.74 ± 0.51	0.37 ± 0.25	0.90 ± 0.57	0.43 ± 0.18
	Absolute Number of Lymphocytes (10e3/µl) Expressing $CD4^+CD8^-$					
1- 0 mg/kg	1.08 ± 0.21	2.47 ± 0.13	2.92 ± 0.24	2.95 ± 0.55	2.83 ± 0.17	2.50 ± 0.29
2- 3 mg/kg[c]	1.25 ± 0.59	1.92 ± 0.25	1.92 ± 0.36	1.43 ± 0.09	1.86 ± 0.48	0.94 ± 0.61
	Absolute Number of Lymphocytes (10e3/µl) Expressing $CD8^+CD4^-$					
1- 0 mg/kg	1.72 ± 0.21	2.28 ± 0.34	3.10 ± 0.48	3.03 ± 0.60	2.73 ± 0.35	2.62 ± 0.59
2- 3 mg/kg[c]	1.54 ± 0.12	1.94 ± 0.45	2.02 ± 0.37	1.17 ± 0.39	2.14 ± 0.36	0.85 ± 0.52

[a] Samples collected approximately 24 hours postdose.

[b] Samples collected predose.

[c] 3 mg/kg on Days 1 and 8; 10 mg/kg on Day 15.

2.6.6.9 Discussion and Conclusions

Pharmacology

Saxagliptin is a potent DPP4 inhibitor being sought for treatment of hyperglycemia in type 2 diabetic patients by Bristol Myers Squibb (IND 63,634, NDA 22-350). DPP4 belongs to a class of proteolytic enzymes with multiple functions in blood in humans and animals. Among multiple activities of DPP4, the most prominent and well characterized feature is degradation of GLP-1 in blood. By inhibiting DPP4, saxagliptin extends the biological activity of GLP-1 which promotes pancreatic β-cells to produce more insulin when blood sugar levels are elevated. Since the physiological effect of GLP-1 is more pronounced under hyperglycemic state, DPP4 inhibitors (via GLP-1) and GLP-1 analogs are thought to produce minimal hypoglycemic state and therefore preferable over antidiabetic drugs that may produced hypoglycemia at peak plasma drug concentrations.

Although saxagliptin is considered selective DPP4 inhibitor (391x DPP8, 75x DPP9), it is not as selective as the approved and marketed sitagliptin therefore, it is highly possible that at high circulating plasma levels, saxagliptin could inhibit off target activities associated with DPP8 and DPP9. However, with significantly greater potency (Ki of 1.3 nM) than sitagliptin (Ki of 18 nM), saxagliptin is likely to exert greater pharmacological effect than sitagliptin. In clinical studies, saxagliptin doses of 2.5 mg (renally insufficient patients) and 5.0 mg (normal patients) were effective in reducing Hb1AC and blood glucose in type 2 diabetic patients. At these doses, saxagliptin was able to inhibit DPP4 activity for up to 24 hrs. Since saxagliptin is metabolized in all species including humans to a highly DPP4 selective active metabolite (BMS-510849), saxagliptin may have an extended pharmacological efficacy.

Pharmacokinetics

Circulating saxagliptin in blood in humans and monkeys was virtually all in the free state (unbound state), while protein binding in rats and dogs was only 5%. Thus, the entire drug in the plasma may exert pharmacological activity, with greatest effect at Cmax. Protein binding can generally extend the pharmacodynamic effect of a drug by making available more free drug thus dampening sharp swings in daily drug levels and possibly pharmacodynamics. With little or no protein binding, one would expect high free saxagliptin at Cmax to result in a rapid rise and fall in DPP4 or other enzyme activity which may not be all desirable. As noted earlier, the active metabolite of saxagliptin may extend the pharmacological effect of the parent and is also more selective for DPP4 (though ~2-fold less potent). Thus, conversion of saxagliptin to BMS-510849 may improve the overall safety profile of saxagliptin. Although saxagliptin is metabolized by CYP3A/4 to BMS-510849 in all species, the degree of BMS-510849 production differ among species. In diabetic humans, the AUC for BMS-510849 was 4 to 7 times greater than AUC for saxagliptin. In animal models, the ratio of BMS-510849 to saxagliptin was near unity or lower (BMS-510849 /saxagliptin AUC in mice: 0.3-0.7, rats: 0.05-0.13, dogs: 0.2-0.35, rabbits: 0.5-0.7, monkeys: 0.4-1). Although in diabetic humans BMS-510849 production is significantly greater than saxagliptin, the safety of BMS-510849 was adequately addressed since rodents and non-rodents received larger doses of saxagliptin to compensate for lower BMS-510849 AUC. It should be noted that in addition to prominent active metabolite, BMS-510849, a large number of small inactive metabolites were also identified in humans. Nearly all these minor metabolites (<10% of parent) were also found in one or more of the nonclinical species thus the safety of all the minor metabolites were also indirectly assessed by testing the parent drug in animals. Since

saxagliptin was substrate to CYP3A/4, the potential inhibitors and inducers of CYP3A/4 could change the abundance of BMS-510849 over saxagliptin in humans. Indeed, ketoconazole, an inhibitor of CYP3A/4 significantly increased AUC for saxagliptin and reduced that of BMS-510849. Interesting, rifampin, a CYP3A/4 inducer, decreased the AUC for saxagliptin but had no notable effect on BMS-510849 AUC, suggesting that saxagliptin metabolism may have been routed to other minor metabolites. Although saxagliptin metabolism may be dependent on drugs that inhibit or induce CYP3A/4, saxagliptin itself was not an inducer or inhibitor of CYP3A/4. Bioavailability of saxagliptin was very good in rats, dogs, cynomolgus monkeys (51 to 76%). The bioavailability of saxagliptin was not determined in humans, but since approximately 75% of total radioactivity in a mass balance study was recovered in urine, suggests that bioavailability was similar to monkeys. The metabolite of saxagliptin had poor bioavailability in rats (<5%), the only species tested. Contrary to good bioavailability in all the species after oral dosing, saxagliptin had low intrinsic permeability (PAMPA) and poor permeability across Caco-2 cell monolayer. Furthermore, saxagliptin was not a substrate to any of the standard cellular uptake transporters, suggesting that exact mechanism by which saxagliptin may transport across cells is unknown. On would expect based on poor intrinsic permeability that the apparent volume of distribution of saxagliptin would be limited to the intravascular compartment like BMS-510849. On the contrary, the apparent Vdss ranged from 5.2 L/kg in rats to 1.3 to 1.8 L/kg in dogs and monkeys suggesting extensive extravascular distribution. Since saxagliptin was nearly free from protein binding, either saxagliptin was getting trapped in the cells or some tissues were accumulating saxagliptin far more than others to result in high apparent Vdss even though single dose autoradiography did not identify any tissues other than liver, kidneys and GI track as the primary sites of radioactivity. Since saxagliptin was only a weak substrate to p-glycoprotein which is extensively distributed in the intestinal epithelium, hepatocytes, renal proximal tubular cells and capillary endothelial cells in the blood brain barrier, the potential for efflux of saxagliptin from the cells in these tissues is likely to be minimal.

Cardiovascular safety

Saxagliptin appeared to have minimal effect on the cardiovascular safety based on *in-vitro* (hERG, Purkinje fiber assay) and in acute *in-vivo* and chronic toxicology studies. Saxagliptin had no significant effect on potassium channel current in the hERG assay or on conduction kinetics in rabbit Purkinje fibers at concentrations up to 30 μM (≥ 200 fold maximum clinical drug concentrations). Single oral doses of saxagliptin administered to conscious telemetered dogs and to monkeys did not significantly alter blood pressure and heart rate or produce electrocardiographic abnormalities at drug concentrations in excess of 100-fold the maximum clinical concentration from a 5mg dose. The sporadic changes in blood pressure and heart rate or QT observed in chronic toxicology studies in rats, dogs and monkeys appeared to be gender specific, non-dose dependent and not progressive. In deed toxicology studies found no notable cardiac histopathology or increase in heart weight in 2-year mouse and rat, 12-month dog or 3-month monkey studies. Exposure to saxagliptin/active metabolite not associated with cardiovascular toxicity in animals was approximately 1000x/300x (lifetime mouse), 2000x/68x (lifetime rat), 43x/11x (12-month dog) and 23x/11x (3-month monkey) the mean human exposure (AUC) at the maximum recommended human dose of 5 mg/day. Although these studies performed in healthy nonclinical animal models may not predict overt cardiovascular safety of saxagliptin in diabetic patient prone to cardiovascular disease, they found no signal to suggest a cardiovascular risk.

Toxicological evaluation of saxagliptin in mice, rats, rabbits, dogs and monkeys had identified several target organs and toxicities: **a)** CNS lesions in male rats at high doses, **b)** skin lesions in

cynomolgus monkeys and foot pad carking in dogs, **c)** nephropathy in monkeys, **d)** immune system related cell suppression, **e)** potential malformation in rat combination study with metformin.

CNS lesions in male rats

Saxagliptin at high doses (≥ 150 mg/kg/d) was found to produce significant CNS lesions in male SD rats. These brain lesions were most commonly found in the corpus callosum but were also present in the caudate-putamen, thalamus, and/or piriform/temporal cortex, 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. These lesions noted in male SD rats were similar to lesions observed with cyanide toxicity. Elaborate studies were carried out by the sponsor, clearly demonstrated that brain lesions were specific to male rats, due to high prevalence of CYP2C11 and cyanide release from saxagliptin metabolism via CYP2C11. In fact it appears that due to high incidence of deaths and early evidence of brain lesions in male rats, the original 2-year rat carcinogenicity study (60/sex/dose) at doses recommended by eCAC (25, 75, 150 and 300 mg/kg/d) was interrupted at several time points and sacrificed at week 82 to explore the saxagliptin related brain lesions in male SD rats. This study found significant incidence of brain lesions in male rats at ≥ 150 mg/kg/d. The survival rate in males at ≤ 150 were similar to females ≤ 300 mg/kg/d suggesting that only male treated with saxagliptin doses ≥ 150 mg/kg/d were susceptible for saxagliptin-induced brain lesions. The higher the dose, greater the number and earlier the incidence of deaths were in male SD rats. Evaluation of the histopathology of brain found seminaries with published reports on cyanide-induced lesions in male rats. It should be noted exposure at ≥ 150 mg/kg/d in male rats with brain lesions were greater than 355x the clinical dose of 5 mg, based on AUC. The deaths appeared to be due to brain lesions caused by cyanide release in male rats since only cyanide was detected in plasma of male rats at ≥ 150 mg/kg/d. There were no such lesions in female SD rats or any gender in any other species tested or in humans. Since brain levels of saxagliptin was not that substantial (brain/plasma ratio of ≤ 0.16), there was no evidence that saxagliptin itself in the brain was the cause of brain lesions. Furthermore, since there were no brain lesions in female rats at 300 mg/kg/d with twice the plasma exposure at the same dose level as male suggest that this was a male specific phenomenon. Additional studies had identified CYP2C11, an androgenic-controlled liver P450 enzyme, abundantly expressed in male SD rats to be the responsible enzyme for cyanide release. When the activity of this enzyme was inhibited by cimetidine or castration, the incidence of CNS lesions were significantly reduced or ameliorated in male rats. Since this enzyme plays a minimal role in other species and analysis of blood for cyanide concentrations found only measurable quantity of cyanide in male SD rat blood but no other species, the CNS lesions were specific to male rats. Although the lesions were very significant in male rats in the 80 week and 104 week study in rats, the exposure at which these lesions were identified occurred at 150 mg/kg/d, 355x the clinical dose of 5 mg, based on AUC. The exposure to BMS-510849 was 21x the clinical exposure. The potential risk to humans is minimal to nonexistent for several reasons: a) cyanide release was only detected in male rats and not in any other species or humans, b) CYP2C11 was prominent in male rats only and could be inhibited by CYP2C11 blocker or castration which tend to ameliorate saxagliptin-induced CNS lesions, c) lesions in male rats occurred at 355x the clinical exposure, d) rapid and predominate metabolism of saxagliptin by CYP3A/4 in humans (4 to 7 fold more BMS-510849 than saxagliptin) more than any other nonclinical species is likely to reduce saxagliptin exposure rapidly thus preventing metabolism by other routes, e) no adverse CNS signs have been observed in other nonclinical models or in more than 3400 individuals

treated with saxagliptin. In the 6-month rat study (2, 20 and 100 mg/kg/d) where the top saxagliptin dose was limited to 100 mg/kg/d (270x MRHD), there were no brain lesions in male SD rats or any other group for that matter, suggesting that any potential risk to humans at 5 mg dose is virtually nonexistent.

Skin lesions and nephropathy in monkeys

Previous experience with DPP4 inhibitors had shown that cynomolgus monkeys develop skin lesions. Since the sponsor had used dog as the non-rodent model, the Division requested a 3 month study with saxagliptin in cynomolgus monkeys. When cynomolgus monkeys were treated with saxagliptin (2, 10, 20/30 mg/kg/d) skin lesions were seen at all doses, however, the incidence was greater and occurred as early as day 6 of the treatment thus leading to dose reduction (30 to 20 mkd on Day 3). The time to incidence decreased while severity increased in a dose-related manner. In two monkeys at 20 mg/kg/d, the tail marked by coagulative necrosis had to be amputated due to severity of lesions. These skin ulcers were noted in tails, digits, face and scrotum. The skin lesions in the face, nose and scrotum were marked visibly by erosions, scabs and microscopically by inflammation and mononuclear cell infiltrations and in the case of tails, coagulative necrosis. Although there were inflammation and mononuclear cell infiltrations in other organs, i.e. pituitary, testes, liver, kidneys and urinary bladder, the most visible toxicological signal was the skin lesions. Since lesions were noted at all doses (NOAEL <2 mg/kg/d, 7-17x MRHD), a new 3-month study was carried out (0.03, 0.3 and 3 mg/kg/d). There were no notable findings $\leq$ 0.3 mg/kg/d in cynomolgus monkeys (1 to 2.5x the clinical dose of 5 mg, based on AUC). However skin lesions were noted in 1/4 male and 4/4 female at 3 mg/kg/d (20-27x MRHD) on tail and foot were marked by vascular hypertrophy (smooth muscle and endothelial cells in microvascular and small arteries) and inflammatory cells infiltration (intramural and perivascular mononuclear cells) and epithelial hyperplasia (possibly reparative response).

In a head to head comparison with two other DPP4 inhibitors (vildagliptin and sitagliptin), both saxagliptin (10 mg/kg/d) and vildagliptin (20 mg/kg/d) had similar toxicological profiles at the end of the 6-week treatment (i.e. skin lesions). Since skin lesions or any of the other toxicological end points were not seen in sitagliptin (except for reactive lymphocytes and lymphoid hyperplasia of the spleen and bone marrow), the incidence of skin ulcers were attributed to less selective DPP4 activity of saxagliptin and vildagliptin. The study fits the hypothesis that sensitive cynomolgus monkeys are responding to potential inhibition of DPP8 and DPP9 since both saxagliptin and vildagliptin are less selective than sitagliptin. It should be noted that all three products had several common features (increased incidence of lymphoid hyperplasia in the spleen and bone marrow and thymic lymphoid depletion and decrease in DPP activity) and there was no measurable levels of cyanide in any of the monkeys. The DPP activity in male and female monkeys were nearly the same for all three products (Emax: 84, 65 and 82% for saxagliptin, vildagliptin and sitagliptin), however, DPP inhibition in saxagliptin group occurred at half the vildagliptin and $1/10^{th}$ of sitagliptin AUC (4448, 8837 and 45250 ng.h/ml, respectively) suggesting that biological activity of saxagliptin *in vivo* fits the Ki value for saxagliptin and saxagliptin is the most potent of the three DPP4. Saxagliptin effect on skin in other species has been more benign although cracking of foot padding was reported dogs but at relatively higher doses of saxagliptin (1 female at 5 mg/kg/d, 19x MRHD and all at 10 mg/kg/d, 34-53x MRHD) in the 12-month study. Whether the mechanism for cracking of food padding is similar to skin lesions in monkeys is not clear, but it is reasonable that they are related. Since these lesions usually have been seen at sites that may come in contact with surrounding environment in monkeys and to some extent in dogs, if they ever occur in humans, one may suspect skin areas

that are frequently under physical or contact stress. Overall the 3 studies in cynomolgus monkeys appear to point out to differences in the three compound's DPP4 selectivity but since the exact mechanism of skin lesion has not been sorted out, we should remain vigilant for any clinical signs of adverse skin reports in humans. Thus far, the incidences of skin-related adverse effects (AEs) in control and treated population have been similar. According to the sponsor, the pooled monotherapy analysis, there was no dose-dependent skin-related AE. The frequency of skin – related adverse events in placebo control, 2.5, 5 and 10 mg were 8.3%, 13.4%, 9.1% and 13.3%, respectively. Rash (2.5% vs. 0.6%) and contact dermatitis (1.2% vs. 0.6%) were more frequent in sax treated patients than controls. Additional finding of interest was two incidence of nephropathy in saxagliptin treated monkeys (1 female at 10 mg/kg/d (21-35x MRHD) and 1 male at 30/20mg/kg). Both these monkeys (but no others) were found to have IgG and/or IgM in glomeruli. Although nephropathy has been seen in saxagliptin treated rats (prone to renal disease with age), the incidences are likely to be drug-related since DPP4 enzyme has been known to preserve renal function thus it is highly possible that severe inhibition of DPP activity may have propagated the multifocal glomerulopathy in a saxagliptin treated males and females. Saxagliptin clearance is greater than GFR, suggesting an active transport mechanism is active in its clearance. A change in renal function may result in drug accumulation; therefore, monitoring of renal function is recommended (note, such monitoring is usually done in the course of treatment of type 2 diabetics).

Potential immune and hyperplasia of lymphoid in spleen/bone marrow

DPP4/CD26 enzyme cleaves several well known peptides (i.e. GLP1, GLP-2 GIP, NPY) and paracrine chemokines like RANTES (regulated on activation of normal T cells expressed and secreted), stromal cell-derived factor and macrophage-derived chemokines) in animals and humans. DPP4 can also serve as a binding protein while maintaining normal renal function (GFR) and modulator of the immune system. As new substrates for DPP4 are identified, its significance as a regulatory enzyme is becoming widely known and appreciated. Among DPP4 inhibitors reaching clinical development, saxagliptin is by far the most potent DPP4 inhibitors. At nearly, 14x the potency of another FDA approved DPP4 inhibitor, saxagliptin is likely to exert greatest DPP4 inhibitory activity even at clinical doses as small as 2.5 or 5 mg. Preclinical studies have consistently shown saxagliptin to reduce platelets and basophils and increase in lymphocyte, neutrophils in rats and monkeys. The changes were generally coincided with thymic lymphoid depletion and lymphoid hyperplasia in spleen and bone marrow. It is possible that thymic source of lymphoid cells were depleted before there was a hyperplasia of lymphoid tissue in bone marrow and spleen, suggesting that DPP4 inhibition was interrupting normal life cycle of lymphocytes in the circulation and thymus. The changes in lymphoid tissues were also seen with two other DPP4 inhibitors suggesting that changes in the lymphoid tissues are due to DPP4 effect. Whether chronic administration of saxagliptin would lead to weaker immune system has been a major clinical concern and has not been sorted out. Furthermore, animal studies consistently found inflammation and mononuclear cell infiltration into tissue identified as target organs (i.e. kidney, liver, epididymides, balder and skin). Depending on the dose and sensitivity of the animal models (Monkey) more tissues were infiltrated with mononuclear cell infiltration. The mononuclear cells infiltrations were generally reversible during the recovery.

In rats, saxagliptin increased IgM and IgG by 2 fold and decreased CD3 and CD4 cell numbers in as few as 14 days (20 to 54x the clinical dose of 5 mg based on AUC). An increase in total IgG and IgM was also noted in monkeys, however there was not detectable antinuclear antibodies to suggest that skin lesions in monkeys were immune mediated. Furthermore, immunohisto-chemistry analysis found only presence of IgG and IgM in the glomerulus or tubular epithelium of

two monkeys with glomerulopathy, suggesting that drug-related skin lesions in monkeys may not be immune related. Furthermore, in *in-vitro* assessments of immune related end points using human lymphocytes (CD26/CD3- dependent T-cell activation or mixed-lymphocyte response), there was no inhibition of T-cell activation at doses equal to the therapeutic levels of saxagliptin. The IC_{50} of saxagliptin for inhibition of CD26/CD3-dependent human T-lymphocyte activation was 1000× that of the IC_{50} of the DPP4 enzyme catalytic activity. In the immunotoxicity assessments in nonclinical studies examining peripheral blood lymphocyte, splenocyte phenotyping, serum immunoglobulin [IgG and IgM] levels, antinuclear antibodies, reactive antibodies to red blood cells and/or or platelets, T- and/or B-cell dependent immune responses, and immune mediator/cytokine, an elevations in serum IgG and IgM were consistently demonstrated in all species, but no phenotypic changes in peripheral blood or splenic lymphocytes were noted. There were no antinuclear antibodies or reactive immunoglobulins to erythrocytes or platelets in monkeys. There was no detectable impairment of T- or B lymphocyte dependent immune responses in rats. Collectively, nonclinical evaluation of immunologic endpoints found no clear saxagliptin related adverse effect on the immune system, even though some immune related parameters such as lymphocyte levels were affected. It is the opinion of the reviewer that if future mechanistic studies discover a link, it will be a DPP4 related effect applicable to all DPP4 inhibitors. Interestingly, the immunological assessment in monkeys found presence of IgG and IgM in glomerulus of monkeys with glomerulopathy. Nephropathy was also observed in rats. Since saxagliptin is actively transported by the kidney and DPP4 play a notable role on GFR maintenance, renal function may be impact. Since clinical studies had found significant increase in saxagliptin exposure in patients with severe renal disease and data from rats show renal tissue to be one of the most exposed organs, saxagliptin may produce renal toxicity in vulnerable diabetic patients with poor renal function. Therefore it is advisable to monitor renal function in diabetic patients receiving saxagliptin chronically.

Reproductive effects

As part of combination development, the sponsor had carried out an embryofetal development study in rats with saxagliptin plus metformin. In the study, saxagliptin (5 and 25 mg/kg/d) in combination with metformin (200 mg/kg/d) were given to pregnant rats between gestation days 6 and 15. The preliminary analysis found incidence of reproductive malformations in 2 fetuses from a single litter treated with 25 mg/kg/d of saxagliptin and 200 mg/kg/d of metformin. In the 15-day non-clinical safety report, the malformations were characterized as craniochischisis (incomplete closure of the skull and spinal column, neural tube defect) with forelimb flexure and absence of renal papillae in 2 fetuses. These malformations occurred at saxagliptin and metformin doses that were 114x and 4x AUC for clinical doses saxagliptin and metformin. One of the fetuses also had cleft palate. The fetal and litter incidences were 0.7 and 4.5%, respectively. There were no malformations at 5/200 mg/kg/d of saxagliptin/metformin with exposure multiples of 21x/4x the clinical dose of saxagliptin/metformin. At this point it is not clear which drug was the culprit or whether it was an incidental or due to pharmacodynamic interaction between saxagliptin and metformin. Since both saxagliptin and metformin have been cleared (Class B) and clean in reproductive toxicology studies, the Division has recommended full report of the combination study and additional studies in rabbits that will include each drug alone and in combination.

The sponsor had reported the fetal malformation in the combination study to metformin since saxagliptin alone given to rats (64, 240 and 900 mg/kg/d, 291x, 1503x and 7986x MRHD) during the same gestation periods found no such signals as seen with the combination. It should be noted that decrease in fetal weight and non significant increase in fetal incidence of hypoplastic parietals

and supraoccipital bone and reduced pelvic ossification occurred at maternally toxic dose of 900 mg/kg/d (8000x the clinical dose). Although there was a dose-related increase in fetuses with reduced pelvic ossification (≥ 240 mg/kg/d, 1503x MRHD), statistically significance was achieved at maternally toxic dose (900 mg/kg/d, 8000x MRHD).
In the rabbits embryofetal development study (8, 40 and 200 mg/kg/d, 31x, 142x, 1432x MRHD), the increase in resorption, post-impanation loss and decrease in live fetus were noted at 200 mg/kg/d. Slight but significant increase in ossification sites per fetus per litter at 200 mg/kg/d was in agreement with the reduced ossification noted in rats. Since there were no notable adverse findings at fetal NOAEL of 40 mg/kg/d (142x MRHD), saxagliptin reduced food intake in a dose-related manner in pregnant rabbits, relation to drug is complicated by poor nutrition status of pregnant rabbits.
The pre- and post-natal development study in rats (40, 100, 250 and 500 mg/kg/d, 174x, 470x, 1629x, 3724x MRHD) found no drug related changes in dams or in off springs at 40 or 100 mg/kg/d. The decrease in body weight of offspring at ≥ 250 mg/kg/d appeared to be related to decrease in maternal weight. The clinical significance of decrease in offspring weight at maternal NOAEL of 100 mg/kg/d (470x clinical dose) is minimal. In the female rat fertility study, a significant decrease in fertility index (83% vs. 92% in control), corpora lutea and implantations were noted in females 750 mg/kg/d (6138x MRHD). There was a 5x increase in pre-implantation loss and 3x post implantation loss and early resorption in some female treated 300 mg/kg/d (2069x MRHD) and 750 mg/kg/d (6138x MRHD). Other changes that were not dose dependent or statistically significant were the decrease in the number of viable embryos, increase in epididymal and testes weight in treated males. Since the findings were likely due to toxicity and the NOAEL for male (200 mkd) and female (125 mg/kg/d) rat fertility studies were nearly 603x and 776x the AUC for 5 mg clinical dose, the clinical relevance to humans given saxagliptin alone is minimal.
In summary, the developmental findings such as delayed or reduced fetal ossification in rats and rabbits occurred at doses that were more than 1500x and 140x the AUC for clinical saxagliptin dose of 5 mg. Overall, saxagliptin alone did not produce malformation in rat and rabbit reproductive toxicology studies and thus was considered not teratogenic. Since TK studies in pregnant rats found significant saxagliptin exposure in milk (~ plasma) suggests that saxagliptin is likely to present in human milk as well. Whether saxagliptin in combination with metformin is teratogenic based on the interim safety report is yet to be determined since it is not clear at this point whether the malformations were coincidental and a pharmacodynamics or metformin related.

Genotoxicity and carcinogenicity

In the initial manufacturing process (Process B and C) the sponsor had used starting material that resulted in some impurities/degradants in saxagliptin (C C) Since the toxicology studies were carried out with the original product with some impurities, the overall safety of the impurities were assessed. These impurities appeared to have contributed to clastogenicity in the *in-vitro* cytogenetics study using human lymphocytes, since neither saxagliptin nor its metabolite, BMS-510849 were genotoxic in Ames assay. No clastogenicity or evidence of DNA damage was observed in rats at doses up to 2000 mg/kg/day for 3 days in a micronucleus assay or in DNA repair study, or 1 month 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 degradant were identified and removed or reduced in the final product manufactured by Process D for marketing. Since saxagliptin produced by the old method was used in toxicology studies in rats and dogs, the safety profile of impurities were covered. Using new different starting material and modification in manufacturing

b(4)

process (process D), the suspected impurities were removed and the remaining was reduced to less than ( ). Overall, the weight of evidence seems to support the idea that saxagliptin manufactured by process D and its' major active metabolite (BMS-510849) are not genotoxic. In the full 2-year carcinogenetic studies in male and female mice (50, 250 and 600 mg/kg/d, 20-32x, 428-376x, 870-1165x MRHD) and in male and female rats (25, 75, 150 and 300 mg/kg/d, 43-108x, 173-380x, 355-1012x, 847-2214x the clinical dose of 5 mg based on AUC) there was no saxagliptin related increase in incidence of tumors in either mice or rats. There was however, significant mortality in male mice at ≥ 250 mg/kg/d and male rats at 300 mg/kg/d in spite of the fact that exposure was 2 fold greater in female mice and rats. Although there were no saxagliptin -related increase in tissue pathology in mice, several target organs were identified in rats (lungs, harderian gland, urinary bladder, liver as well as CNS in male rats) at the end of the 2-year saxagliptin treatment. As noted earlier, the CNS lesions were only seen in the male rats and significant mortality in the HD males appeared to be related to CNS lesions produced by cyanide release. In summary, saxagliptin did not produce neoplasia in mice dosed up to 600 mg/kg/d and in rats up to 300 mg/kg/d at exposure multiples that were ≥ 1000x the clinical dose of 5 mg, based on AUC.

b(4)

2.6.6.10 Table- Safety margins for saxagliptin clinical dose of 5 mg QD

Species	**Dose,** mg/kg/ day	**Saxagliptin** AUC, ng.h/ml	**BMS-510849** AUC, ng.h/ml	Safety margins based on AUC (Animal/Human)	
				Saxagliptin	BMS-510849
2-Wk rat study, NOAEL= 20 mg/kg	2	M:262, F:549	M:60, F:227	M:3, F:7	M:<1, F: 0.5
	20	M:1600, F: 4353	M:506, F: 1227	M:20 , F: 54	M:1, F: 3
	200	M: 26106, F:103861	M:7774, F:15487	M:322, F:1281	M:96, F: 35
3-Month Rat study, NOAEL <300 mg/kg	300	M:106066, F:253300	M:52281, F:122008	M:1309, F: 3127	M:119, F:279
	600	M:238214, F: 517454	M:93214, F:227254	M:2941, F:6388	M:213, F:519
	1200	M:1309910, F: 712535	M:745661, F: 570211	M:16172, F: 8797	M:1702, F: 1302
6-Month rat study, NOAEL= 2 mkd	2	M:217, F:668	M:54, F:333	M:2.7, F:8.2	M:0.1, F:0.8
	20	M:2796, F:6111	M:1345, F:4259	M:35, F:75	M:3, F:10
	100	M:21869, F:48261	M:9464, F:25992	M:270, F:596	M:22, F:59
3-Month dog study, NOAEL=1 mg/kg	0.2	M:165, F: 138	M: 251, F:258	M:2 , F: 2	M:0.6, F: 0.6
	1	M:787, F: 1004	M:1484, F:2124	M: 10, F: 12	M: 3, F: 5
	5	M:5921, F: 4442	M:13388, F:12974	M: 73, F: 55	M:31, F: 30
12-Month dog study, NOAEL= 1 mg/kg	1	M:286, F:415	M:359, F:454	M:4, F:5	M:0.8, F:1
	5	M:1470, F:1544	M:1872, F:1964	M:18, F:19	M:4.3, F:4.5
	10	M:4278, F:2782	M:4767, F:5088	M:53, F:34	M:11, F:12
1-3 Month cynomolgus monkey study, No NAOEL	2	M:578 , F:1367	M:2717, F:3837	M:7, F:17	M:6 , F: 9
	10	M:2857, F:1702	M:15811, F:12387	M:35, F: 21	M: 36, F:28
	30/20	M: 6013 , F:4839	M:28207, F:26087	M:74, F: 60	M: 64, F: 60
3-Month cynomolgus monkey study, NOAEL =0.3 mg/kg	0.03	M: 8.6, F: 8.9	M: 53.9, F:61.6	M: <1, F: <1	M:<1 , F: <1
	0.3	M: 200, F: 79.2	M: 479.7, F: 504	M: 2.5, F: 1	M: 1 , F: 1
	3	M: 1592, F: 2196	M: 4647, F: 4825	M: 20, F: 27	M: 11 , F: 11
104-Week Mouse Carci Study, No tumors	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
104-Week Rat Study, No tumors	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
Fertility and early embryonic development in rats, NOAEL= 200 in males	100 M	M: 16071	M:4376	M:198	M:10
	200 M	M:48899	M:14227	M:603	M:32
	400 M	M: 90186	M:28684	M:1113	M:65
	125 F	F: 62833	F:9951	F:776	F: 23

and 125 mg/kg in females	300 F	F:167578	F:23378	F:2069	F:53
	750 F	F:497194	F:72536	F:6138	F:165
Oral Embryo-fetal development in rats, NOAEL=240 mg/kg	64	F: 23610	F: 6384	F: 291	F: 15
	240	F: 121774	F: 28918	F: 1503	F: 66
	900	F: 646843	F: 143637	F: 7986	F: 328
Oral Embryo-fetal development in rabbits, NOAEL=40 mg/kg	8	F: 2493	F:7407	F: 31	F: 17
	40	F: 12332	F: 47895	F: 152	F: 109
	200	F: 116027	F: 434489	F: 1432	F: 992
Oral rat embryofetal development with **saxagliptin/metformin** combination NOAEL=5/200 mg/kg	5/200	Saxagliptin F: 1630 Metformin F: 85200	Saxagliptin F:658	Saxagliptin F: 20 Metformin F:4	Saxagliptin F: 1.5
	25/200	Saxagliptin F: 8860 Metformin F: 59300	Saxagliptin F: 3510	saxagliptin F: 109 Metformin F:4	saxagliptin F: 8
Pre- and postnatal development in rats, NOAEL=100 mg/kg	40	F: 14100	F:3427	F: 174	F: 8
	100	F: 38061	F: 9573	F: 470	F: 22
	250	F: 131985	F: 23293	F: 1629	F: 53
	500	F: 301680	F: 37728	F: 3724	F: 86
Maximum Recommended Human Dose (MRHD) : Saxagliptin, 5 mg QD (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 in adequate 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. Metformin AUC used in the calculation for maximum therapeutic dose of 1000 mg BID was 20544 ng.h/ml.

OVERALL CONCLUSIONS AND RECOMMENDATIONS

In summary, the nonclinical studies provided by the sponsor support the approval of saxagliptin NDA. The overall safety profile of 5 mg clinical dose was well supported by the chronic nonclinical studies. The two studies recommended by the reviewer should resolve the potential malformation concern found in the rat embryo-fetal development study with saxagliptin in combination with metformin.

Recommendations:

We recommend that the following non-clinical studies be completed as a post-marketing requirement (PMR) as authorized under FDAAA. These studies are intended to clarify findings of neural tube malformations in an embryofetal development study in rats exposed to the combination of saxagliptin and metformin.

- A rat embryofetal development study with a design that includes separate arms for metformin alone, saxagliptin alone, and the combination of saxagliptin + metformin. We recommend analysis of blood glucose, folate, Vit B12 at the time of necropsy to determine the potential mechanism and pharmacodynamic interaction between saxagliptin and metformin.
- A rabbit embryofetal development study with a design that includes separate arms for metformin alone, saxagliptin alone, and the combination of saxagliptin + metformin. We recommend analysis of blood glucose, folate, Vit B12 at the time of necropsy to determine the potential mechanism and pharmacodynamic interaction between saxagliptin and metformin.

2.6.7 TOXICOLOGY TABULATED SUMMARY

Repeat-Dose Toxicity

Report Title: Three-Month Oral Range-Finding Toxicity Study in Mice
Species/Strain: Mouse/CD-1
Initial Age: 5 weeks
Date of First Dose: March 12, 2002
Duration of Dosing: 3 months
Duration of Postdose: None
Method of Administration: Oral (gavage)
Vehicle/Formulation: Benzoate salt in 1.25% Avicel®
Test Article: BMS-477118
Study No. DN02016
Document Control Number: 930004458
Location in Dossier: 4.2.3.2
GLP Compliance: Yes

Special Features: Groups 1-5 (0-600 mg/kg/day) began dosing on March 12, 2002. Groups 10-12 (0-1500 mg/kg/day) began dosing on July 10, 2002. For statistical analysis, Groups 2-5 were compared to Group 1, and Groups 11-12 were compared to Group 10.

No Observed Adverse Effect Level: 300 mg/kg/day

Daily Dose (mg/kg):	(0) Control		30		100		300		600	
Number of Animals:	M: 10	F: 10	M: 10	F: 10	M: 10	F: 10	M: 10	F: 10	M: 10	F: 10
Toxicokinetics (Saxagliptin):										
AUC (ng•hr/mL); Day 29	♦	♦	942	933	4586	4977	29248	20942	81229	42673
Cmax (ng/mL); Day 29	♦	♦	246	310	2616	2287	18970	9026	31572	10970
Toxicokinetics (BMS-510849[a]):										
AUC (ng•hr/mL); Day 29	♦	♦	6391	4846	27922	25508	116352	94758	242069	141548
Cmax (ng/mL); Day 29	♦	♦	2340	2808	13666	12285	55155	29682	71563	29686
Noteworthy Findings										
Died or Sacrificed Moribund	0	1[b]	0	0	1[b]	2[b]	0	1[b]	1[b]	2[b]
Body Weight (%)[c]	36.83 g	27.1 g	-3	+6	-4	+8	+1	+16**	+4	+8
Food Consumption (%)[c]	4.17 g	4.28 g	+9	+7	+15	+6	+25	+7	+30	+10
Water Consumption (mL)	♦	♦	♦	♦	♦	♦	♦	♦	♦	♦
Clinical Observations										
Abdominal distention	--	--	--	--	--	--	--	--	--	--
Activity decreased	--	1	--	--	--	--	--	--	--	--
Collapse	--	--	--	--	--	--	--	--	--	--
Dyspnea	--	--	--	--	--	--	--	--	--	--
Hunched posture	--	--	--	--	--	--	--	--	--	--
Inactive	--	--	--	--	--	--	--	--	--	--
Unkempt appearance	--	--	--	--	--	--	--	--	--	--
Urine-stained coat	--	--	--	--	--	--	--	--	--	--
Ophthalmoscopy	♦	♦	♦	♦	♦	♦	♦	♦	♦	♦
Electrocardiography	♦	♦	♦	♦	♦	♦	♦	♦	♦	♦
Hematology										
Hemoglobin (g/dL)	15.41	15.75	15.23	15.10	15.27	15.79	15.42	15.13	15.31	15.42
Serum Chemistry										
Triglycerides (mg/dL)	128.4	107.9	177.4	93.7	111.0	117.2	147.4	110.3	141.8	109.4
Cholesterol (mg/dL)	191.9	131.9	172.2	129.5	162.0	137.2	163.2	124.8	192.2	144.2
Albumin (g/dL)	3.03	3.14	2.94	3.14	2.98	3.17	2.90	3.02	2.78**	3.18
Globulins (g/dL)	2.38	2.11	2.40	2.16	2.45	2.17	2.35	2.16	2.30	2.27
Albumin/Globulin ratio	1.30	1.52	1.23	1.47	1.24	1.46	1.26	1.41	1.21	1.40*
Urinalysis	♦	♦	♦	♦	♦	♦	♦	♦	♦	♦
Organ Weights (%[c])										
Liver	1.6497 g	1.3192 g	+2	+1	-3	+1	+8	+14	+18**	+11
Spleen	0.0900 g	0.1049 g	-7	+4	+2	-7	+19	+30	+13	+11
Thymus	0.0401 g	0.0454 g	-16	-3	-13	-2	-19	-12	+4	-2
Gross Pathology										
Number Evaluated	10	10	10	10	10	10	10	10	10	10
Hindfeet – Swelling	0	0	0	0	0	0	0	0	0	0
Preputial skin – Ulceration	0	0	0	0	0	0	0	0	0	0
Scrotum – Scab formation	0	0	0	0	0	0	0	0	0	0

Abbreviations: – No noteworthy findings, ♦ Not conducted, NA = Not applicable.
* P<0.05, ** P<0.01 Dunnett's Test
All footnotes are available as table end notes.

Repeat-Dose Toxicity — Test Article: BMS-477118

Document Control Number: 930004458 (Continued) — Study No. DN02016

Daily Dose (mg/kg):	(0) Control		30		100		300		600	
Number of Animals:	M: 10	F: 10	M: 10	F: 10	M: 10	F: 10	M: 10	F: 10	M: 10	F: 10
Spleen – Increased size	0	0	0	0	0	0	0	0	0	0
Urinary bladder – Distention	0	0	0	0	0	0	0	0	0	0
Histopathology										
Number Evaluated	10	10	10	10	10	10	10	10	10	10
Thymus – Atrophy (multifocal)	0	0	0	0	0	0	0	0	0	2
Minimal	0	0	0	0	0	0	0	0	0	1
Mild	0	0	0	0	0	0	0	0	0	1
Lung – Histiocytosis (minimal)	0	0	0	0	0	0	0	0	1	1
Focal	0	0	0	0	0	0	0	0	1	0
Multifocal	0	0	0	0	0	0	0	0	0	1

Repeat-Dose Toxicity — Test Article: BMS-477118

Document Control Number: 930004458 (Continued) — Study No. DN02016

Daily Dose (mg/kg):	(0) Control		1000		1500	
Number of Animals:	M: 10	F: 10	M: 10	F: 10	M: 10	F: 10
Toxicokinetics (saxagliptin):						
AUC (ng•hr/mL); Day 29	♦	♦	208303	209545	263241	350051
Cmax (ng/mL); Day 29	♦	♦	51915	46344	37408	61040
Toxicokinetics (BMS-510849[a]):						
AUC (ng•hr/mL); Day 29	♦	♦	303715	353931	540022	529840
Cmax (ng/mL); Day 29	♦	♦	62495	52729	67822	68786
Noteworthy Findings						
Died or Sacrificed Moribund	0	0	1	0	2[e]	3
Body Weight (%)[c]	38.30 g	30.57 g	+1	-2	-0.3	-0.1
Food Consumption (%)[c]	5.68 g	5.78 g	+7	-6	+29**	-3
Water Consumption (mL)	♦	♦	♦	♦	♦	♦
Clinical Observations						
Abdominal Distention	–	–	–	–	1	–
Activity Decreased	–	–	1	–	2	1
Collapse	–	–	–	–	–	1
Dyspnea	–	–	–	–	1	–
Hunched posture	–	–	–	–	–	1
Inactive	–	–	–	–	1	1
Unkempt appearance	–	–	1	–	1	–
Urine-stained coat	–	–	4	–	3	1
Ophthalmoscopy	♦	♦	♦	♦	♦	♦
Electrocardiography	♦	♦	♦	♦	♦	♦
Hematology						
Hemoglobin (g/dL)	15.73	15.95	14.91	15.73	14.66*	15.12*
Serum Chemistry						
Cholesterol (mg/dL)	202.0	134.3	156.8**	138.4	166.4*	145.1
Albumin (g/dL)	3.04	3.21	2.71**	3.05	2.49**	2.94*

Abbreviations: – No noteworthy findings, ♦ Not conducted, NA = Not applicable.
* P<0.05, ** P<0.01 Dunnett's Test
All footnotes are available as table end notes.

Repeat-Dose Toxicity **Test Article:** BMS-477118

Document Control Number: 930004458 **(Continued)** **Study No.** DN02016

Daily Dose (mg/kg):	**(0) Control**		**1000**		**1500**	
Number of Animals:	**M: 10**	**F: 10**	**M: 10**	**F: 10**	**M: 10**	**F: 10**
Globulins (mg/dL)	2.45	2.29	2.60	2.14	2.85*	2.26
Albumin/Globulin ratio	1.26	1.41	1.08	1.44	0.89**	1.33
Urinalysis	♦	♦	♦	♦	♦	♦
Organ Weights (%[c])						
Liver	1.9115 g	1.4929 g	+6	+5	+19**	+28**
Spleen	0.0947 g	0.1221 g	+60	+1	+101**	+36
Thymus	0.0415 g	0.0566 g	-27	-33	-39*	-45
Gross Pathology						
Number Evaluated	10	10	10	10	10	10
Hindfeet - Swelling	0	0	1	0	0	0
Preputial skin - Ulceration	0	NA	3	NA	3	NA
Scrotum - Scab formation	0	NA	0	NA	1	NA
Spleen - Increased size	0	0	0	0	1	0
Urinary Bladder - Distention	0	0	1	0	3	0
Histopathology						
Number Evaluated	10	10	10	10	10	10
Thymus - Atrophy (multifocal)	0	0	5	2	5	4
Minimal	0	0	3	2	1	1
Mild	0	0	2	0	4	3
Lung - Histiocytosis (minimal)	0	0	2	1	6	4
Focal	0	0	1	1	1	1
Multifocal	0	0	1	0	5	3

[a] The major active metabolite of BMS-477118.

[b] Death deemed accidental.

[c] At the end of dosing period. For controls, group means are shown. For drug-treated groups, percent differences from control are shown (treatment value - control value ÷ control value × 100). Statistical significance is based on actual data (not percent differences).

[d] Both absolute and relative weights differed from controls in the direction indicated. Number indicates percent differences for the absolute organ weights.

[e] One male death deemed accidental at 1500 mg/kg.

Abbreviations: – No noteworthy findings, ♦ Not conducted, NA = Not applicable.
* P<0.05, ** P<0.01 Dunnett's Test
All footnotes are available as table end notes.

Repeat-Dose Toxicity

Report Title: Three-Month Oral Range-Finding Toxicity Study in Rats
Species/Strain: Rat / Harlan Sprague Dawley
Initial Age: 5 weeks
Date of First Dose: February 4, 2003
Duration of Dosing: 3 months
Duration of Postdose: None
Method of Administration: Oral (gavage)
Vehicle/Formulation: Benzoate salt 1.25% Avicel®
Special Features: None
No Observed Adverse Effect Level: Not Identified

Test Article: BMS-477118
Study No. DN03009
Document Control Number: 930004822
Location in Dossier: 4.2.3.2
GLP Compliance: Yes

Daily Dose (mg/kg):	(0) Control		300		600		1200	
Number of Animals:	M: 10	F: 10	M: 10	F: 10	M: 10	F: 10	M: 10	F: 10
Toxicokinetics (saxagliptin):								
AUC (ng•h/mL)								
Day 1	♦	♦	69303	139255	205729	336961	440786	990398
Day 91	♦	♦	106066	253300	238214	517454	1309910[a]	712535
Cmax (ng/mL)								
Day 1	♦	♦	13673	41096	14615	44868	149860	80174
Day 91	♦	♦	13750	45500	20582	106367	97074[a]	106501
Toxicokinetics (BMS-510849[b]):								
AUC (ng•h/mL)								
Day 1	♦	♦	62995	54074	158735	139256	427647	372412
Day 91	♦	♦	52281	122008	93214	227254	745661a	570211
Cmax (ng/mL)								
Day 1	♦	♦	11180	10889	10564	10257	131208	24973
Day 91	♦	♦	7603	15625	10110	30677	57063[a]	56764
Noteworthy Findings								
Died or Sacrificed Moribund	0	0	0	1[c]	3[c,d]	2[c]	6	2
Body Weight (%[e])	407.9 g	236.8 g	-8	-4	-19**	-5	-27**	-7
Food Consumption (%[e])	22.31 g	16.92 g	-7	-8	-18**	-17	-14*	-14
Water Consumption (mL)	♦	♦	♦	♦	♦	♦	♦	♦
Clinical Observations								
Activity decreased	--	--	--	--	--	--	10	4
Respiration abnormal								
Gasping	--	--	--	--	--	--	3	--
Decreased	--	--	--	--	--	--	1	1
Labored	--	--	--	--	--	--	7	--
Tremor	--	--	--	--	--	--	4	--
Ophthalmoscopy	--	--	--	--	--	--	--	--
Electrocardiography	♦	♦	♦	♦	♦	♦	♦	♦
Hematology								
Hemoglobin (g/dL)	15.92	15.31	15.54	14.57*	15.37	14.41**	15.36	14.38**
Mean corpuscular volume (fL)	54.03	54.72	51.54**	51.93*	50.98**	51.45**	52.94	50.24**
Mean corpuscular hemoglobin (pg)	18.24	18.77	17.39**	17.83	17.07**	17.65*	17.50	16.93**
Reticulocytes (%)	2.23	2.19	2.24	2.27	2.73*	3.20	4.06**	3.48*
Leukocytes (10^3/µL)	11.935	8.712	14.710	10.628	18.579**	9.759	19.818**	10.158
Lymphocytes (10^3/µL)	9.967	6.983	12.503	8.963	15.250**	8.100	14.914**	8.135
Neutrophils (10^3/µL)	1.239	1.212	1.456	1.119	2.264*	0.940	3.754**	1.373
Platelets (10^3/µL)	917.6	980.1	766	738.4**	784.9	625.1**	863.4	539.1**
Serum Chemistry								
Cholesterol (mg/dL)	120.0	114.0	104.4	88.1**	104.0	87.4**	105.0	85.5**
Total protein (g/dL)	6.75	6.56	6.79	6.80	6.57	6.47	6.12**	6.69
Albumin (g/dL)	3.40	3.47	3.38	3.36	3.30	3.17**	3.00**	3.16**
Alkaline phosphatase (U/L)	211.0	177.2	261.0	183.7	293.3*	202.6	322.8*	205.0

Abbreviations: – No noteworthy findings, ♦ Not conducted, NA Not applicable.
* P<0.05, ** P<0.01 Dunnett's Test; # P<0.05, ## P<0.01 Fisher's Exact Test
All footnotes are available as table end notes.

Repeat-Dose Toxicity **Test Article:** BMS-477118

Document Control Number: 930004822 **(Continued)** **Study No.** DN03009

Daily Dose (mg/kg):	**(0) Control**		**300**		**600**		**1200**	
Number of Animals:	**M: 10**	**F: 10**	**M: 10**	**F: 10**	**M: 10**	**F: 10**	**M: 10**	**F: 10**
Triglycerides (mg/dL)	98.5	46.3	93.6	58.6	109.0	58.1	233.2**	79.6**
Potassium (mEq/L)	6.41	5.93	5.88**	5.86	5.69**	5.40	5.44**	5.35*
Urinalysis	♦	♦	♦	♦	♦	♦	♦	♦
Organ Weights (%[f])								
Spleen	0.7680 g	0.6220 g	+59**	+43	+67**	+73**	+16	+94**
Thymus	0.3838 g	0.2554 g	-17*	-32**	-31**	-41**	-58**	-51**
Liver	9.7271 g	5.4862 g	+11	+1	+9	+15*	+29*	+39**
Pituitary	0.0101 g	0.0124 g	-16	-23*	-27*	-26**	-18	-45**
Gross Pathology								
Stomach - Red discoloration	0[g]	0	0	0	0	0	7	0
Spleen – Size increased	0	0	0	0	0	0	0	1
Thymus – Size decreased	0	0	0	0	0	0	1	0
Histopathology								
Brain -Degeneration in corpus callosum and/or caudate putamen (minimal)	0[g]	0	0	0	1	0	3	0
Lung - Histiocytosis	1	1	4	9	8	7	8	10
Minimal	1	1	4	9	8	7	6	10
Mild	0	0	0	0	0	0	2	0
Ocular accessory gland - Mononuclear-cell infiltration	1	1	1	6	3	8	1	8
Minimal	1	1	1	4	3	6	1	8
Mild	0	0	0	2	0	2	0	0
Spleen - Lymphoid hyperplasia (minimal)	0	0	9	7	9	2	6	7
Thymus - Lymphoid depletion/necrosis	0	0	0	2	2	9	6	8
Minimal	0	0	0	1	1	3	4	0
Mild	0	0	0	0	1	1	0	0
Moderate	0	0	0	1	0	5	2	7
Marked	0	0	0	0	0	0	0	1
Stomach - Erosions	0	0	0	0	0	0	6	0
Minimal	0	0	0	0	0	0	1	0
Mild	0	0	0	0	0	0	5	0

[a] As a result of a high mortality rate in males at 1200 mg/kg/day, there were a smaller number of toxicokinetic blood sampling times and number of animals per sampling compared to the other groups on Day 91.

[b] The major active metabolite of BMS-477118.

[c] Deaths deemed accidental: 1F at 300 mg/kg, 2M and 2F at 600 mg/kg.

[d] Cause of death for 1M at 600 mg/kg could not be determined.

[e] At end of dosing period. For controls, group means are shown. For treated groups, percent differences from controls are shown (treatment value - control value ÷ control value × 100). Statistical significance is based on actual data (not on the percent differences).

[f] Both absolute and relative weights differed from controls in the direction indicated. Number indicates percent differences for the absolute organ weights.

[g] Incidence of finding (expressed as number of animals)

Abbreviations: – No noteworthy findings, ♦ Not conducted, NA Not applicable.
* P<0.05, ** P<0.01 Dunnett's Test; # P<0.05, ## P<0.01 Fisher's Exact Test
All footnotes are available as table end notes.

Repeat-Dose Toxicity

Report Title: Six-Month Oral Toxicity Study in Rats
Species/Strain: Rat / Harlan Sprague Dawley
Initial Age: 6 weeks
Date of First Dose: March 28, 2002
Duration of Dosing: 6 months
Duration of Postdose: 1 month
Method of Administration: Oral (gavage)
Vehicle/Formulation: Benzoate salt in 1.25% Avicel®
Test Article: BMS-477118
Study No. DN02021
Document Control Number: 930003282
Location in Dossier: 4.2.3.2
GLP Compliance: Yes (except DPP4 analysis)

Special Features: 10 rats/sex/group were necropsied after 3 months of dosing; 20 rats/sex/group after 6 months of dosing, and the remaining rats were necropsied after a 1-month postdose recovery period.
No Observed Adverse Effect Level: 20 mg/kg/day

Daily Dose (mg/kg):	(0) Control		2		20		100	
Number of Animals:	M: 35	F: 35	M: 35	F: 35	M: 35	F: 35	M: 35	F: 35
Toxicokinetics (saxagliptin):								
AUC (ng•h/mL)								
Day 1	♦	♦	181	326	2030	3844	16758	32804
Day 92	♦	♦	183	671	2468	6127	18060	37795
Day 181	♦	♦	217	668	2796	6111	21869	48261
Cmax (ng/mL)								
Day 1	♦	♦	62	127	917	1579	4946	11444
Day 92	♦	♦	77	336	976	2717	4475	14182
Day 181	♦	♦	82	223	1118	2363	4607	18741
Toxicokinetics (BMS-510849[a]):								
AUC (ng•h/mL)								
Day 1	♦	♦	131	211	1871	3251	15317	20130
Day 92	♦	♦	50	307	1300	4738	9148	22995
Day 181	♦	♦	54	333	1345	4259	9464	25992
Cmax (ng/mL)								
Day 1	♦	♦	54	91	738	1219	3552	4332
Day 92	♦	♦	40	184	431	1706	1884	5219
Day 181	♦	♦	40	178	563	1338	1993	5948
Pharmacodynamics (DPP4 activity):								
Emax (% inhibition)[b]								
Day 1	♦	♦	73	72	73	75	72	79
Day 92	♦	♦	81	78	86	82	91	86
Day 181	♦	♦	74	70	84	81	84	77
Noteworthy Findings								
Died or Sacrificed Moribund	2[c]	0	0	1[c]	2[c]	1[c]	0	0
Body Weight (%)[d]	468.8 g	258.6 g	0	-2	-5*	-5	-3	-6*
Food Consumption (%)[d]	23.28 g	15.78 g	+1	-2	+2	+2	-1	-1
Water Consumption (mL)	--	--	--	--	--	--	--	--
Clinical Observations	--	--	--	--	--	--	--	--
Ophthalmoscopy	--	--	--	--	--	--	--	--
Electrocardiography	♦	♦	♦	♦	♦	♦	♦	♦
Blood Pressure (mm Hg)[e]								
Week 1	150.4	124.6	136.8	121.2	121.3*	125.0	123.2*	130.2
Week 13	152.6	123.8	146.6	127.6	143.6	105.0	127.4*	105.2
Week 25	131.0	119.0	131.2	119.8	136.5	126.4	143.4	114.6
Week 30 (post dose period)[f]	147.6	128.8	131.6	124.8	140.0	130.0	140.4	123.2
Heart Rate (beats/minute)	--	--	--	--	--	--	--	--
Hematology	--	--	--	--	--	--	--	--

Abbreviations: -- No noteworthy findings, ♦ Not conducted, Emax = Maximum plasma percent DPP4 inhibition.
* P<0.05, ** P<0.01 All footnotes are available as table end notes.

Repeat-Dose Toxicity **Test Article:** BMS-477118

Document Control Number: 930003282 **(Continued)** **Study No.** DN02021

Daily Dose (mg/kg):	(0) Control		2		20		100	
Number of Animals:	**M: 35**	**F: 35**	**M: 35**	**F: 35**	**M: 35**	**F: 35**	**M: 35**	**F: 35**
Serum Chemistry[e]								
Cholesterol (mg/dL)								
Week 5	117.1	128.1	118.0	123.3	121.1	117.7	101.7**	102.0**
Week 13	124.3	126.7	122.7	122.9	122.1	119.2	104.7**	96.9**
Week 26	147.1	141.4	134.1	132.5	130.9	137.5	108.9**	113.8**
Week 30 (post dose period)[f]	175.4	152.3	138.2	134.0	145.0	132.8	132.0	135.6
Alkaline Phosphatase (U/L)								
Week 5	213.1	169.8	215.5	174.7	237.0**	175.6	240.5**	191.9
Week 13	155.9	128.3	147.6	145.4	181.7**	149.4	182.5**	157.3*
Week 26	168.4	154.1	170.1	172.6	207.9**	188.8*	204.9**	196.7**
Week 30 (post dose period)[f]	173.0	180.0	187.8	177.8	176.0	151.2	193.8	171.0
Urinalysis	--	--	--	--	--	--	--	--
Organ Weights (%[e,g])								
Spleen								
Month 3[h]	0.9499 g	0.6396 g	-17	-13	-16	-7	-2	+12
Month 6[h]	0.8465 g	0.6097 g	+11	-3	+9	-2	+21**	+15*
Gross Pathology	--	--	--	--	--	--	--	--
Histopathology[e]								
Month 3 – Total Examined	10	10	10	10	10	10	10	10
Spleen - Lymphoid hyperplasia	0	0	0	0	1	7	7	9
Minimal	0	0	0	0	1	7	7	6
Mild	0	0	0	0	0	0	0	3
Month 6 – Total Examined	20	20	20	20	20	20	20	20
Spleen - Lymphoid hyperplasia	0	0	0	0	7	13	18	12
Minimal	0	0	0	0	7	13	18	11
Mild	0	0	0	0	0	0	0	1

[a] The major active metabolite of BMS-477118.

[b] For treated groups, percent differences from controls are shown.

[c] All deaths were either accidental or incidental and not considered related to drug.

[d] At end of dosing period. For controls, group means are shown. For treated groups, percent differences from controls are shown (treatment value - control value ÷ control value × 100). Statistical significance is based on actual data (not on the percent differences).

[e] No changes were observed after the 1-month recovery period.

[f] Five rats/sex/group Week 30.

[g] Both absolute and relative weights differed from controls in the direction indicated. Number indicates percent differences for the absolute organ weights.

[h] Ten rats/sex/group Month 3; 20 rats/sex/groups Month 6.

Abbreviations: -- No noteworthy findings, ♦ Not conducted, Emax = Maximum plasma percent DPP4 inhibition.

* P<0.05, ** P<0.01 All footnotes are available as table end notes.

Repeat-Dose Toxicity

Report Title: Three-Month Oral Toxicity Study in Dogs
Species/Strain: Dog / Beagle
Initial Age: 9-25 months
Date of First Dose: April 10, 2002
Duration of Dosing: 3 months
Duration of Postdose: 1 month
Method of Administration: Oral (capsule)
Vehicle/Formulation: Benzoate salt in 1.25% Avicel®
Test Article: BMS-477118
Study No. DN02024
Document Control Number: 930003281
Location in Dossier: 4.2.3.2
GLP Compliance: Yes (except DPP4 analysis)

Special Features: Three dogs/sex/group were sacrificed after 3 months of dosing; the remaining animals were sacrificed after a 1-month postdose evaluation.
No Observed Adverse Effect Level: 1 mg/kg/day

Daily Dose (mg/kg):	(0) Control		0.2		1		5	
Number of Animals:	M: 5	F: 5	M: 5	F: 5	M: 5	F: 5	M: 5	F: 5
Toxicokinetics (saxagliptin):								
AUC (ng•h/mL)								
Day 1	♦	♦	148	118	795	1018	5959	4116
Day 90	♦	♦	165	138	787	1004	5921	4442
Cmax (ng/mL)								
Day 1	♦	♦	64	59	329	419	2350	1763
Day 90	♦	♦	63	61	304	371	2321	1924
Toxicokinetics (BMS-510849[a]):								
AUC (ng•h/mL)								
Day 1	♦	♦	269	263	1587	1611	8614	8938
Day 90	♦	♦	251	258	1484	2124	13388	12974
Cmax (ng/mL)								
Day 1	♦	♦	64	76	364	333	1661	1606
Day 90	♦	♦	62	62	305	394	1903	2081
Pharmacodynamics (DPP4 activity):								
Emax (% inhibition)								
Day 1	♦	♦	89	88	93	93	100	98
Day 90	♦	♦	90	89	93	93	100	100
Postdose Day 27	♦	♦	27	16	15	4	23	14
Noteworthy Findings								
Died or Sacrificed Moribund	0	0	0	0	0	0	0	0
Body Weight (%)[b]	10.64 kg	10.10 kg	-1	-3	-3	-2	-2	-4
Food Consumption (%[c])	100	87.5	96.4	100	85.4*	100	97.8	100
Water Consumption (mL)	--	--	--	--	--	--	--	--
Clinical Observations[d]								
Loose feces	0	0	0	0	0	0	5	5
Red feces	0	0	0	0	0	0	5	5
Ophthalmoscopy	--	--	--	--	--	--	--	--
Electrocardiography	--	--	--	--	--	--	--	--
Hematology[d]								
Eosinophils (10^3/µL) - Week 12	0.280	0.268	0.282	0.720	0.224	0.342	0.758*	0.412
Eosinophils (10^3/µL) - Postdose	0.410	0.150	0.245	0.600*	0.220	0.260	0.195	0.155
Serum Chemistry[d]								
Albumin (g/dL) - Week 12	3.14	3.14	3.28	3.18	3.16	3.22	2.88*	3.08
Albumin (g/dL) - Postdose	3.10	3.10	3.15	3.20	3.20	3.40	3.05	3.30
Phosphorus (mg/dL) - Week 12	5.06	4.42	4.28	4.54	4.00*	4.34	3.60**	4.28
Phosphorus (mg/dL) - Postdose	4.15	4.05	4.05	4.05	4.45	4.25	4.10	4.30
Urinalysis	--	--	--	--	--	--	--	--
Organ Weights (%)	--	--	--	--	--	--	--	--
Gross Pathology	--	--	--	--	--	--	--	--

Abbreviations: -- No noteworthy findings, ♦ Not performed, Emax = Maximum percent inhibition.
* P<0.05, ** P<0.01 All footnotes are available as table end notes.

Repeat-Dose Toxicity **Test Article:** BMS-477118

Document Control Number: 930003281 **(Continued)** **Study No.** DN02024

Daily Dose (mg/kg):	(0) Control		0.2		1		5	
Number of Animals:	M: 5	F: 5	M: 5	F: 5	M: 5	F: 5	M: 5	F: 5
Histopathology[d]								
Liver - Minimal subacute inflammation around central veins	0	0	0	0	0	0	2	0

[a] The major active metabolite of BMS-477118.

[b] At the end of dosing period. For controls, group means are shown. For drug-treated groups, percent differences from control are shown (treatment value - control value ÷ control value × 100). Statistical significance is based on actual data (not percent differences).

[c] At end of dosing period (percent consumed).

[d] All changes reversed during the 1-month recovery period.

Repeat-Dose Toxicity

Test Article: BMS-477118

Report Title: Twelve-Month Oral Toxicity Study in Dogs **Study No.** DN02057

Species/Strain: Dog / Beagle **Duration of Dosing:** 12 months **Document Control Number:** 930008126

Initial Age: 5-6 Months **Duration of Postdose:** None **Location in Dossier:** 4.2.3.2

Date of First Dose: September 12 -13, 2002 **Method of Administration:** Oral (gavage)[a]

Vehicle/Formulation: Benzoate salt in 1.25% Avicel® **GLP Compliance:** Yes (except DPP4 analysis)

Special Features: Three (3) dogs/sex/group were sacrificed after 6 months of dosing for an interim evaluation; the remaining 4 dogs/sex/group were dosed for an additional 6 months.

No Observed Adverse Effect Level: 1 mg/kg/day

Daily Dose (mg/kg):	(0) Control		1		5		10	
Number of Animals:	M: 7[b]	F: 7[b]	M: 7[b]	F: 7[b]	M: 7[b]	F: 7[b]	M: 7[b]	F: 7[b]
Toxicokinetics (saxagliptin):								
AUC (ng•h/mL)								
Day 1	♦	♦	722	754	3688	3582	8979	7723
Day 179	♦	♦	433	461	2406	3583	4141	6944
Day 361	♦	♦	286	415	1470	1544	4278	2782
Cmax (ng/mL)								
Day 1	♦	♦	297	340	1728	1785	3913	2997
Day 179	♦	♦	210	257	1222	1912	1960	3613
Day 361	♦	♦	167	238	749	830	2080	1479
Toxicokinetics (BMS-510849[c]):								
AUC(ng•h/mL)								
Day 1	♦	♦	1072	911	5635	5206	13539	10493
Day 179	♦	♦	428	542	3016	4238	3296	9985
Day 361	♦	♦	359	454	1872	1964	4767	5088
Cmax (ng/mL)								
Day 1	♦	♦	265	259	1439	1585	2998	2497
Day 179	♦	♦	156	188	952	1216	916	2926
Day 361	♦	♦	127	172	666	619	1337	1363
Pharmacodynamics (DPP4 activity):								
Emax (% inhibition)								
Day 1	♦	♦	91.9	92.6	95.7	95.4	96.3	96.2
Day 179	♦	♦	94.3	94.3	96.9	98.0	97.7	98.1
Day 361	♦	♦	97.1	96.6	97.8	98.2	99.1	98.5

Abbreviations: -- No noteworthy findings, ♦ Not conducted, + Present, Emax = Maximum percent inhibition.

* $p<0.05$, ** $p<0.01$ All footnotes are available as table end notes.

Repeat-Dose Toxicity **Test Article:** BMS-477118

Document Control Number: 930008126 (Continued) **Study No.** DN02057

Daily Dose (mg/kg):	(0) Control		1		5		10	
Number of Animals:	M: 7[b]	F: 7[b]	M: 7[b]	F: 7[b]	M: 7[b]	F: 7[b]	M: 7[b]	F: 7[b]
Noteworthy Findings								
Died or Sacrificed Moribund	0	0	0	0	0	0	0	0
Body Weight (%[d])	11.7 kg	8.7 kg	-16	+9	-11	0	-20	-3
Food Consumption (qualitative)	--	--	--	--	--	--	--	--
Water Consumption (mL)	--	--	--	--	--	--	--	--
Clinical Observations								
Abnormally colored feces	--	--	--	--	+	+	+	+
Unformed/mucoid feces	--	--	--	--	+	+	+	+
Foot pad cracking	--	--	--	--	+	+	+	+
Ophthalmoscopy	--	--	--	--	--	--	--	--
Electrocardiography	--	--	--	--	--	--	--	--
Hematology								
Eosinophils (10^3/µL)								
Month 3	0.23	0.24	0.19	0.25	0.34	0.23	0.46	0.58*
Month 6	0.32	0.26	0.29	0.38	0.44	0.45	0.46	0.71**
Month 9	0.32	0.23	0.49	0.49	0.66	0.49	0.33	0.71*
Month 12	0.33	0.35	0.38	0.47	0.59	0.53	0.51	0.78
White Blood Cells (103/µL)								
Month 3	12.84	10.72	11.04	13.35	11.81	16.11	10.28*	12.96
Month 6	12.52	10.09	9.9	11.59	11.89	10.41	10.17	10.07
Month 9	11.12	10.46	10.99	12.46	12.19	11.37	9.59	11.64
Month 12	12.19	11.48	10.49	13.33	16.44	11.81	11.12	13.85
Lymphocytes (103/µL)								
Month 3	3.76	3.77	3.65	3.97	3.36	3.98	3.09	4.06
Month 6	3.20	3.09	2.96	3.34	3.02	3.20	2.51*	3.18
Month 9	3.51	3.74	3.39	3.85	2.94	3.33	2.75	3.86
Month 12	3.23	3.67	3.24	3.91	3.14	3.59	3.42	4.06
Neutrophils (103/µL)								
Month 3	7.79	5.88	6.35	8.11	7.32	10.79	5.88*	7.56
Month 6	8.13	6.04	5.89	7.15	7.78	6.25	6.50	5.65
Month 9	6.62	5.80	6.31	7.39	7.89	6.94	5.89	6.46
Month 12	7.92	6.69	6.16	8.22	11.68*	7.03	6.46	8.24
Serum Chemistry								
Serum Cholesterol (mg/dL)								
Month 3	144	172	145	150	149	152	153	146
Month 6	148	175	157	168	164	158	170	191
Month 9	156	162	173	196	151	168	162	191
Month 12	153	178	175	177	160	169	149	227*
Triglycerides (mg/dL)								
Month 3	36	32	39	36	36	40	33	38
Month 6	38	30	41	35	33	34	35	39*
Month 9	37	30	42	34	30	35	39	33
Month 12	42	36	45	30	40	42	39	60*
Phosphorus (mg/dL)								
Month 3	5.2	5.0	4.9	5.1	4.8	4.7	4.6*	4.5
Month 6	4.3	3.9	4.1	4.1	4.2	3.8	4.0	3.8
Month 9	3.8	3.6	3.4	3.3	3.6	3.2	3.6	3.1
Month 12	3.4	3.5	2.9	3.6	3.0	3.0	2.9	3.0

Abbreviations: -- No noteworthy findings, ♦ Not conducted, + Present, Emax = Maximum percent inhibition.
* $p<0.05$, ** $p<0.01$ All footnotes are available as table end notes.

Repeat-Dose Toxicity **Test Article:** BMS-477118
Document Control Number: 930008126 (Continued) **Study No.** DN02057

Daily Dose (mg/kg):	(0) Control		1		5		10	
Number of Animals:	M: 7[b]	F: 7[b]	M: 7[b]	F: 7[b]	M: 7[b]	F: 7[b]	M: 7[b]	F: 7[b]
Potassium (mEq/L)								
Month 3	4.6	4.5	4.6	4.6	4.7	4.6	4.4	4.4
Month 6	4.5	4.5	4.4	4.5	4.5	4.4	4.2	4.4
Month 9	4.3	4.4	4.1	4.2	4.2	4.3	4.1	4.1*
Month 12	4.6	4.5	4.4	4.6	4.5	4.5	4.2*	4.6
Albumin (g/dL)								
Month 3	3.2	3.4	3.2	3.3	3.2	3.2	3.1	3.2*
Month 6	3.5	3.6	3.5	3.6	3.6	3.6	3.4	3.5
Month 9	3.4	3.4	3.4	3.5	3.4	3.3	3.2	3.4
Month 12	3.5	3.4	3.3	3.4	3.4	3.4	3.0*	3.4
Serum Total Protein (g/dL)								
Month 3	5.4	5.4	5.4	5.4	5.3	5.3	5.4	5.2
Month 6	5.7	5.7	6.0	5.7	5.8	5.6	5.8	5.6
Month 9	5.8	5.5	5.9	5.7	5.6	5.7	5.6	5.6
Month 12	6.0	5.6	5.9	5.5	5.7	5.7	5.3*	5.6
Urinalysis	--	--	--	--	--	--	--	--
Organ Weights (%)	--	--	--	--	--	--	--	--
Gross Pathology - Month 6	--	--	--	--	--	--	--	--
Gross Pathology - Month 12								
Footpads - sores	0	0	0	0	0	1	3	4
Histopathology - Month 6	--	--	--	--	--	--	--	--
Histopathology - Month 12								
Footpads - erosions	0	0	0	0	0	1	3	4
Minimal	0	0	0	0	0	0	0	2
Slight	0	0	0	0	0	1	3	2

[a] Beginning on Day 286, 1 female was capsule dosed.

[b] Three (3) animals at Month 6 and 4 animals at Month 12.

[c] The major active metabolite of BMS-477118.

[d] At the end of dosing period. For controls, group means are shown. For treated groups, percent differences from controls are shown. Statistical significance is based on actual data (not on the percent differences).

Abbreviations: -- No noteworthy findings, ♦ Not conducted, + Present, Emax = Maximum percent inhibition.
* $p<0.05$, ** $p<0.01$ All footnotes are available as table end notes.

Repeat-Dose Toxicity

Report Title: Three-Month Oral Toxicity Study in Monkeys
Species/Strain: Cynomolgus monkey
Initial Age: 25 to 32 months old
Date of First Dose: 24-October-2006
Duration of Dosing: 91 days
Duration of Postdose: 92 days
Method of Administration: Oral (gavage)
Vehicle/Formulation: Acidified water, final pH 5 ± 0.5
Test Article:
Study No.
Document Control Number: 930024646
Location in Dossier: 4.2.3.2
GLP Compliance: Yes (except plasma DPP analysis and urine cystocentesis)

Special Features: Four monkeys were necropsied at the end of the 3-month dosing period, remaining monkeys were necropsied after a 3-month recovery period. Study included detailed observations for appearance, progression, and recovery of skin lesions
No Observed Adverse Effect Level: 0.3 mg/kg/day

Daily Dose (mg/kg):	(0) Control		0.03		0.3		3	
Number of Animals:	M: 7	F: 7	M: 7	F: 7	M: 7	F: 7	M: 7	F: 7
Toxicokinetics (saxagliptin):								
AUC (ng•h/mL)								
Day 1	-	-	9	8	71	79	667	852
Day 36	-	-	9	9	88	79	1137	1762
Day 85	-	-	9	9	200	79	1592	2196
Cmax (ng/mL)								
Day 1	-	-	4	4	30	32	316	360
Day 36	-	-	4	3	34	30	474	533
Day 85	-	-	3	4	58	29	537	657
Toxicokinetics (BMS-510849[a]):								
AUC (ng•h/mL)								
Day 1	-	-	51	53	413	480	3901	4795
Day 36	-	-	53	59	386	503	3669	4806
Day 85	-	-	54	62	480	504	4647	4825
Cmax (ng/mL)								
Day 1	-	-	9	12	117	137	1259	1381
Day 36	-	-	11	12	118	150	1241	1319
Day 85	-	-	9	12	130	141	1474	1362
Pharmacodynamics (DPP Activity)								
Emax (% inhibition)								
Day 1	15.3	26.2	53.8	53.3	68.6	60	64.2	61
Day 36	14.9	23	52.8	52	69.4	58.1	62.5	61.1
Day 85	16.3	16	49.3	52.2	69.8	56.6	64.4	61.3
Emin (% inhibition)								
Day 1	1.7	15.4	9.7	12.3	45.1	22	31	44.5
Day 36	4.3	13.3	12.1	11.7	47.5	17.4	31.3	47.9
Day 85	5.2	13.6	12.8	28	51.6	19.5	29.9	48.4
Noteworthy Findings								
Died or Sacrificed Moribund	--	--[b]	--	--	--	--	--	--
Body Weight (%[c])	3.27 kg	2.87 kg	-3	+2	-3	+3	0	+2
Food Consumption	♦	♦	♦	♦	♦	♦	♦	♦
Clinical Observations								
Scabs, multifocal; feet and/or tail	--	--	--	--	--	--	2	2
Ophthalmoscopy	--	--	--	--	--	--	--	--
Electrocardiography	--	--	--	--	--	--	--	--
Hematology	--	--	--	--	--	--	--	--
Serum Chemistry	--	--	--	--	--	--	--	--
Organ Weights (%)	-	--	--	--	--	--	--	--
Gross Pathology								
Rough skin, ulcers, and/or scabs; multifocal, tail and/or foot	--	--	--	--	--	--	1	1

Abbreviations: -- No noteworthy findings, ♦ Not conducted.
All footnotes are available as table end notes.

Repeat-Dose Toxicity **Test Article:** BMS-477118

Document Control Number: 930024646 **(Continued)** **Study No.** DN06061

Daily Dose (mg/kg):	**(0) Control**				**0.03**				**0.3**				**3**			
Number of Animals:	**M:**	7	**F:**	7	**M:**	7	**F:**	7	**M:**	7	**F:**	7	**M:**	7	**F:**	7
Histopathology																
Skin, tail - microvascular hypertrophy with mononuclear cell infiltration and epithelial hyperplasia																
Minimal	--		--		--		--		--		--		1		--	
Slight	--		--		--		--		--		--		--		1	
Skin, foot - microvascular hypertrophy with mononuclear cell infiltration and epithelial hyperplasia																
Minimal	--		--		--		--		--		--		--		1	
Skeletal muscle - vascular inflammation, non-necrotizing																
Minimal	--		--		--		--		--		--		--		3	
Mammary gland - mononuclear cell infiltration																
Slight	--		--		--		--		--		--		--		3	
Pancreas - inflammation, chronic																
Minimal	--		--		--		--		--		--		1		--	
Spleen/bone marrow/thymus - hyperplasia, lymphoid																
Minimal	--		--		--		--		--		--		1		2	
Postdose Evaluation - Number Evaluated:	3		3		3		3		3		3		3		3	
Clinical Observations	--		--		--		--		--		--		--		--	
Gross Pathology	--		--		--		--		--		--		--		--	
Histopathology	--		--		--		--		--		--		--		--	

[a] The major active metabolite of BMS-477118.

[b] One (1) control female was removed from study on Day 32 due to accidental injury.

[c] At end of dosing period. For controls, group means are shown. For treated groups, percent differences from controls are shown.

b(4)

Genotoxicity: In Vitro

Report Title: Ames Reverse-Mutation Study in Salmonella and Escherichia coli **Test Article:** BMS-477118 (containing 0.98% ( )

Test for Induction of: Reverse mutation in bacterial cells **No. of Independent Assays:** 2 **Study No.** DS01143
Document Control Number: 930000816

Strains: S. typhimurium TA98, TA100, TA1535, TA1537, and E. coli WP2 uvrA **No. of Replicate Cultures:** 2 or 3[b] **Location in Dossier:** 4.2.3.3.1

Metabolizing System: Aroclor induced rat liver S9, 10% **No. of Cells Analyzed/Culture:** Not Applicable

Vehicles: **For Test Article:** Benzoate salt in DMSO **For Positive Controls:** DMSO or Water **GLP Compliance:** Yes[c]

Treatment: Standard plate incorporation followed by 46-50 hour colony formation **Dates of Treatment:** August 14, 2001 and August 28, 2001

Cytotoxic Effects: Yes - strain TA98 (5000 µg/plate) in Assay 2 only

Genotoxic Effects: None

Metabolic Activation	Test Article	Dose Level (µg/plate)	Mean Revertant Colony Counts				
			TA98	TA100	TA1535	TA1537	WP2 uvrA
Without Activation	DMSO	100 µL/plate	15	78	6	7	14
Assay No. 1[c]	BMS-477118	50	8	85	10	6	10
		160	13	93	10	4	9
		500	14	87	9	4	11
		1600	18	74	6	10	20
		5000	12	92	7	6	7
	2-nitrofluorene[d]	2	976	--	--	--	--
Without Activation	sodium azide[d]	2	--	677	521	--	--
Assay No. 1[c]	9-aminoacridine[d]	100	--	--	--	538	--
	methyl methane-sulfonate[d]	2.5 µL/plate	--	--	--	--	458
With Activation	DMSO	100 µL/plate	14	100	15	7	14
Assay No. 1[c]	BMS-477118	50	20	94	8	5	18
		160	23	109	9	4	21
		500	18	106	9	4	16
		1600	24	104	10	6	17
		5000	19	104	6	6	13
	2-aminoanthracene[d]	2.5	1325	1618	238	94	--
	2-aminoanthracene[d]	10	--	--	--	--	570
Without Activation	DMSO	100 µL/plate	10 ± 3	77 ± 10	6 ± 2	3 ± 1	14 ± 7
Assay No. 2	BMS-477118	50	11 ± 2	93 ± 1	9 ± 5	2 ± 3	17 ± 4
		160	5 ± 2	84 ± 9	7 ± 0	2 ± 1	12 ± 6
		500	6 ± 0	81 ± 12	4 ± 1	3 ± 1	15 ± 4
		1600	10 ± 4	81 ± 12	5 ± 2	5 ± 2	19 ± 6
		5000	7[e] ± 4	67 ± 6	9 ± 3	2 ± 2	15 ± 3
	2-nitrofluorene[d]	2	831	--	--	--	--
	sodium azide[d]	2	--	605	536	--	--
	9-aminoacridine[d]	100	--	--	--	739	--
	methyl methane-sulfonate[d]	2.5 µL/plate	--	--	--	--	636

b(4)

Genotoxicity: In Vitro

Test Article: BMS-477118 (containing 0.98% [redacted])

Document Control Number: 930000816 **(Continued)** **Study No.** DS01143

Metabolic Activation	Test Article	Dose Level (µg/plate)	Mean Revertant Colony Counts (± SD)				
			TA98	TA100	TA1535	TA1537	WP2 uvrA
With Activation	DMSO	100 µL/plate	14 ± 5	99 ± 9	8 ± 2	3 ± 2	13 ± 4
Assay No. 2	BMS-477118	50	16 ± 1	85 ± 9	9 ± 2	5 ± 2	16 ± 3
		160	15 ± 3	89 ± 26	11 ± 3	4 ± 2	15 ± 2
		500	15 ± 5	105 ± 20	9 ± 5	3 ± 2	14 ± 2
		1600	16 ± 2	97 ± 7	9 ± 2	5 ± 3	21 ± 3
		5000	13[e] ± 6	76 ± 6	9 ± 1	4 ± 3	15 ± 5
With Activation	2-aminoanthracene[d]	2.5	837	1765	253	49	--
Assay No. 2	2-aminoanthracene[d]	10	--	--	--	--	431

[a] An impurity/degradant

[b] No. of replicate cultures - Assay No. 1: Negative controls, n = 2; Positive controls, n = 2; Test article, n = 2
Assay No. 2: Negative controls, n = 5; Positive controls, n = 2; Test article, n = 3

[c] Assay No. 1 was a non-GLP range-finding assay

[d] Positive controls

[e] Minimal reduction in the bacterial background lawn

Genotoxicity: In Vitro

Report Title: Reverse-Mutation Study in Salmonella typhimurium and Escherichia coli
Test for Induction of: Reverse mutation in bacterial cells
Strains: S. typhimurium TA98, TA100, TA1535, TA1537, and E. coli WP2 uvrA
Metabolizing System: Aroclor induced rat liver S9, 10%
Vehicles: **For Test Article:** Sterile water **For Positive Controls:** DMSO or Water
Treatment: Standard plate incorporation followed by 46-50 hour colony formation
No. of Independent Assays: 2
No. of Replicate Cultures: 2 or 3[b]
No. of Cells Analyzed/Culture: Not Applicable
Test Article: BMS-510849[a]
Study No. DS03079
Document Control Number: 930004892
Location in Dossier: 4.2.3.3.1
GLP Compliance: Yes[c]
Dates of Treatment: April 16, 2003 and July 23, 2003
Cytotoxic Effects: Yes, strains TA100 and TA1537 only at 5000 µg/plate
Genotoxic Effects: None

Metabolic Activation	Test Article	Dose Level (µg/plate)	Mean Revertant Colony Counts (± Std Dev)				
			TA98	TA100	TA1535	TA1537	WP2 uvrA
Without Activation	Sterile water	100 µL/plate	19	123	12	7	20
Assay No. 1[c]	BMS-510849	10	22	126	7	5	26
		50	16	132	10	9	22
		100	18	136	8	7	26
		500	22	137	13	6	28
		1000	16	127	9	5	20
		2500	24	144	13	10	21
		5000	25	144	13	10	24

Abbreviations: DMSO = Dimethyl sulfoxide, -- Not tested.
All footnotes are available as table end notes.

Genotoxicity: In Vitro **Test Article: BMS-510849**

Document Control Number: 930004892 **(Continued)** **Study No.** DS03079

Metabolic Activation	Test Article	Dose Level (µg/plate)	Mean Revertant Colony Counts (± Std Dev)				
			TA98	TA100	TA1535	TA1537	WP2 uvrA
	2-nitrofluorene[d]	2	540	--	--	--	--
Without Activation	sodium azide[d]	2	--	1200	415	--	--
Assay No. 1[c]	9-aminoacridine[d]	100	--	--	--	349	--
	methyl methane-sulfonate[d]	2.5 µL/plate	--	--	--	--	532
With Activation	Sterile water	100 µL/plate	28	148	22	15	18
Assay No. 1[c]	BMS-510849	10	26	175	17	11	24
		50	31	161	16	9	21
		100	30	157	19	8	27
		500	33	168	17	7	24
		1000	31	154	15	9	26
		2500	30	171	13	9	33
		5000	30	166	18	10[e]	24
	2-aminoanthracene[d]	2.5	1299	1839	202	138	--
	2-aminoanthracene[d]	10	--	--	--	--	693
Without Activation	Sterile water	100 µL/plate	12 ± 4	114 ± 14	8 ± 2	5 ± 2	17 ± 7
Assay No. 2	BMS-510849	250	13 ± 5	117 ± 9	6 ± 3	5 ± 3	10 ± 5
		500	13 ± 3	98 ± 2	8 ± 2	6 ± 2	7 ± 2
		1000	15 ± 5	129 ± 10	9 ± 3	8 ± 4	16 ± 5
		1600	14 ± 1	109 ± 10	7 ± 3	7 ± 3	12 ± 3
		3000	15 ± 2	112 ± 15	8 ± 2	5 ± 2	16 ± 4
		5000	12 ± 4	112[e] ± 14	7 ± 1	5[e] ± 3	12 ± 3
	2-nitrofluorene[d]	2	394	--	--	--	--
	sodium azide[d]	2	--	834	278	--	--
	9-aminoacridine[d]	100	--	--	--	585	--
	methyl methane-sulfonate[d]	2.5 µL/plate	--	--	--	--	414
With Activation	Sterile water	100 µL/plate	20 ± 9	140 ± 18	10 ± 3	7 ± 3	17 ± 2
Assay No. 2	BMS-510849	250	21 ± 4	131 ± 14	10 ± 6	9 ± 3	15 ± 2
		500	22 ± 8	133 ± 6	12 ± 3	7 ± 1	13 ± 1
		1000	22 ± 2	112 ± 13	12 ± 4	7 ± 3	10 ± 1
		1600	25 ± 2	128 ± 3	13 ± 7	7 ± 2	13 ± 3
		3000	19 ± 4	110 ± 30	11 ± 7	8 ± 2	15 ± 4
		5000	22 ± 3	126[e] ± 18	13 ± 3	7[e] ± 1	13 ± 2
With Activation	2-aminoanthracene[d]	2.5	1063	1439	242	177	--
Assay No. 2	2-aminoanthracene[d]	10	--	--	--	--	203

[a] BMS-510849 is the major active metabolite of BMS-477118.

[b] No. of replicate cultures - Assay No. 1: Negative controls, n = 2; Positive controls, n = 2; Test article, n = 2
Assay No. 2: Negative controls, n = 5; Positive controls, n = 2; Test article, n = 3

[c] Assay No. 1 was a non-GLP range-finding assay

[d] Positive controls

[e] Minimal reduction in the bacterial background lawn

b(4)

Genotoxicity: In Vitro

Report Title: Cytogenetics Study in Primary Human Lymphocytes

Test Article: BMS-477118 (containing [redacted])

Test for Induction of: Chromosome aberrations

No. of Independent Assays: 2 (duplicate flasks from 2 donors)

Study No. DS01178

Document Control Number: 930002039

Strains: Primary human lymphocytes

No. of Replicate Cultures: 2

Location in Dossier: 4.2.3.3.1

Metabolizing System: Aroclor-induced rat liver S9, 0.4%

No. of Cells Analyzed/Culture: 100

Vehicles: **For Test Article:** Benzoate salt in DMSO **For Positive Controls:** Water

GLP Compliance: Yes

Treatment: Continuous treatment for 24 hours without S9; pulse treatment for 5 hours with S9 and 19 hours recovery time

Date of Treatment: March 6, 2002 (assay 1) and April 24, 2002 (assay 2)

Cytotoxic Effects: Concentration-dependent reduction in mitotic index without S9, none with S9

Genotoxic Effects: Clastogenic at 1000 µg/mL (24 h without S9)

Metabolic Activation	Test Article	Concentration (µg/mL)	Cytotoxicity[b] (% of control)	Aberrant Cells Mean %	Abs/Cell
Without Activation	DMSO	10 µL/mL	100%	1.0 ± 0.6	0.01 ± 0.01
Assay 1	BMS-477118	125	101%	3.0 ± 1.3	0.04 ± 0.02
		250	97%	3.0 ± 1.7	0.03 ± 0.02
		500	85%	3.5 ± 1.0	0.05 ± 0.02
		1000	60%**	6.0 ± 1.4	0.08 ± 0.03
	Mitomycin C[c]	0.1	60%**	46.5 ± 3.4**	0.72 ± 0.07
Without Activation	DMSO	10 µL/mL	100%	3.5 ± 1.7	0.08 ± 0.06
Assay 2	BMS-477118	125	99%	0.5 ± 0.5	0.01 ± 0.01
		250	90%	2.5 ± 1.3	0.07 ± 0.06
		500	78%*	3.0 ± 1.3	0.03 ± 0.01
		1000	47%**	9.5 ± 0.5^	0.10 ± 0.01
	Mitomycin C[c]	0.1	86%	18.0 ± 2.2**	0.20 ± 0.04
With Activation	DMSO	10 µL/mL	100%	2.0 ± 0.8	0.02 ± 0.01
Assay 1	BMS-477118	125	95%	4.0 ± 1.4	0.05 ± 0.01
		250	98%	3.0 ± 0.6	0.03 ± 0.01
		500	91%	1.5 ± 1.0	0.02 ± 0.01
		1000	93%	2.0 ± 0.0	0.02 ± 0.00
	Cyclophosphamide[c]	4	94%	42.5 ± 2.6**	0.72 ± 0.07

[a] An impurity/degradant.

[b] Based on mitotic indices.

[c] Positive control

Abbreviations: DMSO = Dimethyl sulfoxide

Students "t" test: * = P<0.05, ** = P<0.01; ^ Denotes significantly different from control at P< 0.05 by analysis of variance (ANOVA) and least significant difference (LSD).

All footnotes are available as table end notes.

Genotoxicity: In Vivo

Report Title: Oral Micronucleus Study In Male Rats

Test Article: BMS-477118 (with and without C) b(4)

Test for Induction of: Bone-marrow micronuclei

Treatment Schedule: 3 doses

Study No. DS01130

Species/Strain: Rat / Harlan Sprague-Dawley

Sampling Time: 24 hr after last dose

Document Control Number: 930000819

Age: 7 weeks

Method of Administration: Oral (gavage)

Location in Dossier: 4.2.3.3.2

Cells Evaluated: Polychromatic erythrocytes

Vehicle/ Formulation: Benzoate salt 1.25% Avicel®

GLP Compliance: Yes

No. of Cells Analyzed/Animal: 2000

Dates of Dosing: September 4, 5, 6, 2001
October 8, 9, 10, 2001

Special Features: None

Toxic/Cytotoxic Effects: 1 male at 1500 mg/kg/day and 3 males at 2000 mg/kg/day died prior to study termination in the BMS-477118 + ( ) assay b(4)

Genotoxic Effects: None

Evidence of Exposure: None

Test Article	Dose (mg/kg)	No. of Animals	Mean % PCE ±S.D.	% Micronucleated PCEs Mean of 2000 Per Animal ±S.D.
BMS-477118 + ( ) Assay				
Harvest Time-24 hr				
1.25% Avicel®	0	5M	51 ± 3.0	0.09 ± 0.05
BMS-477118 containing (( )	1500[d]	4M[b]	51 ± 7.7	0.09 ± 0.06
	2000	2M[c]	50	0.07
Cyclophosphamide	7	5M	45 ± 3.7	1.92 ± 0.49*
BMS-477118 Assay				
1.25% Avicel®	0	5M	52 ± 6.7	0.13 ± 0.10
BMS-477118	500[e]	5M	51 ± 3.3	0.13 ± 0.09
	1000[e]	5M	53 ± 3.9	0.12 ± 0.10
Cyclophosphamide	7	5M	48 ± 5.2	1.90 ± 0.70*

b(4)

[a] An impurity/degradant.

[b] 1 of 5 rats died prior to evaluation.

[c] 3 of 5 rats died prior to evaluation; data not included in statistical analysis.

[d] Statistical analysis by Student's 't' test since only one drug-treated group was analyzed statistically.

[e] Statistical analysis by ANOVA

Abbreviations: PCE = Polychromatic erythrocyte; *p < 0.01 (Student's 't' test).
All footnotes are available as table end notes.

Genotoxicity: In Vivo

Report Title: Oral DNA Repair Study in Male Rats

Test Article: BMS-477118 (containing C ( ) b(4)

Test for Induction of: Unscheduled DNA synthesis

Treatment Schedule: Single dose

Study No. DS02118

Document Control Number: 930003433

Species/Strain: Rat / Harlan Sprague Dawley

Sampling Time: 2-4 and 12-16 hours postdose

Location in Dossier: 4.2.3.3.2

Age: 9 weeks

Method of Administration: Oral (gavage)

Testing Facility: ( )

Cells Evaluated: Hepatocytes

Vehicle/Formulation: Benzoate salt in 1.25% Avicel

GLP Compliance: Yes b(4)

No. of Cells Analyzed/Animal: 150

Date of Dosing: June 14, 2002

Toxic/Cytotoxic Effects: Drug-related mortalities at 680 and 1500 mg/kg (1 of 6 rats at 1500 mg/kg at 2-4 hour timepoint and 1 of 5 rats at 680 mg/kg and 2 of 6 rats at 1500 mg/kg at 12-16 hr time point).

Genotoxic Effects: None

Evidence of Exposure: None

b(4)

Genotoxicity: In Vivo

Report Title: Oral DNA Repair Study in Male Rats **Test Article:** BMS-477118 (containing C)

Test Article	Dose (mg/kg)	No. of Animals	Time (h)	Nuclear Grain Count Mean ± SD	Cytoplasm Grain Count Mean ±SD	Net Nuclear Grain Count Mean ± SD	Cells in Repair
1.25% Avicel	0	3 M	2-4	6.3 ± 0.4	7.0 ± 1.2	-0.7 ± 0.9	3.6%
BMS-477118	250	3 M	2-4	7.7 ± 2.4	8.6 ± 2.3	-0.9 ± 0.2	4.4%
	500	3 M	2-4	5.7 ± 0.3	7.3 ± 0.3	-1.6 ± 0.6	1.8%
	1000	3 M	2-4	5.6 ± 0.9	7.2 ± 0.7	-1.6 ± 1.2	2.2%
	1500	3 M	2-4	6.0 ± 0.7	7.3 ± 2.2	-1.3 ± 2.0	3.1%
Test Article	**Dose (mg/kg)**	**No. of Animals**	**Time (h)**	**Nuclear Grain Count Mean ± SD**	**Cytoplasm Grain Count Mean ±SD**	**Net Nuclear Grain Count Mean ± SD**	**Cells in Repair**
DMN	35	3 M	2-4	41.4 ± 0.9	6.0 ± 0.7	35.4 ± 1.5	100%
1.25% Avicel	0	3 M	12-16	7.7 ± 2.8	9.6 ± 2.2	-1.9 ± 0.6	3.1%
BMS-477118	340	3 M	12-16	7.1 ± 1.9	8.9 ± 1.3	-1.7 ± 0.6	2.2%
	680	3 M	12-16	7.9 ± 2.1	9.2 ± 0.3	-1.3 ± 1.8	6.4%
	1360	3 M	12-16	8.6 ± 1.2	10.1 ± 1.8	-1.5 ± 1.0	5.3%
	1500	3 M	12-16	7.0 ± 1.1	8.6 ± 0.8	-1.7 ± 0.4	2.4%
DMN	35	3 M	12-16	31.2 ± 1.8	4.1 ± 0.4	27.1 ± 1.7	99.6%

[a] An impurity/degradant of BMS-477118.

Abbreviations: DMN = Dimethylnitrosamine (positive control)

Genotoxicity: In Vivo Chromosome Aberration Assay

Report Title: One-month oral in vivo/in vitro cytogenetics study in rat peripheral blood lymphocytes **Test Article:** BMS-477118
Test for Induction of: Chromosome aberrations **Treatment Schedule:** Daily for 28 days **Study No.** DS05037
Species/Strain: Rat / Harlan Sprague Dawley **Sampling Time:** 24 hr after last dose **Document Control Number:** 930016819
Age: 8 weeks **Method of Administration:** Oral gavage **Location in Dossier:** 4.2.3.3.2
Cells Evaluated: Peripheral blood lymphocytes **Vehicle/ Formulation:** Free base in water (pH 5.5) **GLP Compliance:** Yes
No. of Cells Analyzed/Animal: 500 **Dates of Dosing:** July 13 to August 9, 2005
Special Features: None
Toxic/Cytotoxic Effects: Minimal clinical signs of toxicity
Genotoxic Effects: None
Evidence of Exposure: Toxicokinetics (0.5, 1, 2, 4, 8, and 24 hour time points on Day 28)

Daily Dose (mg/kg/day)	0		150		300		500	
Toxicokinetics: number evaluated	**M: 10**	**F: 10**	**M: 10**	**F: 10**	**M: 10**	**F: 10**	**M: 10**	**F: 10**
BMS-477118								
AUC (ng•h/mL)	♦	♦	23191	90844	46831	201157	98667	357588
C_{max} (ng/mL)	♦	♦	6731	49797	9438	77466	19026	138898
BMS-510849[a]								
AUC (ng•h/mL)	♦	♦	7009	14852	15251	37408	27961	52272
C_{max} (ng/mL)	♦	♦	2057	4644	2721	7921	5148	10804

Abbreviations: ♦= not performed; M = male; F= female; h = hour; SE = standard error of the mean
Dunnett's "t" test: Statistically greater than the corresponding vehicle control, * $p<0.05$
All footnotes are available as table end notes.

Genotoxicity: In Vivo Chromosome Aberration Assay

Report Title: One-month oral in vivo/in vitro cytogenetics study in rat peripheral blood lymphocytes **Test Article:** BMS-477118

Table 2.6.7.9C: Genotoxicity: In Vivo Chromosome Aberration Assay **Test Article:** BMS-477118

Document Control Number: 930016819 **(Continued)** **Study No.** DS05037

Test Article	Dose (mg/kg)	No. of Animals	Group Mean % Structural Aberrations ±SE	Group Mean % Structural Aberrations ±SE
			Males	Females
Water pH 5.5	0	5M / 5F	0.2 ± 0.20	0.2 ± 0.20
BMS-477118	150	5M / 5F	0.2 ± 0.20	0.2 ± 0.20
	300	5M / 5F	0.8 ± 0.37	0.8 ± 0.49
	500	5M / 5F	0.0 ± 0.00	0.6 ± 0.24
Cyclophosphamide	60[b]	5M / 5F	71.4 ± 4.55*	62.8 ± 7.96*

[a] Major active metabolite of BMS-477118

[b] The positive control was dosed on the next to the last day as a single oral dose

Carcinogenicity

Report Title: 104-Week Oral Gavage Carcinogenicity Study in Mice
Species/Strain: Crl:CD-1®(ICR)BR Mice
Initial Age: Males: 38 to 44 Days
Females: 42 to 48 Days
Date of First Dose: 12 January 2004
Duration of Dosing: 104 Weeks
Method of Administration: Oral Gavage
Vehicle/Formulation: Acidified Water
Treatment of Controls: Water
Test Article: BMS-477118
Study No. DN03100
Document Control Number: 930025840
Location in Dossier: 4.2.3.4.1
GLP Compliance: Yes
Basis for High-Dose Selection: Results from the 3-month range-finding study
Special Features: Satellite mice (18M/18F, plus 1 extra per sex) were used for toxicokinetic monitoring and then removed from the study.

Daily Dose (mg/kg):	(0) Control		(0) Control		50		250		600	
Gender:	M	F	M	F	M	F	M	F	M	F
Toxicokinetics (Week 26)										
AUC (0-24) (ng·h/mL)										
Saxagliptin	-	-	-	-	1605	2615	34661	30483	70436	94393
BMS-510849	-	-	-	-	6246	7643	76123	49443	147802	131654
Cmax (ng/mL)										
Saxagliptin	-	-	-	-	1834	2925	20888	36659	66631	92239
BMS-510849	-	-	-	-	5022	7732	32414	31491	65319	59487
Number of Animals										
At Start	60	60	60	60	60	60	60	60	60	60
Died/Sacrificed Moribund	32	47	39	44	38	40	45	44	45	45
Terminal Sacrifice	28	13	21	16	22	20	15	16	15	15
Unadjusted Survival (%)	47[a]	22[b]	35[a]	27[b]	37[a]	33[b]	25[a]	27[b]	25[c]	25[b]
Body Weight (%)[d]	45.1g	34.4g	-2	-1	-4	0	-4	0	-3	+2
Food Consumption	-	-	-	-	-	-	-	-	-	-
Noteworthy Findings										
Histopathology – Neoplastic Lesions										
Adrenal Cortex										
Adenoma	0/59[e]	0/59	0/58	0/60	0/60	0/60	0/59	1/59	0/60	0/60
Adenoma, Subcapsular Cell	0/59	2/59	0/58	1/60	1/60	0/60	1/59	0/59	0/60	1/60
Carcinoma	0/59	1/59	0/58	1/60	0/60	0/60	0/59	0/59	0/60	0/60
Adrenal Medulla										
Pheochromocytoma	0/58	0/58	0/58	1/60	1/60	0/59	0/59	0/58	0/60	2/59
Brain										
Hemangioma	0/60	0/60	0/60	0/60	0/60	0/60	1/60	0/60	0/60	0/60
Cervix										
Leiomyoma	NA	0/57	NA	0/59	NA	2/58	NA	1/58	NA	0/59
Polyp, Endometrial Stromal	NA	1/57	NA	0/59	NA	2/58	NA	1/58	NA	0/59
Sarcoma, Endometrial Stroma	NA	2/57	NA	0/59	NA	1/58	NA	0/58	NA	0/59
Duodenum										
Carcinoma	0/59	0/57	0/57	0/59	0/57	0/57	0/56	0/58	0/56	1/58
Epididymis										
Fibrosarcoma	0/60	NA	1/60	NA	0/60	NA	0/60	NA	0/60	NA
Gallbladder										
Papilloma	0/50	0/53	0/51	0/52	0/53	0/50	1/51	0/48	0/45	1/47
Harderian Gland										
Adenoma	0/60	3/59	0/60	2/59	0/59	2/60	0/60	1/60	0/60	2/60
Carcinoma	0/60	1/59	0/60	0/59	0/59	0/60	0/60	0/60	1/60	0/60
Heart										

Abbreviations: -- No noteworthy findings, NA - not applicable
All footnotes are available as table end notes

Carcinogenicity
Document Control Number: 930025840 (Continued)
Test Article: BMS-477118
Study No. DN03100

Daily Dose (mg/kg):	(0) Control		(0) Control		50		250		600	
Gender:	M	F	M	F	M	F	M	F	M	F
Cervix										
Leiomyoma	NA	0/57	NA	0/59	NA	2/58	NA	1/58	NA	0/59
Polyp, Endometrial Stromal	NA	1/57	NA	0/59	NA	2/58	NA	1/58	NA	0/59
Sarcoma, Endometrial Stroma	NA	2/57	NA	0/59	NA	1/58	NA	0/58	NA	0/59
Duodenum										
Carcinoma	0/59	0/57	0/57	0/59	0/57	0/57	0/56	0/58	0/56	1/58
Epididymis										
Fibrosarcoma	0/60	NA	1/60	NA	0/60	NA	0/60	NA	0/60	NA
Gallbladder										
Papilloma	0/50	0/53	0/51	0/52	0/53	0/50	1/51	0/48	0/45	1/47
Harderian Gland										
Adenoma	0/60	3/59	0/60	2/59	0/59	2/60	0/60	1/60	0/60	2/60
Carcinoma	0/60	1/59	0/60	0/59	0/59	0/60	0/60	0/60	1/60	0/60
Heart										
Rhabdomyosarcoma	0/60	0/59	0/60	0/60	0/60	1/60	0/60	0/60	0/60	0/60
Hemato Neoplasia										
Lymphoma	5/60	10/60	2/60	9/60	2/60	5/60	1/60	13/60	1/60	7/60
Sarcoma, Histiocytic	3/60	6/60	0/60	1/60	0/60	1/60	1/60	5/60	0/60	3/60
Jejunum										
Adenoma	0/58	1/57	0/54	0/58	0/57	0/56	0/54	0/57	0/55	0/58
Kidney										
Carcinoma, Tubular Cell	0/60	0/60	1/60	0/60	0/60	0/60	0/60	0/60	0/60	0/60
Hemangiosarcoma	1/60	0/60	0/60	0/60	0/60	0/60	0/60	0/60	0/60	0/60
Adenoma, Tubular Cell	1/60	0/60	0/60	0/60	0/60	0/60	0/60	0/60	0/60	0/60
Liver										
Carcinoma, Hepatocellular	5/60	0/60	5/60	0/60	1/60	1/60	1/60	0/60	0/60	0/60
Hemangioma	0/60	0/60	0/60	0/60	1/60	1/60	1/60	1/60	1/60	0/60
Hemangiosarcoma	3/60	0/60	3/60	2/60	4/60	0/60	3/60	1/60	1/60	0/60
Adenoma, Hepatocellular	5/60	0/60	2/60	0/60	1/60	0/60	2/60	0/60	1/60	0/60
Lung										
Osteosarcoma	0/60	0/60	0/60	0/60	0/60	1/60	0/60	0/60	0/60	0/60
Carcinoma, Bronchiolar-Alveolar	6/60	0/60	13/60	0/60	5/60	0/60	7/60	0/60	0/60	0/60
Adenoma, Bronchiolar-Alveolar	6/60	8/60	9/60	7/60	3/60	5/60	4/60	5/60	2/60	4/60
Carcinoma	0/60	6/60	0/60	6/60	0/60	9/60	0/60	4/60	0/60	5/60
Mammary, Female										
Fibrosarcoma	NA	1/50	NA	0/52	NA	0/50	NA	0/50	NA	0/51
Carcinoma	NA	1/50	NA	0/52	NA	2/50	NA	0/50	NA	1/51
Muscle, Skeletal										
Fibrosarcoma	1/60	1/60	0/60	1/59	0/60	1/60	0/60	1/60	0/60	1/60
Hemangiosarcoma	0/60	0/60	0/60	0/59	0/60	0/60	0/60	0/60	1/60	0/60
Ovary										

Abbreviations: -- No noteworthy findings, NA - not applicable
All footnotes are available as table end notes

Carcinogenicity **Test Article:** BMS-477118

Document Control Number: 930025840 **(Continued)** **Study No.** DN03100

Daily Dose (mg/kg):	(0) Control		(0) Control		50		250		600	
Gender:	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
Cystadenoma	NA	0/60	NA	0/58	NA	0/59	NA	1/59	NA	1/60
Adenoma	NA	0/60	NA	0/58	NA	0/59	NA	1/59	NA	0/60
Luteoma	NA	2/60	NA	0/58	NA	0/59	NA	1/59	NA	0/60
Pancreas										
Adenoma, Islet Cell	0/60	1/60	0/60	1/60	0/60	0/60	0/60	0/60	0/60	0/60
Pituitary										
Adenoma	0/60	4/59	0/59	2/59	0/60	3/58	1/60	2/58	1/59	2/58
Carcinoma	0/60	1/59	0/59	0/59	0/60	0/58	0/60	0/58	0/59	0/58
Salivary Gland, Mandibular										
Carcinoma	0/60	0/60	1/60	0/59	0/60	0/60	0/59	0/60	0/59	0/60
Skin										
Carcinoma, Basal Cell	0/60	0/59	0/59	0/60	0/59	1/60	0/60	0/60	0/60	0/60
Carcinoma, Squamous Cell	0/60	0/59	0/59	1/60	0/59	0/60	0/60	0/60	0/60	0/60
Fibrosarcoma	0/60	0/59	0/59	1/60	0/59	0/60	1/60	1/60	0/60	0/60
Histiocytoma	0/60	0/59	0/59	0/60	1/59	0/60	0/60	0/60	0/60	0/60
Keratoacanthoma	0/60	0/59	0/59	0/60	0/59	0/60	0/60	1/60	0/60	0/60
Neurofibroma	0/60	0/59	0/59	0/60	0/59	0/60	0/60	0/60	0/60	1/60
Papilloma, Squamous Cell	0/60	0/59	2/59	1/60	0/59	0/60	0/60	0/60	0/60	0/60
Skin and Pinna										
Papilloma, Squamous Cell	0/60	0/0	2/60	0/0	0/60	0/0	1/60	0/0	0/60	0/0
Spleen										
Hemangioma	0/60	0/59	1/60	0/60	2/60	0/60	0/60	0/60	0/60	0/60
Hemangiosarcoma	1/60	0/59	0/60	1/60	0/60	1/60	0/60	0/60	0/60	1/60
Stomach, Glandular										
Adenoma	0/60	0/59	0/60	0/60	1/60	0/60	0/60	0/60	0/60	0/59
Stomach, Nonglandular										
Papilloma, Squamous Cell	0/60	0/59	0/60	0/60	0/60	0/60	0/60	0/60	1/60	1/59
Sarcoma, Spindle Cell	0/60	0/59	0/60	0/60	0/60	0/60	0/60	0/60	0/60	1/59
Testis										
Sertoli Cell Tumor	0/60	NA	1/60	NA	0/60	NA	0/60	NA	0/60	NA
Thymus										
Hemangiosarcoma	0/40	0/51	0/34	0/53	0/44	0/55	1/37	0/50	0/44	0/56
Thyroid										
Adenoma, Follicular Cell	0/60	2/59	0/60	1/59	0/60	0/60	0/60	0/60	0/59	0/60
Tongue										
Papilloma, Squamous Cell	0/60	0/60	0/60	0/60	1/60	0/60	0/60	0/60	0/60	0/60
Urinary Bladder										
Carcinoma, Transitional Cell	0/58	0/60	0/60	0/59	0/58	0/59	0/60	0/60	1/57	0/60
Uterus										
Adenocarcinoma	NA	0/60	NA	0/60	NA	0/60	NA	1/60	NA	0/60
Hemangioma	NA	0/60	NA	0/60	NA	2/60	NA	0/60	NA	0/60
Leiomyoma	NA	0/60	NA	1/60	NA	0/60	NA	0/60	NA	0/60
Polyp, Endometrial, Stromal	NA	1/60	NA	3/60	NA	3/60	NA	1/60	NA	1/60

Abbreviations: -- No noteworthy findings, NA - not applicable
All footnotes are available as table end notes

Carcinogenicity

Document Control Number: 930025840 **(Continued)**

Test Article: BMS-477118
Study No. DN03100

Daily Dose (mg/kg):	(0) Control		(0) Control		50		250		600	
Gender:	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
Sarcoma, Endometrial, Stromal	NA	2/60	NA	3/60	NA	6/60	NA	1/60	NA	4/60
Whole Body										
Hemangioma	0/60	0/60	1/60	0/60	3/60	3/60	2/60	1/60	1/60	0/60
Hemangiosarcoma	5/60	0/60	3/60	3/60	4/60	1/60	4/60	1/60	2/60	1/60
Hemangioma / Hemangiosarcoma	5/60	0/60	4/60	3/60	7/60	3/60	6/60	2/60	3/60	1/60
Penis										
Fibrosarcoma	0/1	NA	0/4	NA	1/5	NA	0/2	NA	0/1	NA
Pinna										
Papilloma, Squamous Cell	0/7	0/2	0/9	0/4	0/9	0/5	1/10	0/4	0/4	0/8
Adipose Tissue										
Mesothelioma	0/0	1/1	0/0	0/2	0/0	0/0	0/0	0/2	0/0	0/0
Bone, Other										
Osteosarcoma	0/0	0/0	0/0	0/0	0/0	1/1	0/0	0/0	0/0	0/0
Subcutaneous Tissue										
Fibrosarcoma	0/0	0/2	0/0	1/1	0/0	0/1	0/0	0/0	0/0	0/0
Rhabdomyosarcoma	0/0	0/2	0/0	0/1	0/0	1/1	0/0	0/0	0/0	0/0

[a] Terminal sacrifice during Week 100
[b] Terminal sacrifice during Week 105
[c] Terminal sacrifice during Week 90
[d] At Week 50. Calculated as the difference from the first control group as follows: Percent difference = treated group – control group ÷ control group x 100
[e] Animal incidence/number of animals evaluated

Carcinogenicity

Report Title: BMS-477118: 104-Week Oral Gavage Carcinogenicity Study in Rats
Species/Strain: Rat / Harlan Sprague Dawley
Duration of Dosing: 104 weeks
Initial Age: Approximately 6 weeks
Method of Administration: Oral gavage
Date of First Dose: 19 January 2005
Vehicle/Formulation: Acidified Water
Treatment of Controls: Water
Basis for High-Dose Selection: Results from the 3-month range-finding study
Special Features:

Test Article: BMS-477118
Study No. DN05004
Document Control Number: 930025839
Location in Dossier: 4.2.3.4.1
GLP Compliance: Yes

Daily Dose (mg/kg):	(0) Control		(0) Control		25		75		150		300	
Gender:	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
Toxicokinetics (Week 26)												
AUC (0-T) (ng·h/mL)												
Saxagliptin	-	-	-	-	3492	8763	13993	30808	28724	81962	68568	179606
BMS-510849	-	-	-	-	1174	2658	3843	7672	9204	15226	28569	29730
Cmax (ng/mL)												
Saxagliptin	-	-	-	-	1893	5600	6153	24733	10863	60800	17600	92333
BMS-510849	-	-	-	-	550	1144	1523	3647	2187	5063	4563	9363
Number of Animals												
At Start	60	60	60	60	60	60	60	60	60	60	60	60

Abbreviations: - N/A; --No noteworthy findings
All footnotes are available as table end notes.

Carcinogenicity **Test Article:** BMS-477118

Document Control Number: 930025839 **(Continued)** **Study No.** DN05004

Daily Dose (mg/kg):	(0) Control		(0) Control		25		75		150		300	
Gender:	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
Died/Sacrificed Moribund	47	34	51	35	39	33	44	30	44	32	46	30
Terminal Sacrifice	13	26	9	25	21	27	16	30	16	28	14	30
Unadjusted Survival (%)	22[b]	43[c]	15[b]	42[c]	35[b]	45[c]	27[b]	50[c]	27[b]	47[c]	23[a]	50[c]
Body Weight (%)[d]	557g	297g	-2	0	-2	-1	-4	-4	-4	-5	-14	-6
Food Consumption (%)	--	--	--	--	--	--	--	--	--	--	--	--
Noteworthy Findings:												
Clinical Signs:												
Recumbent	0	2	3	1	1	0	1	0	1	1	30	0
Tremors, Body	2	1	0	0	0	0	3	0	1	0	7	0
Tremors, Head	1	0	3	0	1	1	0	0	2	0	2	0
Mass	31	49	21	46	36	39	31	41	29	35	5	22
Respiration, Audible	7	1	3	1	2	0	2	3	5	1	13	1
Respiration, Irregular	8	3	10	0	5	4	8	2	7	5	12	6
Respiration, Labored	4	0	6	1	5	0	2	3	8	0	25	1
Noteworthy Findings:												
Histopathology - Non-Neoplastic Lesions												
Brain												
Corpus Callosum Degeneration/ Rarefaction	--	--	--	--	--	--	--	--	10	--	33	--
PAS-Positive Material, Intracytoplasmic, Glial/Gitter Cells	--	--	--	--	--	--	--	--	10	--	32	--
Caudate Putamen Focal/Multifocal, Degeneration/ Rarefaction with Gliosis	--	--	--	--	--	--	--	--	1	--	15	--
PAS-Positive Material	--	--	--	--	--	--	--	--	6	--	40	--
Piriform/Temporal Cortex Focal/Multifocal, Degeneration/Rarefacti on with Gliosis	--	--	--	--	--	--	--	--	0	--	5	--
PAS-Positive Material, Intracytoplasmic, Glial/Gitter Cells	--	--	--	--	--	--	--	--	0	--	4	--
Thalamus Focal/Multifocal Degeneration/ Rarefaction with Gliosis	--	--	--	--	--	--	--	--	1	--	3	--
Lung												
Alveolar Macrophage Infiltrates												
Minimal	12	17	18	21	16	20	22	17	39	10	40	19
Slight	4	3	3	3	1	7	2	12	0	34	8	33
Moderate	3	0	5	0	1	1	3	8	2	14	1	7
Marked	1	0	1	0	1	0	0	0	0	0	0	0
Inflammation												
Minimal	8	6	10	4	6	5	6	16	11	36	4	32
Slight	2	1	5	2	3	2	1	1	1	1	1	0
Moderate	1	0	1	1	0	0	3	0	1	0	0	0
Marked	1	0	0	0	0	1	0	0	0	0	0	0
Urinary Bladder												

Abbreviations: - N/A; --No noteworthy findings
All footnotes are available as table end notes.

Carcinogenicity **Test Article:** BMS-477118

Document Control Number: 930025839 **(Continued)** **Study No.** DN05004

Daily Dose (mg/kg):	(0) Control		(0) Control		25		75		150		300	
Gender:	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
Noteworthy Findings: (Continued)												
Number Evaluated	60	59	59	60	60	60	60	59	59	60	59	59
Lymphocyte Infiltrates												
Minimal	1	0	1	1	2	0	3	6	0	19	12	26
Slight	0	0	1	1	1	0	1	1	0	1	0	4
Harderian Gland												
Lymphocyte/Macrophage Infiltrates												
Minimal	10	12	8	12	9	13	5	14	13	28	15	36
Slight	1	0	0	0	0	1	1	0	0	1	1	3
Epididymis												
Lymphocyte Infiltrates Minimal	7	-	2	-	6	-	5	-	11	-	29	-
Liver												
Lymphocyte/ Macrophage Infiltrates												
Minimal	5	15	6	13	8	9	8	10	16	19	10	13
Slight	0	2	1	1	0	0	0	0	1	0	0	0
Histopathology - Neoplastic Lesions												
Adrenal Cortex												
Adenoma	5/59[e]	1/60	6/60	8/60	3/60	5/60	3/59	6/60	3/60	6/60	1/60[f]	6/60
Carcinoma	1/59	0/60	0/60	0/60	1/60	0/60	0/59	2/60	0/60	1/60	0/60[f]	1/60
Adrenal Medulla												
Malignant Pheochromocytoma	0/59	0/60	0/60	0/60	0/60	0/59	0/59	1/59	0/60	0/60	0/60[f]	1/59
Pheochromocytoma	8/59	3/60	15/60	0/60	15/60	2/59	7/59	1/59	6/60	4/60	0/60[f]	1/59
Body, Whole/Cavity												
Hemangioma	0/60	0/60	0/60	1/60	0/60	0/60	0/60	0/60	0/60	0/60	0/60[f]	0/60
Hemangiosarcoma	0/60	0/60	1/60	0/60	0/60	1/60	1/60	0/60	1/60	0/60	0/60[f]	1/60
Histiocytic Sarcoma	0/60	0/60	0/60	0/60	1/60	0/60	0/60	1/60	2/60	0/60	0/60[f]	0/60
Large Granular Cell Leukemia	1/60	2/60	0/60	0/60	0/60	0/60	0/60	0/60	0/60	0/60	0/60[f]	0/60
Lymphosarcoma	3/60	0/60	2/60	2/60	1/60	0/60	0/60	0/60	0/60	1/60	0/60[f]	0/60
Malignant Mesothelioma	0/60	0/60	0/60	0/60	0/60	0/60	0/60	0/60	0/60	1/60	0/60[f]	0/60
Brain												
Granular Cell Tumor	0/60	0/60	0/60	0/60	0/60	0/60	1/60	0/60	1/60	0/60	0/60[f]	0/60
Malignant Astrocytoma	1/60	0/60	0/60	0/60	1/60	0/60	0/60	0/60	0/60	0/60	0/60[f]	2/60
Malignant Oligidendroglioma	1/60	0/60	0/60	0/60	0/60	0/60	0/60	0/60	0/60	0/60	0/60[f]	0/60
Meningeal Sarcoma	1/60	0/60	1/60	0/60	0/60	0/60	0/60	0/60	0/60	0/60	0/60[f]	0/60
Cavity, Abdominal, Lipoma	0/0	0/1	0/0	0/0	0/0	0/0	1/1	0/0	0/0	1/2	0/0[f]	0/0
Cervix												
Polyp, Endometrial Stromal	-	0/60	-	3/60	-	1/60	-	2/59	-	1/60	-	2/59
Carcinoma	-	1/60	-	0/60	-	1/60	-	0/59	-	0/60	-	0/59
Duodenum												
Fibroma	0/58	0/59	0/59	0/60	0/59	0/57	0/57	0/57	0/57	1/59	0/57[f]	0/59
Sarcoma	0/58	0/59	0/59	0/60	0/59	0/57	0/57	0/57	0/57	1/59	0/57[f]	0/59

Abbreviations: - N/A; --No noteworthy findings
All footnotes are available as table end notes.

Carcinogenicity **Test Article:** BMS-477118

Document Control Number: 930025839 **(Continued)** **Study No.** DN05004

Daily Dose (mg/kg):	**(0) Control**		**(0) Control**		**25**		**75**		**150**		**300**	
Gender:	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
Noteworthy Findings: (Continued)												
Eye, Fibrosarcoma	1/60	0/60	0/60	1/60	0/60	0/59	0/60	0/60	0/60	0/60	0/60[f]	0/60
Heart, Endocardial Schwannoma	0/60	1/60	0/60	0/60	0/60	0/60	0/60	0/60	0/60	0/60	0/60[f]	0/60
Jejunum												
Fibrosarcoma	0/54	0/56	0/56	0/56	0/54	0/55	0/54	0/56	1/55	0/60	0/58[f]	0/60
Carcinoma	0/54	0/56	0/56	0/56	0/54	0/55	1/54	0/56	0/55	0/60	0/58[f]	0/60
Kidney												
Fibrosarcoma	0/60	1/60	0/60	0/60	0/60	0/60	0/60	0/60	0/60	0/60	0/60[f]	0/60
Adenoma, Tubule Cell	0/60	0/60	0/60	0/60	0/60	0/60	0/60	0/60	0/60	0/60	0/60[f]	1/60
Malignant Renal Mesenchyma	0/60	0/60	0/60	0/60	1/60	0/60	0/60	0/60	0/60	0/60	0/60[f]	0/60
Nephroblastoma	0/60	0/60	0/60	0/60	0/60	0/60	0/60	0/60	1/60	0/60	0/60[f]	0/60
Liver, Adenoma, Hepatocellular	1/60	3/60	1/60	3/60	1/60	1/60	0/60	1/60	1/60	0/60	0/60[f]	1/60
Mammary, Female												
Adenoma	-	1/59	-	1/60	-	0/59	-	0/60	-	1/60	-	0/58
Fibroadenoma	-	26/59	-	34/60	-	23/59	-	20/60	-	13/60	-	8/58
Fibrosarcoma	-	0/59	-	0/60	-	0/59	-	1/60	-	0/60	-	0/58
Sarcoma	-	0/59	-	1/60	-	0/59	-	0/60	-	0/60	-	0/58
Schwannoma	-	0/59	-	0/60	-	0/59	-	1/60	-	0/60	-	0/58
Carcinoma	-	11/59	-	4/60	-	2/59	-	0/60	-	3/60	-	1/58
Muscle, Other, Schwannoma	0/0	1/1	0/0	0/1	0/0	0/0	0/0	0/0	0/0	0/0	0/0[f]	0/0
Nerve, Other, Malignant Schwannoma	0/0	0/0	0/0	1/1	0/0	0/0	0/0	0/0	0/0	0/0	0/0[f]	0/0
Ovary												
Leiomyoma	-	0/60	-	0/60	-	0/60	-	0/59	-	0/59	-	1/59
Lutcoma	-	0/60	-	0/60	-	1/60	-	0/59	-	0/59	-	0/59
Malignant Granulosa/Theca	-	0/60	-	0/60	-	0/60	-	2/59	-	0/59	-	1/59
Pancreas												
Carcinoma, Islet Cell	0/60	0/60	0/60	0/60	1/60	1/60	0/59	0/60	0/60	0/60	0/60[f]	0/60
Adenoma, Acinar Cell	1/60	0/60	0/60	0/60	0/60	0/60	1/59	0/60	1/60	0/60	0/60[f]	0/60
Adenoma, Islet Cell	2/60	3/60	2/60	2/60	0/60	1/60	2/59	1/60	0/60	3/60	0/60[f]	0/60
Parathyroid, Adenoma	0/55	0/57	0/60	0/54	0/58	0/58	0/60	0/60	1/59	0/57	0/58[f]	0/58
Pituitary												
Adenoma	9/60	30/60	5/60	37/60	10/60	27/60	6/60	21/60	4/60	17/60	2/60[f]	8/60
Carcinoma	0/60	0/60	0/60	0/60	0/60	1/60	0/60	1/60	0/60	1/60	0/60[f]	0/60
Skin												
Fibroma	0/60	1/60	1/60	0/60	0/60	0/60	1/60	1/60	1/60	0/60	0/60[f]	0/60
Fibrosarcoma	1/60	0/60	1/60	0/60	0/60	1/60	1/60	0/60	0/60	0/60	0/60[f]	0/60
Keratoacanthoma	3/60	0/60	1/60	0/60	0/60	0/60	0/60	0/60	2/60	0/60	0/60[f]	0/60
Leiomyosarcoma	0/60	0/60	0/60	0/60	0/60	0/60	0/60	1/60	0/60	0/60	0/60[f]	0/60
Malignant Basal Cell Tumor	0/60	0/60	0/60	0/60	0/60	0/60	1/60	0/60	0/60	0/60	0/60[f]	0/60
Sarcoma	0/60	0/60	0/60	0/60	1/60	0/60	0/60	0/60	0/60	0/60	0/60[f]	0/60
Skin/SubQ, Other												

Abbreviations: - N/A; --No noteworthy findings
All footnotes are available as table end notes.

Carcinogenicity **Test Article:** BMS-477118

Document Control Number: 930025839 **(Continued)** **Study No.** DN05004

Daily Dose (mg/kg):	**(0) Control**		**(0) Control**		**25**		**75**		**150**		**300**	
Gender:	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
Noteworthy Findings: (Continued)												
Fibrosarcoma	1/11	1/3	1/7	0/5	0/9	0/4	0/10	0/3	0/10	0/6	0/2[f]	0/7
Keratoacanthoma	4/11	1/3	3/7	0/5	0/9	0/4	0/10	0/3	1/10	0/6	0/2[f]	0/7
Papilloma, Squamous Cell	1/11	0/3	0/7	0/5	1/9	0/4	2/10	1/3	0/10	0/6	0/2[f]	0/7
Basal Cell Tumor	0/11	0/3	0/7	1/5	0/9	0/4	0/10	0/3	0/10	0/6	0/2[f]	0/7
Carcinoma, Basal Cell	0/11	0/3	0/7	0/5	1/9	1/4	0/10	0/3	0/10	0/6	0/2[f]	0/7
Stomach, Nonglandular, Carcinoma, Squamous Cell	0/60	0/60	0/60	1/60	1/60	0/60	0/60	0/60	0/60	0/60	0/60[f]	0/60
Tail												
Keratoacanthoma	2/6	0/2	0/7	0/1	2/12	0/0	3/11	0/9	1/11	0/4	0/0[f]	0/4
Leimyosarcoma	0/6	0/2	0/7	0/1	0/12	0/0	0/11	1/9	0/11	0/4	0/0[f]	0/4
Testis, Interstitial Cell Tumor	0/60	-	1/59	-	0/60	-	0/59	-	2/60	-	0/60[f]	-
Thymus, Adenoma	0/57	0/58	0/58	0/59	0/56	0/59	0/58	0/57	0/58	1/58	0/60[f]	0/55
Thyroid												
Carcinoma, C-cell	1/60	0/60	0/60	0/60	1/60	1/60	0/60	2/60	0/60	1/60	0/60[f]	0/60
Adenoma, C-cell	8/60	10/60	5/60	18/60	17/60	22/60	11/60	11/60	11/60	10/60	3/60[f]	13/60
Urinary Bladder, Carcinoma, Transitional Cell	0/60	0/59	0/59	0/60	1/60	0/60	0/60	0/59	0/59	0/60	0/59[f]	0/59
Uterus												
Carcinoma, Squamous Cell	-	1/60	-	0/60	-	0/60	-	0/59	-	3/60	-	0/59
Leimyosarcoma	-	0/60	-	0/60	-	0/60	-	0/59	-	0/60	-	1/59
Polyp, Endometrial Stromal	-	20/60	-	19/60	-	11/60	-	13/59	-	11/60	-	8/59
Sarcoma, Endometrial Strom	-	1/60	-	0/60	-	0/60	-	0/59	-	0/60	-	0/59
Carcinoma	-	1/60	-	1/60	-	0/60	-	2/59	-	1/60	-	3/59
Vagina, Polyp, Endometrial Stromal	-	0/60	-	0/60	-	0/60	-	0/59	-	1/60	-	1/59
Zymbal's Gland												
	0/58	0/54	2/55	0/57	0/59	0/58	0/53	0/54	1/57	0/57	0/52[f]	0/57
	0/58	0/54	0/55	0/57	0/59	0/58	0/53	0/54	0/57	0/57	0/52[f]	1/57

[a] Terminal sacrifice during Week 68

[b] Terminal sacrifice during Week 99

[c] Terminal sacrifice during Week 105

[d] At Day 344. Calculated as the difference from the first control group as follows: Percent difference = treated group – control group ÷ control group x 100

[e] Animal incidence/number of animals evaluated

[f] Due to early termination, males given 300 mg/kg were not included in Peto-Pike analyses.

Reproductive and Developmental Toxicity: Nonpivotal Studies

Test Article: BMS-477118

Species/ Strain	Method of Administration (Vehicle/ Formulation)	Dosing Period	Doses (mg/kg)	No. per Group	Noteworthy Findings	Study No./ Document Control Number
Rat/Crl:CD (SD)IGS BR	Oral gavage (benzoate salt in 1.25% Avicel®)	GD 6 to 15	600, 900, and 1200 mg/kg/day	8	Drug-related changes in the dams at 600 mg/kg/day and higher, whereas effects in the fetuses only at 1200 mg/kg/day. In the dams at 600, 900, and 1200 mg/kg/day, clinical signs of urine-stained coat and/or excess salivation and reduced body-weight gain during the dosing period (10% to 17% lower than controls). In the fetuses at 1200 mg/kg/day, increased resorptions (average of 10% resorptions per litter vs 2% in controls) and reduced body weights (9% less than controls).	DN02012/ 930002469
Rabbit/ Hra: (NZW)SPF	Oral gavage (benzoate salt in 1.25% Avicel®)	GD 7 to 19	25, 50, 100, and 200 mg/kg/day	6	No drug-related changes in the does or the fetuses at 25, 50, or 100 mg/kg/day. In the does at 200 mg/kg/day, reduced maternal body-weight gain and food consumption during the dosing (74% and 33% lower than controls, respectively) and post dosing periods. In the fetuses at 200 mg/kg/day, one aborted litter (GD 28) and reduced body weights (8% less than controls for male fetuses; 6% less than controls for female fetuses).	DN02013/ 930002017
Rat/Crl:CD (SD)IGS BR	Oral gavage Pregnant females: (benzoate salt in 1.25% Avicel®) Males and nonpregnant females: free base in acidified water	Pregnant females: GD 6 to 15 Males and nonpregnant females: Days 1 to 10	Pregnant females: 64, 240, and 900 mg/kg/day Males: 100, 200, and 400 mg/kg/day Nonpregnant females:125, 300, and 750 mg/kg/day	10	Toxicokinetic parameters (AUC and Cmax) of BMS-477118 and its active metabolite BMS-510849 were measured after 10 daily doses on GD 15 (pregnant females) or Day 10 of study (males and nonpregnant females). Data presented in Tables 2.6.7.12 and 2.6.7.13A.	DN05052/ 930016117
Rabbit/ Hra: (NZW)SPF	Oral gavage (benzoate salt in 1.25% Avicel®)	GD 7 to 19	8, 40, and 200 mg/kg/day	5	Toxicokinetic parameters (AUC and Cmax) of BMS-477118 and its active metabolite BMS-510849 were measured after 13 daily doses on GD 19. Data presented in Table 2.6.7.13B.	DN05051/ 930014568

Reproductive and Developmental Toxicity - Fertility and Early Embryonic Development to Implantation

Report Title: Oral Study of Fertility and Early Embryonic Development in Rats
Test Article: BMS-477118
Design similar to ICH 4.1.1? Yes
Duration of Dosing: M: 2 weeks prior to mating & until the day before scheduled necropsy; F: 2 weeks prior to mating & until gestation day 7
Study Number: DN03043
Document Control Number: 930007579
Species/Strain: Rat/Crl:CD(SD)IGS BR
Location in Dossier: 4.2.3.5.1
Initial Age (First Dose): Approximately 17 (males) and 12 (females) weeks
Day of Mating: Gestation Day 0
Date of First Dose: October 20, 2003 (males) September 15, 2003 (females)
Day of C-Section: Gestation Day 15
GLP Compliance: Yes
Method of Administration: Oral (gavage)
Vehicle/Formulation: Water (acidified)
Special Features: Treated females were mated with a population of untreated males; treated males were mated with a population of untreated females. Additionally, sperm evaluations were performed in the control and high-dose males.
No Observed Adverse-Effect Level (NOAEL): F_0 Males: < 100 mg/kg/day (Reproductive NOAEL 200 mg/kg/day); F_0 Females: < 125 mg/kg/day (Reproductive NOAEL 125 mg/kg/day); F_1 Litters: 125 mg/kg/day

Daily Dose (mg/kg) - F_0 Males		0 (Control)	100	200	400
F_0 Males:	Toxicokinetics (BMS-477118):				
	AUC (ng•h/mL)	--	16071	48899	90186
	Cmax (ng/mL)	--	5775	12561	11697
	Toxicokinetics (BMS-510849[a]):				
	AUC (ng•h/mL)	--	4376	14227	28684
	Cmax (ng/mL)	--	1380	2771	2705

Abbreviations: ♦ = Not performed, -- No noteworthy findings, + Mild, ++ Moderate, +++ Marked, M = Males, F = Females
All footnotes are available as table endnotes.

Reproductive and Developmental Toxicity - Fertility and Early Embryonic Development to Implantation

Document Control Number: 930007579 **(Continued)** **Test Article:** BMS-477118

Study No. DN03043

Daily Dose (mg/kg) - F_0 Males	0 (Control)	100	200	400
F_0 Males (Continued):				
No. Evaluated	25	25	25	25
No. Died or Sacrificed Moribund[b]	0	2	0	4
Clinical Observations[c,d]	--	+	++	+++
Necropsy Observations[e]	--	--	+	+
Body Weight (%[f]) Day 15 of Dosing	570.3 g	-2%	-3%	-6%*
Body Weight (%[f]) Day 29 of Dosing	593.0 g	-2%	-4%	-8%**
Body Weight Change (%[f]) Days 1-8 of Dosing	-0.8 g	loss of 6.8 g	loss of 7.9 g*	loss of 14.8 g**
Body Weight Change (%[f]) Days 8-15 of Dosing	14.3 g	-6%	-36%*	-56%**
Body Weight Change (%[f]) Days 1-29 of Dosing	36.3 g	-29%	-49%**	loss of 1.6 g**
Food Consumption (%[f]) Days 1-8 of Dosing	30.3 g/day	-3%	-4%	-8%*
Food Consumption (%[f]) Days 8-15 of Dosing	31.2 g/day	-1%	-1%	-7%
F_0 Males (Continued):				
Food Consumption (%[f]) Days 1-15 of Dosing	30.8 g/day	-2%	-2%	-8%**
Sperm Evaluations[g]:				
Sperm Counts (%[f])	717.1×10^6/g	♦	♦	-3%
Sperm Morphology (mean % abnormal sperm)	0.4	♦	♦	0.5
Sperm Motility (mean % motile sperm)	96.2	♦	♦	96.6
Male Reproductive Organ Weights[h]				
Testis: Right Absolute (g)/Relative (%)	1.81/0.30	1.88/0.32	1.81/0.32	1.83/0.34**
Left Absolute (g)/Relative (%)	1.84/0.31	1.85/0.32	1.78/0.31	1.80/0.33
Epididymis: Right Absolute (g)/Relative (%)	0.71/0.12	0.73/0.13*	0.74/0.13**	0.73/0.13**
Left Absolute (g)/Relative (%)	0.69/0.12	0.71/0.12	0.72/0.13**	0.72/0.13**
Prostate and Seminal Vesicles Absolute (g)/Relative (%)	3.51/0.59	3.59/0.61	3.72/0.65	3.42/0.63
F_0 Males Cohabited with Untreated Females:				
Mean No. Days Cohabited Prior to Mating	2.6	2.8	2.5	2.6
No. of Males that Mated - No. Mated/No. Cohabited (%)	24/24 (100)	21/23 (91.3)	24/25 (96.0)	21/21 (100)
No. of Fertile Males - No. Fertile/No. Mated (%)	23/24 (95.8)	19/21 (90.5)	22/24 (91.7)	17/21 (81.0)
Untreated Females Mated with F_0 Males:				
Mean No. Corpora Lutea	17.4	16.6	17.0	16.4
Mean No. Implantations	16.7	15.4	15.8	15.6
Mean % Preimplantation Loss[i]	4.1	9.0	6.8	4.2
Mean No. Live Conceptuses	15.7	14.4	14.8	14.9
Mean No. Early Resorptions	1.0	1.0	1.0	0.8
Mean % Postimplantation Loss[j]	5.7	5.8	6.1	5.2

Abbreviations: ♦ Not performed, -- No noteworthy findings, + Mild, ++ Moderate, +++ Marked, M = Males, F = Females * $p \leq 0.05$, ** $p \leq 0.01$

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

All footnotes are available as table end notes.

Reproductive and Developmental Toxicity - Fertility and Early Embryonic Development to Implantation

Document Control Number: 930007579 **(Continued)** **Test Article:** BMS-477118

Study No. DN03043

Daily Dose (mg/kg) - F_0 Females	0 (Control)	125	300	750
F_0 Females:				
Toxicokinetics (BMS-477118): AUC (ng•h/mL)	--	62833	167578	497194
Cmax (ng/mL)	–	31479	54221	94088
Toxicokinetics (BMS-510849[a]): AUC (ng•h/mL):	--	9951	23378	72536
Cmax (ng/mL):	--	2524	5504	8008
No. Evaluated	25	25	25	25
No. Died or Sacrificed Moribund[k]	0	2	0	13
Clinical Observations[c,l]	--	+	++	+++
Necropsy Observations[m]	--	--	++	+++
Premating Body Weight (%[f]) Day 15 of Dosing	265.3 g	0%	-1%	0%
Premating Body Weight Change (%[f]) Days 1-15 of Dosing	7.5 g	-41%	-44%	-12%
Gestation Body Weight (%[f]) Gestation Day 15	335.5 g	-1%	-4%	-6%
Gestation Body Weight Change (%[f]) Gestation Days 0-4	17.1 g	-25%	-30%	-25%
Gestation Body Weight Change (%[f]) Gestation Days 4-8	13.3 g	+5%	+2%	-26%
Gestation Body Weight Change (%[f]) Gestation Days 8-15	33.7 g	-1%	-5%	-14%
Gestation Body Weight Change (%[f]) Gestation Days 0-15	64.1 g	-6%	-11%	-20%
Premating Food Consumption (%[f]) Days 1-15 of Dosing	19.5 g/day	-2%	-4%	-6%
Gestation Food Consumption (%[f]) Gestation Days 0-4	22.6 g/day	-5%	+6%	-8%
Gestation Food Consumption (%[f]) Gestation Days 4-8	24.7 g/day	+1%	-11%	-9%
Gestation Food Consumption (%[f]) Gestation Days 8-15	25.8 g/day	+2%	-2%	-2%
Gestation Food Consumption (%[f]) Gestation Days 0-15	24.5 g/day	0%	-1%	-4%
Estrous-Cycling Evaluations (2-Week Treatment Period)[n]:				
No. Evaluated	25	25	25	14
Mean No. (%) of Days in Proestrus	3.7 (26.3)	3.8 (26.9)	3.3 (23.4)	2.8* (19.9)**
Mean No. (%) of Days in Estrus	4.0 (28.3)	3.7 (26.6)	4.1 (29.1)	4.9* (34.7)
Mean No. (%) of Days in Metestrus	2.9 (20.8)	3.0 (21.4)	2.9 (20.8)	2.4 (17.3)
Mean No. (%) of Days in Diestrus	3.4 (24.6)	3.5 (25.1)	3.7 (26.6)	3.9 (28.1)
F_0 Females Cohabited with Untreated Males:				
Mean No. Days Cohabited Prior to Mating	3.1	4.1	3.0	2.4
Females Sperm-Positive - No. Mated/No. Cohabited (%)	25/25 (100)	24/24 (100)	25/25 (100)	12/12 (100)
Pregnant Females - No. Pregnant/No. Mated (%)	23/25 (92.0)	22/24 (91.7)	23/25 (92.0)	10/12 (83.3)
No. Aborted or with Total Resorption of Litter	0	0	1[o]	0
F_0 Females Mated with Untreated Males:				
Mean No. Corpora Lutea	14.7	15.9	14.5[o]	12.9
Mean No. Implantations	14.5	15.6	13.7[o]	11.5
Mean % Preimplantation Loss[i]	1.5	1.9	7.8[o]	11.0
Mean No. Live Conceptuses	13.8	14.8	11.7[o]	9.8
Mean No. Early Resorptions	0.7	0.9	2.0	1.7
Mean % Postimplantation Loss[j]	5.0	5.6	19.8*[o]	17.9

Abbreviations: ♦ Not performed, -- No noteworthy findings, + Mild, ++ Moderate, +++ Marked, M = Males, F = Females * $p \leq 0.05$, ** $p \leq 0.01$

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

All footnotes are available as table end notes.

[a] The major active metabolite of BMS-477118.

[b] Two 400-mg/kg males died within two hours after the first dose. Four additional males (two 100-mg/kg and two 400-mg/kg) were found dead or required euthanasia as the result of dosing accidents (after 4 to 22 daily doses). Each of these rats had clinical signs (rales, gasping, and/or white/foamy perinasal substance) and/or necropsy observations (fluid-filled thoracic cavity, perforations of the esophagus or lung, discoloration and/or mottling of the lungs, and/or axillary mass) consistent with intubation injuries.

[c] No inferential statistical procedures were conducted on clinical observation data.

[d] At 100, 200, and 400 mg/kg/day, drug-related clinical observations in males included perioral and/or perinasal substance. Additional drug-related clinical signs in males at 400 mg/kg/day included collapse (leading to death of two males within 2 hours after the first dose as mentioned above), urine-stained coat, and decreased motor activity.

[e] At 200 and 400 mg/kg/day, drug-related necropsy observations in male rats included visibly enlarged spleens (in two and three males, respectively).

[f] For controls, group means are shown. For treated groups, percent differences from controls are shown. Statistical significance is based on actual data (not on the percent differences).

[g] At scheduled termination (following 29 to 32 daily doses), sperm morphology, motility, and count were assessed on samples collected from the right cauda epididymis, left vas deferens, and left cauda epididymis, respectively.

[h] Although statistically significant increases in the relative weight were noted in the right testis at 400 mg/kg/day and both the right and left epididymides in most rats at all doses, these observations were considered unrelated to BMS-477118 because: 1) values in each treated group were only minimally different as compared with controls (within 0.01 to 0.04%); and 2) the statistical significance resulted from identical or virtually identical standard deviations in all groups (including the control) which mathematically exaggerates differences between groups because statistical estimates of between-group variability are minimized (close to zero).

[i] Preimplantation loss calculated as: [(Corpora lutea - implantations)/corpora lutea] x 100.

[j] Postimplantation loss calculated as: (Early resorptions/implantations) x 100.

[k] Twelve 750-mg/kg females were found dead during the first 2 weeks (11 females) or in the fourth week (1 female) of dosing. These drug-related deaths were preceded by clinical signs of urine-stained coat, perinasal substance, perioral substance, reduced feces, periocular substance, and ungroomed coat. One additional female at 750 mg/kg/day was euthanatized (after 25 daily doses) due to a severe skin lesion which occurred after the initiation of cohabitation and was attributed to aggression of the male rat. At necropsy, all 13 females that died or were euthanatized had one or more gross changes in the spleen (enlarged, mottled, discolored, and/or misshapen). Two 750 mg/kg/day group females were euthanatized after 14 or 21 daily doses as the result of intubation accidents (necropsy revealed fluid-filled thoracic cavity, perforations of the esophagus or lung, discoloration of the lungs, and/or axillary mass).

[l] Drug-related clinical signs consisted of perinasal and/or perioral substance and urine-stained coat at 125 mg/kg/day and higher; reduced feces at 300 and 750 mg/kg/day; and periocular substance and ungroomed coat at 750 mg/kg/day.

[m] Drug-related lesions of the spleen similar to those seen in females found dead or euthanatized at 750 mg/kg/day were noted in rats surviving to scheduled termination and included enlarged spleens in 13 females (10 females at 300 mg/kg/day and 3 females at 750 mg/kg/day) as well as discolored and misshapen spleen in one female at 750 mg/kg/day.

[n] Values for all estrous-cycling parameters during the pretreatment period were omitted from the listings below because they were comparable in all groups and no incidences of prolonged (lasting 3 or more consecutive days) proestrus, estrus, metestrus, or diestrus were noted.

[o] Includes values for one 300-mg/kg female that had a litter consisting of 2 early resorptions. Exclusion of this rat from group calculations will alter the mean value for the following parameters in the 300-mg/kg dose group: corpora lutea (14.8), implantations (14.2), preimplantation loss (4.7%), postimplantation loss (16.1%), and live conceptuses (12.2).

Reproductive and Developmental Toxicity – Effects on Embryo-Fetal Development

Report Title: Oral Study of Embryo-Fetal Development in Rats
Test Article: BMS-477118
Design similar to ICH 4.1.1? Yes
Duration of Dosing: Gestation Days 6 to 15
Study Number: DN02015
Day of Mating: Gestation Day 0
Document Control Number: 930002987
Species/Strain: Rat/Crl:CD(SD)IGS BR
Day of C-Section: Gestation Day 20
Location in Dossier: 4.2.3.5.2
Initial Age: 9 to 10 weeks at receipt
Method of Administration: Oral (gavage)
GLP Compliance: Yes
Date of First Dose: April 16, 2002
Vehicle/ Formulation: Benzoate salt in 1.25% Avicel®
Special Features: None
No Observed Adverse-Effect Level: F_0 Females: 240 mg/kg/day
F_1 Litters: 64 mg/kg/day

Daily Dose (mg/kg)		0 (Control)	64	240	900
Dams:	Toxicokinetics (saxagliptin) AUC (ng·hr/mL):	--	23610	121774	646843
	Cmax (ng/mL):	--	8154	31718	61975
	Toxicokinetics (BMS-510849[a]) AUC (ng·hr/mL):	--	6384	28918	143637
	Cmax (ng/mL):	--	1891	4909	10096
	No. Pregnant/No. Assigned to Study - N/N (%)	20/22 (90.9)	21/22 (95.5)	18/22 (81.8)	21/22 (95.5)
	No. Died or Sacrificed Moribund	0	0	0	1[b]
	No. Aborted or with Total Resorption of Litter	0	0	0	0
	Clinical Observations[c]	--	--	--	++[d]
	Necropsy Observations	--	--	--	--
Body Weight (%[e]) Gestation Day 20		364.3 g	0%	0%	-3%
Body Weight Change (%[e]) Gestation Days 6-9		11.3 g	+1%	-4%	-26%
Body Weight Change (%[e]) Gestation Days 9-12		20.5 g	-11%	-12%	-31%**
Body Weight Change (%[e]) Gestation Days 6-12		31.8 g	-7%	-8%	-29%**
Food Consumption (%[e]) Gestation Days 6-9		25.2 g/day	0%	-2%	-1%
Food Consumption (%[e]) Gestation Days 9-12		27.4 g/day	-3%	-5%	-4%
Food Consumption (%[e]) Gestation Days 6-12		26.6 g/day	-2%	-5%	-3%
Mean No. Corpora Lutea		13.4	14.6*	14.1	13.7
Mean No. Implantations		12.9	13.6	13.2	13.0
Litters (Cesarean-Delivered on Gestation Day 20):					
No. Evaluated		18[f]	20[f]	18	21
Mean No. Resorptions		0.5	0.2	0.1	0.6
No. of Litters with Dead Fetuses		0	0	0	0
Mean % Preimplantation Loss[g]		3.6	6.4	5.3	4.4
Mean % Postimplantation Loss[h]		3.8	1.2	1.0	4.6
Mean Fetal Body Weight/Litter (grams)		3.85	3.86	3.78	3.57**
Fetal Sex Ratios (% male fetuses)		47.0	49.2	49.9	46.8
Summary of Gross External, Visceral, & Skeletal Anomalies[i,j]:					
Total Affected Fetuses/Total Fetuses Evaluated - N/N (%)		19/226 (8.4)	21/270 (7.8)	15/236 (6.4)	19/261 (7.3)
Total Affected Litters/Total Litters Evaluated - N/N (%)		11/20 (55.0)	11/21 (52.4)	9/18 (50.0)	10/21 (47.6)
Percent Affected Fetuses/Litter (Mean %)		8.0	7.4	6.0	7.7
Fetal Gross External Anomalies[j]:					
No. Fetuses Evaluated/ No. Litters Evaluated		226/20	270/21	236/18	261/21
Body: Pale					
	Fetal Incidence N (%)	1 (0.4)	1 (0.4)	0	0
	Litter Incidence N (%)	1 (5.0)	1 (4.8)	0	0
Hematoma					
	Fetal Incidence N (%)	1 (0.4)	1 (0.4)	2 (0.8)	0
	Litter Incidence N (%)	1 (5.0)	1 (4.8)	2 (11.1)	0
Fetal Visceral Anomalies[j]:					
No. Fetuses Evaluated/ No. Litters Evaluated		114/20	133/20[k]	118/18	131/21
Liver: Median lobe, accessory tissue					
	Fetal Incidence N (%)	0	0	1 (0.8)	0
	Litter Incidence N (%)	0	0	1 (5.6)	0

Reproductive and Developmental Toxicity – Effects on Embryo-Fetal Development

Document Control Number: 930002987 **(Continued)** **Test Article:** BMS-477118

Study No. DN02015

Daily Dose (mg/kg)	0 (Control)	64	240	900
Fetal Skeletal Anomalies[j]:				
No. Fetuses Evaluated/ No. Litters Evaluated	112/19[l]	137/21	118/18	130/21
Skull: Parietals, and/or interparietal, hypoplastic				
Fetal Incidence N (%)	1 (0.9)	0	2 (1.7)	3 (2.3)
Litter Incidence N (%)	1 (5.3)	0	2 (11.1)	2 (9.5)
Skull: Supraoccipital, hypoplastic				
Fetal Incidence N (%)	0	1 (0.7)	2 (1.7)	3 (2.3)
Litter Incidence N (%)	0	1 (4.8)	2 (11.1)	1 (4.8)
Skull: Squamosal, hypoplastic				
Fetal Incidence N (%)	0	1 (0.7)	1 (0.8)	0
Litter Incidence N (%)	0	1 (4.8)	1 (5.6)	0
Hyoid: Hypoplastic, incomplete ossification or not ossified				
Fetal Incidence N (%)	10 (8.9)	15 (10.9)	6 (5.1)	10 (7.7)
Litter Incidence N (%)	8 (42.1)	9 (42.9)	5 (27.8)	6 (28.6)
Sternebrae: Misshapen, bifid, or unilateral ossification				
Fetal Incidence N (%)	2 (1.8)	2 (1.5)	1 (0.8)	1 (0.8)
Litter Incidence N (%)	2 (10.5)	2 (9.5)	1 (5.6)	1 (4.8)
Ribs: Hypoplastic, wavy, and/or nodulated				
Fetal Incidence N (%)	0	0	2 (1.7)	0
Litter Incidence N (%)	0	0	1 (5.6)	0
Ribs: 7th cervical				
Fetal Incidence N (%)	0	0	0	1 (0.8)
Litter Incidence N (%)	0	0	0	1 (4.8)
Vertebrae: Thoracic, centra, misshapen or bifid				
Fetal Incidence N (%)	3 (2.7)	2 (1.5)	1 (0.8)	2 (1.5)
Litter Incidence N (%)	2 (10.5)	1 (4.8)	1 (5.6)	2 (9.5)
Vertebrae: Lumbar, centra, bifid				
Fetal Incidence N (%)	0	0	0	1 (0.8)
Litter Incidence N (%)	0	0	0	1 (4.8)
Pelvis: Ischia, incomplete ossification				
Fetal Incidence N (%)	0	0	1 (0.8)	1 (0.8)
Litter Incidence N (%)	0	0	1 (5.6)	1 (4.8)
Pelvis: Pubes, incomplete ossification				
Fetal Incidence N (%)	1 (0.9)	0	4 (3.4)	8 (6.2)*
Litter Incidence N (%)	1 (5.3)	0	3 (16.7)	4 (19.0)
Mean Ossification Sites (per fetus per litter):				
No. Fetuses Evaluated/ No. Litters Evaluated	112/19[l]	137/21	118/18	130/21
Ribs (Total)	13.09	13.08	13.04	13.10
Sternebrae	5.79	5.77	5.71	5.54
Forepaws: Carpals	0.00	0.00	0.00	0.00
Forepaws: Metacarpals	4.00	4.00	4.00	4.05

Abbreviations: ♦ = Not performed to date; -- No noteworthy findings, + = Mild, ++ Moderate, +++ Marked, N = Number, * $p<0.05$, ** $p<0.01$

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

All footnotes are available as table end notes.

Reproductive and Developmental Toxicity – Effects on Embryo-Fetal Development

Document Control Number: 930002987 **(Continued)** **Test Article:** BMS-477118

Study No. DN02015

Daily Dose (mg/kg)	0 (Control)	64	240	900
Mean Ossification Sites (per fetus per litter) (Continued):				
Forepaws: Phalanges	8.98	9.00	9.00	8.99
Hindpaws: Tarsals	0.00	0.00	0.00	0.00
Hindpaws: Metatarsals	5.00	5.00	5.00	4.99
Hindpaws: Phalanges	8.94	9.00	9.01	8.80

[a] Major active metabolite of BMS-477118.

[b] One 900-mg/kg rat died on day 11 of presumed gestation. Reduced food consumption, weight loss after day 8 of presumed gestation, and clinical signs of urine-stained coat, red perioral substance, and reduced feces were noted in this rat. Necropsy revealed an enlarged, mottled spleen, which had autolyzed to an extent that precluded critical microscopic evaluation. The rat was not pregnant.

[c] No inferential statistical procedures were conducted on clinical observation data.

[d] Drug-related clinical signs at 900 mg/kg/day included urine-stained coat and perioral substance.

[e] For controls, group means are shown. For treated groups, percent differences from controls are shown. Statistical significance is based on actual data (not on the percent differences).

[f] Excludes two control and one 64-mg/kg dams which had litters consisting of only 1 or 2 conceptuses.

[g] Preimplantation loss calculated as:[(Corpora lutea - implantations)/corpora lutea] x 100.

[h] Postimplantation loss calculated as:[(Dead + resorbed conceptuses)/implantations] x 100.

[i] One gross external anomaly (protruding tongue) noted exclusively in a control fetus is omitted from the listings below, but is included in the total incidence of affected fetuses and litters and percent affected fetuses/litter.

[j] All percentages were calculated on the basis of the number of live fetuses in each group.

[k] Excludes one litter which consists of a single fetus assigned to skeletal evaluation.

[l] Excludes one litter which consists of a single fetus assigned to visceral evaluation.

Reproductive and Developmental Toxicity – Effects on Embryo-Fetal Development

Report Title: Oral Study of Embryo-Fetal Development in Rabbits **Test Article:** BMS-477118

Design similar to ICH 4.1.1? Yes **Duration of Dosing:** Gestation Days 7 to 19 **Study Number:** DN02034

Day of Mating: Gestation Day 0 **Document Control Number:** 930003036

Species/Strain: Rabbit/Hra:(NZW)SPF **Day of C-Section:** Gestation Day 29 **Location in Dossier:** 4.2.3.5.2

Initial Age: 5.5 - 6 months at receipt **Method of Administration:** Oral (gavage) **GLP Compliance:** Yes

Date of First Dose: May 20, 2002 **Vehicle/Formulation:** Benzoate salt in 1.25% Avicel®

Special Features: None

No Observed Adverse-Effect Level: **F_0 Females:** < 8 mg/kg

F_1 Litters: 40 mg/kg

Daily Dose (mg/kg)	0 (Control)	8	40	200
Does: Toxicokinetics (saxagliptin): AUC (ng·hr/mL)	--	2493	12332	110627
Cmax (ng/mL)	--	963	5711	33923
Toxicokinetics (BMS-510849[a]): AUC (ng·hr/mL)	--	7407	47895	434489
Cmax (ng/mL)	--	2475	16893	101438
No. Pregnant/No. Assigned to Study N/N (%)	22/22 (100)	20/22 (90.9)	22/22 (100)	22/22 (100)
No. Died or Sacrificed Moribund	0	0	0	0
No. Aborted or with Total Resorption of Litter	0	0	0	0
Clinical Observations[b]	--	--	--	--
Necropsy Observations	--	--	--	--
Body Weight (%[c]) Gestation day 20	3.81 kg	0%	-2%	-1%

Abbreviations: ♦ = Not performed to date; -- No noteworthy findings, + = Mild, ++ Moderate, +++ Marked, N = Number, * $p<0.05$, ** $p<0.01$

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

All footnotes are available as table end notes.

Reproductive and Developmental Toxicity – Effects on Embryo-Fetal Development

Document Control Number: 930003036 **(Continued)** **Test Article:** BMS-477118

Study No. DN02034

Daily Dose (mg/kg)	0 (Control)	8	40	200
Does (Continued):				
Body-Weight Change (%[c]) Gestation days 7-20	0.23 kg	-26%	-22%	-17%
Food Consumption (%[c]) Gestation days 13-17	165.1 g/day	-15%	-14%	-16%*
Food Consumption (%[c]) Gestation days 7-20	167.4 g/day	-9%	-9%	-11%
Mean No. Corpora Lutea	9.2	9.1	9.1	8.8
Mean No. Implantations	8.3	8.9	8.7	8.2
Litters (Cesarean-Delivered on Gestation Day 29):				
No. Evaluated	22	20	22	22
No. Live Fetuses	177	175	183	165
Mean No. Resorptions	0.2	0.1	0.4	0.7
No. of Litters with Dead Fetuses	0	0	0	0
Mean % Preimplantation Loss[d]	11.4	3.0	4.8	9.2
Mean % Postimplantation Loss[e]	2.8	1.4	3.8	7.2
Mean Fetal Body Weight/Litter (grams)	44.94	44.79	43.19	44.02
Fetal Sex Ratios (% male fetuses)	49.9	45.7	43.2	56.7
Summary of Gross External, Visceral, & Skeletal Anomalies[f,g]:				
Total Affected Fetuses/Total Fetuses Evaluated - N/N (%)	20/177 (11.3)	13/175 (7.4)	21/183 (11.5)	18/165 (10.9)
Total Affected Litters/Total Litters Evaluated - N/N (%)	11/22 (50.0)	8/20 (40.0)	12/22 (54.5)	12/22 (54.5)
Percent Affected Fetuses/Litter (Mean %)	11.4	6.2	11.3	11.8
Fetal Gross External Anomalies[g]:				
No. Fetuses Examined/No. Litters Examined	177/22	175/20	183/22	165/22
Eyes: Both, open				
Fetal Incidence N (%)	0	1 (0.6)	0	0
Litter Incidence N (%)	0	1 (5.0)	0	0
Fetal Visceral Anomalies[g]:				
No. Fetuses Examined/No. Litters Examined	177/22	175/20	183/22	165/22
Lungs: Accessory lobe absent				
Fetal Incidence N (%)	0	1 (0.6)	1 (0.5)	0
Litter Incidence N (%)	0	1 (5.0)	1 (4.5)	0
Gallbladder: Small				
Fetal Incidence N (%)	0	1 (0.6)	1 (0.5)	0
Litter Incidence N (%)	0	1 (5.0)	1 (4.5)	0
Gallbladder: Absent				
Fetal Incidence N (%)	1 (0.6)	0	0	4 (2.4)
Litter Incidence N (%)	1 (4.5)	0	0	1 (4.5)
Thoracic Cavity: Cyst[h]				
Fetal Incidence N (%)	0	0	0	1 (0.6)
Litter Incidence N (%)	0	0	0	1 (4.5)
Diaphragm: Hernia				
Fetal Incidence N (%)	0	0	1 (0.5)	0
Litter Incidence N (%)	0	0	1 (4.5)	0

Abbreviations: N = Number, * $p<0.05$, ** $p<0.01$

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

All footnotes are available as table end notes.

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Document Control Number: 930003036 **(Continued)** **Test Article:** BMS-477118

Study No. DN02034

Daily Dose (mg/kg)	0 (Control)	8	40	200
Fetal Skeletal Anomalies[g]:				
No. Fetuses Examined/No. Litters Examined	177/22	175/20	183/22	165/22
Skull: Nasals and/or Frontals, supernumerary bone(s) present				
Fetal Incidence N (%)	3 (1.7)	2 (1.1)	8 (4.4)	3 (1.8)
Litter Incidence N (%)	3 (13.6)	1 (5.0)	4 (18.2)	3 (13.6)
Hyoid: Alae, angulated				
Fetal Incidence N (%)	5 (2.8)	0	2 (1.1)	7 (4.2)
Litter Incidence N (%)	3 (13.6)	0	2 (9.1)	6 (27.3)
Sternebrae: Hyperplastic				
Fetal Incidence N (%)	1 (0.6)	4 (2.3)	4 (2.2)	1 (0.6)
Litter Incidence N (%)	1 (4.5)	1 (5.0)	3 (13.6)	1 (4.5)
Sternebrae: Bifid				
Fetal Incidence N (%)	1 (0.6)	1 (0.6)	0	0
Litter Incidence N (%)	1 (4.5)	1 (5.0)	0	0
Ribs: 7th cervical				
Fetal Incidence N (%)	1 (0.6)	0	1 (0.5)	0
Litter Incidence N (%)	1 (4.5)	0	1 (4.5)	0
Ribs: Nodulated				
Fetal Incidence N (%)	0	1 (0.6)	1 (0.5)	0
Litter Incidence N (%)	0	1 (5.0)	1 (4.5)	0
Ribs: Fused and/or Bifurcated				
Fetal Incidence N (%)	1 (0.6)	1 (0.6)	2 (1.1)	1 (0.6)
Litter Incidence N (%)	1 (4.5)	1 (5.0)	1 (4.5)	1 (4.5)
Vertebrae: Cervical, arches, bifid, hypoplastic, and/or incomplete ossification				
Fetal Incidence N (%)	5 (2.8)	4 (2.3)	4 (2.2)	1 (0.6)
Litter Incidence N (%)	3 (13.6)	3 (15.0)	3 (13.6)	1 (4.5)
Vertebrae: Cervical, arches, absent				
Fetal Incidence N (%)	0	0	0	1 (0.6)
Litter Incidence N (%)	0	0	0	1 (4.5)
Vertebrae: Cervical, centra, bifid, misshapen, or unilateral ossification				
Fetal Incidence N (%)	0	0	1 (0.5)	1 (0.6)
Litter Incidence N (%)	0	0	1 (4.5)	1 (4.5)
Vertebrae: Thoracic, arches, fused				
Fetal Incidence N (%)	0	0	0	1 (0.6)
Litter Incidence N (%)	0	0	0	1 (4.5)
Vertebrae: Thoracic, hemivertebrae				
Fetal Incidence N (%)	2 (1.1)	0	1 (0.5)	0
Litter Incidence N (%)	2 (9.1)	0	1 (4.5)	0
Vertebrae: Thoracic, misaligned				
Fetal Incidence N (%)	2 (1.1)	0	1 (0.5)	0
Litter Incidence N (%)	2 (9.1)	0	1 (4.5)	0
Vertebrae: Thoracic, centra, bifid				
Fetal Incidence N (%)	0	0	1 (0.5)	0
Litter Incidence N (%)	0	0	1 (4.5)	0

Abbreviations: N = Number, * $p<0.05$, ** $p<0.01$

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

All footnotes are available as table end notes.

Reproductive and Developmental Toxicity – Effects on Embryo-Fetal Development

Document Control Number: 930003036 **(Continued)** **Test Article:** BMS-477118

Study No. DN02034

Daily Dose (mg/kg)	0 (Control)	8	40	200
Fetal Skeletal Anomalies[g] (Continued):				
Pelvis: Pubes, incomplete ossification				
Fetal Incidence N (%)	0	1 (0.6)	0	0
Litter Incidence N (%)	0	1 (5.0)	0	0
Mean Ossification Sites (per fetus per litter):				
No. Fetuses Evaluated/ No. Litters Evaluated	177/22	175/20	183/22	165/22
Ribs (Pairs)	12.55	12.52	12.72	12.79**
Sternebrae	5.90	5.79	5.91	5.96
Forelimbs: Carpals	0.00	0.00	0.00	0.00
Forelimbs: Metacarpals	4.99	5.00	4.98	4.97
Forelimbs: Phalanges	13.95	13.99	13.89	13.83
Hindlimbs: Tarsals	2.00	2.00	1.99	2.00
Hindlimbs: Metatarsals	4.00	4.00	4.00	4.00
Hindlimbs: Phalanges	12.00	12.00	12.00	11.99

Abbreviations: N = Number, * $p<0.05$, ** $p<0.01$

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

All footnotes are available as table end notes.

[a] Major active metabolite of BMS-477118.

[b] No inferential statistical analyses were conducted on clinical observation data.

[c] For controls, group means are shown. For treated groups, percent differences from controls are shown. Statistical significance is based on actual data (not on the percent differences).

[d] Preimplantation loss calculated as:[(Corpora Lutea - implantations)/Corpora lutea] x 100.

[e] Postimplantation loss calculated as:[(Dead + resorbed conceptuses)/Implantations] x 100.

[f] Anomalies noted exclusively in control fetuses (Kidney: malpositioned and red; Ureter: dilated; Eyes: circumcornea, hemorrhagic; Hyoid: Body, incomplete ossification; Vertebrae: thoracic, arches, hypoplastic; Vertebrae: thoracic, centra, fused) are omitted from the listings below, but are included in the total incidence of affected fetuses and litters and percent affected fetuses/litter.

[g] All percentages were calculated on the basis of the number of live fetuses in each group.

[h] Cyst adhering to the diaphragm and esophagus.

Reproductive and Developmental Toxicity - Effects on Pre- and Postnatal Development, Including Maternal Function

Report Title: Oral Study of Pre- and Postnatal Development in Rats
Test Article: BMS-477118
Design similar to ICH 4.1.2?
Duration of Dosing:
Study No.:
Day of Mating:
Document Control Number: 930024969
Species/Strain:
Method of Administration:
Location in Dossier: 4.2.3.5.2
Initial Age:
Vehicle/Formulation: Free base in Acidified Water
Date of First Dose: November 20, 2006
Culling of Litters:
GLP Compliance:
Special Features:
No Observed Adverse-Effect Level: **F_0 Females:**
F_1 Males & Females:

Daily Dose (mg/kg)	0 (Control)	40	100	250	500
F_0 Generation Females:					
Toxicokinetics (LD4)					
Saxagliptin - AUC (ng•h/mL)	--	14100	38061	131985	301680
Saxagliptin - Cmax (ng/mL)	--	4702	14475	33800	91300
BMS-510849 - AUC (ng•h/mL)	--	3427	9573	23293	37728
BMS-510849 - Cmax (ng/mL)	--	1059	2330	4744	5960
No. Pregnant/No. Assigned to Pre- & Postnatal Evaluations N/N (%)	25/25 (100)	24/25 (96.0)	23/25 (92.0)	21/25 (84.0)	25/25 (100)
Unscheduled Necropsies/Cause of Death or Moribundity:					
Moribund Euthanatized (N)[a]	0	0	0	0	1
Thymic Lymphoma or Mastitis (N)[b]	0	0	1	0	1
Dosing Accident (N)[b]	0	0	1	0	1
No. Pregnant/No. Assigned to Toxicokinetic Evaluations N/N (%)	10/10 (100)	10/10 (100)	10/10 (100)	10/10 (100)	8/10 (80.0)
Unscheduled Necropsies/Cause of Death or Moribundity:					
Severe Urolithiasis (N)[b]	0	1	0	0	0
Dosing Accident (N)[b]	0	0	1	0	0
Clinical Observations	--	--	--	--	--
Necropsy Observations	--	--	--	--	--
Gestation Body Weight (%[c]) - Day 6	264.8 g	0	+1	0	+1
Gestation Body Weight (%[c]) - Day 20	383.2 g	-1	+1	-2	-2
Gestation Body-Weight Gain (%[c]) - Days 6 to 20	118.5 g	-5	+1	-7	-7
Lactation Body Weight (%[c]) - Day 1	288.8 g	-1	+1	-2	-1
Lactation Body Weight (%[c]) - Day 7	306.4 g	-1	+1	-4	-3
Lactation Body Weight (%[c]) - Day 14	334.5 g	-2	+2	-3	-2
Lactation Body Weight (%[c]) - Day 21	348.3 g	-3	-1	-1	-1
Lactation Body-Weight Gain (%[c]) - Days 1-7	17.6 g	-2	-1	-40	-41
Lactation Body-Weight Gain (%[c]) - Days 7-14	28.1 g	-20	+18	+10	+12
Lactation Body-Weight Gain (%[c]) - Days 14-21	13.8 g	-9	-65	+30	+25

Abbreviations: – No noteworthy findings, + Mild, ++ Moderate, +++ Marked; N = Number, GD = Gestation Day, LD = Lactation Day, PND = Postnatal Day
Statistical Analysis: Continuous data were analyzed using Bartlett's Test of Homogeneity of Variances and Analysis of Variance (ANOVA), when appropriate (Bartlett's not significant at $p>0.001$). Dunnett's test was used to identify statistical significance of individual groups. If ANOVA was not appropriate, then the Kruskal-Wallis test was used followed by Dunn's or Fisher's Exact test (≥75% ties). Fisher's Exact test was used for incidence data. Enumeration data were analyzed by using Kruskal-Wallis test as previously described. * $p\leq0.05$, ** $p\leq0.01$

Daily Dose (mg/kg)	0 (control)	40	100	250	500
F_0 Generation Females: (Continued)					
Gestation Food Consumption (%[c]) - Days 6 to 20	26.9 g/day	-1	0	-4	-3
Lactation Food Consumption (%[c]) - Days 1-7	40.2 g/day	-4	+5	-11*	-13**
Lactation Food Consumption (%[c]) - Days 7-14	67.7 g/day	-3	+3	-10**	-7
Lactation Food Consumption (%[c]) - Days 1-14[d]	55 g/day	-3	+3	-11**	-9*
Mean Duration of Gestation (days)	22.8	22.6	22.8	22.8	23.0
No. of Dams with Abnormal Parturition	0	0	0	0	0
Gestation Index (Dams with liveborn pups/No. of pregnant dams)	100%	100%	100%	100%	100%
F_1 Generation Litters: (Preweaning Period)					
No. Litters Evaluated	25	24	23	21	25
Mean Implantation Sites Per Delivered Litter	14.9	14.6	15.9	14.9	15.1
Litters with One or More Stillborn Pups - N (%)	7 (28.0)	3 (12.5)	3 (13.0)	2 (9.5)	3 (12.0)
No. of Litters Without Any Liveborn Pups	0	0	0	0	0
No. of Litters with All Pups Dying Postnatal Days 1-4	0	0	0	0	0
No. of Litters with All Pups Dying Postnatal Days 5-21	0	0	0	1	0
Mean No. Liveborn Pups/Litter	13.5	13.6	14.7	13.5	14.4
Mean No. Stillborn Pups/Litter	0.6	0.1	0.1	0.1	0.2
Pup Survival - Postnatal Days 1 to 4 - % (N/N)[e]	97.0 (327/337)	97.8 (320/327)	97.3 (329/338)	95.8 (271/283)	95.5 (343/359)
Pup Survival - Postnatal Days 4 to 21 - % (N/N)[f]	99.4 (325/327)	100 (320/320)	99.0 (299/302[g])	97.0 (263/271)	97.1 (299/308[h])
Live Litter Size on Postnatal Day 1 - Mean	13.3	13.5	14.6	13.4	14.2
Live Litter Size on Postnatal Day 4 - Mean	13.1	13.3	14.3	12.9	13.7
Live Litter Size on Postnatal Day 7 - Mean	13.0	13.3	14.2	12.8	13.6
Live Litter Size on Postnatal Day 14 - Mean	13.0	13.3	14.3	12.6	13.6
Live Litter Size on Postnatal Day 21 - Mean	13.0	13.3	14.2	13.2	13.6
Pup Body Weights on Postnatal Day 1 - Mean (grams)	6.6	6.4	6.4	6.3	6.2
Pup Body Weights on Postnatal Day 4 - Mean (grams)	8.8	8.6	8.6	8.2	7.8
Pup Body Weights on Postnatal Day 7 - Mean (grams)	12.9	12.4	12.5	11.4	10.8*
Pup Body Weights on Postnatal Day 14 - Mean (grams)	26.7	25.0	24.1	22.7**	22.2**
Pup Body Weights on Postnatal Day 21 - Mean (grams)	42.3	39.2	37.9	36.1**	34.4**
Pup Sex Ratios (% Males/Litter on Postnatal Day 1) - Mean	47.4	54.8	48.4	49.3	52.3
Pup Sex Ratios (% Males/Litter on Postnatal Day 21) - Mean	47.3	55.4	48.3	48.6	54.4
Pup Clinical and Necropsy Signs	--	--	--	--	--
F_1 Generation Males: Postweaning Period					
No. Evaluated Postweaning - N	25	25	25	25	25
No. Died or Sacrificed Moribund - N	0	0	0	1[j]	0
Clinical Observations	--	--	--	--	--
Body Weight (%[c]) - PND 22[i] (Start of Postweaning Period)	47.6 g	-4	-7	-16**	-16**
Body Weight (%[c]) - PND 57[i]	328.8 g	-5*	-3	-7**	-10**

Abbreviations: -- No noteworthy findings, + Mild, ++ Moderate, +++ Marked; N = Number, GD = Gestation Day, LD = Lactation Day, PND = Postnatal Day
Statistical Analysis: Continuous data were analyzed using Bartlett's Test of Homogeneity of Variances and Analysis of Variance (ANOVA), when appropriate (Bartlett's not significant at $p>0.001$). Dunnett's test was used to identify statistical significance of individual groups. If ANOVA was not appropriate, then the Kruskal-Wallis test was used followed by Dunn's or Fisher's Exact test (≥75% ties). Fisher's Exact test was used for incidence data. Enumeration data were analyzed by using Kruskal-Wallis test as previously described. * $p\leq0.05$, ** $p\leq0.01$

Daily Dose (mg/kg)	0 (Control)	40	100	250	500
F_1 Generation Males: Postweaning Period (Continued)					
Body Weight (%[c]) - PND 85[i]	479.2 g	-4	-2	-5*	-7*
Body Weight (%[c]) - PND 114-118[i] (At Termination)	613.8 g	-4	-1	-5	-6
Body-Weight Gain (%[c]) - PND 22-36[i] (1st 2 Weeks Postweaning)	94.8 g	-6	-4	-10**	-13**
Body-Weight Gain (%[c]) - PND 22-85[i] (Weaning to Cohabitation)	431.6 g	-4	-1	-4	-6
Food Consumption (%[c]) - PND 29-85[i] (Weaning to Cohabitation)	29.8 g/day	-2	-1	-3	-1
Sexual Maturation: Preputial Separation					
Age (Postnatal Day) at Preputial Separation (Mean)	46.6	46.6	47.7	48.3	48.4
Body Weight (%[c]) at Preputial Separation[i] (Mean)	237.1 g	-5%	0%	-2%	-4%
Motor Activity[k]	--	--	--	--	--
Sensory Function - Acoustic Startle Habituation[k]	--	--	--	--	--
Learning and Memory - Watermaze	--	--	--	--	--
No. of Days in Cohabitation Prior to Mating - Mean	2.9	3.0	2.9	3.0	2.4
Males that Mated (No. Mated/No. Cohabited) - N/N (%)	25/25 (100)	25/25 (100)	24/24 (100)	24/24 (100)	25/25 (100)
Fertile Males (No. Fertile/No. Mated) - N/N (%)	21/25 (84.0)	17/25 (68.0)	20/24 (83.3)	18/24 (75.0)	19/25 (76.0)
Necropsy Observations	--	--	--	--	--
Organ Weights at Necropsy					
Epididymis - Mean Left/Mean Right (grams)	0.83/0.82	0.82/0.86	0.83/0.82	0.78/0.79	0.76**/0.78
Ratio Epididymis:Body Weight - Mean Left/Mean Right (%)	0.135/0.135	0.138/0.146	0.137/0.136	0.135/0.136	0.133/0.136
Testis - Mean Left/Mean Right (grams)	1.88/1.87	1.88/1.90	1.89/1.85	1.83/1.82	1.77/1.75*
Ratio Testis:Body Weight - Mean Left/Mean Right (%)	0.306/0.305	0.322/0.324	0.312/0.304	0.315/0.313	0.308/0.304
Prostate (grams)	1.36	1.24	1.34	1.24	1.33
Ratio Prostate:Body Weight (grams)	0.223	0.213	0.223	0.212	0.231
Seminal Vesicles With Fluid (grams)	1.92	1.87	1.87	1.81	1.68
Ratio Seminal Vesicles With Fluid: Body Weight (grams)	0.316	0.320	0.306	0.311	0.293
Seminal Vesicles Without Fluid (grams)	0.92	0.89	0.92	0.88	0.85
Ratio Seminal Vesicles Without Fluid: Body Weight (g)	0.151	0.152	0.152	0.152	0.148
F_1 Generation Females: Postweaning Period					
No. Evaluated Postweaning - N	25	25	25	25	25
No. Died or Sacrificed Moribund - N	0	0	0	1[l]	0
Clinical Observations	--	--	--	--	--
Body Weight (%[c]) - PND 22[i] (Start of Postweaning Period)	47.2 g	-7%	-10%	-11%**	-18%**
Body Weight (%[c]) - PND 57[i]	213.7 g	-3%	-1%	0%	-7%**
Body Weight (%[c]) - PND 85[i]	272.0 g	-2%	+1%	0%	-5%*
Body-Weight Gain (%[c]) - 1st Week Postweaning (PND 22-29[i])	36.3 g	-9%*	-7%	-13%**	-16%**
Body-Weight Gain (%[c]) - Premating Period (PND 22-85[i])	224.8 g	-1%	+3%	+2%	-2%
Body-Weight Gain (%[c]) - Gestation Period (GD 0-20)	142.5 g	+9%	+8%	+2%	+6%
Premating Food Consumption (%[c]) - PND 22-85	21.7 g/day	-2%	+2%	+2%	-2%
Gestation Food Consumption (%[c]) - GD 0-20	27.0 g/day	+3%	+7%	+1%	0%

Abbreviations: -- No noteworthy findings, + Mild, ++ Moderate, +++ Marked; N = Number, GD = Gestation Day, LD = Lactation Day, PND = Postnatal Day
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Daily Dose (mg/kg) F_1 Generation Litters: (Preweaning) (Continued)	0 (control)	40	100	250	500
Sexual Maturation: Vaginal Patency					
Age (Postnatal Day) at Vaginal Patency (Mean)	32.6	33.0	33.0	32.9	33.4
Body Weight (%[c]) at Vaginal Patency[i] (Mean)	105.9 g	-5%	-5%	-10%**	-12%**
Motor Activity[k]	--	--	--	--	--
Sensory Function - Acoustic Startle Habituation[k]	--	--	--	--	--
Learning and Memory - Watermaze	--	--	--	--	--
No. of Females Cohabited	25	25	25	24	25
Females that Mated (No. Mated/No. Cohabited) - N/N (%)	25/25 (100)	25/25 (100)	25/25 (100)	24/24 (100)	25/25 (100)
Pregnant Females (No. Pregnant/No. Mated) - N/N (%)	21/25 (84.0)	17/25 (68.0)[m]	21/25 (84.0)	18/24 (75.0)[m]	19/25 (76.0)[m]
Mean No. Days in Cohabitation Prior to Mating	2.9	3.0	2.9	3.0	2.4
Mean No. Corpora Lutea	15.8	16.5	16.8	16.0	15.9
Mean No. Implantations	14.3	15.8	14.8	15.3	15.1
Mean % Preimplantation Loss	10.0	3.7	9.5	4.1	5.7
Necropsy Observations	--	--	--	--	--
F_2 Generation Litters:					
Mean No. Live Fetuses/Litter	13.8	14.9	14.0	14.6	14.3
Mean No. Resorptions	0.4	0.9	0.8	0.8	0.8
No. of Litter with Resorbed Conceptuses N/N (%)	7/21 (33.3)	10/17 (58.8)	9/21 (42.8)	9/18 (50.0)	11/19 (57.9)
No. of Dead Fetuses	0	0	0	0	0
Mean % Postimplantation Loss	3.0	6.2	6.5	4.9	5.4
Fetal Body Weights (g)	3.62	3.68	3.52	3.66	3.65
Fetal Sex Ratios (% Males/Litter)	51.0	48.1	49.6	49.7	43.9
Fetal Gross External Anomalies[n]	--	--	--	--	--

[a] Maternal moribundity was considered drug related.

[b] Not considered drug related due to the absence of a dose-dependent incidence (thymic lymphoma, mastitis, or urolithiasis) or attributed to a gavage accident (signs consistent with intubation trauma were noted at necropsy and/or microscopic evaluation).

[c] For controls, group means are shown. For treated groups, percent differences from controls are shown. Statistical significance is based on actual data (not on the percent differences).

[d] Lactation food consumption data were not evaluated beyond Lactation Day 14, when the pups begin to consume the maternal feed.

[e] Computed as: Number of live pups on Postnatal Day 4/Number of liveborn pups on Postnatal Day 1.

[f] Computed as: Number of live pups on Postnatal Day 21/Number of liveborn pups on Postnatal Day 4.

[g] Excludes a total of 27 pups from 2 litters at 100 mg/kg/day that were euthanatized as required by the protocol following maternal death/euthanasia on Lactation Day 9 (dosing accident) or 14 (thymic lymphoma).

[h] Excludes a total of 35 pups from 3 litters that were euthanatized as required by the protocol following maternal death/euthanasia on Lactation Day 9 (drug-related morbidity), 11 (dosing accident), or 14 (mastitis).

[i] Statistical analysis of F_1-generation body weights and body-weight changes were performed using Analysis of Covariance (ANCOVA), with litter size (at the respective preweaning age) as the covariate for preweaning measures and litter size at weaning (PND 21) as the covariate for postweaning measures.

[j] One F_1-generation male rat at 250 mg/kg/day was euthanatized on Day 32 postweaning due to a broken palate (traumatic injury).

[k] Motor-activity and acoustic startle data were analyzed using an Analysis of Variance with Repeated Measures. In the event of a statistically significant result for the Dose or the Dose x Block interaction, Dunnett's Test was used to identify statistically significant differences between the control and treated groups.

[l] One F_1-generation female rat at 250 mg/kg/day was found dead on Day 2 postweaning. Although no cause of death was identified, it was not considered drug related because it was not a dose-dependent event.

[m] Not considered drug related because the differences between drug-treated and control groups were not dose-dependent. Additionally, fertility rates (number pregnant/number mated) in the drug-treated groups were within the range of the testing facility historical control values (64 to 100%).

[n] There were no drug-related fetal anomalies in the F_2-generation fetuses. Two fetuses (1 at 100 mg/kg/day and 1 at 250 mg/kg/day) had rotation of the left hindlimb; the 100-mg/kg/day fetus also had gastroschisis, rachischisis, cleft palate, and curvature of the body. These observations were not considered drug-related because they were not dose-dependent events. There were no fetal anomalies noted at 40 or 500 mg/kg/day.

Local Tolerance

Test Article: BMS-477118

Species/Strain	Method of Administration	Doses (mg/kg)	Gender and No. per Group	Noteworthy Findings	Study No./ Document Control Number
Bovine Corneal Opacity and Permeability Assay					
Bovine	In vitro	20%	5 corneas	No corneal opacities or effect on permeability.	930027873
Primary Skin Irritation					
Rabbit / New Zealand White	Dermal - topical - semi-occlusive (benzoate salt)	500 mg	3F	Non-irritating to rabbit skin.	930020169
Local Lymph Node Assay					
Mouse / CBA/CaCruBR	Dermal - topical (benzoate salt)	0.5, 1.0, 2.5% 5, 10, 25%	4F 4F	Proliferation indices of 3.2, 7, and 4.1 for concentrations of 0.5, 1.0, and 2.5%, respectively and 9.1, 19.1, and 14.4 for concentrations of 5, 10, and 25%, respectively. Based on the criterion of 3.0 as an indicator of moderate to severe potential to cause skin sensitization, saxagliptin was considered a potential skin sensitizer.	930020171

Other Toxicity Studies

Test Article: BMS-477118

Species/ Strain	Method of Administration	Duration of Dosing	Doses (mg/kg)	Gender and No. per Group	Noteworthy Findings	Study No./ Document Control Number
Genetic Toxicology						
Salmonella typhimurium (TA98 & TA100)	in vitro	NA	5, 16, 50, 160, 500, 1600, and 5000 µg/plate	NA	BMS-477118 was not mutagenic with or without Aroclor-induced rat liver S9 metabolic activation.	DS00162/ 920009473
Immunotoxicology						
Rat / Crl:SD	Oral (gavage) (benzoate salt in 1.25% Avicel®)	28 days (rats dosed with 1 mg KLH, subcutaneously on Day 23)	0, 10, 50, 200	10M/10F	All doses: No drug-related effect on the T lymphocyte-dependent humoral-immune response to KLH. No drug-related effect on the number of splenic pan-T cells, T-helper cells, cytotoxic T cells, or B cells. 50, 200 mg/kg/day: Dose-dependent splenic lymphoid hyperplasia without drug-related splenic-lymphocyte subset changes. 200 mg/kg/day: Mild body weight (males) decrease. Mild decreased platelet counts; increased spleen weight and size with lymphoid hyperplasia; increased mandibular lymphoid node size with lymphoid hyperplasia (mandibular and mesenteric lymph nodes) and plasma-cell hyperplasia (mandibular lymph node); in females, decreased thymus weight and depletion. Bacterial septicemia, thymic lymphoid depletion, splenic and lymph node plasma-cell hyperplasia (1 female).	DS02082/ 930003089
Rat / Fischer 344 Crl (WT) and DuCrj (DPP4 deficient)	Oral gavage (benzoate salt in 1.25% Avicel®)	7 days	40, 200, 600	5F	No differences in systemic exposure to BMS-477118 or BMS-510849 between WT and DPP4-deficient F344 rats across all doses; exposure in both strains of F344 rat similar to those noted previously in HSD rats at comparable doses (≤ 200 mg/kg/day).	DN03164/ 930023459
Rat / Fischer 344 Crl (WT) and DuCrj (DPP4 deficient)	Oral gavage (benzoate salt in 1.25% Avicel®)	1 month	200, 400	8F	Conducted to investigate the potential immunotoxicologic effects of BMS-477118 and their possible relationship with DPP4 inhibition. 200 and 400 mg/kg/day, WT and DPP4-deficient rats: BMS-477118 was equally well tolerated. Minor decreases in body weight and food consumption, were noted primarily in WT rats (at 400 mg/kg/day). Similar, dose-independent, inhibition of plasma DPP4 activity despite reduced number of $CD26^+$ splenocytes in DPP4-deficient rats. Dose-dependent lymphoid changes (increased spleen weights, and lymphoid hyperplasia in lymph nodes) and increases in serum IgG and IgM levels. No effect on functional splenocyte	DS03167/ 930023457

Abbreviations: NA = not applicable, SD = Sprague Dawley, M = Male, F = Female; KLH = keyhole limpet hemocyanin, HSD = Harlan Sprague Dawley, WT = wild type, CN = cyanide, SCN = thiocyanate

Other Toxicity Studies

Test Article: BMS-477118

Species/ Strain	Method of Administration	Duration of Dosing	Doses (mg/kg)	Gender and No. per Group	Noteworthy Findings	Study No./ Document Control Number
					response following mitogenic stimulation. Dose-independent decreases in splenic NK cells. 400 mg/kg/day, WT and DPP4-deficient rats: Decreased thymic weights/size correlating to minimal to moderate thymic cortical lymphocyte depletion. 400 mg/kg/day, DPP4-deficient rats: increased splenic size correlating to minimal splenic extramedullary hematopoiesis.	
Investigative CNS Toxicity Studies: Cyanide (CN) Studies						
Rat/ HSD	Oral (gavage) (free base in acidified water)	54 weeks: 1 control group, 20 rats/sex from 25, 75, 150 mg/kg/day groups, and surviving rats at 300 mg/kg/day; 81/82 weeks: all remaining rats	0, 0, 25, 75, 150, 300	60M/60F	75, 150 and 300 mg/kg/day (through Week 81/82): In females, dose-related increase in the incidence of yellow and/or rough hair coat and pale skin (entire body). 150 mg/kg/day (through Week 81/82): Reduced survival in males, microscopic findings in the brain of 9 of 60 males; most commonly in caudate-putamen, but also in the corpus callosum, frontal cortex, and/or cerebellum (see findings below at 300 mg/kg/day for greater detail). 300 mg/kg/day (through Week 54): Reduced survival in males; decreased mean body weights (~ 11 to 14%) in males and females; increased incidence of pale eyes, red genital discharge, few feces and thin appearance in females. Microscopic findings in brain of 33 of 60 males (most commonly in the corpus callosum, caudate-putamen, thalamus, and/or piriform/temporal cortex) 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 periodic acid Schiff (PAS)-positive material in glial/gitter cells in the corpus callosum, caudate-putamen, piriform/temporal cortex, and thalamus; and increased glinl fibrillary acidic protein immunoreactivity in the corpus callosum, caudate-putamen, and thalamus.	DN03101/ 930005038
Rat/ HSD	Oral (gavage) (free base in water)	Single-dose or 4 weeks (escalating) (5 rats each sacrificed on Day 8 and 14, 10 sacrificed on Day 28)	0, 1000 or 0 Days 1-5: 1000 Days 6-7: 1200 Days 8-13: 1500 Days 9-27: 1800	20M 20M	Single-dose: Mortality (2 rats; Day 1); decreased activity in 5 of 20 rats within approximately 1 to 4 hours of dosing (clinical signs recovered by Day 2). One (1) rat showed no clinical signs prior to death. Escalating-dose: Mortality (2 rats) following 1 or 2 doses of 1000 mg/kg; intermittent decreased activity in most rats throughout study (onset of approximately 1 to 4 hours after dosing with recovery by 24 hours). Additional sporadic clinical signs included labored/increased respiration, tremors, hunched posture, and rough/soiled hair coat. Decreased body-weight gain (6-26%) beginning Day 7. Unilateral, acute infarction in the caudate putamen of 1 rat found dead on Day 2 (2 doses of 1000 mg/kg/day). Microscopically, focal to multifocal acute neuronal necrosis in amygdala (3 of 8), white matter tract vacuolation in caudate putamen (4 of 8), degeneration/demyelination in corpus callosum (1 of 8).	DN04094/ 930010460
Rat / HSD	Oral (free base in water)	5 days	1200 (Days 1/2) 1500 (Days 3/4) 1800 (Day 5)	15M/15F	Drug-related clinical signs in 2 males following 1500 and 1800 mg/kg. Saxagliptin systemic exposures were higher in females than males, but similar in males and females for BMS-510849.	DN05013/ 930012303

Abbreviations: NA = not applicable, SD = Sprague Dawley, M = Male, F = Female; KLH = keyhole limpet hemocyanin, HSD = Harlan Sprague Dawley, WT = wild type, CN = cyanide, SCN = thiocyanate

Other Toxicity Studies

Test Article: BMS-477118

Species/ Strain	Method of Administration	Duration of Dosing	Doses (mg/kg)	Gender and No. per Group	Noteworthy Findings	Study No./ Document Control Number
Rat/ HSD	Oral (gavage) (free base in acidified water)	Single-dose	25, 75, 150, 300	10M/10F	No drug-related clinical signs. Systemic exposure was dose-related with concentrations of saxagliptin and BMS-510849 in the brain relatively low compared to plasma and no gender-related difference.	DN05002/ 930012097
Rat/ HSD	Oral (gavage) (free base in acidified water)	Single-dose or 7 days	1200	10M/10F	Single-dose: In males, decreased activity, ataxia, labored respiration, and inactivity within 20 minutes of dosing. Increased serum glucose and decreased serum bicarbonate. Increased blood CN and thiocyanate (mean of 1.5 and 1.2, µg/mL, respectively) compared to controls (0.05 and 0.4 µg/mL, CN and SCN, respectively); CN levels higher in rats with clinical signs (3.0 µg/mL). 7-day repeat dose: 1 of 10 males necropsied moribund on Day 2. In males, decreased activity, ataxia, labored respiration, cool to touch, hunched posture, tremors by Day 3. Increased reticulocytes, triglycerides, and serum cholesterol in males and females. In males, increased glucose and decreased fibrinogen, total protein, globulins, and bicarbonate. Increased blood CN and SCN (mean, 1.6 and 3.6 µg/mL, respectively) compared to controls (0.04 and 0.3 µg/mL). Increases in CN and SCN were observed to a lesser extent in females (0.07 and 1.2 µg/mL, respectively) compared to males.	DN05020/ 930019069
Rat/ HSD	Oral (gavage) (free base in acidified water)	Single-dose	75, 150, 300, 600, 1000, 1200	5-8M	At 0.5 and/or 2 hours post dose, dose-related increased blood CN at ≥ 150 mg/kg. Two (2) deaths occurred prior to bleeding (1 each at 1000 and 1200 mg/kg). Onset of clinical signs was dose-related and ranged from 8 to 63 minutes. At ≥600 mg/kg, clinical signs included decreased activity, ataxia, collapse, whole-body tremors. At ≥1000 mg/kg, increased respiration, gasping, and splayed posture. Clinical signs were generally associated with blood CN levels ≥ 1.5 µg/mL.	DN05040/ 930012219
Rat/ HSD	Oral (gavage) BMS-477118 (free base in acidified water) Cimetidine (in water)	Single-dose	Cimetidine (300 mg/kg) BMS-477118 (1200 mg/kg) BMS-477118 (1200 mg/kg) + Cimetidine (300 mg/kg)	12M (6M for CN evaluation; 6M for SCN evaluation)	BMS-477118: Clinical signs (usually within 20 minutes of dosing) included mortality, decreased activity, ataxia, labored respiration, inactivity, tremors, and cage biting. Increases in blood CN concentrations at 0.5 (2.2 µg/mL) and 2 hours (1.5 µg/mL) post dose compared to cimetidine-treated controls (no blood CN detected at either time point) and increased serum SCN concentrations at 0.5 (4.3 µg/mL control) and 2 hours (9.1 µg/mL) after dosing. BMS-477118 + cimetidine: No adverse clinical signs or blood CN detected. Increased serum SCN concentrations at 0.5 (2.9 µg/mL) and 2 hours (6.5 µg/mL) post dose.	DN05038/ 930016837
Rat/ HSD	Oral (gavage) BMS-477118 (free base in acidified water) Vildagliptin (in water)	Single-dose	0 (intact) BMS-477118: 1200 (intact) BMS-477118: 1200 (castrated) Vildagliptin: 1200 (intact)	10M 15M 10M 5M	BMS-477118 (intact): 6 of 15 rats (with tremors and/or collapsed) were necropsied in moribund condition 30 to 60 minutes after dosing. Clinical changes in moribund or surviving rats included decreased activity, increased/labored respiration, and ataxia. Increased mean (0.5 and 2 hours) blood CN (1.1µg/mL) and serum SCN (6.9 µg/mL) compared to control and castrated rats. BMS-477118 (castrated): 1 of 10 rats (with tremors and ataxia) was necropsied in moribund condition 45 minutes after dosing (prior to blood collection for CN assessment). Decreased activity, increased respiration, and ataxia in other rats (3 of 10 unaffected). Increased mean (0.5 and 2 hours) blood CN (0.2 µg/mL) and serum SCN (6.8µg/mL)	DN05048/ 930020745

Abbreviations: NA = not applicable, SD = Sprague Dawley, M = Male, F = Female; KLH = keyhole limpet hemocyanin, HSD = Harlan Sprague Dawley, WT = wild type, CN = cyanide, SCN = thiocyanate

Other Toxicity Studies

Test Article: BMS-477118

Species/ Strain	Method of Administration	Duration of Dosing	Doses (mg/kg)	Gender and No. per Group	Noteworthy Findings	Study No./ Document Control Number
					compared to control rats. Relative to intact rats, blood mean CN levels of castrated rats were 83% lower at 0.5 hours and undetectable at 2 hours. Vildagliptin: None	
Mouse/ CD-1	Oral (gavage) (free base in acidified water)	Single-dose	300, 600, 1000, 1500, 2000	12M/12F + extra 10M at 2000 mg/kg (for blood collection at onset of clinical signs)	CN not detected at any dose.	DN05043/ 930012407
Rat/ HSD	BMS-510849 (Subcutaneous)	5-day	300, 600	3M/3F	Dose-dependent increases in systemic exposure, no gender-related difference. No clinical observations.	DN05024/ 930012330
Rat/ HSD	BMS-510849 (Subcutaneous)	1-month	0, 10, 50, 200	10M/10F	All doses: In males, increased blood CN levels (0.056 to 0.315 µg/mL versus 0.05 µg/mL in controls) in the absence of clinical signs of toxicity. Increased serum SCN (2.3 to 7.1 µg/mL versus 2.2 µg/mL in controls) and urine SCN (0.61 to 59 µg/mL versus 0.39 µg/mL in controls) concentrations at exposures equivalent to those achieved at a neurotoxic dose of BMS-477118 (CN levels associated with BMS-477118 toxicity were generally ≥ 1.5 µg/mL). ≥ 50 mg/kg/day: In females, minimally decreased body-weight gain. In males, minimally decreased platelets, APTT, and urine volume. 200 mg/kg/day: Minimally decreased body-weight gain (males), food consumption, and serum cholesterol (males). Minimal to mild pulmonary histiocytosis in lungs of all rats and minimal lymphoid hyperplasia in spleen of 2 males (observed previously with BMS-477118). Brain lesions not observed at any dose of BMS-510849.	DN05028/ 930010508
Investigative CNS Toxicity Studies: Vasoconstriction Studies						
Rat/ HSD	Oral (gavage) (free base in acidified water)	Single-dose	800 (pilot study) 0, 300	3M 4-5M	800 mg/kg: Death in 2 of 3 rats (1 to 2.5 hours post dose). 300 mg/kg: Insignificant heart rate increase (to 40 beats/minute) and blood pressure decrease (< 10 mm Hg) changes.	DT05021/ 930018446
Rat/ HSD	Oral (gavage) (free base in acidified water)	4 weeks (escalating)	0 or Days 1-5: 1000 Days 6-7: 1200 Days 8-13: 1500 Days 9-27: 1800	10-12M	No differences in brain lesion development between rats sorted by low and high endogenous plasma DPP4 activity. High and Low endogenous DPP4 Groups: Tremors, respiratory changes, abnormal posture, ataxia, cage-biting, and decreased body weight gain. Decrease in plasma DPP4 activity (76 to 89% of control). Brain lesions of mild to marked degeneration and necrosis of myelin with periodic acid Schiff (PAS)-positive microglia in the caudate putamen and corpus callosum, with similar incidence regardless of endogenous DPP4 levels. Low Endogenous DPP4 Group: Death of 11 of 12 rats. Cortical infarcts (4 of 12 rats) and necrotizing duodenal enteritis (3 of 12 rats). High Endogenous DPP4 Group: Death of 6 of 12 rats.	DN05018/ 930019176
Rat/ HSD	Oral (gavage) (free base in acidified water)	4 weeks (escalating)	0 or Days 1-5: 1000 Days 6-7: 1200	6M (control) 30M	CN-related clinical signs, dose and MRI-detectable bilateral brain lesions (unrelated to vascular perfusion deficits, including infarction) in 8 of 12 rats. Microscopically, MRI lesions were characterized by myelin	DN05033/ 930019560

Abbreviations: NA = not applicable, SD = Sprague Dawley, M = Male, F = Female; KLH = keyhole limpet hemocyanin, HSD = Harlan Sprague Dawley, WT = wild type, CN = cyanide, SCN = thiocyanate

Other Toxicity Studies

Test Article: BMS-477118

Species/ Strain	Method of Administration	Duration of Dosing	Doses (mg/kg)	Gender and No. per Group	Noteworthy Findings	Study No./ Document Control Number
			Days 8-13: 1500 Days 9-27: 1800		degeneration/ necrosis in the caudate-putamen and corpus callosum. A single rat had a MRI-detected perfusion deficit which correlated to severe necrosis with cell dropout without water content (edema) in the caudate putamen changes, (inconsistent with infarction). No microscopic lesions were observed in the 4 treated rats negative for brain lesions by MRI. Moreover, no brain lesions were detected either by MRI or histology in control rats.	
Oral Investigative Monkey Studies (Saxagliptin)						
Monkey/ Cynomolgus	Oral (free base in acidified water)	3 months 4-6 weeks 1-3 month	2 10 30/20	3M/3F 3M/3F 5M/5F	At all doses: Ulcerative and erosive tail, nose, digit, and/or scrotum skin lesions noted at all doses. Tail amputation required (2; 1 monkey found dead after amputation). Drug-related minimal to severe clinicopathologic changes were transient and included decreased hematocrit, hemoglobin, RBC count; increased reticulocytes and neutrophils; and, thrombocytopenia with recovery (1 male; 30/20 mg/kg/day) after drug cessation; no recrudescence upon rechallenge), decreases in serum albumin and increases in serum globulins. No blood CN was detected at any dose in monkeys. Microscopic findings included minimal to mild splenic and bone marrow lymphoid hyperplasia and minimal to moderate perivascular mononuclear cell infiltrates/inflammation in skin, genitourinary tract, secretory glands (salivary, mammary, and thyroid glands), visceral organs, choroid plexus (brain), and peripheral nerve. Microvascular vasculitis without immunoglobulin deposition in the skin, genitourinary tract, gastrointestinal tract, thyroid gland, skeletal muscle, and lung. Toxicologically insignificant, low levels of SCN (30/20 mg/kg/day). 10 and 30/20 mg/kg/day: The 30 mg/kg/day dose was acutely toxic and resulted in moribund sacrifice (2 females; after 1 or 2 doses) and subsequent decrease in dose to 20 mg/kg/day. Generalized edema (1 female; 10 mg/kg/day) associated with glomerulopathy and local scrotal edema (2; 1 with glomerulopathy; 30/20 mg/kg/day). Minimal to moderate increases in monocytes, lymphocytes, fibrinogen, total protein and decreased total protein (2 with glomerulopathy). No immune-mediated mechanisms established based on non-recurrence of thrombocytopenia following drug re-challenge (30/20 mg/kg/day); absence of anti-platelet, anti-RBC, or anti-nuclear antibody titers at any dose; no evidence of immune-complex deposition in the skin, kidney, or vasculature.	DN05063/ 930019299
Monkey/ Cynomolgus	Oral (gavage) (free base in acidified water)	5 days	0.03, 0.1, 0.3, 1	1M/1F	Dose-dependent increases in plasma DPP inhibition with Emax of 50-80%. Sustained inhibition over 24 hours only observed at 1 mg/kg/day.	DN06055/ 930023730
Oral Investigative Studies (Other DPP4 Inhibitors)						
Monkey/ Cynomolgus	Oral (gavage) Saxagliptin: free base in acidified water Vildagliptin: free base in water	Ascending (3 daily doses/dose level with 4-5 day washout in between)	3, 10, 30, 60, 100	1M/1F	Saxagliptin, vildagliptin, and sitagliptin were clinically well-tolerated when given as an ascending dose up to 30 mg/kg/day or following a 50 mg/kg single dose (vildagliptin and sitagliptin). Most clinical findings (all compounds) occurred at doses ≥ 60 mg/kg/day and included skin lesions (saxagliptin and vildagliptin) emesis, salivation, transient lameness, and/or decreased activity. Common	DN06007/ 930022854

Abbreviations: NA = not applicable, SD = Sprague Dawley, M = Male, F = Female; KLH = keyhole limpet hemocyanin, HSD = Harlan Sprague Dawley, WT = wild type, CN = cyanide, SCN = thiocyanate

Other Toxicity Studies

Test Article: BMS-477118

Species/ Strain	Method of Administration	Duration of Dosing	Doses (mg/kg)	Gender and No. per Group	Noteworthy Findings	Study No./ Document Control Number
	Sitagliptin: phosphate salt in water)	Single dose	50	1M/1F	clinical pathologic changes included decreased serum albumin and A/G ratios (saxagliptin and vildagliptin) at doses ≥ 30 mg/kg/day and increased urine ketones (vildagliptin and sitagliptin). Microscopic changes correlative to clinically observed skin lesions included focal epidermal ulceration/erosions, inflammation and/or scabs (saxagliptin and vildagliptin), and lymphoid hyperplasia in the spleen (saxagliptin), thymus and/or bone marrow (sitagliptin).	
Monkey/ Cynomolgus	Oral (gavage) Saxagliptin: free base in acidified water Vildagliptin: free base in water Sitagliptin: phosphate salt in water	6 weeks	BMS-477118: 10 Vildagliptin: 40/30/20 Sitagliptin: 40	3M/3F	10 mg/kg/day (saxagliptin): Skin lesions observed in 5/6 monkeys. Tail edema and necrosis (1 female) ultimately required partial tail amputation. Localized genital edema adjacent to abrasion or ulcer) in 2 monkeys. Transient clinical signs included red or translucent nasal discharge, tremor, lameness, and audible respiration. Minimal to moderate transient clinicopathologic changes included: decreased hematocit, hemoglobin, and red blood cell count with increased reticulocytes; decreased platelet counts; increased total white blood cell, monocyte, and lymphocyte counts; neutrophil count fluctuations; increased total serum protein and globulins; decreased A/G ratios; in 2 monkeys, increased urine protein and decreased blood albumin due to mild glomerulopathy. Microscopic lesions included focal moderate to severe, necrotic skin lesions with ulcers and microvascular smooth muscle and endothelial-cell hypertrophy and mural perivascular mononuclear inflammatory-cell infiltrates in the tail, digits, and/or genital area, nose and nasal cavity; minimal to mild multitissue perivascular and periglandular mononuclear cell infiltrates/ inflammation in secretory glands, visceral organs, genitourinary tract, choroid plexus (brain), and peripheral nerve; minimal to slight subacute non-necrotizing vasculitis in skeletal muscle; splenic and bone marrow lymphoid hyperplasia; mild thymic lymphoid depletion; and mild glomerulopathy with ultrastructural features of glomerular podocyte foot process fusion and effacement, aggregated podocyte phagosome granules, and intravascular mononuclear leukocytes. 40/30/20 mg/kg/day (vildagliptin):Severe, dose-limiting, diffuse edema of scrotum, hindfeet, and forehands with marked palmar cracking in 1 male and in the forehands, hindfeet, and tail of 1 female at 40 mg/kg/day. Mild to moderate edema was also observed in forehands, hindfeet, and scrotum in an additional 3 of 6 monkeys. Dose reduced (Days 3 [males] and 2 [females]) to 20 mg/kg/day to improve tolerability and reduce edema in all monkeys, although 1 monkey developed tail edema with severe serosanguinous discharge 2 days later. Increased dose to 30 mg/kg/day on Days 8 (males) and 7 (females) which exacerbated focal to generalized edema and resulted in bloody nasal discharge in a 1 monkey. Dose decreased to 20 mg/kg/day for all monkeys until study termination; intermittent scrotal, tail, and/or hands/feet edema remained in 4 of 6 monkeys. In 2 monkeys, focal necrotic tail lesions subsequently required partial to full tail amputation; 1 monkey failed to recover from surgery and was euthanatized. Remaining monkeys at 20 mg/kg/day, had transient nasal red to translucent discharge, tremor, lameness, and/or audible respiration. In 2 monkeys with severe skin lesions, and	DN06018/ 930024807

Abbreviations: NA = not applicable, SD = Sprague Dawley, M = Male, F = Female; KLH = keyhole limpet hemocyanin, HSD = Harlan Sprague Dawley, WT = wild type, CN = cyanide, SCN = thiocyanate

Other Toxicity Studies

Test Article: BMS-477118

Species/ Strain	Method of Administration	Duration of Dosing	Doses (mg/kg)	Gender and No. per Group	Noteworthy Findings	Study No./ Document Control Number
					minimal to moderate clinicopathologic changes consistent with and included changes associated with hemorrhage and systemic inflammation (increased leukocytes), decreased total serum protein and albumin, and increased or decreased serum globulin. Drug-related microscopic findings included mild to severe focal to diffuse necrosis and ulceration of the skin with microvascular mural disruption and perivascular hemorrhage and edema in the tail, digits, genitalia, and nose; thoracic and abdominal subcutaneous edema; mild splenic and bone marrow lymphoid hyperplasia; and minimal thymic lymphoid depletion. 40 mg/kg/day (sitagliptin): Minimal to slight splenic and bone marrow lymphoid lymphoid hyperplasia in 3 of 6 monkeys.	
Monkey/ Cynomolgus	Oral (gavage) Saxagliptin (free base in acidified water) Vildagliptin (free base in water) Sitagliptin (phosphate salt in water) BMS-767778: (acidified water)	Single-dose 7 weeks total (modified cross-over design)	BMS-477118: 0.1, 0.3, 1, 3, 10 Vildagliptin: 0.1, 0.3, 1, 3, 10, 30 Sitagliptin: 0.3, 1, 3, 10, 40 BMS-767778: 0.1, 0.3, 1, 3, 10, 30	24 total	Drug-related clinical signs (30 mg/kg vildagliptin) included slight hand/foot edema in 3 monkeys and slight tremor on the day of dosing in 2 monkeys. Dose-dependent increases in systemic exposure and DPP inhibition. Sustained maximal inhibition (Emax comparable to Emin) with saxagliptin at ≥ 3 mg/kg, vildagliptin at 30 mg/kg, sitagliptin at 40 mg/kg, and BMS-767778 at 30 mg/kg.	DN06044/ 930025897
BMS-510849 Analytical Bridging Studies						
Mouse/ CD-1	Oral (gavage) (free base in acidified water)	7 days	50, 250, 600	36M/36F	Mortality at 50 and 600 mg/kg/day (1 male and 1 female, respectively). Both had an axillary mass or swelling. Mean toxicokinetic parameters derived from the original assay of BMS-510849 were generally higher (up to 24.5% for Cmax and 20.8% for AUC) versus the new assay with no gender-related differences.	DN07014/ 930024595
Rat/ HSD	Oral (gavage) (free base in acidified water)	7 days	2, 25, 75, 300	12M/12F	Mortality in 1 male at 300 mg/kg/day. Mean toxicokinetic parameters derived from the original assay of BMS-510849 were generally higher (up to 25.3% for Cmax and 42.7% for AUC) versus the new assay with greater differences in females compared to males.	DN07011/ 930025376
Pregnant Rat/ Crl:SD	Oral (gavage) (benzoate salt in 1.25 Avicel®)	10 days	64, 240, 900	10F	Mortality in 2 rats dosed with 900 mg/kg/day. Mean toxicokinetic parameters derived from the original assay of BMS-510849 were generally higher (up to 1.6% for Cmax and 2.7% for AUC) versus the new assay.	DN07015/ 930025618
Pregnant Rabbit/ NZW	Oral (gavage) (benzoate salt in 1.25 Avicel®)	13 Days	8, 40, 200	5F	Mean toxicokinetic parameters derived from the original assay of BMS-510849 were generally higher (up to 10% for Cmax and 11.1% for AUC) versus the new assay.	DN07016/ 930025662
Dog/ Beagle	Oral (gavage) (benzoate salt in 1.25 Avicel®)	7 days	1, 5, 10	3M/3F	Mean toxicokinetic parameters derived from the original assay of BMS-510849 were generally higher (up to 30.8% for Cmax and 36.2% for AUC) versus the new assay with no gender-related differences.	DN07012/ 930025730
Monkey/ Cynomolgus	Oral (gavage) (free base in acidified water)	7 days	0.03, 0.3, 3, 10	3M/3F	Mean toxicokinetic parameters derived from the original assay of BMS-510849 were generally higher (up to 23.0% for Cmax and 15.1% for AUC) versus the new assay with similar gender-related differences.	DS07029/ 930025307

Abbreviations: NA = not applicable, SD = Sprague Dawley, M = Male, F = Female; KLH = keyhole limpet hemocyanin, HSD = Harlan Sprague Dawley, WT = wild type, CN = cyanide, SCN = thiocyanate

Other Toxicity Studies

Test Article: BMS-477118

Species/ Strain	Method of Administration	Duration of Dosing	Doses (mg/kg)	Gender and No. per Group	Noteworthy Findings	Study No./ Document Control Number
Studies on Impurities/Degradants						
Salmonella typhimurium Strains TA98, TA100, TA1535, and TA1537) and *Escherichia coli* Strain WP2 *uvrA* (Qualifying Ames Reverse-Mutation)	In vitro (benzoate salt)	NA	150, 300, 600, 1200, 2500, 5000 µg/plate containing ( ) and ( ) (± S9)	NA	Not mutagenic	«StudyNumber»/ 930005651
Salmonella typhimurium Strains TA98, TA100, TA1535, and TA1537) and *Escherichia coli* Strain WP2 *uvrA* (Qualifying Ames Reverse-Mutation)	In vitro (benzoate salt)	NA	200, 400, 800, 1600, 3000, 5000 µg/plate containing ( ) and ( ) (± S9)	NA	Not mutagenic	DS06118/ 930019013
Rat / Crl:SD (Qualifying Micronucleus)	Oral (free base in acidified water)	3 days	250, 500, 1000 containing ( )	6M	No evidence of genotoxicity	DS07036/ 930024704
Rat / HSD (3-month Qualifying)	Oral (free base in acidified water)	3 months	300 300 containing ( ) 300 containing ( )	10M/10F	Toxicity profile of BMS-477118 not altered by ( )	DN07006/ 930025076
Other Toxicity Studies						
Mouse / CD-1	Oral (benzoate salt in 1.25% Avicel® and free base in water)	2 weeks	30, 300, 1000	21M/21F	No difference in systemic exposure. No observed toxicity.	DN03028/ 930005006
Rat / HSD	Oral (benzoate salt in 1.25% Avicel® and free base in water)	2 weeks	300, 600, 1200	12M/12F	No difference in systemic exposure. Doses of ≥ 600 mg/kg/day resulted in overt toxicity and death.	DN03033/ 930005000
Rat / HSD	Oral (free base in water) Batches: 3D6848, 3D65912, and 3D65913	1 or 3 days	300 for 3 days 1200 for 1 day	10M	No major differences in toxicity attributed to batch.	DN03113/ 930008664
Monkey / Cynomolgus	Intravenous	Single dose	5	1M	Conducted to investigate an unexpected death in a pharmacokinetic study. Decreased blood pressure (~50 mm Hg), but responded well to therapy. Marked elevations of serum AST, LDH, and CPK after 22 hours. Microscopically, punch biopsy indicated moderate, acute myofiber degeneration in skeletal muscle.	NA/930000876
Monkey/ Cynomolgus	Intravenous (free base)	M: 2 doses (24 hour washout between) F: 1 dose	M: 3.4 and 6.8 F: 3.4	1M/1F	Conducted to determine whether acute mortality in monkeys was due to hemodynamic changes and/or immunologic or prothrombogenic mechanisms. Male: Sacrificed 5 days post 6.8 mg/kg dose with pale skin/mucous membranes and cool to touch. No hemodynamic or ECG changes or clinical pathology changes suggestive of prothrombogenic effect.	DS05194/ 930018371

Abbreviations: NA = not applicable, SD = Sprague Dawley, M = Male, F = Female; KLH = keyhole limpet hemocyanin, HSD = Harlan Sprague Dawley, WT = wild type, CN = cyanide, SCN = thiocyanate

b(4)

b(4)

Other Toxicity Studies

Test Article: BMS-477118

Species/ Strain	Method of Administration	Duration of Dosing	Doses (mg/kg)	Gender and No. per Group	Noteworthy Findings	Study No./ Document Control Number
					Female: Decreased mean arterial blood pressure (30 mm Hg) and increased in heart rate (30 bpm) starting approximately 45 minutes after dosing. Blood pressure oscillated from 1 post dose and eventually fell to critically low levels (< 40 mm Hg) approximately 4 to 4.5 hours post dose. Monkey was subsequently sacrificed. No ECG changes or clinical pathology changes suggestive of prothrombogenic effect.	
Monkey/ Cynomolgus	Intravenous (free base)	Single dose (escalating in second male, with 7-day washout between doses)	M1: 5 M2: 5, 10, 20 F: 2,5	2M/2F	Based on a working hypothesis that acute saxagliptin toxicity in monkeys was due to altered vascular tone, this study was conducted to potentially define an animal model by identifying a subpopulation of monkeys susceptible to acute toxicity. The objective of this study was to determine the time course of clinical pathology findings including potential immune mediators after a single intravenous dose of saxagliptin. Ultimately, due to a high variability in inter-animal response an animal model to test the hypothesis of altered vacular tone-mediated acute toxicity could not be established. 5 mg/kg, Male 1: sacrificed in moribund condition 7 days after dosing. Clinical signs from Day 1 through 8 included: pale appearance, decreased activity, preputial and scrotal swelling with black and blue bruising and urine scalding, transient decreased body temperature, lameness of 1 hindlimb, and tremor. Decreased red cell parameters: red blood cell count, hemoglobin, and hematocrit with compensatory increased absolute and relative reticulocyte count correlating with decreased MCH and MCHC, increased RDW, polychromasia, and anisocytosis on Day 8 immediately prior to necropsy. Increased total white blood cell count; and neutrophil count on Day 8 immediately prior to necropsy. Decreased clinical chemistry parameters: total protein and albumin on Day 1 (4 hours post dose) and Day 8 (immediately prior to necropsy) and decreased albumin to globulin ratio and increased globulin on Day 8 immediately prior to necropsy. Decreased serum total protein and albumin on Day 1 (4 hours post dose), and alpha-1 globulins on Day 1 (0.5 to 4 hours post dose). Increased beta-1 globulins on Day 1 (0.5 to 4 hours post dose). Presence of blood in urine on Days 2, 3, 6, and 8. Marked preputial and scrotal ulceration correlating clinically to black and blue bruising, macroscopically to red discoloration, and microscopically to hemorrhage, edema, necrosis, fibrin thrombi, and inflammation. 5, 10, and 20 mg/kg, Male 2: No clinical signs at 5 or 10 mg/kg. Tremors at 20 mg/kg. 2.5 mg/kg, 2 Females: In both females, decreased red cell parameters: red blood cell count, hemoglobin, and hematocrit, increased total white blood cell, neutrophil associated with increased number of bands, and monocyte counts on Day 1 (2, 4, and/or 6 hours post dose), and in 1 of 2 females, decreased lymphocyte count on Day 1 (1 to 2 hours post dose).	DN06017/ 930028066

Abbreviations: NA = not applicable, SD = Sprague Dawley, M = Male, F = Female; KLH = keyhole limpet hemocyanin, HSD = Harlan Sprague Dawley, WT = wild type, CN = cyanide, SCN = thiocyanate

Other Toxicity Studies

Test Article: BMS-477118

Species/ Strain	Method of Administration	Duration of Dosing	Doses (mg/kg)	Gender and No. per Group	Noteworthy Findings	Study No./ Document Control Number
Monkey/ Cynomolgus	Oral (suspensions of clinical tablets)	27 days	3 (Day 1 and 8) 10 (Days 15 and 22)	3M/3F	Conducted to evaluate potential transient decreases in lymphocytes. No drug-related clinical signs of immunotoxicity. No drug-related lymphocyte decreases. Larger, but non-statistically significant, lymphocyte subpopulation decreases in monkeys with tail lesions. Potential drug relationship in monkeys with tail lesions unclear. Drug-related clinicopathologic changes included: increased hematocrit in 2F monkeys and decreased erythrocyte count and hemoglobin (1F) and hemoconcentration (1M, 2F). Skin (tail tip and/or back) scabs and sores (1M, 2F) with secondary systemic inflammatory response (2 of 3 monkeys with tail lesions). Skin lesions resolved after study termination (2 of 3); tail amputation required (1M). Stress-related leukograms (decreased lymphocytes and eosinophils, and increased neutrophils) occurred sporadically during study.	DN05054/ 930014662

Abbreviations: NA = not applicable, SD = Sprague Dawley, M = Male, F = Female; KLH = keyhole limpet hemocyanin, HSD = Harlan Sprague Dawley, WT = wild type, CN = cyanide, SCN = thiocyanate

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/s/

Fred Alavi
6/1/2009 03:28:40 PM
PHARMACOLOGIST
Saxagliptin final review
Saxagliptin NDA review

Todd Bourcier
6/1/2009 03:31:05 PM
PHARMACOLOGIST
I concur with Dr. Alavi's recommendations.

45 Day NDA Filing Meeting Checklist
NONCLINICAL PHARMACOLOGY/TOXICOLOGY

NDA #: 22-350 DRUG: saxagliptin, Sponsor: Bristol-Myers Squibb

ITEM	YES	NO	COMMENT
1) Does this section of the NDA appear to be organized (according to 21 CFR 314 and current guidelines for format and content) in a manner that would allow a substantive review to be completed?	x		
2) Is this section of the NDA indexed and paginated in a manner to enable a timely and substantive review?	x		
3) Is this section of the NDA sufficiently legible so that a substantive review can be done? Has the data been presented in an appropriate manner (consider tables, graphs, complete study reports, inclusion of individual animal data, appropriate data analysis, etc.)?	x		
4) Are all necessary and appropriate studies for this agent, including special studies/data requested by the Division during pre-submission communications/discussions, completed and submitted in this NDA? (Please itemize the critical studies included and indicate any significant studies that were omitted from the NDA - e.g., safety pharm, genotox, reprotox, chronic tox, carcinogenicity)	x		Have electronic files of the carcinogenicity studies been submitted for statistical review? SAS transport data for the rat and the mouse carcinogenetic studies were not submitted with the electronic NDA. Data was requested from the sponsor on Aug 25,08 and should be available in the next few days.

ITEM	YES	NO	COMMENT
5) Were the studies adequately designed (ie., appropriate number of animals, adequate monitoring consistent with the proposed clinical use, state-of-the art protocols, etc.)?	x		
6) If the formulation to be marketed is not identical to the formulation used in the toxicology studies (including the impurity profiles), has the sponsor clearly defined the differences and submitted reviewable supportive data (ie., adequate repeat studies using the marketed product and/or adequate justification for why such repetition would not be necessary)?	x		
7) Does the route of administration used in animal studies appear to be the same as the intended human exposure route? If not, has the sponsor submitted supportive data and/or an adequate scientific rationale to justify the alternative route?	x		
8) Has the proposed draft labeling been submitted? Are the appropriate sections for the product included and generally in accordance with 21 CFR 201.577? Is information available to express human dose multiples in either mg/m2 or comparative serum/plasma AUC levels?	x		

ITEM	YES	NO	COMMENT
9) From a pharmacology/toxicology perspective, is this NDA fileable? If not, please state in item # 10 below why it is not.	x		
10) Reasons for refusal to file:			

Fred Alavi, Ph.D.
Reviewing Pharmacologist

Todd Bourcier, PhD.
Supervisory Pharmacologist

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

/s/

Fred Alavi
8/27/2008 11:13:02 AM
PHARMACOLOGIST
No pharmtox NDA filing issues- except for the missing SAS transport file for carci studies. The missing SAS data was communicated to the sponsor and should be available soon
45-day filling check list for PharmTox

Todd Bourcier
9/8/2008 02:51:19 PM
PHARMACOLOGIST
Concur- NDA is filable for pharm/tox