One- to Three-Month Oral Toxicity Study in Monkeys

Key study findings:

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

Study no.: DN05063
Volume #, and page #: eNDA
Conducting laboratory and location: BMS, One Squibb Drive, New Brunswick, NJ
Date of study initiation: August 30, 2005
GLP compliance: No
QA report: yes ( ) no (x)
Drug, lot #, and % purity: Batch 4K85994 with free base purity of 93.5%
Methods
Intended as a one month dose-ranging study, observations of severe skin lesions altered the study design to investigate the nature of the drug-induced toxicities in monkeys. Doses and treatment duration are listed in the following table:

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

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

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

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

Doses: 2, 10, 30/20 mg/kg (free base used in the study)
Species/strain: cynomolgus monkeys from b(4)
Route, formulation, volume, and infusion rate: oral gavage
Age: 23 to 38 months old
Weight: 2.4 to 4.7 kg
Sampling times: PK data for saxagliptin and active metabolite were collected on Day 1, 28, 84/85 at 0.5, 1, 2, 4, 8 and 24 hrs post dose. Prior to freezing, an aliquot of plasma was taken for DPP4 inhibition evaluation. Plasma samples were analyzed at b(4)
Unique study design or methodology: Animals were acclimated for at least 21 days and had ad lib access to water. They received daily ration of approximately 12 biscuits each. Cyanide and thiocyanate levels were also measured during the first 2 TK time points on Day 1 and Day 28. Peripheral blood lymphocyte phenotying was also made in samples collected before and on week 2, 3, 4 and 8. The sponsor also investigated serum immunoglobulin (Ig) levels, antinuclear antibody (ANA) levels and Ig reactivity to red blood cells and platelets. Attempts were made to look at skin and kidney samples using electron microscopy. Based on histopath findings, paraffin-embedded tail, nose, scrotum, foot, skin (face and torso), urinary bladder, kidney, tongue, lung, vagina, testes, skeletal muscle intestine (jejunum) were stained for IgG, IgM and IgA deposition by immunohistochemistry staining.

Observations and times:
Mortality: daily
Clinical signs: twice daily
Body weights: weekly
Food consumption: daily
Ophthalmoscopy: before and at weeks 4 and 8
ECG: before and at weeks 4 and 8
Hematology: Blood samples were collected from fasted monkeys twice prior to first dose and at week 2, 3, 4, 5, 6, 8, 10 and 13
Clinical chemistry: The same as above
Urinalysis: before and at week 4, 6 and 13
Gross pathology: at the end of each phase (treatment and recovery).
Organ weights: standard list with greater emphasis on the skin lesions to determine NOAEL.
Histopathology: Adequate Battery: yes (x)
Peer review: yes (x)

Results

Mortality and clinical signs:

2mg/kg (3 month dosing duration)
Five of the six monkeys developed skin 'abrasions/ulcerations' on the tail and digits during the dosing period. Lesions developed within 13 days of dosing. The lesions largely resolved despite continued dosing, with only one female harboring minimal lesions at the end of 3 months of treatment.

10mg/kg (6 weeks dosing duration)
Four of six monkeys developed abrasions/ulcerations on the tail, digits, and scrotum within 13 days of dosing. While some lesions resolved, there remained ulcerative, inflamed lesions of the tail and multifocal scabs of the skin/subcutis in some animals at the end of 6 weeks treatment.

30/20mg/kg (Dose reduction at day 2/3, then 4 & 6 weeks at 20mg/kg)
Two females (#s 4202 & 4205) were sacrificed after the first or second dose after becoming acutely moribund (ataxia, collapse). Skin lesions were not observed in these two acutely ill females. The dose was reduced in remaining animals to 20mg/kg for an additional 4 or 6 weeks duration.
Severe ulcerative lesions were identified in 7 of 8 monkeys as early as day 6. Surgical amputation of the tail was required in one male and female; the female was found dead the following morning. An additional male was sacrificed moribund on day 39. Edema was feature of the skin lesions.

Body weights: No drug-related change in BW
Food consumption: No drug-related change in food intake
Ophthalmoscopy: No drug-related change

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

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

Hematological changes relative to pre-dose levels at 2 mg/kg
- Decrease in RBC, Hgb, HC, decrease in platelets
- Compensatory 2.4x increase in reticulocyte on Days 35 to 56, increase in neutrophils
- Presence of reactive lymphocytes (typical of lymphoid activation) on Day 35; and,

Hematological changes at 10 mg/kg
- Decreased in RBC, Hgb, HCt and decrease in platelet count
- Increase in reticulocytes and WBC (predominantly due to increased neutrophil count) and monocytes (1.8 to 2.9x) and increase in lymphocytes (1.4x),

Hematological changes at 30/20 mg/kg,
- Decrease in RBC, hemoglobin, Hct and decreased in platelet
- Increase in reticulocyte, Neutrophils, monocytes
- Presence of reactive lymphocytes
- Additionally 1 (4102) developed severe thrombocytopenia (-77 to -94%) at 2weeks leading to temporary halt in treatment until platelet counts recovered. This animal was re-challenged with drug without recurrence of the thrombocytopenia.

Hematological change in cynomolgus monkeys relative to pretest values

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

Clinical chemistry:

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

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

Cyanide levels:

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

Peripheral-Blood Lymphocyte Phenotyping and Immunology Assessment:

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

Saxagliptin: One-Month Oral Toxicity Study in Monkeys
Summary of Absolute Number of Lymphocytes (10e^3 /µL)
for Male Monkeys at Prestudy

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

Female Monkeys at Prestudy

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

Saxagliptin: One-Month Oral Toxicity Study in Monkeys
Summary of Absolute Number of Lymphocytes (10e^3 /µL)
for Male Monkeys at Day 22

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

Female Monkeys at Day 21

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

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

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