

thyroid) and target organs included kidney (males only), lymph nodes, eye, heart and thyroid. The sponsor thus chose 60 mg/kg/day as the highest dose for the 26 week study.

Observation and Times:

- Clinical signs: Once daily pretreatment, during recovery and on the day of necropsy, twice (pre-treatment and 4 hour post-treatment) daily during treatment, for mortality, moribundity and gross abnormality.
- Body weights: Prior test and weekly during treatment and weekly during recovery period.
- Food consumption: Once weekly during the acclimation, dosing and recovery periods for each cage (n=2/cage). Daily average food consumption per animal was calculated for each week.
- Ophthalmoscopy: Once on all animals prior to the start of treatment and on surviving Groups 1 and 4 during Week 13 and 26, and during Week 30 for all recovery animals.
- EKG: Not performed.
- Hematology: During Weeks 14 and 26 (all groups) and during Week 30 (or, Recover Week 4 [R4], recovery groups). Blood samples also were collected from the vena cava upon necropsy (PN [D183] and R5 [D211, the last day of R4]: main and recovery animals, respectively).
- Clinical chemistry: During Weeks 14 and 26 (all groups) and during Week 30 (or, Recover week 4 [R4], recovery groups). Blood samples also were collected from the vena cava upon necropsy (PN [D183] and R5: main and recovery animals, respectively).
- Urinalysis: During Weeks 13 and 25 (all groups) and during Week 30 (R4, recovery groups), urine was collected for ~ 4 hours.
- Bone marrow smears: From the primary and recovery necropsies. Samples were not examined microscopically.
- Gross pathology: Scheduled sacrifice: Day 183 or Day 210 (or R29, recovery groups).
- Organ weights: See Histopathology inventory
- Histopathology: Day 183 (Note: the samples from the recovery animals were not examined). All tissues collected from all animals were preserved. The microscopic examinations were performed on the control and the high dose animals (not including the animal found dead and the moribund sacrifices). The uterus was examined for all females (including Groups 2 and 3, as well as recovery females). See inventory list for organs examined.
- Toxicokinetics: Blood samples (0.5 mL) were collected from all TK animals on Days 1 (WK1), 28 (WK 4) and 154 (WK 22), at 0.5, 1, 2, 6, and 24 hours post-dose (n=2/ time point). A pre-dose sample was also collected on D28 and D154. LLOQ was 5 ng/mL.
- Genomic analysis: Exploratory, non-GLP study. Blood samples (2.5 mL) and tissues collected from satellite groups on D183 were saved for pharmacogenomics and toxicogenomics assessments (data not reported)

Results:

Mortality:

No treatment-related deaths. The following animals were found moribund, and found dead or sacrificed afterwards: #194 (D76), #191 (D77) and #201 (recovery animal, D87); all of them were Group 4 females. The cause of death was most likely due to dosing accident (ulceration/perforation of the stomach and/or esophagus). However, decreased cellularity in thymus was found in #194 (minimal) and #201 (severe); although similar findings were also seen in the control (minimal in severity) (data not shown). There were no other remarkably different findings in these rats in comparison with surviving rats. Also one control female #133 was moribund sacrificed on R23, and the cause of death was uncertain.

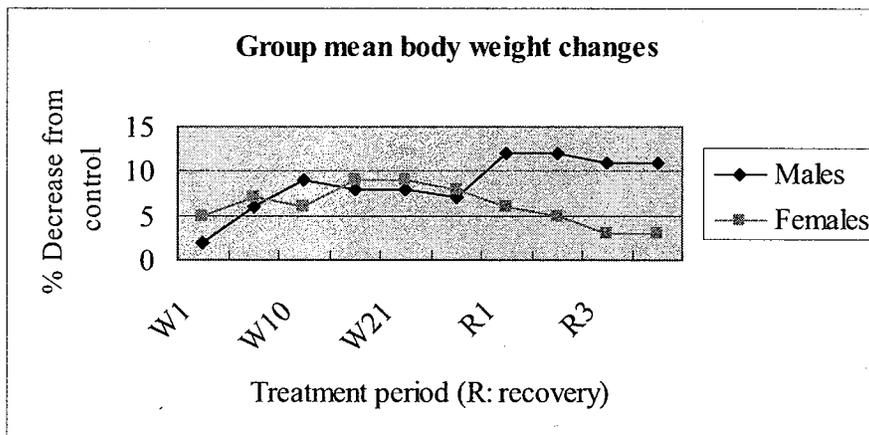
Clinical signs:

AMN107-related clinical signs were mainly increased incidences of oral discharge and/or excessive salivation in Groups 3 and 4 males and Group 4 females. The observations were mostly after Week 5 (earliest Day 13) and resolved during recovery period. The change was sporadic, about 1-8 times/animal. The frequency and severity followed a trend of increase with doses. In males, the severity increased from trace to slight in the control and trace to moderate in Groups 3 and 4. Females followed a similar trend. The incidences are summarized in the table below:

Group	Males				Females			
	G1	G2	G3	G4	G1	G2	G3	G4
Number of animals affected	2	2	9	13	0	1	1	20
Frequency (days/animals)	1	1-2	1-3	1-5	0	1	1	1-8

Body weights:

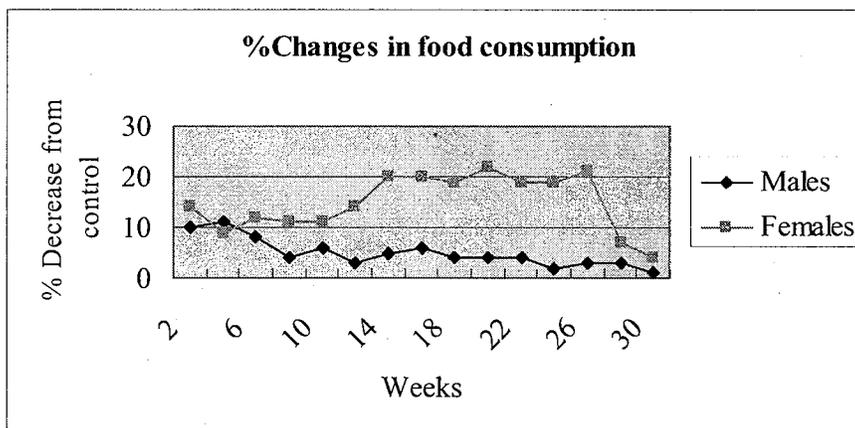
Significant reductions of group mean body weights in Group 4 (both males and females), in comparison with the control, were observed through the treatment period. The largest reduction was 9% from the control in both males and females. In males, although not statistically significant, the % decrease from the control remained 11-12% and did not recover. Findings in mean body weights in Group 4 females resolved. The results are summarized in the figure below:



There were no significant changes in weight gain in comparison to the control.

Food consumption:

Decreased food consumption occurred in Group 4, especially females. The finding resolved. The % decrease from the control throughout the study period are summarized in the figure below:



Note: The data were not statistically analyzed.

Ophthalmoscopy: Not remarkable

EKG: Not performed

Hematology:

The % changes (statistically significant) from the vehicle control (Group 1) were summarized in the table below:

	Week 14				Week 26			
	Males		Females		Males		Females	
Group	G3	G4	G3	G4	G3	G4	G3	G4
Number of animals	18	29	18	27	20	30	19	27
Retic % ↑		9*		17		7*		18
Retic (Ab) ↑		5*		6*		6*		11*
RBC ↓		3*		9			5	
HGB ↓				5				3
HCT ↓				7				
MCV ↑		4				3		2
MCH ↑		3		4				3
MCHC ↑				2				
RBC distribution width % ↑				3				
Mean platelet volume ↑	5	7				6		
WBC ↑			17	15*		9*		60
Lympho (Ab) ↑								36
Lympho (%) ↓								9
Mono (Ab) ↑				33			54	93
Mono (%) ↑		8*		14*		21*	25	20*
Neut (Ab) ↑		6*		36		13*	48	165
Neut (%) ↑		13*		22*				41
Baso (Ab) ↑						15*	53	20*
Baso (%) ↓				17*				30

Group	Week 14				Week 26			
	Males		Females		Males		Females	
	G3	G4	G3	G4	G3	G4	G3	G4
Eosino (Ab) ↑								15*
Eosino (%) ↓				6*				26

* Not statistically significant

Group	D183§				Week R4	Week R5§
	Males		Females		Males	
	G3	G4	G3	G4	G4	G4
Number of animals	16	18	17	15	10	8
Retic % ↑		26		31		14
Retic (Ab) ↑		26		24		
HGB ↑						4
HCT ↑						4
MCV ↑						4
WBC ↑		43	53	95		
Lympho (Ab) ↑	30	56		103		
Lympho (%) ↓		10				
Mono (Ab) ↑			58	139		
Mono (%) ↑		33*				
Neut (Ab) ↑		89		81		
Neut (%) ↓		26				
Baso (%) ↓		44*	43	46	47	
Eosino (%) ↓		40				

§: blood samples from vena cava at necropsy, D183 for main study groups and R5 for recovery groups.

Comment:

The findings of decreased erythroid parameters and increased white blood counts (total and differentiated), that became more distinguished with duration of treatment, resolved.

Clinical chemistry:

The % changes (statistically significant) from the control (Group 1) were summarized in the table below:

Group	Week 14				Week 26			
	Males		Females		Males		Females	
	G3	G4	G3	G4	G3	G4	G3	G4
Number of animals	20	30	20	27	20	30	20	27
BUN ↑				10	4*	7		
Creatinine ↓			10	10				
Total cholesterol ↑				35		19	21	27
Triglyceride ↑				37				
Albumin ↓		5	4	4				
Globulin ↑				5				6
A/G ratio ↓				7				7
Chloride ↓				2				11

	D183§				Week R4		Week R5§	
	Males		Females		M	F	M	F
	G3	G4	G3	G4	G4	G4	G4	G4
Number of animals	20	20	19	18	10	9	10	9
BUN ↑		17	23	11		12		23
Total cholesterol ↑		21		48		13*		25*
Triglyceride ↑				135		76	↓ 42	53*
Total bilirubin ↑				50				
Albumin ↓		4						
Globulin ↑						9		10
A/G ratio ↓		6				10		10
Inorganic phosphorus ↑	20	26	30	28	9			62
Chloride ↓		1		1				3

* Not statistically significant;

§: blood samples from vena cava at necropsy, D183 for main study groups and R5 for recovery groups.

Comments:

◇ The main findings, including decreased A/G ratio (a result of ↓albumin and ↑ globulin), increased total cholesterol, triglyceride, BUN and inorganic phosphorus (at the end of treatment period), as well as decreased chloride and creatinine, may not be due to abnormality of hepatic or renal function, since there were no supportive histopathological findings (see below). The sponsor considered the findings of decreased A/G ratio and chloride secondary to decrease in food consumption and not a direct effect of AMN107. This was supported by the higher severity of these findings in females and the coincidentally lesser food consumption in females. Of note, the findings did not resolve in females, despite the recovery of food consumption in females.

Urinalysis: not remarkable.

Gross pathology:

The gross pathological findings were summarized in the table below:

Sex	Males		Females	
Group	G3	G3	G4	
No. of animals	20	20	20*	
GI (stomach and/or esophagus)				
Ulceration/perforation				2
Liver				
Nodule	1			
Lymph node (axillary, tracheal)				
Enlargement				1
Uterus, body				
Distention		3		5

*: 2 of rats were moribund sacrificed and findings in them were indicated in bold. One moribund sacrifice female recovery animal (#201) also had the same finding, in addition to distention of the uterus.

Findings resolved in recovery animals

Organ weights:

Organ weight changes (absolute or relative body weight, % change from the control) were summarized in the following table:

Group	Males				Females				
	G4	G3	G4	G4R	G4	G4R	G3	G4	G4R
Parameter	gm	% BW	% BW	% BW	gm	gm	% BW	% BW	% BW
Number of animals	20	20	20	10	18	9	20	18	
Adrenal ↑								26	
Brain ↑								9	
Heart ↑	11	12	16				12	19	
Kidney ↑			10	10				12	
Liver ↑		9	15		14	22		29	28
Ovary ↑					57	18*		76	23*
Pituitary ↑				45					35

gm: absolute weight, % BW: relative to body weight

Histopathological findings:

The following table is the summary of incidence and severity (expressed as incidence/group mean of severity) of drug-related histological findings:

Sex	Males		Females			
	1	4	1	2	3	4
Group	No. of animals		No. of animals			
Heart	20	20	20	a	a	18
Mononuclear cell infiltration		3/1.33				1/1
Pericardium polygranuloma, pigment						1/1
Kidney, left						
Degeneration/regeneration						1/1
Kidney, right						
Mononuclear cell infiltration, perivascular		3/1				
Dilated tubule		1/1				
Mononuclear cell infiltration		1/1				1/2
Hyperplasia, epithelium, pelvis						1/2
Lacrimal gland, left						
Mononuclear cell infiltration		1/1				1/1
Liver						
Hyperplasia	1/1					2/1
Mononuclear/polymorphonuclear cell infiltration with spindle cell proliferation, perivascular		1/1				
Lymph node, mandibular						
Plasmacytosis	5/1.6	4/2	7/1.6			7/2
Hyperplasia, paracortical area	1/2	3/2	2/2			3/1.7
Increased intracellular pigment			1/1			3/1.3
Lymph node, mesenteric						
Increased intracellular pigment		3/1	3/1			6/1.2
Erythrocytosis	5/1.2	12/1	3/1			7/1.4
Increased mast cells, sinusoids						1/1
Lymph node, tracheal						
Pyogranuloma						1/1
Lymph node, axillary						
Follicular cysts						1/1
Mammary gland, left						
Polymorphonuclear cell infiltration		1/5				

Sex	Males		Females			
Group	1	4	1	2	3	4
No. of animals	20	20	20	a	a	18
Ovary						
Cyst, present						2
Salivary gland, right						
Mononuclear/polymorphonuclear cell infiltration interstitium						1/3
Mineralization						1/1
Spleen						
Increased extramedullary hematopoiesis		1/1				
Increased, brown pigment						1/1
Stomach						
Increase, polymorphonuclear cell infiltration, mucosa					2/1	3/1.7
Degeneration					2/1	3/1.7
Trachea						
Tracheitis						1/3
Uterus				(20)	(20)	
Dilation						5/2.4

a: not microscopically examined, unless otherwise indicated by numbers in the parenthesis.

Severity: 1: slight, 2, mild, 3: moderate, 4: marked, 5: severe

Histopathological findings in the pre-schedule deaths (all Group 4 females):

Findings	#191	#194	#201
Esophagus, thoracic			
Pyroglanduloma, adventitia			Present
Ulceration		Slight	
Kidney, left			
Tubular cast, medulla	Minimal		
Mineralization, cortex or medulla	Minimal	Minimal	
Kidney, right			
Mineralization, cortex	Minimal		
Liver			
Mononuclear cell infiltration, perivascular/sinusoidal			Minimal
Lung/Bronchi			
Polymorphonuclear cell infiltration, pleural			Minimal
Lymph node, mesentery or mandibular			
Hyperplasia, cortical area	Slight	Minimal	
Plasmacytosis	Minimal		
Spleen			
Brown pigment, increase			Slight
Stomach			
Ulceration, multifocal, nonglandular mucosa	Slight		
Mononuclear/polymorphonuclear cell infiltration		Slight	
Thymus			
Decreased cellularity		Minimal	Severe
Uterus, body			
Dilation	Slight		

Adequate Battery: yes (x) (note: no recovery data except uterus), no ()—explain

Peer review: yes (x), no ()

Comments:

- ✧ According to the sponsor and the peer review pathologist, the main finding was dilation of the uterine body (mild to moderate), which was characterized by increased diameter of the uterine lumen and decreased thickness of the wall. The clinical relevance of the finding was not certain. This finding was not shared by Groups 2 and 3, or the recovery females.
- ✧ There was no information regarding the reversibility of other findings.

Toxicokinetics:

The C_{max} and AUC_{0-24hr} , dose-normalized parameters and T_{max} (hr) were summarized in the tables below:

Males:

Group	Day 1			Day 28			Day 154		
	G2	G3	G4	G2	G3	G4	G2	G3	G4
Dose (mg/kg/d)	6	20	60	6	20	60	6	20	60
C_{max} (ng/mL)	722	2710	3890	795	2860	5730	1590	2770	5840
$C_{max}/dose$	120	136	65	133	143	96	265	139	97
AUC (ng x h/mL)	3960	21400	45700	4000	29500	54300	9200	29200	80200
AUC/dose	660	1070	762	667	1480	905	1530	1460	1340
T_{max} (hr)	2	2	2	2	2	2	2	2	6

Females:

Group	Day 1			Day 28			Day 154		
	G2	G3	G4	G2	G3	G4	G2	G3	G4
Dose (mg/kg/d)	6	20	60	6	20	60	6	20	60
C_{max} (ng/mL)	1200	3520	8830	1290	3790	7140	2370	12100	11200
$C_{max}/dose$	200 (1.7)	176 (1.3)	147 (2.3)	215 (1.6)	190 (1.3)	119 (1.2)	395 (1.5)	605 (4.4)	187 (1.9)
AUC (ng x h/mL)	6870	29800	64200	8230	35900	86700	15300	146000	61842
AUC/dose	1150 (1.7)	1490 (1.4)	1070 (1.4)	1370 (2.1)	1800 (1.2)	1450 (1.6)	2550 (1.7)	7300 (5)	1030 (0.8)
T_{max} (hr)	2	2	2	2	2	2	2	6	2

Note: the numbers in the parenthesis indicate fold increase of C_{max} and AUC in females in comparison to males.

Reviewer's note:

According to the sponsor, there were detectable AMN107 levels in the sera of control animals (13 of the 68 controls including males and females, data not shown). Although some of the findings could be due to *ex vivo* contamination, some serum AMN107 was attributable to exposure to the drug. The study director did not determine the source of the contamination or evidence of administration by mistakes. The exposure (AUC) of two female controls (#115 and #120) was in between the exposure values of Group 2 and Group 3. The individual hematology and histopathological findings of these two animals were not remarkable, except that the platelet count of #120 was higher (1149 versus group mean of the control: 781). The sponsor did not provide the summary of C_{max} or AUC values of the control, but claimed that the findings did not have impact on the TK results since the C_{max} and AUC appeared to follow a dose-proportional increase.

Study summary and discussion:

See Section 2.6.7 "Toxicology tabulated summary".

- The treatment of AMN107 in rats, dosed up to 60 mg/kg/day, reduced body weight/food consumption, affected hematological parameters (decreased erythroid parameters, increased erythrocytes and white cell counts) and clinical chemistry parameters (increased total cholesterol and triglycerides), and increased organ weights (liver, kidney, heart, brain, ovary and adrenal). The main target organ was uterus (distention/dilation).
- The moribund sacrifice of three high dose females and one control female was due to dosing trauma, and not due to drug effect.
- It was noted that females showed more reduction in food consumption, but weight loss in males persisted even in the recovery period despite the observation of recovering food intake.
- Clinical signs and clinical chemical evidences may be due to indirect drug effects. The former were potentially due to an unpleasant taste. The clinical chemistry findings, including decreased A/G ratio and chloride, were probably secondary to decrease in food consumption.
- Toxicokinetics:
 - ✧ Orally administered AMN107 was rapidly absorbed. Detectable serum levels of AMN107 were found 0.5 hr after administration (serum concentration-time profile not shown). The T_{max} was 2-6 hours.
 - ✧ The dose normalized C_{max} and AUC on Days 1 and 28 was in general proportional, but C_{max} /dose decreased at 60 mg/kg, a suggestion of saturated absorption, or an induction in metabolism.
 - ✧ Dose normalized AUC on Day 154, especially males and females at 6 mg/kg and females at 20 mg/kg, were generally higher. This may indicate accumulation of AMN107 after repeat administration.
 - ✧ Females demonstrated a higher exposure than the males (approximately 2 fold, but 4-5 fold at 20 mg/kg on Day 154), and also showed more susceptibility to the treatment, as evidenced in food consumption, hematological and clinical chemistry findings.

Study title: 4-Week oral (gavage) toxicity study in dogs with a 4-week recovery period.

Key study findings:

- AMN107-related findings included weight loss in female dogs at 45 mg/kg/day.
- The target organs/tissues of toxicity were liver (Kupffer cells and bile duct), gall bladder, and kidney, and minor changes in lung and spleen.

Note: This study was reviewed in IND 69764 (Review #1) . The IND review is reformatted and incorporated in this NDA review.

Study no.: 0370147

Volume#, and Page number: Volume #5 and page #8-1104 to 8-1299

(Note: in the NDA submission: Electronic module (pharmtox\tox\0370147.pdf))

Conducting laboratory and location: Novartis Pharmaceuticals Corporation,
One Health Plaza East, Hanover, NJ 07936-1080.

Date of study initiation: November 26, 2003

GLP compliance: yes.

QA report: yes (x) no ().

Drug, lot #, and % purity: AMN107 hydrochloride, lot #0351002, purity: █████

Methods:

Species: Beagle dog

n: 3/sex/group, plus 2 in the control and 45 mg/kg group as recovery animals.

Age/Weight: 13-14 months/8.7-14.3 kg

Doses: 0 (control), 5, 15, and 45 mg/kg (groups 1, 2, 3 and 4, respectively).

Schedule: Once daily for 28 consecutive days. The recovery animals (Groups 1 and 4) were dosed for 28 days followed by a 28-day recovery phase (no treatment).

Route: Oral by gavage.

Formulation/vehicle: A solution in vehicle: 0.5% w/v hydroxypropyl methylcellulose (HPMC). The concentration was calculated to give a constant dose volume of 2.5 mL per kg body weight for each dose level (see table below).

Table 3-1 Study design, animal allocation and test article doses

Group	Number/sex	Animal Numbers		Dose* (mg/kg/day) Base/Salt	Concentration (mg/mL) Salt
		males	females		
1	3	1001-1003	1501-1502 1504**	0	0
Control	+2 recovery	1004-1005	1503**, 1505		
2	3	2001-2003	2501-2503	5/5.3	2.1
Low					
3	3	3001-3003	3501-3503	15/16.0	6.4
Mid					
4	3	4001-4003	4501-4503	45/46.1	19.2
High	+2 recovery	4004-4005	4504-4505		

*Doses are not corrected for active moiety. Salt/Base ratio is 1.068.

**Animal no. 1504 was reassigned from the recovery group to the non recovery group due to a wound on it's tail and was sacrificed with the rest of the animals at scheduled necropsy. Animal no. 1503, a non recovery animal, was reassigned as a recovery animal.

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Dose justification:

Dose levels were selected based upon previously conducted 2-week oral (gavage) dose range-finding toxicity study (██████████). For dogs treated with 6, 20 and 60 mg/kg/day (n=1/sex for the first two doses and n=2/sex for the high dose), weight loss was seen at 60 mg/kg/day (reduction compared to control: 13% in one male and 10-26% in two females). Findings in clinical chemistry, including increased ALP, ALT and total bilirubin (3 fold, 2 fold and 12 fold increase compared to the control, respectively), occurred in 1/2 females at 60 mg/kg/day. Histopathological findings were also observed at 60 mg/kg/day (3/4 animals), and mainly involved liver: periportal/sinusoidal inflammatory cell infiltration, Kupffer cell hypertrophy/hyperplasia and centrilobular bile stasis. The sponsor thus selected 45 mg/kg/day as the high dose in the 4 week study.

Observations and times:

Clinical signs: Twice daily for mortality, moribundity and gross abnormality. Detailed physical examination: twice daily on each day of dosing days and at least once on non-dosing days.

Body weights: Once prior to treatment and weekly thereafter.

Food consumption: Estimated daily pre-study and throughout the remainder of the study.

Ophthalmoscopy: On all animals during pretest and on all control and Group 4 animals during week 4 with an indirect ophthalmoscope.

Electrocardiography: On all animals during pretest and during week 4, performed approximately 1.5 to 2 hours after administration.

Hematology: In Week 4 and on Day 57.

Clinical chemistry: In Week 4 and Day 57.

Urinalysis: Up to 1 mL urine samples were collected in Week 4 and Day 57.

Gross pathology (necropsy): Scheduled sacrifice: Day 29 or after recovery period (D57).

Organ weights: At scheduled sacrifice. See Histopathology inventory

Histopathology: Day 29 or Day 57. All tissues collected from all animals.
Note: Bone marrow smears were collected but not evaluated.

Toxicokinetics: Blood samples (2 mL) were collected from non-recovery animals on Day 1/2 and Day 22/23, at 0.5, 1, 2, 6, and 24 hours post-dose (n=3/ time point). LLOQ was 4.98 ng/mL.

Genomic analysis: Blood (2.5 mL, in week 4), liver and mesenteric nodes samples were collected and used for investigational gene expression analysis.

Results:

Mortality /Moribundity	No mortality or moribundity.
Clinical signs	Mostly in Group 4 females (see table below). Most of findings resolved, except reduced feces: 2/2 recovery Group 4 males and females.
Body weights	Reduction in group mean body weight from the control was seen in Group 4 females at the end of treatment (Day 29). At the beginning of recovery period the reduction worsened and only partially resolved at the end of recovery period. (see table below).
Food consumption	Decreases in estimated food consumption (25-50% food consumed, in all Group 4 females) and inappetence (0 % food consumed, in 3/5 Group 4 females) was observed between days 2-31 and days 23-30, respectively. The finding resolved in the recovery period. Note: the statement was cited from the sponsor, no individual or summarized data available in the submission.

Clinical signs:

	Males				Females			
	Group 1 (5)	Group 2 (3)	Group 3 (3)	Group 4 (5)	Group 1 (5)	Group 2 (3)	Group 3 (3)	Group 4 (5)
Thin appearance							1	2
Red ears		1	1	4		3		4
Feces: reduced feces	2			5			2	5
Absence of feces								3
Dark urine				1				3
Salivation		1	1	1				1

Emesis: food	2	2	2	1	1	1	1	3
Apparent compound							1	2

- Reduction (%) of group mean body weight in AMN107-treated Group 4 females as compared to the control:

Dates/Dose	Group 4
D8	
D15	3
D22	6
D29	9
D37*	30
D44*	17
D58*	12

Note: the numbers reflect % reduction compared to the control. *recovery period.

Ophthalmoscopy: not remarkable.

Electrocardiography: not remarkable.

Hematology: not remarkable

Clinical chemistry:

	Males			Females		
	Week 4			Week 4		
	Group 2	Group 3	Group 4	Group 2	Group 3	Group 4
ALT ↑			36			217
ALP ↑			79	33	33	200
TBIL ↑						900
CHOL ↑						84

Note: Numbers indicate % changes from the control.

Comment:

- Changes in ALT, ALP, bilirubin and cholesterol occurred mainly in Group 4, especially females. Elevation of ALP was observed in male #4003 and #4004, but in all female Group 4 animals. Female #4501, 4503, 4504 showed 200-400% increase of ALT from the control. Bilirubin increased in all Group 4 females, especially #4501.
- All findings resolved.

Unanalysis:

Mostly Unremarkable, except that minimal to marked bilirubinuria was seen in all Group 4 females, which was concurrent with elevated serum bilirubin.

Organ weights:

- Liver: in AMN107-treated males there was a trend of increase related to the dose:

	Absolute weight (gm)		% Body weight	
	Mean	↑ % Control	Mean	↑ % Control
Group 1	324.7		2.78	

Group 2	328.7	1	2.82	1
Group 3	370.9	14	3.08	11
Group 4	373.5	15	3.13	13

- All the recovery animals showed comparable organ weights to the control.

Macroscopic findings:

	Males				Females			
	G1	G2	G3	G4	G1	G2	G3	G4
Liver: yellow focus Mottled discoloration			1					1
Gall bladder: black-brown content Black mucosa focus			1				1 1	
Heart: cyst, dark Thickened right AV valve		1	1					
Pituitary: clear cyst						1	1	1
Lung: tan focus		1						
Mesentery: accessory spleen, adipose tissue		1						
Thyroid: enlarged			1					
Spleen: dark raised area White focus							1 1	1
Urinary bladder: irregular red mucosa area							1	
Vagina: vulve swollen							1	

Comment:

- Most findings were not dose-related.
- Changes in liver (discoloration) and gall bladder (brown content and black focus) were correlated with increased amount of mucosa in lumen of the gall bladder and Kupffer cell hypertrophy, respectively. The raised area found in the spleen was related to hemorrhage in this area.
- Most of the macroscopic findings resolved in the recovery animals. However, a female recovery animal (#4505) showed findings in mesentery (accessory spleen, adipose tissue) and lymph nodes (mediastinal and mesenteric, discoloration).

**Appears This Way
On Original**

Histopathological findings:

Histopathology	Group	Group 2		Group 3		Group 4		Recovery (G4)	
		Sex		M	F	M	F	M	F
		Number of animals		3	3	3	3	3	2
Heart									
minimal arterial focal mesothelial cell proliferation									
minimal to slight coronary artery medial hypertrophy				1	1	1			
slight focal ventricle fibrosis		1							
Adrenal									
minimal to slight zona fasciculata vacuolation					3				
Pituitary									
minimal lymphocytic infiltrate									
minimal to slight cyst*		2	2		2	2	2		
Lung									
slight chronic active pneumonitis		1							
minimal perivascular cuffing		1	1	1		1	1		
slight focal medial artery hypertrophy						1			
minimal focal hemosiderosis							1		
minimal focal macrophage accumulation			1				1		
Duodenum									
minimal focal glandular dilatation				1		1			
Ileum									
minimal Peyer's patch hyperplasia						1			
Mesentery									
accessory spleen		1							1
Tongue									
minimal focal lymphocytic infiltrate		1		1	1		1		
Gall bladder									
slight lymphocytic inflammation			1						
slight lymphoid hyperplasia							1		
increased luminal mucus				1	1		2		
Liver									
minimal focal extramedullary hematopoiesis								1	1
minimal focal leukocytic inflammation						1			
minimal to slight mononuclear infiltrate*		1	2	2	1	2	2		
minimal to marked Kupffer cell hypertrophy				1	2	1	3		2
minimal to moderate Kupffer cell hyperplasia				1	1		3		
slight hemosiderosis					1				
slight increased focal hepatocellular glycogen				1					
slight centrilobular bile inspissation							2		
slight focal bile duct proliferation					1			1*	
minimal to slight bile duct proliferation							2		

Kidney							
minimal focal fibrosis							
minimal to slight focal mineralization*	2	3	2	2	2	3	2
minimal focal glomerulus lipidosis		1			1		
minimal tubular pigment			1				
minimal focal lymphocytic infiltrate*			1	3			
minimal focal medulla fibrosis		1				1	
minimal focal glomerulus fibrosis	1		2				
minimal focal tubular basophilia				1	1	1	
minimal to slight focal tubular dilatation							1
minimal focal tubular cast			1	1	1	1	1*
sligh to moderate increased tubular vacuolation				1		1	
Bone marrow							
moderate hemosiderosis				1			
Lymph node: Mandibular							
slight pigment	1			1	1	1	
slight lymphoid hyperplasia		1					
Lymph node: Mediastinal							
slight to marked erythrophagocytosis						1	1
Lymph node: mesenteric							
slight lymphoid hyperplasia						1	
minimal to moderate erythrophagocytosis						1	1
Nerve: optic							
minimal focal vacuolation					1		
Spinal cord							
minimal to focal hemorrhage	1						
minimal arterial axonal swelling					1		
Pancreas							
minimal to slight focal lymphocytic inflammation				1	1		
slight acinar degranulation					1		
Spleen							
minimal to slight fibrosiderotic nodule	1			1	1	2	
slight focal hemosiderosis						1	
moderate hemorrhage				1		1	1
slight focal capsule fibrosis					1		
Thyroid							
minimal to slight cyst		1	1			1	
Epididymides							
slight sperm granuloma			1				
Ovary							
minimal focal mineralization		1					
marked luteal cyst				1			
Uterus							
minimal pseudocyst				1			
Vagina							
physiologic hypertrophy				1			

Note: * observed also in the control.

Comments:

- The most prominent, drug-related histopathological lesions were observed in liver (especially Kupffer cells and bile ducts), gall bladder, and kidney. It appears that the changes in liver and gall bladder were more frequent in female dogs.
- The finding of hyperplastic and hypertrophic Kupffer cells in the two Group 4 females (#4501 and 4503) was accompanied with bile duct proliferation, centrilobular bile inspissation. These dogs together with one Group 3 male and female showed both changes in Kupffer cells and gall bladder (increased luminal mucus). Macroscopically, brown-black focus or discoloration was seen in liver and gall bladder of these dogs. Minimal Kupffer cell hypertrophy and bile duct proliferation was still seen two Group 4 female recovery animals, while changes in gall bladder resolved in recovery animals.
- The increased vacuolation in nephron tubule in medullary ray found in two female dogs (#3502 and #4501) was Oil Red O positive, indicating increased intracellular neutral lipid content (according to the sponsor).

Toxicokinetics:

Males:

Group	Day 1			Day 22		
	Group 2	Group 3	Group 4	Group 2	Group 3	Group 4
Dose (mg/kg/d)	5	15	45	5	15	45
C _{max} (ng/mL)	280	582	446	370	1241	648
C _{max} /dose	56	38.8	9.91	74	82.7	14.4
AUC _{0-24h} (ng•h/mL)	1070	5685	3092	1560	11505	3879
AUC _{0-24h} /dose	214	379	68.7	312	767	86.2

Females:

Group	Day 1			Day 22		
	Group 2	Group 3	Group 4	Group 2	Group 3	Group 4
Dose (mg/kg/d)	5	15	45	5	15	45
C _{max} (ng/mL)	357	662	878	296	983	2039
C _{max} /dose	71.4	44.1	19.5	59.2	65.5	45.3
AUC _{0-24h} (ng•h/mL)	2400	3570	6300	1680	5625	25700
AUC _{0-24h} /dose	480	238	140	356	375	571

- In male dogs, there was a rise at the middle dose and a drop at high dose in AUC/dose on both Day 1 and Day 22. Female dogs showed a decreasing trend in AUC/dose on Day 1, but a slightly increasing trend on Day 22.
- The C_{max}/dose decreased at 45 mg/kg in both male and female dogs on both Day 1 and Day 22.
- The observations mentioned above may not be meaningful, since there was a great variation within the dose group, especially at the high dose.

Summary of the individual study:

- Overall, the oral treatment of AMN107 in the dose range of 5-45 mg/kg/day was tolerable. Most of toxicity, such as weight loss, reduced food consumption, clinical chemistry and histopathological findings resolved at the end of recovery period. These effects were seen more in the female dogs.
- There were unremarkable changes in hematology.
- There were no histopathological findings in liver to support the changes in clinical chemistry parameters (i.e., elevated ALT and ALP).
- The histopathological findings of centrilobular bile inspissation and bile duct proliferation support the increase of serum total bilirubin in this group of females. Also bilirubinuria did reflect elevated serum level of total bilirubin.
- The targeted organs of AMN107 toxicity included: liver (Kupffer cells and bile duct), gall bladder, kidney, spleen and lung. Most changes were minimal and resolved during recovery period.

Study title: AMN107: A 39-week oral (gavage) toxicity study in cynomolgus monkeys with a 4-week recovery period

Key study findings:

- The AMN107 related toxicity (at ≥ 200 mg/kg) included: GI clinical signs, decreased food intake (males at 600 mg/kg)/body weights, hematological effects (\downarrow HGB and Hct), increased ALT and total cholesterol.
- The main target organ was the liver (bile duct).
- One male recovery animal was euthanized on Day 59, due to pneumonia.

Study no.: #0580157 (— .053.11)

Volume #, and page #: Electronic module (pharmtox\tox\0580157.pdf)

Conducting laboratory and location:

Date of study initiation: April 7, 2005

GLP compliance: Yes

QA report: yes (X) no ()

Drug, lot #, and % purity: AMN107, Lot # 0523025, purity: —

Methods

Doses: 0 (control), 30, 200 and 600 mg/kg (free base) (as Groups 1, 2, 3 and 4)

Species/strain: Cynomolgus monkeys (*Macaca fascicularis*)

Number/sex/group or time point:

Main study: n=4/sex/group; these animals were also used for toxicokinetics

Recovery animals: n=2/sex in Groups 1 and 4

There were no separate satellite groups.

Route, formulation, volume: oral gavage at dose volume of 5 mL/kg

- Formulation: AMN107 suspensions at dose concentrations of 1.2, 4 and 12 mg/mL for Groups 2, 3, and 4, respectively. (Note: Doses were corrected for percent active AMN107 moiety (96.4%). Salt/base ratio for AMN107 was 1.103.)
- Vehicle: 0.5% (w/v) hydroxypropyl-methylcellulose (0.5% HPMC) in water for injection, USP.

Age: 2.5-7.5 years

Weight: ~ 2-6 kg

Schedule: Once daily for 39 consecutive weeks. The main study was followed by a 4-week recovery period.

Dose justification: Dose selection is based on a previous rising-dose toxicity study (), as well as a 4-week dose range-finding study () in monkeys (daily at 100, 200, 400 and 600 mg/kg, for 8 days or for 4 weeks, respectively). Oral administration of 600 mg/kg resulted in decreased erythroid parameters (approximately 30% decrease in RBC, HGB and Hct) and increased bilirubin (13 fold) and creatinine (88%), and decreased albumin (9%) in the 8-day treatment, while proteinuria (moderate), glucosuria (mild) and hematuria (gross) were found in individual animals in the 4-week study. The histopathological findings included renal tubular basophilia and dilation in the 8-day study, and atrophy in thymus and hemorrhage and fibrosis in femur in the 4-week study. The sponsor thus chose 600 mg/kg/day as the highest dose in the 39 week study.

Observation and Times:

- Clinical signs: Once daily pretreatment, during recovery and on the day of necropsy, twice (pre-treatment and 4 hour post-treatment) daily during treatment, for mortality, moribundity and gross abnormality.
- Body weights: Prior test and weekly during treatment and weekly during recovery period, as well as at necropsy.
- Food consumption: Estimated food consumption was recorded daily (in the morning and in the afternoon).
- Ophthalmoscopy: Once prior to the start of treatment and once in Week 39 (D268) at a minimum of 3 hours prior to dosing for all animals, but only data of all surviving controls and high dose animals were reported. No measurements were performed in recovery animals.
- EKG: Once prior test (Day -12), and once during Weeks 13 (D86), 26 (D177) and 39 (D267), at a minimum of 2 hours after dosing. Data were reported from all surviving controls and high dose animals. No measurements were performed in recovery animals.
- Hematology: Once prior to the test (Day -6), during Weeks 7 (D48), 10 (D69), 13 (Day 86) and 39 (D268), and during the last week of recovery (Day R27).
- Clinical chemistry: Once prior to the test (Day -6), during Weeks 7 (D48), 10 (D69), 13 (Day 86) and 39 (D268), and during the last week of recovery (Day R27).

Urinalysis: Urine (minimum 5 mL) was collected for all animals once prior to the test (Day -6), during Weeks 7 (D48), 10 (D69), 13 (Day 86) and 39 (D268), and during the last week of recovery (Day R27).

Bone marrow smears: Samples from femur were prepared, but no examination was performed.

Gross pathology: Scheduled sacrifice: Day 274 or Day 303 (or R29, recovery groups).

Organ weights: See Histopathology inventory

Histopathology: Day 274 and Day 303. All tissues collected from all animals were preserved. The microscopic examinations were performed on all animals (including the animal found dead and the moribund sacrifices).

Toxicokinetics: Blood samples (2 mL) were collected from all TK animals on Days 1/2, 27/28 (WK 4), 90/91 (WK13), 181/182 (WK 26) and 272/273 (WK 39), at 0.5, 1, 2, 6, and 24 hours post-dose (n=2/ time point). A pre-dose sample was also collected on D28 and D154. LLOQ was 2.5 ng/mL.

Genomic analysis: Blood samples (5 mL) and tissues (for gene expression analysis, including liver, kidney, spleen, heart, thyroid, stomach, duodenum, jejunum, urinary bladder, testis and adrenal) collected from all animals at necropsy were saved for pharmacogenomics and toxicogenomics assessments (data not reported).

Results:

Mortality:

There was one drug-unrelated moribund sacrifice (Group 4 male, #40, recovery animal) on Day 59.

Clinical signs:

AMN107-related clinical signs were observed mainly in animals dosed at 200 and 600 mg/kg, and most of them were fecal changes. The findings (number of animals affected)/duration of findings* through the study [including recovery period]) were summarized below.

Sex	Males				Females			
Groups	G1	G2	G3	G4	G1	G2	G3	G4
No. of animals	6	4	4	6	6	4	4	6
Eye								
Swollen eyelids				1/1.0			1/7.0	
Postual/status								
Hunched		1/6.0	1/1.0	4/8.3			3/1.3	3/6.7
Feces								
Liquid	1/1.0	1/8.0	2/11.5	2/18.5	2/2.5	1/2.0	2/10	4/7.3
Soft	2/5.0	4/10.6	4/10	5/9.2	5/5.0	3/2.0	3/12.7	6/10
Dry	1/1.0	1/1.0	2/1.0	3/1.0	1/1.0	2/1.5	3/1.7	4/1.8
Bloody		1/1.0	1/2.0	1/2.0			1/4.0	1/1.0
Abnormal color	1/1.0	1/1.0		1/7.0				3/2.3
Emesis								
Treat-like emesis							1/1.0	2/1.0
Test article like emesis		2/3.5	2/1.0	2/2.0			2/3.0	3/2.3

*: duration of the finding is indicated as mean number of weeks with the observation.

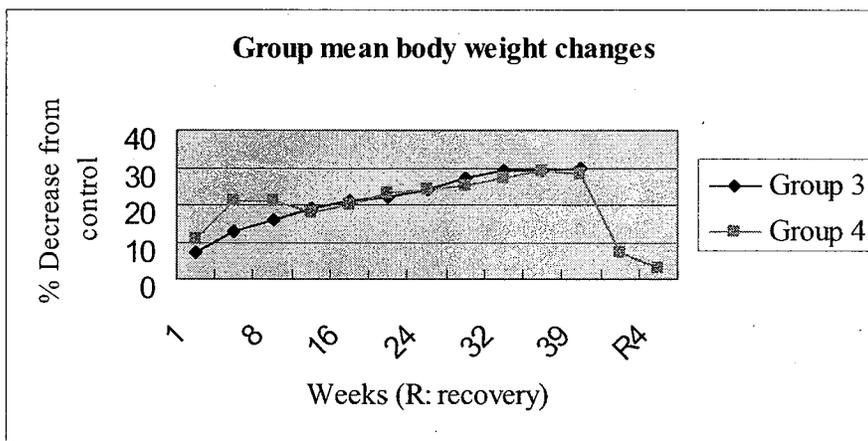
Point to discuss: findings: number of animals/duration of the finding

The female recovery animal #37 had moderate to marked soft feces and moderate test article-like or food-like emesis during recovery period (R24-R27). The rest of recovery animals did not have findings during recovery period.

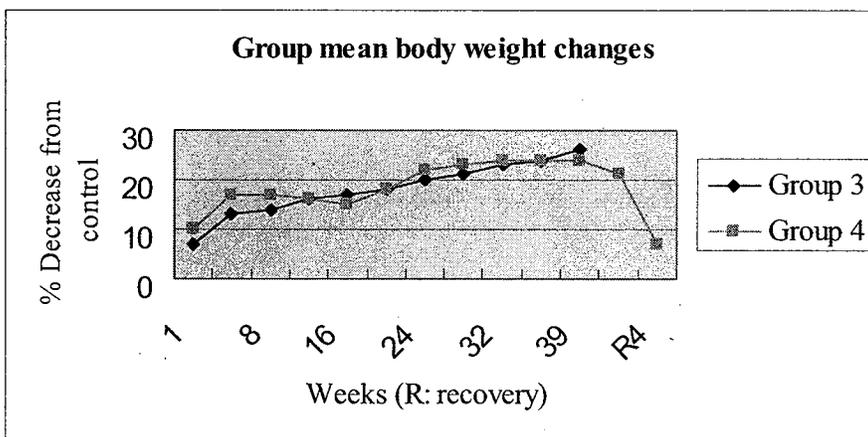
Body weights:

AMN107-induced decrease in body weight was mainly observed in Groups 3 and 4, in both males and females. There was no apparent difference in terms of severity of the findings between the two dose groups, or between genders. However, the recovery was slower in females.

Males:



Females:



There were no remarkable changes in weight gains. Weight gain changes were in general fluctuating and not dose-related.

Food consumption:

Decreased food consumption (more biscuits remained) was found mainly in Group 4 males (data not shown). Starting on Day 3 until Day 58, the largest numbers of biscuits remained in each dose groups were 2, 2, 4 and 6, for Groups 1, 2, 3, and 4, respectively. After Weeks 8-9, the reduced food consumption in this group was sporadic throughout the treatment period. The longest period of greater than 3 biscuits remained was 3 days (D101-D103) and

largest number was 4 biscuits remained. The finding resolved. In females, there was no apparent treatment-related reduction in food consumption.

Electrocardiography: Not remarkable

Ophthalmology: Not remarkable.

Hematology:

The effects of AMN107 on hematological parameters were not severe, and limited in platelet counts (↑) and erythroid parameters (↓ in HGB, Hct, and slightly RBC count) in Groups 3 and 4 animals. The % changes from the control were summarized in the table below:

	Week 7				Week 10			
	Males		Females		Males		Females	
	G3	G4	G3	G4	G3	G4	G3	G4
Number of animals	4	6	4	6	4	6	4	6
RBC ↓				4				9
HGB ↓	7	11	7	10	12	11	8	12
Hct ↓	4	6	8	9	9	4	10	11
Platelet ↑	18	46	26	49	13	48	38	40

	Week 13				Week 39			
	Males		Females		Males		Females	
	G3	G4	G3	G4	G3	G4	G3	G4
Number of animals	4	6	4	6	4	6	4	6
RBC ↓				6				5
HGB ↓	7	8	5	10	13	15	5	10
Hct ↓	6	3	8	8	12	10	7	9
Platelet ↑	15	39		29	19	33	44	26

Comment:

- ✧ Not every individual in the same dose group was affected: erythroid parameters were not changed in #34 (G4 M) and only #22 (G3 M) and #21 (G3 F) showed changes in these parameters.
- ✧ The changes fluctuated through the treatment and the duration of the effect varied among the animals. However, according to the sponsor, the erythroid changes in group means reached statistical significance.
- ✧ There were no compensative changes in reticulocyte counts (relative or absolute), except for one Group 4 male (#32) in that at least 10 fold increase of absolute reticulocyte counts occurred on Day 46. The suppression of erythroid parameters after all was small, and no histopathological lesions were observed in bone marrow.
- ✧ The RBC counts, HGB and Hct returned to control in the male recovery animals. RBC counts in females resolved too. However, HGB and Hct decreased 12% and 9%, respectively, compared to the control in female recovery animals.
- ✧ APTT was prolonged in certain males (% changes from individual pre-test): #16 (G2, 20% in WK 13), #30 (G4, 31% in WK 13, 17% in WK 39), and #32 (G4, 39% in WK 39).
- ✧ One Group 4 female #29 had a 7 fold increase in eosinophil count. It returned to pre-test level by the end of treatment.

Clinical chemistry:

The % changes (group means) from the control were summarized in the table below:

	Week 7				Week 10			
	Males		Females		Males		Females	
	G3	G4	G3	G4	G3	G4	G3	G4
Number of animals	4	6	4	6	4	6	4	6
ALT ↑	157	330	118	127	159	363	194	178
Total cholesterol ↑	49	57	12	13	36	63		11
Total bilirubin ↑		33		20		25		
ALP ↓	31	51		36	30	54		38
Triglyceride ↑	96	11			86	31		

	Week 13				Week 39			
	Males		Females		Males		Females	
	G3	G4	G3	G4	G3	G4	G3	G4
Number of animals	4	6	4	6	4	6	4	6
ALT ↑	156	356	68	156	77	212		143
Total cholesterol ↑	45	76	12		35	64		10
Total bilirubin ↑		33				17		
ALP ↓	29	53		35	18	41		27
Triglyceride ↑	93				39			

AMN107-related findings, including increase in ALT and total cholesterol, occurred mainly in groups 3 and 4, especially in males. However, not every individual in the same dose group showed changes. For instances, animals did not have remarkable changes in ALT including #26 (G3 M), #40 (G4 M), #21 and #27 (G3 F), and #43, #33, #35 (G4 F).

Urinalysis: not remarkable.

Gross pathology:

Not remarkable, except findings in liver in Groups 3 and 4:

- ◇ Enlarged: Group 4 (males: #30, #32, #36; females: #29)
- ◇ Prominent lobular markings: Group 3 (male, #22; females: #23, #27), Group 4 (males: #30, #32, #36; females: #29)
- ◇ Enlarged bile duct: #36
- ◇ Findings resolved.

Organ weight:

Not remarkable based on the following reasons:

- ◇ In general, the relative organ weights were increased in most organs examined in all treated animals, except lung and prostate/seminal vesicles in males. These increases were often not dose-related and most likely secondary to decreased body weights.
- ◇ The decrease of relative thymus and prostate/seminal vesicle weight was not dose-dependent and without histopathological evidence of lesions, so this was not biologically relevant.

Histopathological findings:

The following table is the summary of incidence and severity (expressed as incidence/group mean of severity) of drug-related histological findings. Shaded columns were data of the recovery animals.

	Sex		Males				Females					
	Group	No. of animals	1	2	3	4	1	2	3	4	4	
Adrenal		1				4						
Deposition, brown pigment, corticomedullary		4				1*						
Mineralization, corticomedullary		(3)										
Brain, meninges												
Hemorrhage, acute												
Eye (eye ball, ciliary body/choroid)												
Infiltration, mononuclear cell (left)				2/1		1/1						
Infiltration, mononuclear cell (right)					1/1				1/1			
Femur (bone marrow core)												
Deposition, brown pigment						1/2						
Sternum												
Hypercellularity, granulocytic precursors												
Esophagus												
Infiltration, mononuclear cell		1/1	1/1	2/1	1/1					1/1	2/1	
Heart												
Hemorrhage, subepicardial (septum, atrium)						1/2						
Hemorrhage, subepicardial (ventricle)						1/2						
Infiltration, mononuc. cell (septum, atrium)		1/1	4/1.3	1/1	1/1	1/1	1/1	1/1	2/1	1/1	1/1	
Infiltration, mononuclear cell (ventricle)		1/1	3/1.3	2/1	2/1	1/1	1/1	1/1	2/1	2/1	2/1	
Infiltration, neutrophilic, focal						1/1						
Colon												
Infiltration, mononuc. cell (mucosa, diffuse)						1/1						
Rectum						1/1						
Infiltration, lymphocytic (mucosa)						1/1						
Kidney												
Basophilic; tubular epithelium						1/2				1/1	1/1	
Degeneration, vacuolar; proximal convoluted tubules						1/3						
Left						1/3						
Right						1/3				1/1		

Sex	Group	Males								Females			
		No. of animals											
Degeneration/atrophy; distal convoluted tubules	Left	1	2	3	4	4	4	4	1	2	3	4	4
	Right	4	4	4	4	4	1*	1	4	4	4	4	2
Fibrosis, interstitial, focal/segmental	Left												
	Right		1/1	1/1		1/1		1/1			1/1	1/1	
Glomerulosclerosis	Left												
	Right				1/1								
Infiltration, mononuclear cell	Left												
	Right												
Mineralization	Left												
	Right												
Tubular protein	Left												
	Right												
Vacuolation, cytoplasmic, transitional epithelium	Left												
	Right												
Lacrimal gland	Left												
	Right												
Infiltration, mononuclear cell	Left												
	Right												
Liver (left lobe)	Left												
	Right												
Aggregation, cytoplasmic; sinusoidal cells	Left												
	Right												
Congestion	Left												
	Right												
Degeneration, vacuolar; hepatocyte	Left												
	Right												
Fibrosis, periductal; around gall bladder	Left												
	Right												
Fibrosis, periductal	Left												
	Right												
Hyperplasia	Left												
	Right												
Bile duct epithelium around gall bladder	Left												
	Right												
Bile duct epithelium	Left												
	Right												
Hypertrophy/hyperplasia, sinusoidal cells	Left												
	Right												
Infiltration, mononuclear cell, multifocal	Left												
	Right												
Pigment, brown, cytoplasmic, sinusoidal	Left												
	Right												
Vacuolation, cytoplasmic, hepatocyte	Left												
	Right												
Vacuolation, cytoplasmic, sinusoidal cells	Left												
	Right												

	Sex		Males				Females					
	Group	No. of animals	1	2	3	4	4	1	2	3	4	4
Liver (right lobe)												
Aggregation, cytoplasmic, sinusoidal cells		1										
Congestion		4										
Degeneration, vacuolar, hepatocyte					2/1	3/3,3	1/3	1/2				1/4
Fibrosis, periductal; around gall bladder						1/1	1/1					
Fibrosis, periductal												
Hyperplasia						2/2						
Bile duct epithelium												
Hypertrophy/hyperplasia, sinusoidal cells				2/1	1/1	4/1,5			1/1	1/1	1/4	
Infiltration, mononuclear cell, multifocal					2/1	3/3,3			1/2	2/1	3/2	
Pigment, brown, cytoplasmic, sinusoidal					4/1,3	3/1,3	1/1	1/1	4/1,3	1/1	1/4	2/1
Vacuolation, cytoplasmic, hepatocyte					1/1	4/1,8				4/1	2/1,5	
Vacuolation, cytoplasmic, sinusoidal cells						3/3,3			2/1	1/1	1/4	1/1
Gall bladder												
Infiltration, mononuclear cell, submucosal			3/1	1/1	2/1	1/1		1/1		1/1	3/1	
Lung												
Congestion							1/4					
Edema							1/3					
Infiltration, lymphocytic, bronchial/peribronchial							1/1					
Infiltration, neutrophilic, intra-alveolar							1/1					
Inflammation, interstitial, focal/multifocal							1/2					
Pigment, anthraco-silicotic, multifocal			4/1	4/1	4/1,3	4/1	1/1	1/1		1/1	1/1	
Lymph node, mandibular								1/1				
Hematopoiesis, extramedullary								1/3				1/2
Pancreas												
Atrophy, acinar cell								1/2				
Degeneration, acinar cell								1/2				
Inflammation, mixed inflammatory cell, focal								1/3				
Prostate												
Degeneration/regeneration, focal; glandular epithelium												
Inflammation, neutrophilic, focal												
Skeletal muscle												
Degeneration/necrosis; myocyte							1/2					

Sex	Males		Females				
	Group	No. of animals	1	2	3	4	4
Spleen		4	4	4	4	4	4
Deposition, hyaline, white pulp		4	4	4	1*	1	1
Hypocellularity, lymphoid cells; Malpighian corpuscles							
Stomach							
Degeneration/regeneration; gastric glands (fundus)			1/2	1/2	1/1	1/1	1/1
Infiltration, lymphocytic, mucosal (pylorus)					1/1	1/1	1/1
Thymus							
Cyst							
Necrosis, lymphocytic; cortex					1/2		1/2
Thyroid							
Fibrosis, interstitial						1/3	1/2
Hyperplasia, follicular cells						1/3	1/2
Infiltration, mononuclear cell							
Tongue							
Granuloma							1/1
Trachea							
Infiltration, mononuclear cells							1/1

* Male #40: pre-schedule moribund sacrifice on Day 59.

Severity of findings: 1: very slight, 2: slight, 3: moderate, 4: marked, P: non-graded change.

Numbers in the parentheses: the number of animals actually examined

Adequate Battery: yes (x) (note: no recovery data except uterus), no ()—explain

Peer review: yes (x), no ()

Comments:

✧ The main target organ was liver and bile duct. The findings did not resolve completely.

Toxicokinetics:

The C_{max} and AUC_{0-24hr} , dose-normalized parameters and T_{max} (hr) were summarized in the tables below:

Males:

Group	Day 1			Day 27			Day 90		
	G2	G3	G4	G2	G3	G4	G2	G3	G4
Dose (mg/kg/d)	30	200	600	30	200	600	30	200	600
C_{max} (ng/mL)	340	728	855	559	1590	3250	644	2920	2800
$C_{max}/dose$	11.3	3.6	1.4	18.6	8.0	5.4	21.5	14.6	4.7
AUC (ng x h/mL)	3600	8240	9580	7430	20100	52500	8980	34600	35800
AUC/dose	120	41.2	16	248	101	87.5	299	173	59.7
t_{max} (hr)	1.8	2	2	4	3	4	4	4	3

Group	Day 181			Day 272		
	G2	G3	G4	G2	G3	G4
Dose (mg/kg/d)	30	200	600	30	200	600
C_{max} (ng/mL)	585	1330	1250	632	1370	1630
$C_{max}/dose$	19.5	6.7	2.1	21.1	6.9	2.7
AUC (ng x h/mL)	8460	18000	17900	9770	19900	26200
AUC/dose	282	90	29.8	326	99.5	43.7
t_{max} (hr)	3	3	4	5	4	6

Females:

Group	Day 1			Day 27			Day 90		
	G2	G3	G4	G2	G3	G4	G2	G3	G4
Dose (mg/kg/d)	30	200	600	30	200	600	30	200	600
C_{max} (ng/mL)	419	742	924	585	1670	2530	702	1680	1800
$C_{max}/dose$	14	3.7	1.5	19.5	8.4	4.2	23.4	8.4	3
AUC (ng x h/mL)	5530	7870	10600	9380	15900	30300	9690	24400	22600
AUC/dose	184	39.4	17.7	312	79.5	50.5	323	122	37.7
t_{max} (hr)	3	1.5	3	6	3	3	1.8	4	2.8

Group	Day 181			Day 272		
	G2	G3	G4	G2	G3	G4
Dose (mg/kg/d)	30	200	600	30	200	600
C_{max} (ng/mL)	701	768	1740	756	965	1830
$C_{max}/dose$	23.4	3.8	2.9	25.2	4.8	3.1
AUC (ng x h/mL)	10100	12100	25300	11600	14900	24400
AUC/dose	337	60.5	42.2	387	74.5	40.7
t_{max} (hr)	4	4	6	1.8	3.8	3.8

Comments:

- ✧ There was a less than unity increase in dose normalized C_{max} and AUC through the dosing period, a sign of saturation of absorption or induction of metabolism. Also, these values increased with the length of treatment indicating accumulation of AMN107.

- ◇ The inter-individual variations of C_{max} , AUC and T_{max} increased with length of treatment (data not shown).
- ◇ There were no detectable levels of AMN107 in the controls. AMN107 was rapidly absorbed. Detectable serum levels of AMN107 were found 0.5 hr after administration (serum concentration-time profile not shown). The T_{max} was 1.5-6 hours.
- ◇ There was no gender difference of the TK profiles.

Study summary and discussion:

See Section 2.6.7 "Toxicology tabulated summary".

- The treatment of AMN107 in monkeys, dosed up to 600 mg/kg/day, induced GI clinical signs (feces changes and emesis), reduced weight/food consumption, hematological (decreased erythroid parameters and increased platelet counts), coagulation (\uparrow APTT in certain males) and clinical chemistry findings (increased ALT and total cholesterol), and the main target organs were liver (bile duct), kidney, pancreas and thyroid.
- The cause of death of the high dose male (#40) subjected to moribund sacrifice was pneumonia, as evidenced by the histopathological findings in the animal. Since no other treated animals exhibited similar findings, pneumonia was not a direct drug effect. Other findings in this monkey also included:
 - ◇ Clinical signs: vomiting (white substance), not eating on the previous day.
 - ◇ Clinical pathology: \uparrow neutrocyte (absolute count: 22,760 neutrophils), also \uparrow liver enzymes (ALT, AST), total bilirubin, creatinine, CPK, BUN, and glucose.
 - ◇ Gross pathology: heart (pale), liver (lobular markings)
 - ◇ Organ weights: \uparrow lung (42.2 gm versus reference range of 8.77-29 gm).
 - ◇ Histopathology: lung (pulmonary congestion and edema, neutrophilic infiltrate, presence of inhaled foreign material), kidney (proximal convoluted tubules: vacuolar degeneration; distal convoluted tubules: degeneration/atrophy, tubular lumens: protein accumulation), liver (centrilobular congestion), heart (subepicardial and epicardial acute hemorrhage), and lymphoid tissues (spleen: hypocellularity, and thymus: atrophy/necrosis).
- The lesions in liver, kidney, pancreas and thyroid partially recovered.
- Fibrosis and mineralization in kidney still existed in the recovery animals. The lesion to thyroid and pancreas appeared to be late-onset.
- AMN107 did not induce remarkable changes in electrocardiography under the study condition.

Histopathology inventory

Study (Duration)	0370146 (4-wk)	0580158 (26-wk)	0370147 (4-wk)	0580157 (39-wk)
Species	Rat	Rat	Dog	Monkey
Adrenals	x*, §	x*, §	x, §	x, §
Aorta	x*	x*	x	x
Bone Marrow	x*	x*	x	x

Study (Duration)	0370146 (4-wk)	0580158 (26-wk)	0370147 (4-wk)	0580157 (39-wk)
Species	Rat	Rat	Dog	Monkey
smear				
Bone (femur)	x*	x*	x	x
Brain	x*, §	x*, §	x, §	x, §
Cecum	x*	x*	x	x
Cervix	x	x	x	
Colon	x*	x*	x	x
Duodenum	x*	x*	x	x
Epididymis	x*, §	x*, §	x, §	x, §
Esophagus	x*	x*	x	x
Eye	x*	x*	x	x
Fallopian tube				
Gall bladder	x*	x*	x	x
Gross lesions	x	x	x	x
Harderian gland	x*	x*		
Heart	x, §	x*, §	x, §	x, §
Ileum	x*	x*	x	x
Injection site				
Jejunum	x*	x*	x	x
Kidneys	x, §	x*, §	x, §	x, §
Lacrimal gland	x*	x*	x	x
Larynx				
Liver	x, §	x*, §	x, §	x, §
Lungs	x*	x*	x	x, §
Lymph nodes, bronchial	x		x	
Lymph nodes mandibular	x	x	x	x
Lymph nodes, mesenteric	x	x	x	x
Mammary Gland	x*		x	x
Nasal cavity				
Optic nerves	x*	x*	x	x
Ovaries	x, §	x*, §	x, §	x, §
Pancreas	x*		x	x
Parathyroid	x*	x*, §	x	
Peripheral nerve				
Pharynx				
Pituitary	x*, §	x*, §	x, §	x, §
Prostate	x*, §	x*, §	x, §	x, §
Rectum	x*	x*	x	x
Salivary gland	x*	x*	x	x, §

Study (Duration)	0370146 (4-wk)	0580158 (26-wk)	0370147 (4-wk)	0580157 (39-wk)
Species	Rat	Rat	Dog	Monkey
Sciatic nerve	x*	x*	x	x
Seminal vesicles		x*		
Skeletal muscle	x*	x*	x	x
Skin	x*	x*	x	x
Spinal cord	x*	x*	x	x
Spleen	x*, §	x*, §	x, §	x, §
Sternum	x*	x*	x	x
Stomach	x*	x*	x	x
Testes	x*, §	x*, §	x, §	x, §
Thymus	x*, §	x*, §	x	x, §
Thyroid	x, §	x*, §	x, §	x, §
Tongue	x*	x*	x	x
Trachea	x*	x*	x	x
Urinary bladder	x*	x*	x	x
Uterus	x, §	x	x, §	x, §
Vagina	x	x*	x	x
Zymbal gland				

x, histopathology performed;

* performed only in Groups 1 and 4 non-recovery animals.

§ organ weight obtained

2.6.6.4 Genetic toxicology

Study title: Mutagenicity test using *Salmonella typhimurium* strains TA98 and TA100

This is a non-GLP study (#0258040), and no raw data were included in the submission to be reviewed. According to the summary, AMN107 at concentration up to 1500 µg/plate (precipitated at 5000 µg/plate) was not mutagenic in Ames test using *Salmonella typhimurium* TA98 and TA100 in the absence and presence of S9.

Study title: Mutagenicity test using *Salmonella typhimurium*

Key study findings:

- AMN107 at concentrations 4-2500 µg/plate, with or without S-9 mix, was not mutagenic in *Salmonella typhimurium* TA98, TA97a, TA100, TA102 and TA1535.

Study no.: #0412001

Volume #, and page #: Electronic module (pharmtox\tox\0412001.pdf)

Conducting laboratory and location: Preclinical Safety Europe, Novartis Pharma AG, Basel, Switzerland.

Date of study initiation: January 21, 2004

GLP compliance: Yes (OECD)

QA reports: yes (x) no ()

Drug, lot #, and % purity: AMN107, Batch # 0351002, Purity: —

Formulation/vehicle: DMSO

Methods:

Strains: *Salmonella typhimurium* TA97a, TA98, TA100, TA1535 and TA102

Dose selection criteria

Basis of dose selection: based on previous experiments whose data were not available in this submission. However, the investigator followed ICH S2 guidance and employed the highest feasible concentration of AMN107 as the highest concentration in the study. AMN107 precipitated on the test plates at ≥ 2500 $\mu\text{g}/\text{plate}$, and no bacteriotoxicity evidence was observed at the highest feasible concentration.

Test agent stability: Stable

Metabolic activation system: Aroclor 1254 induced rat liver microsome S-9 mix

Controls:

Vehicle: DMSO (100 $\mu\text{L}/\text{plate}$ for plate incorporation test and 10 $\mu\text{L}/\text{plate}$ for preincubation test)

Negative controls: vehicle control

Positive controls:

With S-9: TA98, TA100 and TA1535: 2-aminoanthracene (3 $\mu\text{g}/\text{plate}$), TA97a and TA102: 2-aminoanthracene (10 $\mu\text{g}/\text{plate}$), TA98: Benzo[a]pyrene (3 $\mu\text{g}/\text{plate}$)

Without S-9: TA98: 2-nitrofluorene (2 $\mu\text{g}/\text{plate}$), TA 97a: 9-aminoacridine (100 $\mu\text{g}/\text{plate}$), TA100 and TA1535: sodium azide (3.0 $\mu\text{g}/\text{plate}$), TA102: mitomycin C (0.5 $\mu\text{g}/\text{plate}$).

Exposure conditions:

Incubation and sampling times:

- Plate incorporation: 3 days
- Pre-incubation method: Pre-incubation for 20 min, incubation for 3 days

Doses used:

- Experiment 1: 4, 20, 100, 500, 2500 $\mu\text{g}/\text{plate}$.
- Experiment 2: 78.125, 156.25, 312.5, 625, 1250 $\mu\text{g}/\text{plate}$
- Experiment 3: 1250 and 2500 $\mu\text{g}/\text{plate}$ (conducted because no precipitation concentration was reached in Experiment 2)

Study design: Plate incorporation for initial test (Experiment 1); Pre-incubation method for two confirmatory studies (Experiment 2 and 3)

Analysis:

No. of replicates: 3 plates for each test compound concentration

Counting method: automated colony counter or manually (in case of precipitation at highest concentration used 2500 $\mu\text{g}/\text{plate}$).

Result:Study validity:

The study is considered valid, because:

- Tester strain integrity was documented in the report.
- Both negative (vehicle) and positive control data were within the laboratory historical range; except for vehicle control in test strain TA1535 in Experiment 2 in the presence of S9 (see table).
- The mean positive control value (\pm S9-mix) exhibited at least three fold increase over the respective mean vehicle control value for each tester strain, except for TA102. In Experiment 2, mitomycin C induced revertant colonies were only 1.37 fold of the vehicle control. The deviation was small; besides, the other positive control produced expected result. (see table of experiment result, below)
- There was a minimum of three nontoxic dose levels (\leq 50% reduction in mean number of revertants/plate relative to the mean vehicle control value) in each tester strain, both in the absence and presence of S9-mix.

However, all tester strain culture titers (i.e., 10^8 cells/mL) were less than conventionally recommended titers (3×10^8 cells/mL).

Study outcome:

● Experiment 1:

The numbers of revertant colonies/plate at each test compound concentration, and vehicle and positive controls are summarized in the table below:

Treatment	Concentration (μ g/plate)	Revertant colonies/plate (mean \pm SD, n=3)				
Without S-9						
		TA1535	TA97a	TA98	TA100	TA102
DMSO	100 μ L/plate	32 \pm 0	178 \pm 22	36 \pm 1	146 \pm 1	265 \pm 13
AMN107	4	24 \pm 1	179 \pm 16	36 \pm 1	130 \pm 12	264 \pm 13
	20	27 \pm 6	177 \pm 13	37 \pm 1	129 \pm 13	227 \pm 13
	100	25 \pm 5	197 \pm 12	34 \pm 2	140 \pm 3	263 \pm 9
	500	25 \pm 8	179 \pm 3	32 \pm 2	134 \pm 2	243 \pm 13
	2500*	21 \pm 2	197 \pm 1	31 \pm 1	139 \pm 1	285 \pm 25
Positive control	(see above for + controls)	1333 \pm 29	2413 \pm 443	198 \pm 19	862 \pm 1	774 \pm 74
With S-9						
DMSO	100 μ L/plate	24 \pm 1	195 \pm 21	30 \pm 1	123 \pm 6	254 \pm 3
AMN107	4	19 \pm 4	194 \pm 17	32 \pm 1	136 \pm 13	217 \pm 42
	20	23 \pm 9	183 \pm 12	32 \pm 1	142 \pm 9	228 \pm 19
	100	19 \pm 4	181 \pm 4	32 \pm 1	114 \pm 15	211 \pm 25
	500	21 \pm 2	172 \pm 5	32 \pm 1	116 \pm 6	242 \pm 10
	2500*	20 \pm 1	207 \pm 1	29 \pm 1	130 \pm 4	226 \pm 37
Positive control	(see above for + controls)	357 \pm 15	2096 \pm 278	1788 \pm 3 118 \pm 5	1840 \pm 159	1136 \pm 232

*: manual counting because precipitation of AMN107

Two positive controls for TA98 (with S9): the upper value is 2-aminoanthracene (3 μ g/plate) and lower is benzo[a]pyrene (3 μ g/plate). This was similar to Experiment 2 and Experiment 3.

Experiment 2:

Treatment	Concentration ($\mu\text{g}/\text{plate}$)	Revertant colonies/plate (mean \pm SD, n=3)				
Without S-9						
		TA1535	TA97a	TA98	TA100	TA102
DMSO	10 $\mu\text{L}/\text{plate}$	26 \pm 2	220 \pm 2	42 \pm 4	138 \pm 14	270 \pm 5
AMN107	78.125	19 \pm 2	219 \pm 6	40 \pm 9	151 \pm 17	293 \pm 3
	156.25	20 \pm 0	210 \pm 4	40 \pm 1	153 \pm 4	289 \pm 4
	312.5	29 \pm 6	216 \pm 11	28 \pm 1	152 \pm 14	294 \pm 5
	625	30 \pm 1	219 \pm 9	25 \pm 2	142 \pm 13	295 \pm 7
	1250	23 \pm 1	216 \pm 12	37 \pm 2	137 \pm 7	296 \pm 8
Positive control	(see above for + controls)	1132 \pm 97	2687 \pm 428	420 \pm 3	745 \pm 52	371 \pm 7
With S-9						
DMSO	10 $\mu\text{L}/\text{plate}$	31 \pm 1 (a)	217 \pm 9	40 \pm 1	135 \pm 5	223 \pm 1
AMN107	78.125	21 \pm 5	220 \pm 25	38 \pm 1	122 \pm 9	236 \pm 5
	156.25	19 \pm 6	229 \pm 16	38 \pm 1	135 \pm 12	232 \pm 5
	312.5	21 \pm 5	207 \pm 5	38 \pm 0	147 \pm 2	237 \pm 11
	625	30 \pm 1	245 \pm 6	36 \pm 1	140 \pm 9	235 \pm 3
	1250	34 \pm 4	252 \pm 6	37 \pm 1	134 \pm 10	234 \pm 3
Positive control	(see above for + controls)	334 \pm 8	1830 \pm 194	1597 \pm 140 254 \pm 36	1590 \pm 146	2256 \pm 8

(a): higher than the laboratory's historical range (range 10-29)

Experiment 3

Treatment	Concentration ($\mu\text{g}/\text{plate}$)	Revertant colonies/plate (mean \pm SD, n=3)				
Without S-9						
		TA1535	TA97a	TA98	TA100	TA102
DMSO	10 $\mu\text{L}/\text{plate}$	23 \pm 5	205 \pm 8	21 \pm 3	176 \pm 3	209 \pm 2
AMN107	1250	25 \pm 2	193 \pm 21	26 \pm 2	184 \pm 6	216 \pm 2
	2500*	21 \pm 1	189 \pm 1	22 \pm 1	187 \pm 2	213 \pm 3
Positive control	(see above for + controls)	955 \pm 15	1399 \pm 2	284 \pm 1	824 \pm 4	393 \pm 38
With S-9						
DMSO	10 $\mu\text{L}/\text{plate}$	21 \pm 4	215 \pm 6	33 \pm 2	142 \pm 1	222 \pm 1
AMN107	1250	22 \pm 2	219 \pm 7	29 \pm 1	137 \pm 6	233 \pm 1
	2500*	25 \pm 6	223 \pm 2	28 \pm 1	142 \pm 1	233 \pm 2
Positive control	(see above for + controls)	312 \pm 2	1481 \pm 3	1560 \pm 5 181 \pm 1	1253 \pm 5	733 \pm 69

Study title: Chromosome aberration test with cultured human peripheral blood lymphocytes**Key study findings:**

- AMN107 did not show clastogenic potential with or without S9-mix under the conditions of the study.

Study no.: #0412101**Volume #, and page #:** Electronic module (pharmtox\tox\0412101.pdf)**Conducting laboratory and location:** Preclinical Safety Europe, Novartis Pharma AG, Basel, Switzerland.

Date of study initiation: January 7, 2004

GLP compliance: Yes (OECD)

QA reports: yes (x) no ()

Drug, lot #, and % purity: AMN107, Batch # 0351002, Purity: —

Formulation/vehicle: DMSO

Methods:

Cells: Primary human lymphocytes

Dose selection criteria

Basis of dose selection: by examination of the depression of mitotic index during the chromosomal aberration test. A total of five experiments were performed, and only three were valid. The rest of two were excluded due to a high toxicity at all tested doses.

(See below "doses used in the experiments"). Plates treated with a concentration showing cytotoxicity (i.e., a decrease in mitotic index between 30% and 50% of control mitotic indices) were the highest concentration selected for analyses. Additionally, two lower concentrations were selected for analyses.

Dose range finding studies: none.

Test agent stability: Stable

Metabolic activation system: Aroclor 1254 induced rat liver microsome S-9 mix

Controls:

Vehicle: DMSO (100 µL/mL)

Negative (DMSO/RPMI) controls: DMSO diluted with the culture medium RPMI

Note: Untreated controls (RPMI only) were not included in the study.

Positive controls:

With S-9: Cyclophosphamide (CP, Experiment C: 55 µM)

Without S-9: Ethyl methanesulfonate (EMS, Experiment B: 8 mM; Experiment E: 12.1 mM)

Exposure conditions:

Incubation and sampling times:

- Experiments C and E: Pulse treatment 3 hr and recovery time 17 hr without (Exp. E) or with S9 (Exp. C)
- Experiment B: Continuous treatment 20 hr without S9

Doses used in the Experiments:

- Experiment B (without S9): 10.0, 14.3, 20.4, 29.2, 41.8, 59.8, 85.5, 122.3, 174.8 and 250 µg/mL, and cultures treated with concentrations of 10.0, 29.2 and 41.8 µg/mL were analyzed.
- Experiment E (without S9): 5.0, 6.8, 9.3, 12.6, 17.1, 23.3, 31.7, 43.2, 58.8 and 80.0 µg/mL, and 6.8, 12.6 and 17.1 µg/mL were analyzed
- Experiment C (with S9): 5.0, 6.5, 8.3, 10.8, 13.9, 18, 23.2, 30.0, 38.7, and 50.0 µg/mL, and 13.9, 18.0, and 23.2 µg/mL were analyzed.
- There were two other experiments not included in the results, the concentrations were:
Experiment A: 30.0, 38.0, 48.1, 60.8, 77.0, 97.4, 123.3, 156.1, 197.5, and 250 µg/mL (with S9, 3 hr treatment, 17 hr recovery)

Experiment D: 20.0, 27.0, 36.5, 49.3, 66.6, 90.0, 121.6, 164.3, 222, and 300 $\mu\text{g/mL}$ (without S9, 3 hr treatment, 17 hr recovery). In this experiment, AMN107 precipitated at concentrations $\geq 90.0 \mu\text{g/mL}$.

Study design: Counting the % cells with chromosome aberration in metaphase

- Only structural aberrations were counted. Numerical aberrations were not determined by this protocol, but the occurrence of polyploidy or endoreduplication were scored and used as a potential indication for numerical aberration.
- Cytotoxicity was based on mitotic index (% of mitotic cells within the total population of mitotic and non-mitotic cells)
- Statistics: A statistical analysis was not performed, because aberration values did not exceed the historical control range.

Analysis:

No. of replicates: Duplicate cultures for each test compound concentration, vehicle and positive controls.

Counting method:

- Observation under the microscope. The following structural aberrations were recorded: chromatid breaks (deletions), isolocus breaks, chromosome breaks, all forms of chromatid exchanges, decentrics, tracentrics, ring chromosomes and interstitial deletions; but did not include cells with only gaps.
- Cells with more than five aberrations were recorded as multiple aberrant cells.
- Cell with chromosome counts ≥ 69 were classified as polyploids.
- For test compound, 200 metaphases (100/code, 2 codes for each concentration) were analyzed for each concentration; for positive controls, 50 metaphases were analyzed.

Assay acceptance criteria:

- There were at least 160 analyzable metaphases located from slides at each concentrations selected for metaphase analysis.

Result:

Study validity:

The study is considered valid, because:

- There were at least 160 (~200) analyzable metaphases located from slides at each concentrations selected for metaphase analysis.
- There was an apparent increase (no statistical analysis) in percent aberrant cells in the positive control relative to the solvent control in each assay, with or without S9.
- The vehicle control data were within the laboratory historical range.
- The high concentrations selected for analysis had mitotic index in the range between 30-50% of the corresponding vehicle control.

Study outcome:

● Experiment B and E (without S9)

Treatment	Concentration (µg/mL)	Mean MI* (% of negative control)**	Aberrant cells (mean %)	Exchange cells (mean %)	% polyploidy cells
Experiment B					
DMSO/RPMI	100 µL/mL	7.4* (100)**	2.0	0.0	0.0
AMN107	10.0	70.1**	2.5	0.0	2.0
	14.3	66.7**	ND	ND	ND
	20.4	44.9**	ND	ND	ND
	29.2	51.7**	2.5	0.0	4.3
	41.8	46.9**	2.5	0.0	2.0
	59.8	44.9**	ND	ND	ND
	85.5	36.1**	ND	ND	ND
	122.3p	34.7**	ND	ND	ND
	178.4p	23.1**	ND	ND	ND
	250.0p	20.4**	ND	ND	ND
EMS	8 mM	2.2*	46.0	6.0	0.0
Experiment E					
DMSO/RPMI	100 µL/mL	5.1* (100)**	2.0	0.0	1.0
AMN107	5.0	104.0**	ND	ND	ND
	6.8	110.9**	2.5	0.0	2.4
	9.3	72.3**	ND	ND	ND
	12.6	73.3**	2.5	0.0	1.0
	17.1	45.5**	1.0	0.0	1.0
	23.3	34.7**	ND	ND	ND
	31.7	24.8**	ND	ND	ND
	43.2	ND	ND	ND	ND
	58.8	ND	ND	ND	ND
	80.0	ND	ND	ND	ND
EMS	12.1 mM	2.3*	40.0	18.0	0.0

**MI: mitotic index; **: mitotic indices as % of the negative control.

P: precipitation occurred.

ND: not determined

● Experiment C (with S9)

Treatment	Concentration (µg/mL)	Mean MI* (% of vehicle control)**	Aberrant cells (mean %)	Exchange cells (mean %)	% polyploidy cells
DMSO/RPMI	100 µL/mL	4.2* (100)**	2.5	0.0	0.5
AMN107	5.0	139.8**	ND	ND	ND
	6.5	120.5**	ND	ND	ND
	8.3	113.3**	ND	ND	ND
	10.8	115.7**	ND	ND	ND
	13.9	102.4**	2.0	0.0	1.5
	18.0	80.7**	3.0	0.0	1.0
	23.2	44.6**	2.5	0.0	2.0
	30.0	48.2**	ND	ND	ND
	38.7	ND	ND	ND	ND
	50.0	ND	ND	ND	ND
CP	55 µM	1.8*	38.0	2.0	2.0

Study title: Oral bone marrow micronucleus test in rats

Key study findings:

- AMN107 was negative in the rat bone marrow micronucleus assay.

Study no.: #0512401

Volume #, and page #: Electronic module (pharmtox\tox\0512401.pdf)

Conducting laboratory and location: Preclinical Safety Europe, Novartis Pharma AG, Basel, Switzerland.

Date of study initiation: January 25, 2005

GLP compliance: Yes (OECD)

QA reports: yes (x) no ()

Drug, lot #, and % purity: AMN107, Batch # 0442004, Purity: —

Formulation/vehicle: 0.5% (w/v) hydroxypropylmethylcellulose (HPMC)

Methods:

Species: Rat/ —:WI (Han)

Dose selection criteria:

The dose selection was based on the dose-range finding study performed according to Mackay and Elliot (Mutat. Res. 271: 97-99, 1992). Orally (gavage) administered AMN107 at doses of twice 1250, and 2000 mg/kg (administration volume: 10 mL/kg and 20 mg/kg, respectively) were given to male and female rats in a dose range-finding study. The following findings were observed after both administrations and at both doses: piloerection, reduced motor activity, hunched posture and decreased body weight. In the 200 mg/kg group, increased startle response was also seen. There were no gender differences in response; thus, only male rats were used in the micronucleus assay.

Dose range finding studies: see above.

Test agent stability: Stable

Metabolic activation system: not applicable

Controls: (not used in the dose range-finding study)

Vehicle: 0.5% (w/v) hydroxypropylmethylcellulose (HPMC)

Negative controls: Vehicle control

Positive controls:

Cyclophosphamide (Endoxan-Lyophilisate, 2 mg/mL in vehicle for lyophilisate [water for injection]): Twice oral gavage administration (24 hr apart) at 10 mL/kg.

Study design:

➤ Rats:

✧ in the dose range-finding: n=3/sex/dose, ~ 7-8 weeks, weights: 160-252 g

✧ in the micronucleus assay: males only, ~7-8 weeks, weight: 209-252 g.

Micronucleus analysis: males only, n=7/dose (n=8 in 2000 mg/kg/d group, with additional 1 rat as the recovery animal); positive control: n=3

➤ Dose schedule: twice at 0, 200, 630, and 2000 mg/kg (as Groups 1, 2, 3 and 4), two doses 24 hour apart. Dosing volume: 20 mL/kg.

➤ Micronucleus assay:

✧ Bone marrow harvest time points: ~ 48 hr after first dosing for negative and positive controls, as well as AMN107 treated rat.

- ✧ Slide analysis: The bone marrow cells collected from one femur of each rat were used to prepare cytospin slides for automatic micronuclei analysis (Romagna, Mytat. Res. 206: 307-309, 1988; Romagna and Staniforth, Mutat. Res. 213: 91-104, 1989). Two slides and one additional reserve slide were prepared for each animal. Cells on slides were stained, and scored for micronuclei and the PCE to NCE cell ratio.
- ✧ Micronucleus frequency (% micronucleated cells) were determined by analyzing 4000 PCEs per animals (2000 PCE/slide x 2)
- ✧ Historical background frequency of micronuclei was not reported.

Assay acceptance criteria:

- Levene’s test for homogeneity of variance was performed on absolute deviations. A one-way ANOVA was performed to detect significant differences between groups. A Dunnett test (heterogenous at the 0.05 level) and a Kruskal Wallis test (heterogenous at the 0.01 level) was used to determine whether the test article and the positive control induced a significant difference from the vehicle control.
- A positive response was the detection of a statistically significant increase in the micronucleated PCEs above the control level.

Result:

Study validity:

The study is considered valid, because:

- Acceptable controls: the vehicle control had less than the published limit of 0.5% (5/1000) PCEs for vehicle control (CRC Handbook of Toxicology, Derelanko and Hollinger Editors) and ratio of PCE/total erythrocytes (PCE+NCE) within the range (0.12-0.85%) for male rats, while the positive control, administered via the same route as the test article, had a statistically significantly higher number of micronucleated PCEs than the vehicle control.
- Acceptable high dose: the high dose used 2 x 2000 mg/kg, was the highest dose of dose range-finding study.

Study outcome:

- ✧ In the 630 mg/kg group, one rat (#42) was found dead at ~22 hr after the second administration. No micronucleus data were available on this rat. The investigator did not discuss the cause of death or any related findings on this animal. Since there were no other deaths, the reviewer considered the finding not dug-related.
- ✧ Micronucleus analysis (mean ± SD) at 48 hr harvest time:

Dose (mg/kg)	0	200	630	2000	Positive control
N	7	7	6	7	3
Frequency (%) Micronucleated PCEs (a)					
48 hr	0.14 ± 0.03	0.11 ± 0.05	0.19 ± 0.06	0.14 ± 0.07	3.64 ± 0.78*
Frequency (%) PCEs (b)					
48 hr	44.0 ± 5.5	49.3 ± 6.5	51.9 ± 7.2*	47.0 ± 11.2	32.9 ± 9.4*

(a) Means of MPCEs x 100 /number of PCEs per animal

(b) Means of PCEs x 100/number of (PCE+NCE)

*: significantly different from the vehicle control

Study title: Comet assay *in vitro* with L5178Y mouse lymphoma cellsSummary:

This was a non-GLP study (Study #0259011, study report date: December 5, 2002) with no original data in the report. The study and result were summarized in the following:

- Test system: Comet assay (to determine the DNA strand-breaking potential) with L5178Y cells (3 hr incubation) in the absence or presence of S9-mix.
- AMN107 concentrations: see tables below.
- Controls:
 - Negative (vehicle) control: DMSO
 - Positive controls: methyl methanesulfonate (MMS 25 µg/mL, -S9), 2-amino anthracene (2-AA 19 µM, +S9)
- Results: tables from the sponsor

Table 1. Summary of experimental results without S9

Experiment 1	DNA damage	Viability (ATP content)	Cells with non- detectable nuclei
Treatment (3 hr, -S9)	Tail Moment	% of control	% cells
RPMI/DMSO	0.13	100	0.0
55.0 µg/ml	0.12	87	3.0
85.0 µg/ml	0.13	79	6.0
120.0 µg/ml	0.15	63	3.0
150.0 µg/ml	0.17	60	11.0
180.0 µg/ml	0.17	52	8.0
210.0 µg/ml*	0.23	52	10.0
MMS, 25 µg/ml	2.14	100	14.0

MMS: methyl methanesulfonate

RPMI: medium

DMSO: dimethyl sulphoxide

Bold indicates positive effect

*Precipitation

Table 2. Summary of experimental results with S9

Experiment 3	DNA damage	Viability (ATP content)	Cytotoxicity
Treatment (3 hr, +S9)	Tail Moment	% of control	% cells with non- detectable nuclei
RPMI/S9/DMSO	0.17	100	0.0
50.0 µg/ml	0.15	50	7.0
85.0 µg/ml	0.19	49	6.0
120.0 µg/ml	0.21	41	5.0
150.0 µg/ml	0.14	43	5.0
210.0 µg/ml*	n.d. [†]	30	n.d.
2-AA, 19 µM	4.08	64	27.0

2-AA: 2-amino anthracene

RPMI: medium

DMSO: dimethyl sulphoxide

n.d. not determined

Bold indicates positive effect[†] Due to high percentage of dead cells (ATP content) the tail moment was not determined.

* Precipitation

- According to the summary table, AMN107 did not increase DNA migration in the comet assay with or without S9-mix. AMN107 thus did not induce DNA damage under the conditions of the study.

Genotoxicity of Impurities:

Study #0358098: Ames test: _____

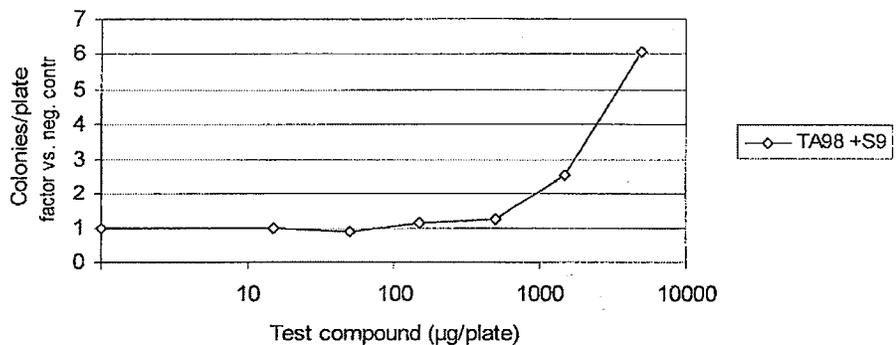
Key study finding: _____ showed a mutagenic potential in TA98 in the presence of metabolic activation.

Summary of the study

- ◇ Non-GLP study
- ◇ Test system (plate incorporation): _____ (batch: #08402DA, _____ #A62809) at 15, 50, 150, 500, 1500 and 5000 µg/plate were incubated with *Salmonella typhimurium* (3 days) in the absence or presence of S9-mix.
Experiment 1: TA100 (± S9)
Experiment 2: TA98 (+ S9)
- ◇ Negative control: DMSO; positive controls were included but not described.
- ◇ No precipitation or signs of bacteriotoxicity were found up to 5000 µg/plate.
- ◇ _____ treatment induced a concentration-dependent increase in the number of revertants in TA98 (with S9) up to 6 fold of the vehicle control.

(Figure from the sponsor)

Study 0358098 (Ames screen)



Study# 0358099: Ames test: _____

Key study finding: _____ was negative for mutagenicity under the conditions of the study.

Summary of the study:

- ◇ Non-GLP study
- ◇ Test system (plate incorporation): _____ (batch: TRD-1058-142-21) at 15, 50, 150, 500, 1500 and 5000 µg/plate were incubated with *Salmonella typhimurium* TA98 and TA100 (3 days) in the absence or presence of S9-mix.

- ◇ Negative control: DMSO; positive control was included but not described.
- ◇ No precipitation was found up to the highest concentration. In TA98 (+S9) and TA100 (-S9), signs of bacteriotoxicity were found at 5000 µg/plate.
- ◇ [REDACTED] was negative for mutagenicity under the conditions of the study.

Study# 0513508: Ames test: [REDACTED]

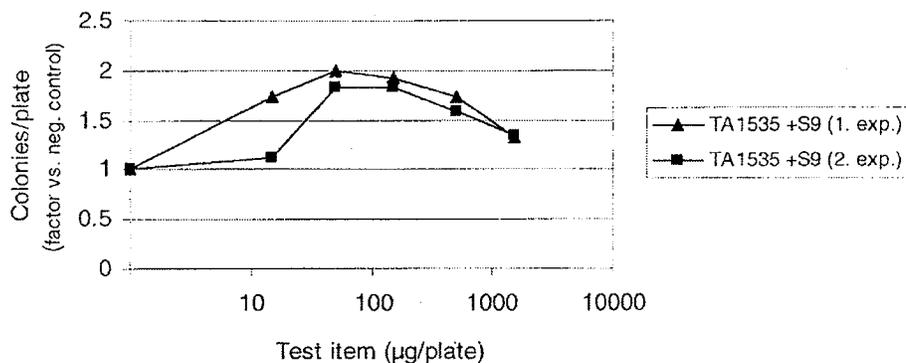
Key study finding: [REDACTED] showed a mutagenic potential (up to 2 fold increase in number of revertants) in TA1535 in the presence of metabolic activation.

Summary of the study:

- ◇ Non-GLP study
- ◇ Test system (plate incorporation): [REDACTED] ([371-03], batch: CHAD0406) at 15, 50, 150, 500, 1500 and 5000 µg/plate were incubated with *Salmonella typhimurium* (3 days) in the absence or presence of S9-mix.
Experiment 1: TA98 (± S9), TA100 (± S9) and TA1535 (± S9)
Experiment 2: TA1535 (+ S9), TA97a and TA102 (± S9)
- ◇ Negative control: DMSO; positive controls (e.g., 2-aminoanthracene) were included but not described.
- ◇ No precipitation was found up to the highest concentration. Signs of bacterio-toxicity were found at 5000 µg/plate (in TA102 at ≥ 500 µg/plate).
- ◇ The mutagenicity effects of [REDACTED] in TA97a, TA98, TA100 and TA102 were not reported in the study.
- ◇ [REDACTED] treatment at 50 mg/plate increased the number of revertants in TA1535 (with S9) up to a maximum factor of 2.

(Figure from the sponsor)

Study 0513508



Study title: Mutagenicity test using *Salmonella typhimurium*

Key study finding:

- [REDACTED] (a synthesis intermediate) at concentrations 4-5000 µg/plate, with or without S-9 mix, was not mutagenic in *Salmonella typhimurium* TA98, TA100 (+S9) and TA1535 (-S9).

Study no.: #0412011

Volume #, and page #: Electronic module (pharmtox\tox\0412011.pdf)

Conducting laboratory and location: Preclinical Safety Europe, Novartis Pharma AG, Basel, Switzerland.

Date of study initiation: November 4, 2004

GLP compliance: Yes (OECD)

QA reports: yes (x) no ()

Drug, lot #, and % purity: [REDACTED] Batch # 20040912
(=CHAD0401), Purity: [REDACTED]

Formulation/vehicle: DMSO

Methods:

Strains: *Salmonella typhimurium* TA97a, TA98, TA100, TA1535 and TA102

Dose selection criteria

Basis of dose selection: The investigator followed ICH S2 guidance and employed 5 mg/plate of [REDACTED] as the highest concentration in the study. [REDACTED] precipitated on the test plates at 5000 µg/plate in Experiment 3. Occasionally, bacteriotoxicity evidence ($\leq 60\%$ of the negative control level in the number of colonies per plate) was observed (at 5000 µg/plate in TA102 without S9-mix, 20% colonies of the control, in Experiment 2).

Dose range finding studies: none.

Test agent stability: Stable (see Study # 04301019)

Metabolic activation system: Aroclor 1254 induced rat liver microsome S-9 mix

Controls:

Vehicle: DMSO (100 µL/plate for plate incorporation test and 10 µL/plate for preincubation test)

Negative controls: vehicle control

Positive controls:

With S-9: TA98, TA100 and TA1535: 2-aminoanthracene (3 µg/plate), TA97a and TA102: 2-aminoanthracene (10 µg/plate), TA98: Benzo[a]pyrene (3 µg/plate)

Without S-9: TA98: 2-nitrofluorene (2 µg/plate), TA 97a: 9-aminoacridine (100 µg/plate), TA100 and TA1535: sodium azide (3.0 µg/plate), TA102: mitomycin C (0.5 µg/plate).

Exposure conditions:

Incubation and sampling times:

➤ Plate incorporation: 3 days

➤ Pre-incubation method: Pre-incubation for 20 min, incubation for 3 days

Doses used:

- Experiment 1: 5, 50, 500, 5000 µg/plate.
- Experiment 2: 8, 40, 200, 1000, 5000 µg/plate
- Experiment 3: 212.5, 425, 850, 1700, 3400 µg/plate*

Note*: The originally selected doses were 312.5, 625, 1250, 2500 and 5000 µg/plate. The actual concentrations were corrected according to the analysis of dose formulation via HPLC. A 32% lower concentration was found with the test solution. The investigator attributed the cause of lower concentration to precipitation of _____ that was observed when the test article was dissolved in the vehicle. However, the investigator did not explain why precipitation did not occur in Experiment 1 and 2.

Study design: Plate incorporation for Experiment 1 and 2); Pre-incubation method for two confirmatory studies (Experiment 3)

Analysis:

No. of replicates: 3 plates for each test compound concentration

Counting method: automated colony counter (_____) or manually (in case of precipitation at highest concentration used 2500 µg/plate).

Criteria of positive results:

- The test article was considered mutagenic if it produced, in at least one concentration and on strain, a response equal to twice (or more) the negative control incidence, with the exception in TA102 (see below).
- The result had greater significance if a concentration-dependent increase in the number of revertant colonies was observed.

Result:**Study validity:**

The study is considered valid, because:

- Tester strain integrity was documented in the report.
- Both negative (vehicle) data were within the laboratory historical range; except for vehicle control in test strain TA1535 in Experiment 2 in the presence of S9 (see table).
- The mean positive control value (\pm S9-mix) exhibited at least three fold increase over the respective mean vehicle control value for each tester strain, except for TA102.
- There was a minimum of three nontoxic dose levels (\leq 50% reduction in mean number of revertants/plate relative to the mean vehicle control value) in each tester strain, both in the absence and presence of S9-mix.

However, all tester strain culture titers (i.e., 10^8 cells/mL) were less than conventionally recommended titers (e.g., 3×10^8 cells/mL).

Study outcome:

- Experiment 1:

The numbers of revertant colonies/plate at each test compound concentration, and vehicle and positive controls are summarized in the table below:

Treatment	Concentration (µg/plate)	Revertant colonies/plate (mean ± SD, n=3)
Without S-9		
		TA100
DMSO	100 µL/plate	120 ± 8
	5	126 ± 17
	50	141 ± 11
	500	130 ± 15
	5000	119 ± 10
Positive control	(see above for + controls)	719 ± 60
With S-9		
DMSO	100 µL/plate	131 ± 12
	5	118 ± 5
	50	140 ± 7
	500	191 ± 7
	5000	198 ± 20
Positive control	(see above for + controls)	1189 ± 140

Experiment 2:

Treatment	Concentration (µg/plate)	Revertant colonies/plate (mean ± SD, n=3)				
Without S-9						
		TA1535	TA97a	TA98	TA100	TA102
DMSO	100 µL/plate	25 ± 4	170 ± 13	26 ± 10	133 ± 8	322 ± 24
Test article	8	23 ± 3	162 ± 11	24 ± 5	136 ± 15	311 ± 14
	40	26 ± 3	175 ± 11	28 ± 3	123 ± 8	291 ± 13
	200	31 ± 9	174 ± 9	26 ± 7	140 ± 14	285 ± 9
	1000	32 ± 8	173 ± 21	27 ± 2	127 ± 11	242 ± 17
	5000	34 ± 10	159 ± 6	33 ± 2	132 ± 12	66 ± 18 (0.2)
Positive control	(see above for + controls)	1089 ± 43	2542 ± 729	175 ± 2	850 ± 31	909 ± 31
With S-9						
DMSO	100 µL/plate	19 ± 8	182 ± 8	43 ± 3	127 ± 8	310 ± 13
Test article	8	15 ± 3	197 ± 9	41 ± 9	118 ± 14	318 ± 22
	40	19 ± 2	200 ± 5	43 ± 8	144 ± 3	266 ± 22
	200	20 ± 7	197 ± 12	43 ± 3	166 ± 5	299 ± 42
	1000	17 ± 1	225 ± 6	42 ± 8	258 ± 13	319 ± 5
	5000	14 ± 2	212 ± 2	43 ± 3	227 ± 9	191 ± 13
Positive control	(see above for + controls)	346 ± 30	2747 ± 342	3407 ± 330 139 ± 18	1236 ± 7	663 ± 150

Two positive controls for TA98 (with S9): the upper line value is 2-aminoanthracene (3 µg/plate) and lower line is benzo[a]pyrene (3 µg/plate)

Number in parenthesis and in bold: fold change in revertant colonies compare to the vehicle control.

Experiment 3

Treatment	Concentration ($\mu\text{g}/\text{plate}$)	Revertant colonies/plate (mean \pm SD, n=3)				
Without S-9						
		TA1535	TA97a	TA98	TA100	TA102
DMSO	10 $\mu\text{L}/\text{plate}$	22 \pm 5	189 \pm 13	27 \pm 4	163 \pm 18	264 \pm 16
Test article	212.5	27 \pm 4	187 \pm 14	40 \pm 2	154 \pm 6	263 \pm 29
	425	26 \pm 10	190 \pm 12	35 \pm 7	166 \pm 23	274 \pm 23
	850	27 \pm 6	181 \pm 10	41 \pm 5	162 \pm 14	213 \pm 12
	1700	38 \pm 5	176 \pm 14	31 \pm 6	168 \pm 20	209 \pm 19
	3400*	45 \pm 5 (2.05)	180 \pm 9	38 \pm 4	171 \pm 19	194 \pm 18
Positive control	(see above for + controls)	1254 \pm 27	2841 \pm 406	158 \pm 12	883 \pm 45	729 \pm 43
With S-9						
DMSO	10 $\mu\text{L}/\text{plate}$	18 \pm 1	196 \pm 31	46 \pm 4	153 \pm 15	301 \pm 48
Test article	212.5	17 \pm 2	224 \pm 8	52 \pm 7	205 \pm 35	312 \pm 15
	425	21 \pm 3	230 \pm 18	59 \pm 6	201 \pm 13	291 \pm 17
	850	21 \pm 4	233 \pm 9	67 \pm 4	214 \pm 6	299 \pm 5
	1700	16 \pm 2	230 \pm 24	58 \pm 9	225 \pm 17	244 \pm 57
	3400*	22 \pm 2	239 \pm 19	49 \pm 8	203 \pm 17	305 \pm 44
Positive control	(see above for + controls)	373 \pm 20	2957 \pm 137	2430 \pm 247 221 \pm 11	1561 \pm 139	522 \pm 61

*: precipitation

Conclusion:

Based on the data, it is indicated that _____ was not positive for mutagenicity (TA100 +S9 and TA1535 –S9) in the Ames *Salmonella typhimurium* test under the conditions of the study. Although two fold increase of mutants in TA102 and TA1535 in comparison to the control were observed occasionally, the findings were not consistent. In the case of TA1535, in the plates with two fold increase of mutants, precipitation of test article was also observed.

Summary of genetic toxicity studies:

- AMN107 was negative in Ames test, *in vitro* chromosome aberration assay (in human peripheral lymphocytes) and micronucleus test in rats.
- Impurities of AMN107 _____ were mutagenic in the Ames test.
- _____

2.6.6.5 Carcinogenicity

Not conducted.

2.6.6.6 Reproductive and developmental toxicology

Fertility and early embryonic development

Study title: An oral (gavage) fertility and early embryonic development study in rats

Key study findings:

- AMN107 induced dose-dependent embryotoxicity, including increases in post-implantation loss and early (total) resorptions and decreased fetal viability.
- AMN107 treatment, up to 180 mg/kg/day, did not affect male or female fertility, mating index or pregnancy in females.
- Decreased fetal viability was observed at ≥ 20 mg/kg.
- Reduction in gestation body weights (5-7%) and weight gains (30-40%) occurred at ≥ 60 mg/kg, while reduced food intake (9-15%) were observed in all treated dams. However the maternal toxicity of AMN107 could not be assessed in this study, since net gestation body weight changes were not corrected for gravid uterine weights.

Study no.: #0570152

Volume #, and page #: Electronic module (pharmtox\tox\0507152.pdf)

Conducting laboratory and location: Novartis, East Hanover Facility, NJ

Date of study initiation: June 17, 2005

GLP compliance: Yes

QA reports: yes (x) no ()

Drug, lot #, and % purity: AMN107, Batch # 0422007, Purity:

Methods

Doses: 0 (control), 20, 60 and 180 mg/kg (as Groups 1, 2, 3 and 4)

Species/strain: IGS Wistar Hannover rats; WI(GlxBRL/Han)IGS BR

Number/sex/group or time point: 25/sex/group

No separate satellite groups used for TK study; all surviving study animals were analyzed for TK.

Route, formulation, volume: oral gavage at dose volume of 10 mL/kg

- **Formulation:** AMN107 suspensions at dose concentrations of 2, 6 and 18 mg/mL for Groups 2, 3, and 4, respectively. (Note: Doses were corrected for percent active AMN107 moiety (99.6%). Salt/base ratio for AMN107 was 1.103.)
- **Vehicle:** 0.5% (w/v) hydroxypropyl-methylcellulose, aqueous solution (0.5% HPMC)

Study design:

Males- dosed once daily for 4 weeks prior to mating, during the 2 week mating period, and until terminal necropsy that was following the completion of the mating period.

Females- dosed once daily for 2 weeks pre-mating period, during mating and through gestation day (GD) 6. Sperm-negative females were sacrificed.

Mating: Animals in each dose group were co-housed one female: one male and mating continued for up to two weeks. Note: Both male and female rats were dosed in this fertility study. The sponsor did not mate dosed male and female rats separately.

Note: Gestation Day (GD) 0: the day when evidence of mating was identified; while Study Day 1 was designated to the day on which AMN107/vehicle treatment started (i.e., two weeks before mating).

Dose justification: dose selection was based on the result of 4 week repeat dose study in rats (#0370146). The main toxicity was weight loss and maternal target organs included kidney, lymph node and thyroid.

Parameters and endpoints:

Clinical signs:	Mortality and moribundity (twice daily), clinical signs (twice daily: predose and ~3 hr postdose).
Body weights:	Males: Twice weekly on Days 1, 4, 8, 11, 15, 18, 22, 25, etc., until Day 50. Females: Twice weekly on Days 1, 4, 8, 11, 15, etc., until mated or sacrificed, and additionally on GD 0, 3, 6, 9 and 13 if pregnant.
Food consumption:	Weekly and additionally on GD 0, 3, 6, 9 and 13 for females.
Vaginal cytology:	Estrous cycle
Gross and histopathology:	At scheduled necropsy. Males following the completion of the mating period, females: GD 13
Toxicokinetics:	On Days 1-2 and Days 14-15 at 0.5, 1, 3, 6, and 24 hr postdose; n=3/sex/group.
Reproductive parameters:	Males: weights of testes and epididymis, sperm counts, and % motile sperm. <i>Note: the sperm motility was determined manually from the videotaped images of sperm samples collected from the vas deferens. The investigator did not report detailed parameters of analysis, such as average path velocity, straight line velocity, curvilinear velocity or straightness, in the study protocol.</i> Females: mating indices, corpora lutea, pregnancy rate, viable fetuses, live fetal sex and weights, early/late reabsorption, pre/post-implantation loss <i>Note: the following categories were listed above but not performed in the study, because the females were sacrificed on GD 13: late resorption, dead fetuses, fetal sexes and fetal body weights.</i>

Statistical analyses: group means were compared against controls by employing the following methods:

- One-way ANOVA followed by Duncan's Multiple range test: body weights and food consumption
- ANOVA followed by Dunnett's t-Test: body weights and food consumption during gestation period and reproductive parameters

- Fisher's Exact Test: maternal examination data and reproductive parameters calculated as percent

Results

Mortality: None

Clinical signs:

Salivation occurred 2 weeks after dosing: data expressed as frequency/# of animals.

Dose (mg/kg)	0	20	60	180
Males	0/0	4/3	18/7	142/14
Females (a)	0/0	0/0	0/0	1/1
Females (b)	0/0	0/0	1/1	5/3

(a): during pre mating, (b): during gestation period.

Body weight:

● Males

- ◇ Body weights: There were statistically significant decreases in group mean body weights in Group 4, starting on Day 11 and through Day 50 when all males were sacrificed. The % decrease in group means in comparison to the control was in the range of 3-8%.
- ◇ Changes in weight gain were summarized in the table below (n=25/group):

Study Day	1-4	4-8	8-11	11-15	15-18	18-22	22-25	25-29	29-32	32-36	36-39
Control	1	7	7	9	5	10	6	9	1	5	5
20 mg/kg	-1	7	7	9	5	8	7	7	1	7	5
60 mg/kg	-2	7	6	7	5	7	6	7	1	5	5
180 mg/kg	-6	4	6	4	6	6	5	6	2	6	3

Statistically significant changes, compared to the control, were indicated by bold numbers.

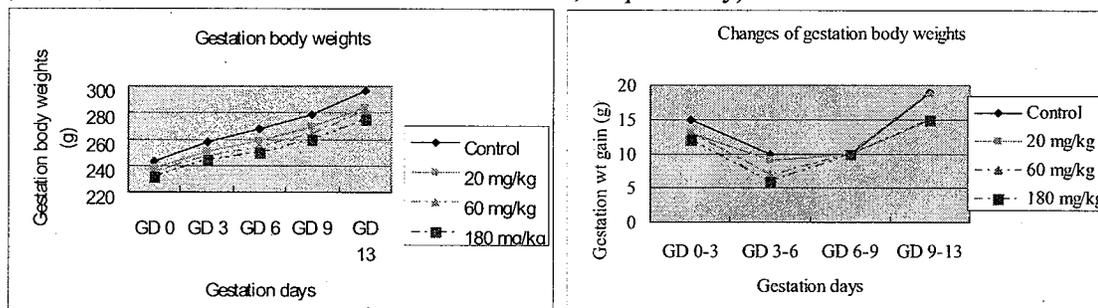
● Females

- Premating and during mating (Study Days 1-29, pregnant females were not included): (Point to discuss)
- ◇ Body weights: By the end of mating period (Day 28) significant reduction in group mean body weights was seen in all AMN107-treated females, with % reduction of 10-11%, 7% and 8%, for Groups 2, 3, and 4, respectively.
- ◇ Changes in weight gain were summarized in the table below (n=25/group or as indicated):

Study Day	1-4	4-8	8-11	11-15	15-18	18-22	22-25	25-29
Control	-3	6	2	3	13; n=8	20; n=2	12; n=2	16; n=1
20 mg/kg	-5	5	2	3	15; n=5	11 ; n=3	8; n=3	-9; n=1
60 mg/kg	-7	4	2	4	7; n=9			
180 mg/kg	-8	4	2	1	8; n=11			

- Dams (gestation body weights):

Decreased group mean body weights were seen in dams in Group 3 (on GD6 and GD9) and Group 4 (through out gestation). Gestation body weights and gestation weight gains are shown below. Dams in Groups 3 and 4 exhibited statistically significant reduction in both gestation body weights (GD 6-13, 5% in Group 3 and 6-7% in Group 4) and weight gains (GD3-6, -30% and -40% relative to the control, respectively).



Note: numbers of pregnant rats: n=21, 23, 21, 22 in Groups 1, 2, 3, and 4, respectively.

Comment:

The gestation body weights were not corrected for gravid uterine weights. Therefore, it was not certain that there were net body weight deficits in the dams, and hence drug-related toxicity in maternal body weights.

Food consumption:

AMN107 induced dose-dependent decreases of food consumption which were observed in both males (Group 4, D1-D22) and females (Groups 3 and 4, D1-D8). The reduction was 8-12% in males and 11-16% in females. During gestation, reduced food consumption was also seen in all groups of females. Data of group mean food consumption (g/animal/day), and % deviation from controls wherever the deviation reached statistical significance are presented in the table below.

Gestation day	GD 0-3	GD 3-6	GD 6-9	GD 9-13
Control	71	80	83	109
20 mg/kg	67	73 (-9%)	79	103
60 mg/kg	68	72 (-10%)	74 (-11%)	109
180 mg/kg	68	68 (-15%)	74 (-11%)	107

Toxicokinetics:

Blood samples collected on prematuring days (on Days 1 and 14) were analyzed using LC-MS/MS system, with the lower limit of quantification (LLOQ) at 5 ng/mL.

The data of means of C_{max} , AUC and T_{max} are summarized in tables below

Day 1:

	C_{max} ($\mu\text{g/mL}$)	AUC _{1-24h} ($\mu\text{g}\cdot\text{hr/mL}$)	$C_{max}/$ dose	AUC ₁₋₂₄ /dose	T_{max} (hr)
Males					
20 mg/kg	2.4	21	0.12	1.04	3
60 mg/kg	4.7	48	0.08	0.81	3
180 mg/kg	6.8	97	0.04	0.54	3

Females					
20 mg/kg	5.3	61	0.27	3.03	3
60 mg/kg	7.0	110	0.12	1.83	3
180 mg/kg	9.4	194	0.05	1.08	3

Day 14:

	C_{max} ($\mu\text{g/mL}$)	AUC_{1-24h} ($\mu\text{g}\cdot\text{hr/mL}$)	$C_{max}/$ dose	AUC_{1-24} /dose	T_{max} (hr)
Males					
20 mg/kg	2.4	31	0.12	1.57	3
60 mg/kg	7.2	99	0.12	1.65	6
180 mg/kg	12	162	0.07	0.9	6
Females					
20 mg/kg	4.9	58	0.24	2.89	3
60 mg/kg	9.1	125	0.15	2.08	6
180 mg/kg	16.9	238	0.09	1.32	3

- The C_{max} and AUC increased dose-dependently, but the increase of dose normalized C_{max} and AUC was less than unity on both Day 1 and Day 14.
- After repeated administration, the dose-normalized C_{max} and AUC on Day 14 appeared greater than those on Day 1.
- Females exhibited a greater systemic exposure than males.

Necropsy:

Gross pathology:

Small renal papilla (kidney) was found in 2/25 males and 1/25 females in Groups 3 and 4.

Fertility parameters (mating/fertility index, corpora lutea, preimplantation loss, etc.):

- Male reproductive parameters:
 - ✧ Testis and epididymal weights: No significant difference was found in testis weights among control and treated groups. However, mean epididymal weight of Group 4 (1.46 g) was significantly smaller than the control (1.58 g).
 - ✧ No remarkable findings in sperm counts, sperm motility or precoital intervals.
- Female reproductive parameters:
 - ✧ Vaginal cytology and mating indices: There were no remarkable findings in estrous cycle or mating indices (precoital intervals).
 - ✧ Fertility/fecundity:
 - Mating parameters: no remarkable effects

	Control	20 mg/kg	60 mg/kg	180 mg/kg
Number of females mated	25	25	25	25
Number positive for mating	24	24	25	25
Number of females pregnant (fertility index %)	21 (87.5)	23 (95.8)	21 (84)	22 (88)
Aborted	0	0	0	0
Premature births	0	0	0	0
Pregnant at C-section (on GD 13)	21	23	21	22
Dams with viable fetuses	21	21	21	21
Dams with no viable fetuses (all absorbed)	0	2	0	1

- Uterine/implantation data: The AMN107 treatment did not induce remarkable changes in corpora lutea or pre-implantation loss, but induced dose-dependent increases in post-implantation loss and early (total) resorptions. Viable fetal numbers were affected accordingly. The data are summarized in the table below:

	Control	20 mg/kg	60 mg/kg	180 mg/kg
Number of females pregnant	21	23	21	22
Corpora lutea				
Total	283	294	268	268
Average/animal (mean)	13.5	12.8	12.8	12.2
Implantation sites				
Total	258	244	234	218
Average/animal (mean)	12.3	10.6	11.1	9.9
Preimplantation loss (%)	8.8	17.0	12.7	18.7
Postimplantation loss (%)	5.4	15.2	24.8	56.4
Resorptions (early resorptions)				
Total	14	37*	58*	123*
% (resorptions/implantation sites x 100%)	5.4	15.2	24.8	56.4
Average/animal (mean)	0.7	1.6	2.8*	5.6*
Viable fetuses				
Total	244	207*	176*	95*
% (viable/total fetuses x 100%)	94.6	84.8	75.2	43.6
Average/animal (mean)	11.6	9.0* (78%)	8.4* (72%)	4.3* (37%)

* Statistically significant. Numbers in the parentheses: % of the control.

Summary of individual study findings:

- AMN107 treatment induced body weight loss (both absolute BW and weight gain) and decreased food consumption in both male and female rats during pre-mating period. Deficits in absolute body weights, weight gains and reduced food consumption were observed in the dams during gestation. However, the gestation body weights were not corrected for gravid uterine weights.
- Other than decreased epididymal weights in Group 4, AMN107 did not induce remarkable effects on male fertility.
- In females, AMN107 did not exhibit remarkable effects on estrous cycle, mating indices and pregnancy.
- Although no changes in corpora lutea or pre-implantation loss, AMN107 induced dose-dependent increases in post-implantation loss and early (total) resorptions. Such effects were observed at ≥ 20 mg/kg. Fetal viability decreased dose-dependently (litter size: 78-37% of the control), starting at ≥ 20 mg/kg.
- AMN107 at highest dose used, 180 mg/kg, did not affect fertility or mating in both sexes.

Embryofetal development

Study title: An oral embryo-fetal development study in rats

Key study findings:

- Dose dependent embryonic toxicity (increased resorption and decreased viable fetuses and litter size) and fetal toxicity (external, visceral and skeletal malformations and/or variations) were observed from 30 mg/kg of AMN107, but most of the findings were at 100 mg/kg.
- Dams treated at 100 mg/kg were associated with maternal toxicities, based on deficits in corrected gestation weight gain (23%) and decrease in food consumption (13% to 19%).
- At 30 mg/kg, embryo-fetal toxicities were observed in the absence of maternal toxicities under the conditions of the study.

Study no.: #0570057**Volume #, and page #:** Electronic module (pharmtox\tox\0507057.pdf)**Conducting laboratory and location:** Novartis, East Hanover Facility, NJ**Date of study initiation:** March 24, 2005**GLP compliance:** Yes**QA reports:** yes (x) no ()**Drug, lot #, and % purity:** AMN107, Batch # 0422007, Purity: —**Methods****Doses:** 0 (control), 10, 30 and 100 mg/kg (as Groups 1, 2, 3 and 4)**Species/strain:** IGS Wistar Hannover rats; —:WI(GlxBRL/Han)IGS BR**Number/sex/group or time point:** 25/group

Satellite groups used for TK study: n=3 (Group 1) or n=5 (Groups 2, 3, and 4).

Route, formulation, volume: oral gavage at dose volume of 5 mL/kg

- Formulation: AMN107 suspensions at dose concentrations of 2.21, 6.62 and 22.06 mg/mL for Groups 2, 3, and 4, respectively. (Note: Doses were corrected for percent active AMN107 moiety (96.5%). Salt/base ratio for AMN107 was 1.103.)
- Vehicle: 0.5% (w/v) hydroxypropyl-methylcellulose, aqueous solution (0.5% HPMC)

Study design: Dated-pregnant females were treated during gestation days (GDs) 6-17. The animals in the main study were sacrificed on GD21, while the TK animals on GD 18. The animals arrived to the lab on GD 1 or 2.

Dose justification: dose selection was based on the result of 2 week and 4 week repeat dose study in rats (— #0370146). The latter is reviewed and the main toxicity was weight loss and target organs included kidney, lymph node and thyroid. No dose justification is necessary, since maternal toxicity and embryo-fetal toxicity was reached in this study.

Parameters and endpoints evaluated:**Clinical signs:** Mortality and moribundity (twice daily), clinical signs (twice daily: predose and ~3 hr postdose; not for TK animals).**Body weights:** Main study animals on GDs 3, 6, 9, 12, 15, 18 and 21

Food consumption:	TK animals on GDs 0, 6, 9, 12 and 15 (data were not reported) Main study animals on GDs 0, 3, 6, 9, 12, 15, 18 and 21; not for TK animals.
Gross pathology:	At scheduled necropsy: major viscera of all main study animals including gross evaluation of placenta
Histopathology:	<u>All organs/tissues were considered normal unless otherwise indicated</u>
Toxicokinetics:	On Day 17 at 1, 3, 7, and 24 hr postdose; n=3/sex/group (data from non-pregnant animals #701 [control] and #711 [10 mg/kg] were excluded).
Cesarean section:	GD 21
Reproductive parameters:	Dams: gravid uterine weight, uterine site description (live fetus, early or late resorption), corpora lutea (main study animals) Fetal examination (live fetuses): weights, sexes, external findings, visceral examination at ~0.5 hr on approximately 50% of the fetuses from each litter, skeletal examination on the rest of 50% fetuses)

Statistical analyses: group means were compared against controls by employing the following methods:

- ANOVA followed by Duncan's t-Test: body weights, food consumption and reproductive parameters
- Fisher's Exact Test: fetal and maternal examination data and reproductive parameters calculated as percent

Results

Mortality (dams): None

Clinical signs (dams): Not remarkable

Body weights (dams) and gravid uterine weights:

- ◇ Body weights: Treatment-related body weight reduction occurred at 100 mg/kg. The % deviations from the control mean group weights were 4%, 8% and 11%, on GD 15, 18 and 21, respectively.
- ◇ Mean group body weights in these dams were still smaller than the control after cessation of AMN107 treatment on GD 17.
- ◇ Weight gains in dams (before C-section): Deficits of weight gains were seen in Group 4.
- ◇ Data of weight gains of dams, gravid uterine weights, carcass weights (GD21 body weight minus gravid uterus weight) and net body weight change of dams (carcass weight on GD21 minus body weight on GD0) are summarized in the table below:

	Control	10 mg/kg	30 mg/kg	100 mg/kg
N (number of gravid females)	25	21	24	25
Mean body weight on GD0 (g)	205	205	203	203
Mean body weight on GD21 (g)	310	315	311	275
Weight gain (g) GD0-GD3	5	5	5	6

GD3-GD6	13	12	13	13
GD6-GD9	7	7	3	0
GD9-GD12	11	12	13	12
GD12-GD15	14	13	11	10
GD15-GD18	24	26	25	13
GD18-GD21	32	35	37	20
Mean total weight gain (g, BW _{GD21} – BW _{GD0})*	105.2	109.3	108.1	72.9
Gravid uterine weights (g) (% deviation from the control)	56.4	64	65.1	34.7 (-38%)
Carcass weights (g) (% deviation from the control)	253.3	250.7	245.6	240.7 (-5%)
Net body weight changes from GD 0 (g)	48.8	45.2	43.0	37.8 (-23%)

Bolded numbers indicated statistically significant changes compared to the control. The numbers in the parentheses represented % reduction from the control (group mean).

* Weight gains in dams without correction with gravid uterine weights.

Food consumption (dams)

Decreased food consumption occurred mainly in Group 4 during GD 6 and GD18; statistically significant decrease was also seen in Group 3 on GD 6-9. The finding resolved after cessation of AMN107 on GD17. Mean food consumption (g) was summarized in the table below.

	Control	10 mg/kg	30 mg/kg	100 mg/kg
GD 3-6	19	18	20	20
GD 6-9	21	20	19 (-10%)	17 (-19%)
GD 9-12	23	22	21	20 (-13%)
GD 12-15	24	23	22	22
GD 15-18	25	24	23	21 (-16%)
GD 18-21	25	24	25	23

Numbers in the parentheses represented % deviation from the control.

Toxicokinetics:

Blood samples collected on GD 17 were analyzed using LC-MS/MS system, with the lower limit of quantification (LLOQ) at 5 ng/mL.

The means of C_{max}, AUC and T_{max} are summarized in the table below:

	C _{max} (µg/mL)	AUC _{1-24h} (µg·hr/mL)	C _{max} / dose	AUC ₁₋₂₄ /dose	T _{max} (hr)
10 mg/kg (n=4)*	2.6	30.4	0.3	3.0	3
30 mg/kg (n=5)	5.3	72.7	0.18	2.4	3.8
100 mg/kg (n=5)	14.6	204	0.15	2.0	6.2

* excluding #711 because this dam was not pregnant.

- The C_{max} and AUC increased dose-dependently, but the increase of dose normalized C_{max} was less than unity. The dose-normalized AUC followed an approximately linear fashion.

Terminal and necropsic evaluations:C-section data (implantation sites, pre- and post-implantation loss, etc.):

There were no AMN107-related necropsy findings.

The AMN107-related reproductive effects were seen at doses ≥ 30 mg/kg wherein post-implantation loss (%) and number of early/total resorption were increased dose-dependently. Further toxicities in the high dose group (100 mg/kg) were demonstrated as significantly increased number of dams with total resorption (no viable fetuses) and decreased total and

average number of viable fetuses. The caesarian and fetal parameters for gravid rats were summarized in the table below:

	Control	10 mg/kg	30 mg/kg	100 mg/kg
Females mated	25	25	25	25
Number of females pregnant (%)	25 (100)	21 (84)	24 (96)	25 (100)
Aborted	0	0	0	0
Premature birth	0	0	0	0
Pregnant at C-section	25	21	24	25
Dams with viable fetuses	25	20	23	20
Dams with all resorption	0	1 (a)	1 (b)	5
Corpora lutea				
Total	245	218	269	257
Average/animal (mean)	9.8	10.4	11.2*	10.3
Implantation sites				
Total	204	190	220	230
Average/animal (mean)	8.2	9.0	9.2	9.2
Preimplantation loss (%)	16.7	12.8	18.2	10.5
Postimplantation loss (%)	5.4	5.3	9.1	55.7
Dead fetuses	0	0	0	0
Total Resorptions				
Total	11	10	20	128*
% (resorptions/implantation sites x 100%)	5.4	5.3	9.1	55.7
Average/animal (mean)	0.4	0.5	0.8	5.1*
Early resorptions				
Total	11	9	17	125*
% (resorptions/implantation sites x 100%)	5.4	4.7	7.7	54.3
Average/animal (mean)	0.4	0.4	0.7	5.0*
Late resorptions				
Total	0	1	3	3
% (resorptions/implantation sites x 100%)	0	0.5	1.4	1.3
Average/animal (mean)	0	0	0.1	0.1
Viable fetuses				
Total	193	180	200	102*
% (viable/implantation sites x 100%)	94.6	94.7	90.9	44.3
Average/animal (mean)	7.7	8.6	8.3	4.1*
Viable male fetuses (%)	97 (50.3)	93 (51.7)	105 (52.5)	47 (46.1)
Live fetal body weight (g) (mean)	5.3	5.3	5.4	5.1
Mean male fetal weight (g)	5.4	5.4	5.5	5.2
Mean female fetal weight (g)	5.0	5.2	5.3*	5.1

*: Statistically significant

(a): # 77 with one implantation site and (b): #147 with two implantation sites; no viable fetuses were found in either dam.

Comment:

- Decreased gravid uterine weight in Group 4 was supported by increased resorption and decreased viable fetuses at this dose level (100 mg/kg).
- Total live fetal weights were decreased in Group 4, but not statistically significant.
- The investigator considered the finding of all resorption in Group 2 (#77) and Group 3 (#147) incidental, because total resorption numbers in these two groups were comparable to the control and the finding was mostly attributable to smaller number of implants in these individuals.

Offspring (malformations, variations, etc.):

The incidence of fetal external/visceral and skeletal malformations and variations was shown in the tables below.

● External malformation and variations:

Group (mg/kg)	Fetus				Litter			
	0	10	30	100	0	10	30	100
Number evaluated	193	180	200	102	25	20	23	20
Malformation								
Cleft palate (%)	0 (0)	0 (0)	0 (0)	1 (1)	0 (0)	0 (0)	0 (0)	1 (5)
Total incidence (%)	0 (0)	0 (0)	0 (0)	1 (1)	0 (0)	0 (0)	0 (0)	1 (5)
Variations								
Skin, pale (%)	0 (0)	0 (0)	0 (0)	2 (2)	0 (0)	0 (0)	0 (0)	2 (10)
Edema (%)	0 (0)	0 (0)	1 (0.5)	5* (4.9)	0 (0)	0 (0)	1 (4.3)	5* (25)
Total incidence (%)	0 (0)	0 (0)	1 (0.5)	7* (6.9)	0 (0)	0 (0)	1 (4.3)	7* (35)

● Visceral malformation and variations:

Group (mg/kg)	Fetus				Litter			
	0	10	30	100	0	10	30	100
Number evaluated	91	84	94	47	23	20	23	18
Variations								
Renal papilla, small (%)	0 (0)	0 (0)	3 (3.2)	2 (4.3)	0 (0)	0 (0)	1 (4.3)	2 (11.1)
Ureter, dilated (%)	0 (0)	0 (0)	0 (0)	1 (2.1)	0 (0)	0 (0)	0 (0)	1 (5.6)
Total incidence (%)	0 (0)	0 (0)	3 (3.2)	2 (4.3)	0 (0)	0 (0)	1 (4.3)	2 (11.1)

Note: Small renal papilla and dilated ureter were found in the same fetus (litter # 05-00203, fetus # 6), and another fetus showed small renal papilla (litter # 05-00165, fetus # 6). Thus, the total incidence in fetus/litter was accounted for these two fetuses.

● Skeletal malformations and variations:

Group (mg/kg)	Fetus				Litter			
	0	10	30	100	0	10	30	100
Number evaluated	102	96	106	55	25	20	23	20
Malformation								
Maxilla, fused (%)	0 (0)	2 (2.1)	4 (3.8)	12* (21.8)	0 (0)	2 (10)	4* (17.4)	10* (50)
Zygomatic, fused (%)	0 (0)	2 (2.1)	4 (3.8)	12* (21.8)	0 (0)	2 (10)	4* (17.4)	10* (50)
Total incidence (%)	0 (0)	2 (2.1)	4 (3.8)	12* (21.8)	0 (0)	2 (10)	4* (17.4)	10* (50)
Variations								
Frontal, incomplete ossification (%)	0 (0)	1 (1)§	3 (2.8)	6* (10.9)	0 (0)	1 (5)§	2 (8.7)	4* (20)
Forepaw phalanx, unossified (%)	2 (2)	2 (2.1)	3 (2.8)	9* (16.4)	2 (8)	2 (10)	3 (10)	7* (35)
Sternebra, misshapen (%)	2 (2)	5 (5.2)	13* (12.3)	29* (52.7)	2 (8)	5 (25)	9* (39.1)	18* (90)
Sternebra, fused (%)	0 (0)	0 (0)	2 (1.9)	21* (38.2)	0 (0)	0 (0)	2 (8.7)	14* (70)
Sternebra, bipartite ossification (%)	0 (0)	0 (0)	1 (0.9)	12* (21.8)	0 (0)	0 (0)	1 (4.3)	8* (40)
Cervical vertebra, incomplete ossification (%)	0 (0)	0 (0)	1 (0.9)	5* (9.1)	0 (0)	0 (0)	1 (4.3)	5* (25)
Hindpaw phalanx, unossified (%)	16 (15.7)	12 (12.5)	21 (19.8)	25* (45.5)	10 (40)	7 (15)	11 (47.8)	15* (75)

Group (mg/kg)	Fetus				Litter			
	0	10	30	100	0	10	30	100
Number evaluated	102	96	106	55	25	20	23	20
Total incidence (%)	17 (16.7)	14 (14.6)	30 (28.3)	49* (89.1)	10 (40)	9 (36)	15* (65.2)	20* (100)

§: incidental and within historical values of the laboratory.

Comments:

- Fetal developmental toxicities, such as edema, small renal papilla and skeletal malformations (fused maxilla/zygoma) and variations (incomplete ossification of the frontals, misshapen sternebra), was observed from 30 mg/kg. Further toxicities in sternebra and delayed, incomplete ossification in cervical vertebra were seen in the high dose groups, and may be accounted for reduced (not statistically significant) fetal weights in this group.
- The comparison between means of net body weight gains (with correction of gravid uterine weights) of dams that gave birth to fetuses with skeletal malformations and the group means of the respective groups is tabulated below:

	Control	10 mg/kg	30 mg/kg	100 mg/kg
Group means (g)	48.8 (n=25)	45.2 (n=21)	43.0 (n=24)	37.8 (n=25)
Means of affected dams		57.6 (n=2)	47.9 (n=4)	40.2 (n=10)

Thus, the dams that gave birth to fetuses with skeletal malformations did not exhibit more maternal toxicity (in term of net weight gains) than their group mates. However, treatment of AMN107 at 100 mg/kg resulted in not only deficits in net weight gain, but also higher fetal and litter incidences of skeletal malformations.

Summary of individual study findings:

- AMN107 treatment induced decreased gestation body weights, net weight changes (from GD0, with correction for gravid uterine weight) and decreased food consumption in Group 4 (100 mg/kg) dams. The gravid uterine weights, net body weight changes (weight gains) and food consumption, were significantly different from the control.
- Both embryonic and fetal toxicities were observed from 30 mg/kg of AMN107. However, most of findings were mainly in Group 4 (100 mg/kg).
 - ✧ AMN107 induced increased number of dams with all resorption (↑ early resorption), increased post implantation loss, decreased total number of viable fetuses as well as decreased litter size (↓ viable fetuses/dam), while it did not affect pregnancy, corpora lutea or implantation sites.
 - ✧ Fetal toxicities included edema, and skeletal malformations (fused maxilla/zygoma) and variations (incomplete ossification of the frontals, misshapen sternebra, and delayed, incomplete ossification in cervical vertebra).
 - ✧ In Group 4, the embryo-fetal toxicity was accompanied with maternal toxicity.

Study title: An oral embryo-fetal development study in rabbits

Key study findings:

- AMN107 induced dose-dependent maternal (deaths, deficits in body weight gain and decreased food consumption), embryonic toxicity (abortion, increased resorption and decreased post implantation sites) and fetal toxicity (skeletal variations). These toxicities were observed at 300 mg/kg.

Study no.: #0570058

Volume #, and page #: Electronic module (pharmtox\tox\0507058.pdf)

Conducting laboratory and location: Novartis, East Hanover Facility, NJ

Date of study initiation: April 15, 2005

GLP compliance: Yes

QA reports: yes (x) no ()

Drug, lot #, and % purity: AMN107, Batch # 0422007, Purity: —

Methods

Doses: 0 (control), 30, 100 and 300 mg/kg (as Groups 1, 2, 3 and 4)

Species/strain: Rabbits/New Zealand White; Hra:(NZW)SPF

Number/sex/group or time point: 20/group

Satellite groups used for TK study: n=3 (Group 1) or n=5 (Groups 2, 3, and 4).

Route, formulation, volume: oral gavage at dose volume of 5 mL/kg

- **Formulation:** AMN107 suspensions at dose concentrations of 6.62, 22.06 and 66.18 mg/mL for Groups 2, 3, and 4, respectively. (Note: Doses were corrected for percent active AMN107 moiety (100.4%). Salt/base ratio for AMN107 was 1.103.)
- **Vehicle:** 0.5% (w/v) hydroxypropyl-methylcellulose aqueous solution (0.5% HPMC)

Study design: Dated-pregnant females were treated during gestation days (GDs) 7-20. The animals in the main study were sacrificed on GD29, while the TK animals on GD 21. The animals arrived to the lab on GD 1 or 2.

Dose justification: dose selection was based on the result of a dose range-finding study in pregnant rabbits —. Oral doses of 50, 150, 300, 400 and 500 mg/kg were administered during GD7-21. Severe maternal toxicity (deceased or no stool, decreased body weight gains (56%, 95% and 113% relative to the control at 150 mg/kg, 300 mg/kg and 400 mg/kg, respectively) and food consumption at ≥ 150 mg/kg) and abortion (2/3, on GD 18 and 21) in 500 mg/kg group led to early termination of treatment at this dose level. Increased resorption and decreased viable fetuses (≥ 400 mg/kg) were the main embryo-fetal findings.

Parameters and endpoints evaluated:

Clinical signs: Mortality and moribundity (twice daily), clinical signs (twice daily: predose and ~3 hr postdose; not for TK animals).

Body weights: Main study animals on GDs 0, 5, 7, 10, 14, 17, 21, 24 and 29
TK animals on GDs 0, 7, 10, 14 and 17 (data were not reported)

Food consumption:	Calculated based on feeder weights collected on GD 5 through GD 29, expressed as g/animal/day for main study animals; not required for TK animals.
Gross pathology:	At scheduled necropsy: major viscera of all main study animals including gross evaluation of placenta
Histopathology:	<u>All organs/tissues were considered normal unless otherwise indicated</u>
Toxicokinetics:	Blood samples collected on Day 20 at 0.5, 1, 2, 6, and 24 hr postdose, n=3/sex/group; also tissue homogenate samples were prepared from live fetuses.
Cesarean section:	GD 29
Reproductive parameters:	Dams: gravid uterine weight, uterine site description (live fetus, early or late resorption), corpora lutea (main study animals) Fetal examination (live fetuses): weights, sexes, external findings, visceral examination and skeletal examination

Statistical analyses: group means were compared against controls by employing the following methods:

- ANOVA followed by Duncan's t-Test: body weights, food consumption and reproductive parameters
- Fisher's Exact Test: fetal and maternal examination data and reproductive parameters calculated as percent

Results

Mortality (dams):

Two Group 4 dams were euthanized moribund: #149 on GD17 and #137 on GD18. These animals were sacrificed due to a severe decrease in food intake (anorexia) and weight loss. Animal #137 aborted on GD18. The deaths were attributed to the test article.

Two other rabbits were sacrificed due to gavage accidents: #91 (100 mg/kg) on GD14 and TK animal #613 (30 mg/kg) on GD21 after blood sampling for TK study.

Clinical signs (dams):

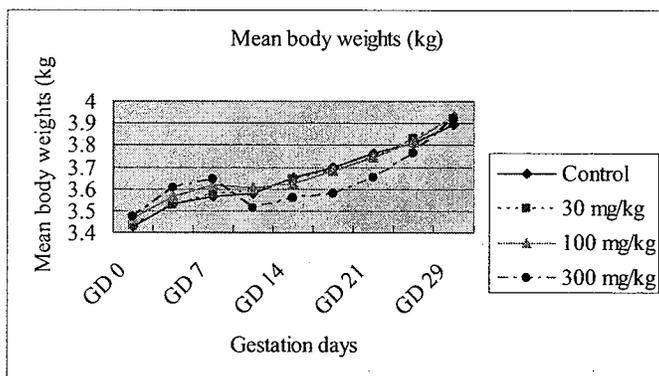
Findings were mainly at doses ≥ 100 mg/kg, including no and/or soft stool, and red stains in the cage (data expressed as frequency/# of animals).

Dose (mg/kg)	100	300
Stool		
Decreased	16/7	39/11
No	0/0	2/2
Soft	6/3	13/6
Red stains in pan	0/0	8/3

Two of three Group 4 animals with red stains in the cage were associated with complete resorption.

Body weights (dams) and gravid uterine weights:

Treatment-related body weight reduction occurred at 300 mg/kg. During treatment (GD7-GD20), there was approximately 3% reduction in mean body weights when compared to the control. Mean group body weights in all AMN107 treated animals rebounded on GD 29 to exceed the control after cessation of AMN107 treatment on GD20.



Deficits of weight gains were also seen in Group 4. Data of weight gains of dams, gravid uterine weights, carcass weights (GD29 body weight minus gravid uterus weight) and net body weight change of dams (carcass weight on GD29 minus body weight on GD0) are summarized in the table below. No remarkable findings were seen for gravid uterine weights or net body weight changes.

	Control	30 mg/kg	100 mg/kg	300 mg/kg
N (number of gravid females)	20	19	18	20
Mean body weight on GD0 (g)	3425	3434	3464	3474
Mean body weight on GD29 (g)	3893	3924	3933	3924
Weight gain (g)				
GD 0-GD5	104	92	99	128
GD 5-GD7	38	49	54	39
GD7-GD10	11	8	-12	-132*
GD10-GD14	72	62	22	48
GD14-GD17	43	39	44	19
GD17-GD21	68	57	56	38
GD21-GD24	43	90*	77	116*
GD24-GD29	87	93	115	159*
Mean total weight gain (g, BW _{GD29} - BW _{GD0})*	467.8	489.6	487.3	453.5
Gravid uterine weights (g)	476.8	535.2	515.9	491.3
Carcass weights (g)	3416	3388.7	3417.4	3432.3
Net body weight changes from GD 0 (g)	-9	-46	-29	-38

Bolded numbers (*) indicated statistically significant changes compared to the control.

#45 (Group 2), #95 and #103 (Group 3) were excluded because they were not pregnant.

*** Weight gains in dams without correction with gravid uterine weights.**

Thus, decreases in gestation body weight gains were mainly seen in Group 4 between GD7-GD10 (statistically significant).

Food consumption (dams)

Statistically significant decreases in food consumption occurred mainly in Group 4 during GD 7 and GD21. Percent decreases from the control ranged from -43% to -17%. There was

a rebound in food consumption in Groups 3 and 4 after cessation of AMD107 administration and this finding supported rebound of body weights.

Comment:

Although significant deficits in weight gain occurred only between GD7-GD10, the reduction in food consumption continued until cessation of AMN107 on GD20 in Group 4. With the moribund sacrifices in both Group 3 (1/20) and Group 4 (2/20), the NOAEL of maternal toxicity was 30 mg/kg.

Toxicokinetics:

Blood samples collected on GD 17 were analyzed using LC-MS/MS system, with the lower limit of quantification (LLOQ) at 2.5 ng/mL for serum and 3 ng/g for fetal tissue homogenate.

	C _{max} (ng/mL)	AUC _{1-24h} (ng·hr/mL)	C _{max} / dose	AUC ₁₋₂₄ /dose	T _{max} (hr)
30 mg/kg (n=4)*	194	2190	6.48	73	3
100 mg/kg (n=5)	494	5980	4.94	59.8	4.4
300 mg/kg (n=5)	1100	17100	3.67	57	8

* excluding #613 because moribund sacrifice. T_{max} of #647 (Group 4) was approximately 24 hr.

Mean fetal tissue concentrations were detectable only in three of the four fetuses in Group 4, the rest of samples were under LLOQ:

Dose (mg/kg)	0 (n=3)	30 (n=4)	100 (n=5)	300 (n=4)
Tissue concentration (ng/g)	0	0	0	28.9

- The fetal tissue homogenate collected from the litter of Dam #647, which had a long T_{max}, was one of the litters with detectable AMN107 in tissues.

Terminal and necropsic evaluations: C-section data (implantation sites, pre- and post-implantation loss, etc.):

Other than the moribund sacrifices, all rabbits survived until caesarian section. There were no AMN107-related necropsy findings in these animals.

The AMN107-related reproductive effects were seen at 300 mg/kg wherein post-implantation loss (%) and number of early/late/total resorption were increased. Two dams at this dose had all resorption. However, the changes did not reach statistical significance. AMN-107 did not have remarkable effects on total and average number of viable fetuses, fetal sex ratio or fetal weights. The caesarian and fetal parameters for gravid rats were summarized in the table below:

	Control	30 mg/kg	100 mg/kg	300 mg/kg
Females mated	20	20	20	20
Number of females pregnant (%)	20 (100)	19 (95)	18 (90)	20 (100)
Mortality	0	0	1	2
Aborted	0	0	0	1
Premature birth	0	0	0	0
Pregnant at C-section	20	19	17	18
Dams with viable fetuses	20	19	17	16
Dams with all resorption	0	0	0	2
Corpora lutea				

	Control	30 mg/kg	100 mg/kg	300 mg/kg
Total	187	202	176	178
Average/animal (mean)	9.4	10.6	10.4	9.9
Implantation sites				
Total	166	180	156	167
Average/animal (mean)	8.3	9.5	9.2	9.3
Preimplantation loss (%)	11.2	10.9	11.4	6.2
Postimplantation loss (%)	7.8	3.9	2.6	12.6
Dead fetuses	0	0	0	0
Total Resorptions				
Total	12	7	4	21
% (resorptions/implantation sites x 100%)	7.2	3.9	2.6	12.6
Average/animal (mean)	0.6	0.4	0.2	1.2
Early resorptions				
Total	10	5	3	16
% (resorptions/implantation sites x 100%)	6	2.8	1.9	9.6
Average/animal (mean)	0.5	0.3	0.2	0.9
Late resorptions				
Total	2	2	1	5
% (resorptions/implantation sites x 100%)	0.2	1.1	0.6	3
Average/animal (mean)	0.1	0.1	0.1	0.3
Viable fetuses				
Total	153	173	152*	146
% (viable/implantation sites x 100%)	92.2	96.1	97.4	87.4
Average/animal (mean)	7.7	9.1	8.9	9.1
Viable male fetuses (%)	72 (47.1)	86 (49.7)	73 (48)	76 (52.1)
Live fetal body weight (g) (mean)	43.6	41.7	40.8	41.9
Mean male fetal weight (g)	44.7	41.5*	40.9*	41.7
Mean female fetal weight (g)	42.7	41.6	40.5	41.5

*: Statistically significant

Comment:

- AMN107 treatment did not affect reproductive competence (i.e., fertility) in females but induced embryotoxicity at 300 mg/kg.

Offspring (malformations, variations, etc.):

There were no AMN107-related external malformations/variations or visceral malformation/variations. Animal #171 (Group 4) had several findings in kidney (absent, malpositioned, and misshapen), gallbladder (absent), and ureter (absent). These findings were considered as spontaneous due to a singular incidence. Other commonly shared visceral variations were small or enlarged gallbladder and small renal papilla (#115 in Group 3 and #167 in Group 4).

There were no treatment related fetal skeletal malformations under the study condition too. However, the incidence (not statistically significant) of several skeletal variations increased in Group 4. The incidence was summarized in the table below:

Group (mg/kg)	Fetus				Litter			
	0	30	100	300	0	30	100	300
Number evaluated	153	173	152	146	20	19	17	16
Variations								
Hyoid, incomplete								

Group (mg/kg)	Fetus				Litter			
	0	30	100	300	0	30	100	300
Number evaluated	153	173	152	146	20	19	17	16
ossification (%)	6 (3.9)	10 (5.8)	9 (5.9)	16* (11)	5 (25)	3 (16)	4 (23.5)	6 (37.5)
Hyoid, bent (%)	8 (5.2)	4 (2.3)	7 (4.6)	11 (7.5)	6 (30)	3 (16)	4 (23.5)	10 (62.5)
Nasal, extra ossification site (%)	0 (0)	0 (0)	2 (1.3)	5* (3.4)	0 (0)	0 (0)	2 (11.8)	3 (18.8)
Sternebra, incomplete ossification (%)	63 (41)	40* (23)	55 (36)	61 (42)	16 (80)	14 (74)	13 (77)	14 (88)
Sternebra, extra ossification site (%)	0 (0)	1 (0.6)	1 (0.7)	5* (3.4)	0 (0)	1 (5.3)	1 (5.9)	2 (12.5)
Rib, supernumerary, short, detached (%)	15 (9.8)	11 (6.4)	13 (6.6)	18 (12)	10 (50)	9 (48)	10 (59)	13 (81)
Rib, cervical (%)	0 (0)	0 (0)	0 (0)	3 (2.1)	0 (0)	0 (0)	0 (0)	1 (6.3)
Cervical centrum, incomplete ossification (%)	0 (0)	0 (0)	0 (0)	1 (0.7)	0 (0)	0 (0)	0 (0)	1 (6.3)
Thoracic centrum, bipartite ossification (%)	0 (0)	0 (0)	0 (0)	1 (0.7)	0 (0)	0 (0)	0 (0)	1 (6.3)
Pubis, incomplete ossification (%)	0 (0)	1 (0.6)	1 (0.7)	2 (1.4)	0 (0)	1 (5.3)	1 (5.9)	1 (6.3)
Hindpaw phalanx, unossified (%)	0 (0)	0 (0)	0 (0)	1 (0.7)	0 (0)	0 (0)	0 (0)	1 (6.3)

Summary of individual study findings:

- AMN107 treatment induced moribund sacrifice in Group 4 (2/20). Statistically significant reduction of weight gain in Group 4 was observed between GD7-GD10. The gravid uterine weights and net body weight changes (from GD0) were not significantly different from the control.
- In addition, significant reduction in food consumption was observed between GD7-GD21 at the dose of 300 mg/kg. The NOAEL for maternal toxicity was 100 mg/kg.
- Decreased food consumption was observed in Group 4. A rebound increase in mean body weights and food consumption were seen after cessation of AMN107 treatment.
- AMN107 induced embryonic and fetal toxicities in Group 4 (300 mg/kg).
 - ◇ AMN107 treatment resulted in abortion in one dam, two dams with all resorption, increased early/late/total resorption, and increased post implantation loss. There were no effects on total number of viable fetuses or litter size (viable fetuses/dam), or fetal sex ratio and fetal weights. It did not affect reproductive functions (female fertility), corpora lutea or implantation sites. The observation of reproductive competence in females was the same as that in rats.
 - ◇ Fetal toxicities were mainly skeletal variations (incomplete ossification of the hyoid, bent hyoid, supernumerary short detached ribs, and additional ossification sites in nasal and sternebra) found in Group 4.
- The NOAEL for embryo-fetal toxicities was 100 mg/kg under conditions of the study.
- The sponsor concluded that there was no evidence of teratogenicity of AMN107 in rabbits under the study condition. The reviewer concurs.

Prenatal and postnatal development

No studies performed.

2.6.6.7 Local tolerance

No studies were reviewed.

2.6.6.8 Special toxicology studies

Study title: UV/vis absorption spectrum for initial phototoxicity assessment

Key study findings: AMN107 significantly absorbed light within the UV-B and –A range that is within the range of natural sunlight.

Study no.: #0517003

Volume #, and page #: Electronic module (pharmtox\tox\0517003.pdf)

Conducting laboratory and location: Department of Safety Europe, Novartis Pharma AG, Basel, Switzerland.

Date of study initiation: March 23, 2005

GLP compliance: Yes (OECD)

QA reports: yes (x) no ()

Drug, lot #, and % purity: AMN107, Batch #AMN107 K CK 15 2004, Purity: —

Formulation/vehicle: DMSO, physiological saline buffer (PBS, pH=7.4) or 0.1 N HCl

Methods:

Concentration:

- Stock solutions: AMN107 in DMSO at 100 mM was sequentially diluted and the diluted stock solutions were measured to receive absorption maxima in the range of 0.5 to 2.5 OD.
- Aqueous solutions in PBS or 0.1 N HCl were prepared from the DMSO stock solutions (1% of a 100x AMN107 in DMSO), to make 100 µM of AMN107 in DMSO/PBS and in DMSO/HCl (DMSO content: 0.1%).

Study design:

- The UV/vis spectra of AMN107 in various solvents were assessed.
- Spectra: UV-B (290-320 nm) and UV-A (320-400 nm) range, and visible light range (400-700 nm)
- Molar absorption coefficient: $\epsilon = A_{\text{absorbance}} / (C_{\text{concentration}} \times d_{\text{path length}})$
- Reference materials: $K_2Cr_2O_7$, holmium glass

Results:

Calculated molar absorption coefficients for absorption maxima or for the limit of 290 nm were summarized (table from the sponsor):

Table 4-1 Calculated molar absorption coefficients (maxima, 290 to 700 nm)

Conditions	Wavelength [nm]	Absorbance	ϵ [l/mol/cm]
100 μ M AMN107 in DMSO	290	~2.1	21000
100 μ M AMN107 in PBS/DMSO 1%	290	~1.2	12000
100 μ M AMN107 in 0.1 N HCl/DMSO 1%	339	0.255	2550
	290	1.2	12000

AMN107 significantly absorbed light within the UV-B and -A range. Strong increase of absorption towards 290 nm was noted, but no discrete absorption maxima could be detected.

Study title: *In vitro* 3T3 NRU phototoxicity assay

Key study findings: AMN107 showed phototoxicity in the *in vitro* 3T3 NRU assay.

Study no.: #0520056 (— #100159)

Volume #, and page #: Electronic module (pharmtox\tox\0520056.pdf)

Conducting laboratory and location: _____

Date of study initiation: July 4, 2005

GLP compliance: Yes (OECD)

QA reports: yes (x) no ()

Drug, lot #, and % purity: AMN107 (salt:base ratio 1.103), Batch #0523025, Purity: _____

Formulation/vehicle: 1% DMSO (v/v) in Earl's balanced salt solution (EBSS)

Methods:

Concentration:

- Maximum concentration according to OECD Guidance 432: 1000 μ g/mL. AMN107-DMSO/EBSS solution precipitated at ≥ 50 μ g/ μ l.
- Concentrations used in pre test (1st valid experiment): 100 to 0.032 μ g/mL, and in the main test (2nd valid experiment): 100 to 0.39 μ g/mL.
- The following was the requirement of the highest test item concentration: pH 6.5-7.8 and osmolality < 10 mM, and also monitoring signs of precipitation.
- Positive control concentrations: chlorpromazine (CPZ) (in EBSS)
 - With irradiation: 316 to 0.032 μ g/mL
 - Without irradiation: 10 to 0.003 μ g/mL

Study design:

- Test system: Balb/c 3T3 cells (clone 31).
- Solutions of AMN107, CPZ or vehicle (1% DMSO) (referred as incubation mixtures) were incubated with the 3T3 cell cultures ($\sim 1 \times 10^4$ cells/well) for 1 hr at 37°C.
- The microtiter plates were irradiated with artificial sunlight (1.7 ± 0.1 mW/cm² UVA resulting in a radiation dose of 4.6 ± 0.3 J/cm² UVA) for 45 min. Or the plates were

incubated in the dark for 45 min (-UV). After removal of the incubation mixtures, the plates were further incubated for 18-22hr.

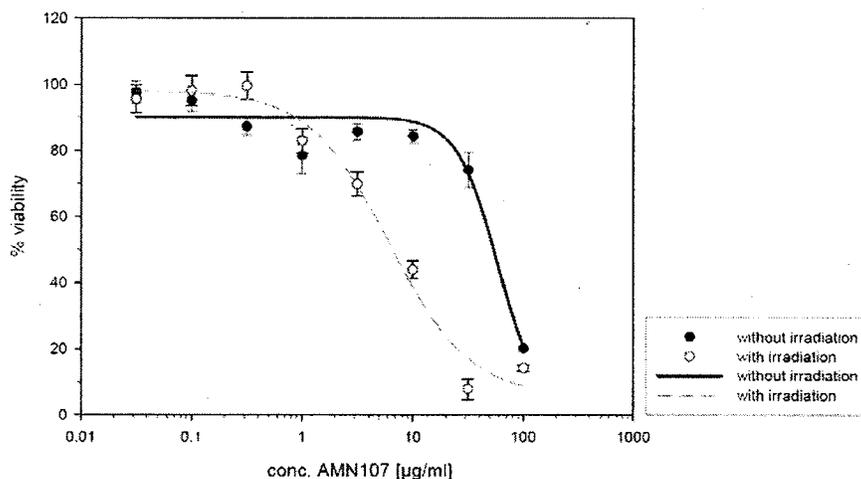
- Measurement of the UVA irradiance: spectral sensitivity (315-400 nm), and measuring range (0-199.9 mW/cm²).
- Reference materials: K₂Cr₂O₇, holmium glass
- Definitions:
 - Viability = mean OD₅₄₀ (sample replicates)-mean OD₅₄₀ (blank replicates)
 - EC₅₀: the concentration reducing cell viability to 50% compared to the untreated controls.
 - RIF (photo irradiation factor)= EC₅₀(-UV)/EC₅₀(+UV);
 - ✓ RIF < 2.0: no phototoxic potential could be predicted
 - ✓ 2.0 < RIF < 5.0: a probable phototoxic potential could be predicted
 - ✓ RIF > 5.0: a phototoxic potential could be predicted
- Acceptance criteria:
 - Positive control CPZ: EC₅₀(+UV): 0.1-3.0 µg/mL, EC₅₀(-UV): 5.0-90.0 µg/mL, RIF ≥ 6.
 - Radiation sensitivity: the viability of the irradiated negative control compared to the non-irradiated negative control (OD₅₄₀ ≥ 0.2) was at least 80%.
 - Deviation within negative controls ≤ 15%.

Results:

In both 1st and 2nd valid experiments, all acceptance criteria were fulfilled.

First valid experiment:

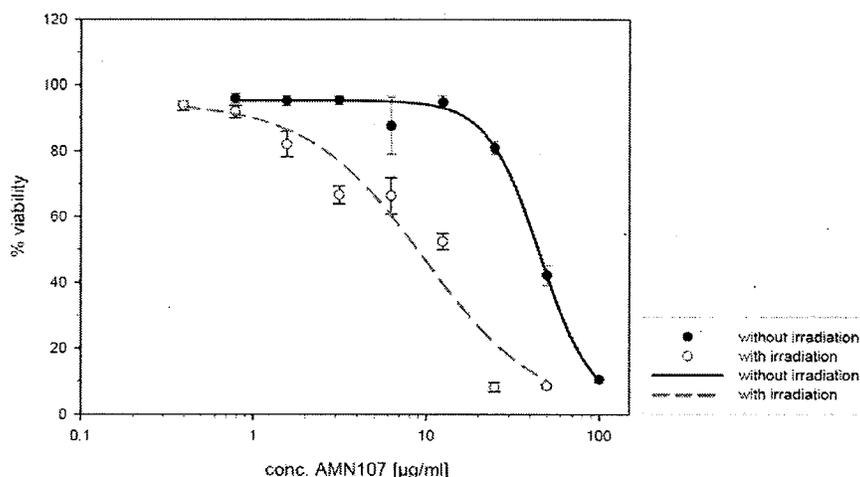
The viability of 3T3 cells was plotted against AMN107 concentrations (figure from the sponsor):



And the resulting parameters: EC₅₀(-UV): 59.2 µg/mL, EC₅₀(+UV): 6.2 µg/mL, RIF 9.6

Second valid experiment:

(Figure from the sponsor)



And the resulting parameters: EC₅₀(-UV): 45.5 µg/mL, EC₅₀(+UV): 9.7 µg/mL, RIF 4.7

Study title: Assessment of photosensitizing potential with the murine local lymph node assay (LLNA tier 1)

Key study findings: AMN107 did not show a photosensitizing potential in the murine UV-LLNA assay.

Study no.: #0517020

Volume #, and page #: Electronic module (pharmtox\tox\0517020.pdf)

Conducting laboratory and location: _____

Date of study initiation: July 28, 2005

GLP compliance: Yes (OECD)

QA reports: yes (x) no ()

Drug, lot #, and % purity: AMN107 (salt:base ratio 1.103), Batch #0523025, Purity: _____

Formulation/vehicle: 0.5% (w/v) hydroxypropylmethylcellulose (0.5% HPMC)

Methods:

Doses:

- AMN107: 50, 150, 400 mg/kg, once daily for three days.
- Positive control: Lomefloxacin HCl (200 mg/kg), once daily for three days.

Study design:

- Test system: Female mice (BALB/CBYJICO)
- Administration route: oral gavage (20 mL/kg).
- Animal allocation:

Group	1	2*	3*	4	5	6	7	8	9	10**
n	6	6	6	6	6	6	6	6	6	2
Dose (mg/kg)	0	200	200	50	50	150	150	400	400	---
UVA	-	-	+	-	+	-	+	-	+	-

* Positive control, ** reserve animals

- Light exposure: Mice in Groups 3, 5, 7, and 9 were exposed to a UVA light dose of $\geq 10\text{J}/\text{cm}^2$ within 75 min after treatment of all animals on each treatment day.
- Study activity and schedule (table from the sponsor):

Major activities	Time point, animals examined
Mortality	At least twice daily during the dosing period.
Clinical signs	At least twice daily during the dosing period.
Body weight	On the first day of treatment before administration and on the day of necropsy.
Toxicokinetic blood sampling	On the day of necropsy, before starting necropsy
Necropsy	One day after treatment period

- TK study: the AMN107 levels in serum and tissues were analyzed by HPLC/MS/MS. LLOQ: 2.5 ng/mL for serum and 2.5 ng/g for tissues
- The following tissues were obtained immediately after animal death:
 - From both ears, circular pieces (diameter of 8 mm) were excised and weighed.
 - Pairs of auricular lymph nodes (LN) were excised, weighed and prepared in suspensions for LN cell counts and lymphocyte phenotyping (CD4/CD8 (T cells), I-A/B220 (B cells), CD4/CD25) via FACS.
 - Left eye: fixed for histopathological examination.
- Data evaluation:
 - Thresholds of test parameters (based on historical data): ear weight index (control =1): 1.05, LN weight: 1.2, and LN cell count: 1.3.
 - Values which exceeded these thresholds were considered positive, if statistical significance or a clear dose-dependence existed.
 - Positive in phototoxicity in LLNA assay (tier 1):
 - ✓ A LN-activation potential (LN hyperplasia, changes in lymphocyte subpopulation) was high in the absence of any ear skin irritation.
 - ✓ LN hyperplasia and ear skin irritation were detected.
 - ✓ The finding of ear skin irritation without any LN hyperplasia.

Results: (Tables from the sponsor)

- No AMN107-related mortality
- Serum and tissue AMN107 levels:
Detectable tissue levels of AMN107 were found in skin and eye.

Table 4-1 Mean concentrations of AMN107 in mouse serum

Group	1	4	5 (UVA)	6	7 (UVA)	8	9 (UVA)
AMN107 dose mg/kg base	0	50	50	150	150	400	400
Mean concentration (ng/mL) (n=6)	0	295	272	300	695*	2560	2870
SD	-	228	213	334	689	1080	1960
CV%	-	77.3	78.3	111	99.1	42.2	68.3

-: not applicable; LLOQ = 2.5ng/mL; *: 4 animals, only

Table 4-2 Mean concentrations of AMN107 in mouse tissues

Group	1	4	5 (UVA)	6	7 (UVA)	8	9 (UVA)
AMN107 dose mg/kg (base)	0	50	50	150	150	400	400
Mean AMN107 concentration in skin (ng/g)	0.433**	-	-	-	-	638	1850
SD	-	-	-	-	-	205	1580
CV%	-	-	-	-	-	32.1	85.4
Ratio of skin to serum mean levels*	-	-	-	-	-	0.25	0.64
Mean AMN107 concentration in eye (ng/g)	0.708***	-	-	-	-	403	833
SD	-	-	-	-	-	127	569
CV%	-	-	-	-	-	31.5	68.3
Ratio of eye to serum mean levels*	-	-	-	-	-	0.16	0.29

-: not applicable; * based on the assumption that 1g tissue is equivalent to 1 mL serum; **: in one control animal; ***: in two control animals; LLOQ = 2.5ng/g

- **Body weights and tissue information:**

The positive control lomefloxacin showed phototoxic potential in the parameters of ear weight, LN weight and LN cell counts. Lomefloxacin also induced skin erythema on ears and tail following UVA exposure. AMN107 was negative in these parameters.

Table 4-3 Summary of results

Group	Substance	b.w. gain [g] (mean ± SD)	Ear weight index	LN weight index	Cell count index
1	Placebo	0.60±0.49	1.00 [21.02]	1.00 [5.21]	1.00 [11.67]
2	200mg/kg Lomefloxacin	0.28±0.64	0.99	0.94	1.01
3	200mg/kg Lomefloxacin / UVA	-0.18±0.51	1.59*\$	2.13*\$	2.97*\$
4	50mg/kg AMN107	0.57±0.48	1.01	0.93	1.05
5	50mg/kg AMN107 / UVA	0.23±0.61	1.03	0.95	1.12
6	150mg/kg AMN107	0.35±0.53	1.03	0.87	0.84
7	150mg/kg AMN107 / UVA	0.02±0.75	1.03	0.85	0.98
8	400mg/kg AMN107	0.22±0.68	1.01	0.85	0.89
9	400mg/kg AMN107 / UVA	0.00±0.38	1.05	0.92	1.02
	Threshold concentration	N/A		N/A	

Body weight gain (±SD) was calculated from values of day 4 before necropsy and day 1 before treatment start. Ears in pairs weight (apical area: 0.5cm²), weight and cell count data of LN pairs are represented as mean values (N=6) in relation to the control group 1 (index = 1). *: P<0.05, test groups vs respective vehicle control group 1. Values in brackets show mean values (weight in [mg], cell count in [E+06/LN pair]).

- **Lymphocyte surface markers: not remarkable**

2.6.6.9 Discussion and Conclusions

A full battery of toxicology studies that supported the safety evaluation for the NDA of nilotinib were conducted in *in vitro* systems as well as in rats, rabbits, dogs and monkeys. The target organs of nilotinib are liver, bile duct, gall bladder, kidney, spleen, pancreas and thyroid. Findings of the latter two were seen after longer exposure. The general toxicology studies were conducted in appropriate animal models, following administration route and dosing regimens that adequately addressed safety concerns in human usage.

The rodent and nonrodent species in general did not demonstrate much difference in susceptibility to nilotinib treatment. Prolonged treatment did not identify different target organs either, except thyroid and pancreas in the monkey. There were toxicities attributable to the pharmacological effects of nilotinib, such as hematological findings. The common features of hematological findings in rats and monkeys included suppressed erythroid parameters (RBC, HGB, and Hct) with or without an increase in reticulocyte counts, and increased white blood cell counts (total and differentiated). Literature reports have associated targeting c-Kit and PDGFR and toxicity in hematopoiesis (Griswold *et al.*, Blood 104: 2912-2918, 2004). Increased white counts could be likely the consequences of inflammation, as evidenced by the observation of leukocyte infiltration in multiple organs, and findings in spleen and lymph nodes. Increased platelet counts and prolonged APTT were seen in monkeys. It was noted that there were no remarkable hematological findings in the dog. Also, there were no remarkable histopathological findings in bone/bone marrow in all tested species.

Toxicities associated with lesions in the liver and bile duct, were more noticeable in the non-rodent species dogs and monkeys, but minimal in the rats, according to the dose levels (on the basis of body surface area). However, according to the PK data, there was high distribution of nilotinib radioactivity to the liver and in the bile in rats. The hepatobiliary toxicities included increased levels of ALT, total bilirubin, cholesterol, and triglycerides, increased liver weight, as well as gross and histopathological findings (enlargement, discoloration, Kupffer cell hypertrophy, hyperplasia, vacuolation, proliferation and hyperplasia in bile ducts). There were findings in gall bladder in dogs (increased luminal mucus).

The finding of hyaline droplet formation in the renal proximal tubules was a unique toxicity in male rats. However, such findings were not observed in the chronic (i.e., 26-week) study. Other renal toxicities of nilotinib in dogs and monkeys were tubular cast, basophilia and vacuolation, fibrosis, mineralization and mononuclear cell infiltration. These toxicities were mild and low in incidence. nilotinib treatment generally did not affect electrolyte homeostasis. In the 26 week study in rats, nilotinib decreased plasma chloride levels (at 60 mg/kg) but increased inorganic phosphorus levels (at ≥ 20 mg/kg).

The hematological, hepatic and renal toxicities were also seen in animals treated with imatinib and dasatinib. Like nilotinib, these two tyrosine kinase inhibitors also inhibit Bcr-Abl, c-Kit and PDGF receptors. Other than the link to effects on hematopoiesis, the PDGF inhibition of imatinib was associated with lesions of lymphoid tissues, such as lymphoid atrophy and depletion, and hyperplasia in spleen. In nilotinib treated animals, lymphoid hyperplasia was found in lymph nodes and spleen. However, no remarkable findings were seen in the thymus.

Cardiac and ocular toxicities have been associated with tyrosine kinase inhibitors. There were reports of patients who developed severe congestive heart failure while on imatinib, and a parallel manifestation of left ventricular contractile dysfunction in mice treated with imatinib. Researchers have proposed that imatinib-related toxic cardiomyopathy may be triggered by inhibition of c-Abl, a mechanism that results in mitochondrial abnormalities

(e.g., collapse of the mitochondrial membrane potential, release of cytochrome C, reduced ATP and cell death) (Kerela *et al.*, Nature Medicine 12:908-916, 2006). Other investigators have also reported that inhibition of ErbB2 caused mitochondrial dysfunction in cardiomyocytes, implications for herceptin-induced cardiomyopathy (Grazette *et al.*, J Am Coll Cardio., 44: 2231-2238, 2004). The cardiovascular effects of nilotinib, also an inhibitor of c-Abl, as assessed in the safety pharmacology studies and general toxicology studies, did not show remarkable cardiotoxicity. Nilotinib caused minimal cardio-myopathy in rats ($\geq 120 \text{ mg/m}^2$ for 4 weeks, but no findings in the 26 week study), minimal focal mesothelial cell proliferation, coronary medial hypertrophy and fibrosis in dogs ($\geq 100 \text{ mg/m}^2$ for 4 weeks), and slight hemorrhage in monkeys (7200 mg/m^2). Neither dogs nor monkeys showed remarkable results in electrocardiography, as similarly shown in the negative telemetry test in conscious dogs. However, positive results were obtained in the *in vitro* studies: inhibition of hERG tail currents at IC_{50} of 0.13 μM , possible prolongation in APD, triangulation ($\geq 3 \mu\text{M}$) and instability (beat-to-beat variability, $\geq 18 \mu\text{M}$) in isolated rabbit hearts, and coronary vasoconstriction in rabbit heart and isolated human coronary artery segments. In general, ocular toxicities were not significant in animals treated with nilotinib. In rats, histopathological findings in eyes included anterior chamber exudates, keratitis, retrobulber hemorrhage and inflammation, at low incidence but with moderate severity. The high radioactivity distribution of nilotinib to the uveal duct supports that the eye is one of target organs of nilotinib in rats. Mononuclear cell infiltration was found in eye balls/choroid in male monkeys.

Other minor nilotinib target organs in animals, that also found in imatinib and dasatinib in the laboratories, included lung (focal hemosiderosis, macrophage accumulation, perivascular cuffing in dogs, and interstitial inflammation, edema, leukocytic infiltration in monkeys), and thyroid (fibrosis, hyperplasia, mononuclear cell infiltration). Pancreas (acinar atrophy and degeneration, and inflammation in dogs and monkeys), however, was a unique target to nilotinib treatment. The lesion to thyroid and pancreas appeared to be late-onset in monkeys, and these findings were not shared with the chronic study in rats. In the clinical trial, increased lipase and amylase (pancreas lesion) and hyperthyroidism (or hypothyroidism) have been reported. Nilotinib induced GI-related clinical signs such as salivation, oral discharge, fecal changes (including diarrhea) and emesis, in rats, dogs and monkeys, but no hard evidence of lesions in the GI tract upon histopathological examination. Other than uterine dilation in 26 week study in rats, nilotinib was devoid of toxicities in male or female reproductive organs. Based on the tissue distribution of radio-labeled nilotinib, the compound showed little passage through the blood-brain, or blood-testis barriers, and it was supported by little toxicities found in testis or in the brain.

There are repeated dose toxicity studies which are not reviewed. The results are summarized in the table from the sponsor in Section 2.6.7. There were no additional histopathological findings in these studies. Increased heart weights were seen in rats treated with nilotinib at doses $\geq 20 \text{ mg/kg/day}$ (4 week oral in feed dose range-finding study in rats).

AMN107 was negative in bacterial Ames test, *in vitro* chromosomal aberration test in human peripheral lymphocytes, Comet test and *in vivo* micronucleus test in rats. However, impurity

_____ were positive in the bacterial mutagenicity test. Nilotinib did not affect male or female fertility or pregnant index. Nilotinib demonstrated dose-dependent embryotoxicity in fertility and early embryonic development test in rats, where both parents were treated, and in embryofetal development tests in rats and rabbits, where only the mothers were treated during organogenesis period. In theory, there would be no nilotinib-related embryonic or early fetal toxicity, if untreated F0 female rats have been mated with treated males, since nilotinib was not expected to cause toxicity in male reproductive organs. The lack of male sexual toxicity was supported by the repeated dose toxicity studies as well. Nilotinib increased post-implantation loss and early (and/or total) resorption, as well as decreases in viable fetuses and litter size. When treatment in rats started pre-mating, the embryotoxicity was noted at 120 mg/m² (Study #0570152). Such toxicities started at ≥ 180 mg/m², if treatment started after mating (during gestation period) (Study #0570057). In rats, the embryo-fetal toxicity included edema, and skeletal malformations (fused maxilla and zygomatic) and variations (incomplete ossification of the fontals, misshapen sternbra, delayed and incomplete ossification in cervical vertebra). The embryofetal development study in rabbits, although showing abortion and death in the dam, as well as embryotoxicity (two dams with all resorption, increased early/total resorption and post-implantation loss), the fetotoxicity was milder compared to that in the rats. Only skeletal variations (such as incomplete ossification of the hyoid, bent hyoid, supernumerary short detached ribs) were found. Nilotinib was not teratogenic.

Nilotinib increased light absorption within the range of normal sunlight, and nilotinib showed phototoxicity (RIF 9.6 and 4.7 in 1st and 2nd valid experiment, respectively) in the *in vitro* 3T3 NRU assay. However, AMN107 did not show a photosensitizing potential in the murine UV-LLNA assay. Thus, although pigmented tissues (such as eyes and skin) showed high nilotinib radioactivity distribution, the potential of phototoxicity of nilotinib in humans is not certain.

2.6.6.10 Tables and Figures

See text of review for pertinent tables and figures.

2.6.7 TOXICOLOGY TABULATED SUMMARY

General toxicology

Single Dose Toxicity Studies					
Species	Route	N/sex/ dose	mg/kg	mg/m ²	Significant findings
Rat	IV (bolus or infusion)	2	20	120	Not remarkable
		2	9	54	Not remarkable
Rat	IV bolus	5	9	54	Not remarkable
Repeat Dose Toxicity Studies					
Species	Route	N/sex/ dose	mg/kg/ day	mg/m ² /day	Significant findings
Rat	Oral Daily 4 week	10	60 20 6	360 120 36	360 mg/m ² /d: salivation, ↓ BW (♂): -6% from control, ↑ white counts (total and differentiated), ↑ reticulocytes (♂), ↓ prostate weights, ↑ multiple organ weights, histopathological findings: kidney (hyaline droplet formation in the proximal tubules (♂)), eye (anterior chamber exudates, keratitis, retrobulber

					hemorrhage, inflammation), heart (minimal cardiomyopathy), lymph node (erythrocyte accumulation, hyperplasia, pigment), spleen (lymphoid hyperplasia, congestion). <u>120 mg/m²/d</u> : ↓ prostate weights, ↑ spleen, thymus, thyroid weights, histopathological findings: kidney (hyaline droplet formation in the proximal tubules (♂)), heart (minimal cardiomyopathy), lymph node (erythrocyte accumulation) <u>36 mg/m²/d</u> : kidney: hyaline droplet formation in the proximal tubules (♂)
Rat	Oral Daily 26 week	20	60 20 6	360 120 36	<u>360 mg/m²/d</u> : oral discharge, salivation, ↓ BW (-5-9%), ↓ food intake (♀: -20%), ↓ RBC, HGB, Hct, ↑ reticulocytes, ↑ white counts (total and differentiated), ↑ total cholesterol, TG, ↓ A/G ratio, ↑ multiple organ weights, Histopathological findings: uterus (dilation of uterine body: ↑ diameter and ↓ wall thickness), lymph nodes (hyperplasia, pigment), kidney (dilated tubule, mononuclear cell infiltration), liver (hyperplasia, mononuclear cell infiltration) <u>120 mg/m²/d</u> : oral discharge, salivation, ↑ white counts (total and differentiated), ↑ heart & liver weights
Dog	Oral Daily 4 week	3	45 15 5	900 300 100	<u>900 mg/m²/d</u> : thin appearance, reduced or no feces, dark urine, salivation, emesis (mainly ♀), ↓ BW (♀): -3-30% (not resolved), ↓ food intake (♀): -25-50%, ↑ ALT, ALP, total bilirubin & cholesterol (mainly ♀), bilirubinuria (♀), ↑ liver weights (♂), liver (discoloration, Kupffer cell hypertrophy, hyperplasia), bile duct (inspissation, proliferation), gall bladder (increased luminal mucus, lymphoid hyperplasia), heart (focal mesothelial cell proliferation), lung (focal hemosiderosis, macrophage accumulation, perivascular cuffing), kidney (tubular cast, basophilia & vacuolation, mineralization), pancreas (acinar degranulation, inflammation), spleen (fibrosiderotic nodule, hemorrhage, fibrosis), lymph node (lymphoid hyperplasia, erythrophagocytosis) <u>300 mg/m²/d</u> : liver (discoloration, Kupffer cell hypertrophy, hyperplasia), bile duct (proliferation), gall bladder (brown content and black focus, increased luminal mucus, lymphoid hyperplasia), heart (coronary medial hypertrophy), kidney (tubular cast, basophilia & vacuolation, mineralization, fibrosis), spleen (fibrosiderotic nodule, hemorrhage) <u>100 mg/m²/d</u> : heart (fibrosis), kidney (mineralization, fibrosis)
Monkey	Oral Daily 39 week	4	600 200 30	7200 2400 360	<u>7200 mg/m²/d</u> : moribund sacrifice (1/6♂, due to pneumonia); swollen eyelid, hunched, fecal changes emesis, ↓ BW: -20-30%, ↓ food intake (♂), ↓ RBC, HGB, Hct, ↑ platelets, ↑ APTT, ↑ ALT, total bilirubin & cholesterol, ↑ TG, histopathological findings: liver (enlarged, cytoplasmic aggregation, hypertrophy, pigment, and vacuolation in sinusoidal cells, hepacytic vacuolation, congestion, fibrosis, mononuclear cell infiltration), bile duct (enlarged, hyperplasia), heart (hemorrhage in 1/6 ♂), lung (interstitial inflammation; 1/6 ♂: congestion, edema, WBC infiltration), kidney (tubular basophilia & vacuolar degeneration, mineralization, fibrosis), pancreas (acinar atrophy & degeneration, inflammation), spleen (white pulp: hyaline deposition, lymphoid hypocellularity), thyroid (fibrosis, hyperplasia, mononuclear cell infiltration) <u>2400 mg/m²/d</u> : hunched, fecal changes emesis, ↓ BW: -20-30%, ↓ HGB, Hct, ↑ platelets, ↑ ALT, total bilirubin & TG,

					histopathological findings: liver (cytoplasmic aggregation, hypertrophy, pigment, and vacuolation in sinusoidal cells, hepacytic vacuolation, fibrosis, mononuclear cell infiltration), bile duct (hyperplasia), kidney (tubular protein, mineralization) 360 mg/m ² /d: hunched, fecal changes emesis, liver (fibrosis), bile duct (hyperplasia), kidney (tubular protein, mineralization)
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Non-pivotal general toxicology studies: Table from the sponsor (Section 2.6 Nonclinical summary, 2.6.7 toxicology tabulated summary). These studies were not reviewed in the NDA.

2.6.7.6 Repeated-dose toxicity

Non-pivotal studies

Species/ strain	Method of administration (vehicle/ formulation)	Duration of dosing	Doses (mg/kg)	Gender and no. per group	NOAEL ² (mg/kg)	Noteworthy findings	Study number
Mice OF1	Oral by gavage (10% NMP/90% PEG300)	2 weeks	0, 50, 150, 450	5M (0 mg/kg) 6M (other groups)	No NOEL establi- shed	Severe clinical signs of toxicity at 450 mg/kg with mortality and discontinuation of treatment on day 6; transiently reduced activity and rough coat at 150 mg/kg Liver effects at doses > 50 mg/kg, including elevations in alkaline phosphatase and glucose and hepatocellular hypertrophy; mild elevations in bilirubin at 150 mg/kg Plasma concentrations 24 hours after last dose indicated a more than dose proportional increase with 150 mg/kg (450 mg/kg not evaluated due to early termination)	[02R143]
Mouse CD- 1(ICR)	Oral (feed) admixture with powdered rodent diet	4 weeks	0, 20, 60, 180 base	10/sex per group plus 12/sex per group for toxico- kinetics	Not establi- shed (< 20 mg/kg)	180 mg/kg: Single premature sacrifice (toxicokinetic animal), decreased body weight gain (males, only), dehydration, decreased red blood cell parameters, increases in total, direct and indirect bilirubin and urea, increased incidence of tubular basophilia in kidneys 60 and 20 mg/kg: decreased body weight (males, only), dehydration, decreased red blood cell parameters, increases in total, direct and indirect bilirubin and urea 20 mg/kg: decreased body weight (males, only), dehydration, decreased red blood cell parameters, increases in total, direct and indirect bilirubin	[0580231]
Rat Wl(Glx/ BRL/Han) IGS BR	Oral by gavage, 0.5% (w/v) hydroxyl- propylmethyl- cellulose (0.5% HPMC)	3 and 4 days	0, 50, 250, 500, 750	2/sex/ group or 1/sex/ group (control)	250	500 mg/kg: Reduced feces, red discoloration of mesenteric lymph nodes 750 mg/kg: Slight body weight loss, red discoloration of mesenteric lymph nodes corresponding to hemorrhage within medullary sinuses, chronic inflammation, single cell necrosis of hepatocytes in liver	[0370053]

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Rat ; Wl(Glx/ BRL/Han) IGS BR	Oral by gavage. 0.5% (w/v) hydroxyl- propylmethyl- cellulose (0.5% HPMC)	2 weeks	0, 30, 100, 300	5/sex/ group	Not establi- shed (< 30 mg/kg)	Decreases in body weight and food consumption at all doses. clinical pathology alterations at ≥ 100 mg/kg (increases in total white blood cell and lymphocyte counts, decreases in red blood cell counts, hemoglobin, hematocrit with regenerative responses in red blood cells, increases in bilirubin, cholesterol), hemorrhage, angiectasis, erythrophagocytosis within medullary sinuses with red discoloration of mesenteric lymph nodes, ovarian follicular or luteal cysts, increased ovarian weights at ≥ 30 mg/kg	[0370138]
Rat IGS Wistar Hanover Rat. Wl (Han)	Oral (feed) admixture with powdered rodent diet	4 weeks	0, 20, 60, 180 (base)	5/sex per group	Not establi- shed (< 20 mg/kg)	180 mg/kg: decreased body weight gain and food consumption, increases in urea, adrenal and heart weights, adrenal cortical hypertrophy (both sexes), increased urine volume/decreased specific gravity, increased hyaline droplets in kidneys (males, only), increased liver weights, increases in white blood cell and lymphocyte counts, cholesterol, globulin and sodium (females, only) 60 mg/kg: decreased body weight gain and food consumption, increases in urea, heart weights, adrenal cortical hypertrophy (both sexes), increased adrenal weight and hyaline droplets in kidneys (males, only), increases in white blood cell and lymphocyte counts, cholesterol, globulin and sodium (females, only) 20 mg/kg: increased heart weights and adrenal cortical hypertrophy (both sexes), increased adrenal weights (males, only)	[0580230]
Dog Beagle	Oral (gavage) 0.5% HPMC	3-4 days	0, 100, 300, 600	1/sex/ group	300	600 mg/kg: emesis, feces with apparent compound, slight body weight loss, reduced food consumption, reduced feces, chronic inflammation in liver with hypertrophic Kupffer cells, bile stasis	[0370052]
Dog Beagle	Oral (gavage) 0.5% HPMC 1, 3, 4, 3, 12, 8 mg/mL salt	2 weeks	6, 20, 60 base	1/sex per group in control, low, mid 2/sex in high dose group	20	60 mg/kg: reddened ears, body weight loss, ↑ALT, ↑ALP, ↑ Total bilirubin. Microscopic changes in liver included periportal/sinusoidal inflammatory cell infiltration, Kupffer cell hypertrophy, centrilobular bile stasis	[0370139]
Cynomol- gus monkey Macaca fascicula- ris)	Oral (gavage) 0.5% HPMC	3 (rising dose phase) or 8 days	100, 200, 400, 600	1/sex/ group	400	600 mg/kg: decreased red cell parameters, increased bilirubin concentrations	[0470193]
Cynomol- gus monkey Macaca fascicula- ris)	Oral (gavage) 0.5% HPMC	4 weeks	0, 100, 200, 400, 600	2/sex/ group (600 mg/kg), 1/sex/ group /all other doses)	600	No relevant findings	[0570038]

Footnotes: ⁰¹ = no observed adverse-effect level

Genetic toxicology:

In Vitro Studies			
Study #	System	Concentrations	Results
0412001	Bacterial Ames test: <i>Salmonella typhimurium</i> : TA98, TA97a, TA100, TA102 and TA1535	Experiment #1: 4-2500 µg/plate Experiment #2: 78.125-1250 µg/plate Experiment #3: 1250 and 2500 µg/plate	Negative in mutagenicity. With or without S9-mix
0412101	Chromosome aberration: Human peripheral blood lymphocytes	- S9: 10-250 µg/mL, or 5-80 µg/mL + S9: 5-500 µg/mL	Negative in clastogenicity
0259011	Comet assay: L5178Y mouse lymphoma cells	- S9: 55-210 µg/mL + S9: 50-210 µg/mL	Negative, with or without S9-mix
Impurities			
0358098	Bacterial Ames test: <i>Salmonella typhimurium</i> : TA98	15-5000 µg/plate	Positive in mutagenicity with S9-mix

0358099 —	Bacterial Ames test: <i>Salmonella typhimurium</i> : TA98 and TA100	15-5000 µg/plate	Negative in mutagenicity with or without S9-mix
0513508 —	Bacterial Ames test: <i>Salmonella typhimurium</i> : TA97a, TA98, TA100, TA102 and TA1535	15-5000 µg/plate	Positive in mutagenicity in TA1535 with S9-mix
0412011 /	Bacterial Ames test: <i>Salmonella typhimurium</i> : TA97a, TA98, TA100, TA102 and TA1535	Experiment #1: 5-5000 µg/plate Experiment #2: 8-5000 µg/plate Experiment #3: 212.5 -3400* µg/plate	Negative is mutagenicity with or without S9
<i>In Vivo Study</i>			
512401	Micronucleus assay: Rat bone marrow	Oral doses at 0, 200, 630, 2000 mg/kg , twice (24 hr apart) ➤Dose range-finding: n=3/sex/dose ➤Micronucleus assay: males only, n=7/dose	Negative

* Precipitation

Reproductive toxicology:

Study	Route	Duration	Dose (mg/kg/d)	Results
Rat (0570152)	PO	Males for 4 weeks prior to mating, 2-week mating, and until euthanized. Females for 2 week prior to mating and then up until GD 13.	20, 60 and 180 (Or, 120, 360 and 1080 mg/m ²)	<ul style="list-style-type: none"> ➤ Parental toxicity: ↓ body weight (including gestation body weight) and food consumption. ➤ No effects on fertility in both genders, mating index or pregnancy in females. ➤ Induced dose-dependent embryotoxicity: ↑ post-implantation loss, early (total) resorption, and ↓ fetal viability. ➤ NOAEL for fetal viability: 20 mg/kg/d
Rat (0570057)	PO	Females dosed GD 6-17 inclusive	10, 30, and 100 (Or, 60, 180 and 600 mg/m ²)	<ul style="list-style-type: none"> ➤ Maternal toxicity: ↓ gestation body weights, food consumption, and gravid uterus weights. ➤ Dose-dependent embryonic toxicity (≥ 30 mg/kg): ↑ resorption, ↓ viable fetuses and litter size. ➤ Dose-dependent fetal toxicity (≥ 30 mg/kg/d): visceral and skeletal malformations and variations. ➤ NOAEL for maternal and fetal: 10 mg/kg/d
Rabbit (0570058)	PO	Females dosed GD 7-20 inclusive	30, 100 and 300 (Or, 360, 1200 and 3600 mg/m ²)	<ul style="list-style-type: none"> ➤ Maternal toxicity: death, ↓ gestation body weights and food consumption. ➤ Dose-dependent embryonic toxicity (mainly 300 mg/kg): abortion, ↑ resorption, ↓ post-implantation sites. ➤ Fetal toxicity (mainly 300 mg/kg/d): skeletal variations. ➤ NOAEL for maternal toxicity: 30 mg/kg/d, and embryo-fetal toxicity: 100 mg/kg/d

OVERALL CONCLUSIONS AND RECOMMENDATIONS

Conclusions:

The non-clinical studies of AMN107 (nilotinib) identified the target organs/tissues of toxicity to be liver, bile duct, gall bladder, kidney, heart, lung, spleen, thyroid and pancreas. AMN107 demonstrated potentially pro-arrhythmic, as evidenced by inhibition of hERG current, prolongation of APD, and induction of triangulation and beat-to-beat variability in the *in vitro* assay systems. It is not mutagenic or clastogenic; but is of teratogenic potential because it induced dose-dependent fetal toxicities in rats. AMN107 is both an inducer and inhibitor of CYP isoenzymes.

Unresolved toxicology issues (if any): None

Recommendations:

Recommend that nilotinib (Tasigna) is approvable, with the preclinical data adequately addressing the non-clinical safety requirements.

Suggested labeling:

A separate review for labeling will be conducted.

Signatures (optional):

Reviewer Signature _____

Supervisor Signature _____ Concurrence Yes ___ No ___

APPENDIX/ATTACHMENTS

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/s/

Shwu-Luan Lee
8/21/2007 04:43:41 PM
PHARMACOLOGIST

John Leighton
8/27/2007 11:17:59 AM
PHARMACOLOGIST